MEASURING RESPONSE TO PAIN MITIGATION
FOR OVARIECTOMY IN \textit{Bos taurus} YEARLING BEEF HEIFERS

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By

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ABSTRACT

Modern society is concerned about the humane treatment of food animals. Pain mitigation is desirable and expected when surgical procedures are performed on cattle. More than a million *Bos taurus* and *Bos indicus* female cattle are surgically ovariectomized (spayed) annually in areas where extensive grazing management is practiced around the world, most commonly using the dropped ovary technique (DOT) which accesses the ovaries via a colpotomy (trans-vaginal) approach. This procedure prevents estrus cycling and pregnancy in cull females that will end up fattened for meat production rather than breeding stock. Producers can realize financial benefits, and heifers may experience better welfare when they are not pregnant on arrival at the feedlot. However, no analgesia for bovine ovariectomy has been described in the literature.

The purpose of this research was to design an effective pain mitigation protocol for ovariectomy. Analgesic drugs that were easily administered, rapidly effective and did not cause recumbency were essential to minimize stress and enable administration while heifers were in the handling system for ovariectomy. Two experiments were performed, each using an injectable combination of the analgesic drugs butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg) and ketamine (0.04 mg/kg) (BXK) that was administered by intramuscular injection while heifers waited in the chute race and the oral anti-inflammatory meloxicam (1 mg/kg) that was administered in the squeeze chute where ovariectomy was performed. To determine the efficacy of the protocol, behavioural (experiments 1 and 2) and physiological (experiment 2) data were collected. The objectives were to 1) determine the degree of stress and pain that ovariectomy causes in yearling beef heifers, 2) to determine whether BXK administered 5 min prior to ovariectomy could mitigate the procedural and immediate post-operative pain of ovariectomy, 3) to determine whether oral meloxicam administered at the time of ovariectomy could mitigate the post-surgical inflammatory pain of ovariectomy and 4) whether it was possible to administer the drug protocol in a ranch setting without hindering the spaying process.

Experiment 1 was a randomized controlled clinical trial with a 2 × 2 factorial design that took place on a cattle ranch using 120 yearling beef heifers (302 ± 27 kg) scheduled for ovariectomy. Heifers were weighed, identified with ear tags and had activity data loggers affixed to a hind limb prior to spaying on d -1. Heifers were spayed on d 0 and randomly
allocated to 4 treatment groups: control (no treatment), BXK, meloxicam, or BXK and meloxicam; then observed for the next 5 d. Behavioural data collected included visual analog scale (VAS) scoring and pain-related behaviour recording by observers during ovariectomy, and stride length, gait score, standing/lying behaviour and video recording for feeding duration measurement after ovariectomy. More BXK treated heifers \( (P = 0.002) \) walked after leaving the squeeze chute and lay down for a larger percentage of time \( (P < 0.01) \) at 4 h after ovariectomy compared to those not treated with BXK, and meloxicam treated heifers stood for a larger percentage of time at 7 h \( (P = 0.02) \), 8 h \( (P = 0.001) \) and 9 h \( (P = 0.02) \) after spaying compared to those not treated with meloxicam. On the day of ovariectomy, groups that had BXK \( (P = 0.03) \) and BXK and meloxicam \( (P = 0.002) \) had longer feeding event durations compared to controls. On the day after ovariectomy, heifers treated with meloxicam stood for a smaller percentage of the day \( (P = 0.01) \) and for shorter periods of time \( (P = 0.03) \) compared to heifers not treated with meloxicam. Behavioural measurements provided minimal evidence of pain from ovariectomy and its alleviation.

Experiment 2 took place at a research facility using 45 yearling beef heifers \( (322 \pm 27 \text{ kg}) \) randomly allocated to 3 treatment groups: PALP (control group palpated but not spayed), SPAY (spayed without analgesia) and BXKM (spayed with analgesia). Continuous data was gathered over 7 d using accelerometers for activity and the GrowSafe System for feeding behaviour. Saliva and blood samples were harvested, rectal temperature was taken, and recording of flight speed, stride length and gait occurred on d -1, d 0 (time of palpation/ spaying), and 1, 2, 4, 24, 48, 96 and 168 h after palpation/spaying. Salivary cortisol concentrations were lower in BXKM heifers than SPAY heifers at 1 h \( (P = 0.01) \) and 2 h \( (P = 0.004) \) after ovariectomy. Serum haptoglobin concentrations were lower in BXKM heifers than SPAY heifers at 48 h \( (P = 0.01) \), 96 h \( (P < 0.001) \), and 168 h \( (P = 0.008) \) and lower than PALP heifers at 96 h \( (P < 0.001) \) after ovariectomy. Serum amyloid A concentrations were higher in BXKM heifers than PALP heifers \( (P = 0.04) \) at 1 h after ovariectomy and at 24 h after ovariectomy PALP heifers had lower concentrations than BXKM \( (P = 0.02) \) and SPAY \( (P = 0.05) \) heifers. Rectal temperatures were greater in BXKM heifers than PALP and SPAY heifers at 1 h \( (P < 0.001) \) and 2 h \( (P = 0.004) \) after ovariectomy. Results suggest that ovariectomy is acutely stressful and painful, BXK mitigated the procedural and immediate post-surgical pain of ovariectomy and meloxicam reduced the post-surgical inflammation and inflammatory pain for up to 7 days after ovariectomy.
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I would also like to thank the funding sources that made the clinical trials possible. Frolek Cattle Co. Ltd. supplied their heifers, infrastructure and human resources for the first experiment. The Canada/Alberta Livestock Research Trust funded the purchase of heifers for the second experiment. The British Columbia Cattlemen’s Association allocated funds to the research via the Beef Cattle Industry Development Fund Project #520. The Interprovincial Graduate Student Fund enabled me to devote my time to this research that I hope will improve the welfare of cattle.

Lastly I would like to thank my family and friends for their support and understanding in knowing that I needed to spend some time to try to make a difference in the lives of our beef animals. In Canada, extensively grazed cattle enjoy better welfare than most food animals. But as my husband John says, “we can always do better”.

Thank you all!
DEDICATION

I have two pictures of my late Danish “Farfar” (father’s father) taken about a hundred years ago. In one, he is riding a horned bull and in the other, he has the bull harnessed to a buggy. My father inherited this interest in livestock, vaccinating our ranch calves at branding time until he was 90, and perhaps this is where my interest also originated. My husband and sons are excellent stockmen too – our sons are the fifth generation to care for the cattle on the family ranch. I dedicate this manuscript to all of these stockmen in my life – my grandfather Karl Hansen, my father Knud (Ken) Hansen, my husband John, and our sons Adam, Erik and Ian.
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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Worldwide, there are over a million feeder heifers surgically ovariectomized every year (Williams & Page, 2014); however, no pain mitigation protocols for this procedure have been published to date. Current social conscience and professional veterinary obligation dictates that pain mitigation should be provided for elective surgical procedures. Most heifers destined for ovariectomy are extensively grazed rather than intensively managed, and therefore are more likely to find human contact stressful (Grandin, 1997). Consequently, a reasonably priced pain control treatment administered at the time of surgery without increasing handling time is more humane for the heifers and more likely to be adopted by producers.

Immediate procedural pain is induced when performing an ovariectomy, and the resulting tissue trauma induces inflammatory pain that diminishes as healing progresses. A pain mitigation strategy needs to target both procedural and inflammatory pain to be effective. The most commonly used technique for ovariectomy employs a colpotomy (trans-vaginal) approach that triggers visceral neural pain pathways that are different from pain pathways stimulated by surgery involving somatic tissues. Behavioural and physiological measurements of response to analgesia provide evidence for veterinarians, researchers and producers to make science-based decisions regarding provision of pain mitigation. The processes that produce pain and the mode of action of pharmaceuticals in reducing pain are important to understand in order to develop an appropriate analgesic protocol.

In this chapter, the literature is reviewed for the reasons and techniques for ovariectomizing beef heifers, the animal welfare issues associated with the procedure, the physiology of pain, the methods of measuring pain and response to analgesia, and analgesic pharmaceuticals. Chapter 2 describes a study of response to analgesia for pain of ovariectomy using behavioural measurements in a ranch setting. Chapter 3 describes a study of response to analgesia using both behavioural and physiological measurements.

The objectives of this research were to document the degree to which heifers experience pain of ovariectomy, to measure the effectiveness of a combination of butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg) and ketamine (0.04 mg/kg) in providing analgesia for procedural and
immediate post-surgical pain of ovariectomy, and to measure the effectiveness of using oral meloxicam (1 mg/kg) for post-procedural analgesia by reducing inflammation after ovariectomy.

1.2 Terminology

The terms ovariectomy and ovariotomy are used synonymously to describe the surgical removal of the ovaries. “Ovariectomy” is defined as “excision of an ovary” (Blood, Studdert, & Gay, 2007) and “surgical removal of one or both ovaries; oophorectomy” (Martin 2015). “Ovariotomy” is defined as “surgical removal of an ovary, or removal of an ovarian tumor” (Blood, Studdert, & Gay, 2007), and “literally, incision of an ovary. However, the term commonly refers to surgical removal of an ovary (oophorectomy)” (Martin, 2015). Using either term implies that the ovaries are surgically removed from the body. Ovaries can be removed via surgical approaches either through a left flank laparotomy or a trans-vaginal colpotomy. Using the colpotomy approach requires directing any one of three specially designed instruments through the dorsal vaginal fornix into the abdominal cavity. The Willis ovariotome is the most commonly used colpotomy instrument, but rather than removing the ovary it severs the ovarian pedicle and allows each ovary to drop into the abdomen where it undergoes ischemic necrosis. The term “ovariotomy” seems fitting for this technique because the ovaries remain in the body; however, the term “ovariectomy” is used to describe a laparoscopic surgery in weanling fillies in which their ovaries are dropped intra-abdominally rather than removed from the body (Shoemaker et al., 2004).

Veterinarians and producers both use the term “spay” to refer to the ovariectomy procedure in cattle. An ovariectomized heifer is called a spayed heifer, or a “spay”.

In view of the common usage and evolution of the procedure, the terms “ovariectomy” and “spay” are both used to describe the ovariectomy technique performed on heifers in this thesis.

1.3 Global Prevalence of Ovariectomy in Cattle

The number of cattle ovariectomized annually is difficult to determine as to date no surveys have been published with this information. Generally, heifers are spayed in areas that practice extensive grazing management. The World Organization for Animal Health (Organisation mondiale de la santé animale, OIE) defines extensive beef cattle production systems as those “where cattle have the freedom to roam outdoors, and where the cattle have
some autonomy over diet selection (through grazing), water consumption and access to shelter” (OIE; Terrestrial Animal Health Standards Commission, 2017).

In Canada, spaying is more common in British Columbia in the interior plateau area, where a few thousand animals may be spayed per year depending on market conditions (Kamloops Large Animal Veterinary Clinic Ltd., personal communication). An on-line livestock auction offers and advertises spayed heifers for sale, however their total sales database does not distinguish between spayed and intact heifers, so numbers sold through this system are not retrievable (The Electronic Auction Market (TEAM), Calgary, personal communication).

Globally, the U.S.A. and Australia spay the most heifers. A Nebraska veterinarian spayed over 49,000 heifers in 2017 and knows of other veterinarians on the north central plains and in the western states that spay an undetermined number (Daryl Meyer DVM, personal communication). An Australian review of cattle management procedures estimated that one million heifers and cows are spayed annually in that country (Williams & Page, 2014).

South America and southern Africa are other areas where heifers are spayed (Petherick et al., 2013). Although Central America is not mentioned, an ovariectomy instrument designer disclosed that a veterinarian in Panama is using his instrument to teach the procedure to veterinary students (Harry Disney DVM, personal communication). No data could be found for the numbers of animals ovariectomized in these regions.

1.4 Indications for Ovariectomy

Only a proportion of heifer calves born in any given calf crop have the desirable physical traits and temperament to retain for breeding as replacement heifers in the beef cow herd. Those heifers that are not kept for breeding are culls to be marketed for human consumption and are candidates for ovariectomy. Reasons for performing ovariectomy depend upon cattle management and market conditions, and vary with location.

1.4.1 North America

In North America, there are two industry segments that can benefit from ovariectomizing heifers. Ranchers that utilize grazing resources for stockers between weaning and sale to feedlots spay heifers for financial and management benefits. Feedlots acquiring spayed heifers realize benefits during finishing compared to feeding intact heifers (Sharman et al., 2011).

Spaying heifers on the ranch for management purposes has been done for over a century (Sharman et al., 2011). Rupp and Hamilton (1995) published a photograph taken in 1909 in
Wyoming showing heifers suspended by the hind legs and ovariectomy being performed through the ventral midline. On British Columbia ranches, yearling heifers have been spayed for approximately one hundred years. My late father-in-law, Joseph William Lauder, left us a photograph of spaying taken in 1923 showing Guichon Ranch and Douglas Lake Ranch cowboys with heifers tethered on their right sides between sets of two posts in a corral (Figs. 1.1,1.2). One cowboy on horseback roped a heifer by the head and another cowboy roped the hind feet. A front leg was placed in the head loop to prevent asphyxiation. Once restrained and recumbent, a short log was placed under a heifer’s belly to elevate her left flank area where a cowboy surgeon made the incision into the abdomen. There are no clippers or disinfectant visible in the photograph and it is not clear how the incision was closed. Animal welfare considerations were about a half-century in the future. Considering the hard physical work involved and the unknown losses to surgical complications, ranch managers must nevertheless have considered the procedure as financially beneficial.

In modern times, yearling beef heifers are often turned out onto grass before going to feedlots for finishing. In the USA, weaned heifers may be on winter grazing pastures before grazing summer pastures as yearlings (Sharman et al., 2011). On Canadian ranches yearling heifers may be turned out after a winter backgrounding period, either with bulls if they are replacements or as stockers if they are culls. There are both management and financial benefits to ranchers that graze extensively to spay their cull yearling heifers before they are turned out onto grass.

Management benefits include the option to graze spayed heifers with steers or the cowherd, because they are anestrous and are not a distraction to other cattle. This improves pasture management and eliminates the expense for extra fencing and additional bulls. Intact heifers will display estrus every 21 days until they are bred and on extensive range are difficult to fence away from bulls. Bulls diverted from the cowherd or from neighbouring herds to breed cull heifers may negatively impact cow pregnancy rates unless additional bulls are purchased. Fence integrity on rangeland is often compromised by falling trees, fires, wildlife or livestock damage and gates inadvertently left open, therefore intact heifers can often access bulls in adjoining properties. Invariably if cull heifers are left intact, a proportion of them will become pregnant.
Spayed heifers have reduced growth rate and deposit fat earlier than intact heifers, but when implanted, consistently outperform their intact counterparts (Rupp & Hamilton, 1995). A Canadian study demonstrated a 12 kg advantage over 120 days of grazing in implanted spayed heifers when compared to both implanted and non-implanted intact heifers on pasture (Zobell et al., 1993). If 850 pound heifers are sold for $1.75 per pound (Canfax, 2018), a benefit of $26.20 would be realized per head for spayed heifers over intact heifers after considering $20 per head cost for spaying and implanting.

There may also be a financial benefit to ranchers when offering spayed heifers for sale. Feedlot buyers know that compared to steers, intact heifers gain less per day and finish at lighter weights (Zobell et al., 1993), and factor in the cost of estrus suppression and pregnancy management for intact heifers, discounting the price paid to the producer accordingly. A guarantee that heifers will not be pregnant on arrival to the feedlot may boost the price paid to the producer. An economic risk assessment study in the USA determined that $10.99 more per head could be paid for guaranteed non-pregnant heifers (Buhman et al., 2003).

Once in the feedlot, spayed heifers incur lower costs when compared to intact heifers. The steroid progestin analog melengesterol acetate (MGA) is often added to the feed of intact heifers to prevent cycling for more consistent feed intake, better gain and reduced potential bruising and injury from estrous activity (Sides et al., 2009). It is not necessary to add MGA to the feed of spayed heifers because they are in a state of anestrus. Intact heifers also incur the costs of pregnancy diagnosis and abortifacient administration. A proportion of them will require treatment for abortion or calving complications such as retained fetal membranes, prolapsed uterus, obturator nerve paralysis, and caesarian section and are at a higher risk of mortality (Buhman et al., 2003; Rademacher et al., 2015).

Pregnant heifers have lower average daily gain and lower feed efficiency when adjusted for the weight of the pregnancy, which amounted to a mean of 25.4 kg and 34 kg in two studies and a decreased dressing percentage at slaughter compared to non-pregnant heifers (Rademacher et al., 2015). As a result, heifers are generally pregnancy tested and administered a prostaglandin abortifacient if they are pregnant on arrival to the feedlot. A study on economic risk assessment of pregnant feedlot heifers determined that if groups of intact heifers were likely to have pregnancy rates of $\geq 43\%$, the best returns would be realized if the abortifacient was administered to the entire group without determining pregnancy status (Buhman et al., 2003). In one Canadian
study, aborted heifers returned $26.41 more per head than pregnant heifers but heifers that were never pregnant returned $39.94 more per head than aborted heifers and $66.35 more per head than pregnant heifers (Jim et al., 1991). Abortifacients are not 100% effective, with reported failure rates of 5.0 to 7.5% (Rademacher et al., 2015) and heifers retaining their pregnancies would still represent higher risk and potential loss to the feedlot. Spayed implanted heifers could therefore realize the best possible returns for the feedlot compared to intact heifers.

There is also a human cost to feeding intact heifers. Dealing with abortion or calving in the feedlot is stressful for staff and negatively affects morale (Buhman et al., 2003; Rademacher et al., 2015). Losing baby calves is understandably distressing to employees. Calves born in the feedlot have an estimated survivability of 50% (Buhman et al., 2003) because the high stocking intensity and lack of a dedicated calving facility is not favourable to successful neonatal management. There is also a health risk to employees as the prostaglandin abortifacients can be cutaneously absorbed and cause bronchospasm or miscarriage, limiting their handling ideally to non-asthmatic male employees (Rademacher et al., 2015).

Ovariectomy has been investigated as a means of inducing abortion once pregnant heifers are placed in the feedlot but is only a reliable method of abortion if the pregnancy is less than 150 days, and carries a higher mortality risk because of the increased blood supply to the reproductive tract. In a study comparing methods of abortion in feedlot heifers, ovariectomy in four pregnant heifers resulted in termination of both 60-day pregnancies but not in either of the 150-day pregnancies (Horstman et al., 1982). Spaying is therefore most beneficial to the feedlot when done on the ranch to avoid any potential complications from abortion and calving.

Another indication for ovariectomy is to simplify movement of heifers across jurisdictions. In the USA spayed heifers are exempt from the expense of Brucellosis testing when transporting cattle between states (United States Department of Agriculture, 2018). The Canadian Food Inspection Agency (CFIA) provides an exemption for Brucellosis testing of spayed heifers imported into Canada (Canadian Food Inspection Agency, n.d.).

1.4.2 Australia

Australian cattle production involves extensive grazing on large tracts of land especially in the northern part of the continent. It is difficult to gather cattle in such vast expanses that may include wetlands and bulls are therefore difficult to control. Ovariectomizing females is mainly done to prevent pregnancy and improve cull heifer marketability (Petherick et al., 2011).
Spaying increases heifer survival rate, as fewer are lost to dystocia in areas where it is impossible to observe them during calving. Spaying adds value to females, as a large part of the industry relies on exporting live animals. Intact females comprise less than 5% of the live export market because of unwanted disruption from estrus activity and the possibility of calving during transport, so spayed females realize a price premium (de Witte et al., 2006; Niethe & Holmes, 2007).

In Australia aged cull cows are also ovariectomized to prevent pregnancy and lactation. This allows them to fatten on grass before marketing (Niethe & Holmes, 2007), which differs somewhat from North America where cull cows may be fattened in feedlots prior to slaughter.

1.5 Animal Welfare Issues

Two welfare issues surround heifer ovariectomy. The first concern is the pain and stress created by the surgery itself and how best to manage it. The second concern is the welfare of spayed heifers during the grazing and feedlot periods compared to their intact cohorts.

1.5.1 Societal Conscience and Concerns

Globally, animal welfare is the “single most important concern” the public has regarding the cattle industry, and survey results indicate that consumers value it sufficiently to pay a premium for farm animal products indicated to be humanely raised (de Grassi, 2001). The OIE’s 2017 edit of its Terrestrial Animal Health Code lists ovariectomy as a painful husbandry procedure and outlines the following recommendations:

Ovariectomy of heifers is sometimes required to prevent unwanted pregnancies under extensive rangeland conditions. Surgical spaying should be performed by veterinarians or by highly trained operators. Producers should seek guidance from veterinarians on the availability and advisability of analgesia or anaesthesia for spaying of beef cattle. The use of analgesia or anaesthesia should be encouraged.

Retailers have taken notice of food animal welfare concerns and want to assure consumers that they supply humanely sourced products, for example by becoming members of the National Farm Animal Care Council (NFACC) in Canada. The NFACC also has producers, government, animal welfare groups and enforcement as partners, together creating codes of practice such as the Code of Practice for the Care and Handling of Beef Cattle (National Farm Animal Care Council, 2013).

Treating pain in animals is an ethical duty of veterinarians (Gaynor, 2010). Ovariectomy, like any surgical manipulation, causes tissue trauma and therefore some degree of pain and good
welfare practices dictate that this pain should be mitigated. Since 2003 pain has been included as the fourth vital sign after temperature, pulse and respiration by the American Animal Hospital Association (Hellyer et al., 2007). The NFACC Code of Practice (2013) regarding managing pregnant heifers in the feedlot states:

**RECOMMENDED PRACTICES**

- a. prevent pregnancy in heifers destined for feedlots. If possible, inform feedlot buyers if there is a chance that heifers have been exposed to a bull
- b. consult a veterinarian if considering spaying to prevent pregnancy in heifers destined for the feedlot. Spaying is a very infrequent practice; however, if done, it should be carried out by a veterinarian using appropriate pain management.

The Code of Practice recognizes that spaying is used by some Canadian producers and recommends pain management for the procedure. Unfortunately to date no studies on pain mitigation for ovariectomy in heifers exist in the literature, although several assessments of analgesic strategies for castration of bull calves have been published (Coetzee et al., 2010; Meléndez et al., 2017; Molony et al., 1995). A Canadian review and an Australian report both recommend research on welfare issues and pain mitigation for ovariectomy (Pinner, 2006; Williams & Page, 2014). An Australian study of welfare outcomes of spaying in *Bos indicus* cattle found mean free plasma cortisol concentrations higher in spayed cows (7.76 ± 0.04 nmol/L) compared to physically restrained cows (3.61 ± 0.04 nmol/L) for eight hours after spaying, prompting a recommendation that “spaying should not be conducted without measures to manage the associated pain and stress” (Petherick et al., 2013).

Mortality resulting from surgical complications is always a possibility, but is anecdotally reported to be rare in ovariectomized heifers. An Australian training manual suggests mortality rates are as low as 0.1% under well managed conditions and when performed by a skilled experienced person (de Witte et al., 2006). In Canada, a mortality rate of 0.26% was reported in a group of 384 heifers ovariectomized by a skilled operator (Habermehl, 1993), however the producer was responsible for monitoring losses and it is unclear how heifers were monitored. A study by Jubb et al. (2003) in *Bos indicus* and *Bos indicus* cross heifers reported mortality rates from DOT ovariectomy of 0% (0/44) when performed by an experienced operator, and 2.6% (2/76) and 2% (1/51) when performed by inexperienced operators, although only one heifer was found dead and the other two were missing and presumed dead. McCosker et al. (2010) reported a DOT ovariectomy mortality rate in heifers of 0.5% (3/574) with two deaths (one in a pregnant
heifer) occurring within 48 hours and the third animal found dead four days after spaying. In the same study, mortality rate in a second group of heifers was reported as 1.5% (3/200) but all of the deaths had occurred between 10 and 21 days after spaying during a period of less intensive monitoring and it was suggested that these animals may have succumbed to Clostridial disease after another animal was found showing clinical signs of tetanus. There was no mention of antibiotics administered to heifers at the time of spaying in that study. For comparison, mortality rates could not be found for castration of bulls and bull calves, but 1% of producers responding to a New Zealand survey listed death as a complication of castration (Stafford et al., 2000).

1.5.2 Welfare of Spayed versus Intact Cull Heifers

The UK Farm Animal Welfare Council (UFAWC) suggests that an animal’s Quality of Life (QoL) is determined by the balance between its lifetime positive and negative experiences (Mellor, 2016). The UFAWC QoL scale denotes the category of “A life worth living” as one with a positive experience balance and the highest category, “A good life”, as one with a strongly positive experience balance. Shaped during its thirty year evolution as a science, animal welfare currently recognizes that an animal’s welfare status continually varies depending on its circumstance (Mellor, 2016). Not all heifers in a herd are suitable as breeding replacements and these cull animals will ultimately be fattened for slaughter. In spite of enduring the negative experience of ovariectomy, I propose that a spayed cull heifer has more positive cumulative experiences in her lifetime than an intact pregnant cull heifer, living a good life while extensively grazed and having an overall life worth living.

Spaying prevents pregnancy and therefore ensures that grazing heifers do not endure the adverse welfare of an unpleasant death from unsupervised calving complications. In Australia, heifer calving mortality is reported to be prevented by spaying, as heifers may be marketed a year after ovariectomy, longer than the nine month bovine gestational period (Niethe & Holmes, 2007). Calving mortality prevention by spaying also applies in the U.S.A. where spayed heifers may be grazed during the winter as well as summer for up to ten months (Sharman et al., 2011). In Canada, this is less of a concern, as summer grazing is generally seven months or less and spaying heifers in the spring identifies any pregnant heifers that can be diverted for better surveillance. In three groups of yearling heifers observed spayed in March to May of 2017, the pregnancy prevalence was 0 to 3%, as 0/45, 2/70 and 2/120 yearling heifers were diagnosed pregnant on rectal palpation (personal observation). Early pregnancies undetectable by palpation
during spaying will not be maintained so all properly spayed heifers should be non-pregnant
(open) when sold to the feedlot (Horstman et al., 1982).

Cessation of estrus cycling allows for alternate management options for spayed heifers
such as grazing them with the cowherd because they will not distract the bulls. Cattle are
extremely social animals, and this option allows them better welfare because they have the
potential to maintain a relationship with their mothers, as cows are reported to have relationships
with successive offspring (Watts & Stookey, 2000).

Extensive grazing conditions that spayed heifers experience prior to their sale to feedlots
align with the “natural living” orientation of thought regarding animal welfare. Positive affective
experiences are recognized as those that allow an animal to engage in rewarding behaviours
(Mellor, 2016), such as those that occur on the spacious and stimulus rich environment of
extensive pastures. They can select appealing forages and seek their choice of conditions in
which to rest, socialize and decide where they find the most thermal comfort. Although intact
heifers are also often extensively grazed, a proportion of them will invariably become pregnant
and they will likely endure the negative experience of pregnancy termination and abortion or
calving under feedlot conditions. It is unknown whether intact heifers that do not become
pregnant and undergo repeated estrus every three weeks may experience the negative affect of
frustration, one in a list of several negative affects that includes loneliness, boredom, fear and
anxiety (Mellor, 2016). A study in 37 dairy cows in the Netherlands concluded that estrus itself
is a cause of stress, as sampling four times per day for plasma cortisol showed that the diurnal
ebb in cortisol concentration was obliterated during estrus and maximum cortisol concentrations
occurred 24 hours prior to behavioural estrus scores (Lyimo et al., 2000). The study noted that
restraining cows during estrus increased their stress; this suggests that extensively grazed heifers
in estrus may not experience stress to the same degree as dairy cows. Eliminating estrus cycling
in yearling heifers may promote better welfare by reducing riding activity and potential riding
injury (Prado et al., 2016). Mating injuries are potentially also prevented, but literature on
mating trauma reports injury sustained by bulls, and no incidence of injury to heifers was found.

After the grazing season, spayed heifers continue to benefit from improved welfare
because they are not pregnant on arrival at the feedlot. The majority of yearling heifers arriving
at North American feedlots are intact, and inevitably a percentage of them are pregnant even if
they were not intentionally exposed to bulls. Beef cattle have been selected for breeding
efficiency and the drive to mate can overcome inadequate fencing. One source cites on-arrival pregnancy prevalence in heifers at 3 to 20% (Buhman et al., 2003). A summary citing a 2011 US Department of Agriculture randomized survey of ≥1000 head capacity feedlots in 12 states reported an on-arrival heifer pregnancy prevalence of 7.6% and a survey of Colorado feeders in 1983 reported pregnancy prevalence at 16.5%. The same study summary lists pregnancy prevalence at slaughter for 120,699 heifers from 12 different packing plants between 1984 and 1990 at 3.9% to 23.3% (Rademacher et al., 2015). The critical value for pregnancy prevalence that would negatively impact the QoL equation for a group of intact heifers has been unexplored.

Pregnancy in heifers represents a financial loss to the feedlot and packing plant and therefore most feedlots have abortion protocols in place for arriving heifers (Rademacher et al., 2015). Literature on heifers in North American feedlots focuses on abortion strategies (Barth et al., 1981; Horstman et al., 1982) and the economics of feeding heifers (Buhman et al., 2003) and no studies could be found examining the degree of stress or distress heifers experience as a result of pregnancy termination and abortion or calving and its complications in the feedlot. A summary of feedlot pregnant heifer management acknowledges that public perception in this area deserves consideration and that “it is important that as an industry we try to move toward a system in which few or no pregnant heifers are placed on feed to begin with” (Rademacher et al., 2015). Spaying more cull heifers would be a step towards this goal.

As humans, we accept as common knowledge that birth is a painful event, and there is some evidence that calving is painful to cows and heifers. Cattle spend a longer time in active (second stage, expulsive) labour than other domestic animals, reported as 96 ± 31 minutes (Nagel et al., 2016) and arguably therefore experience a longer period of parturition pain. Nagel’s study reported that compared to pre-calving levels in 12 dairy cows, there was an increase in heart rate and salivary cortisol concentrations during the second stage of labour that indicated a stress response and inferred that pain was experienced. A study on 80 buffalo cows and heifers showed that those animals that experienced dystocia, presumably a more painful event than normal calving, had behavioural differences of increased frequency of pawing, looking at the flank and hunching of the back in the first stage of labour and overall increased heart rates, respiratory rates and serum cortisol concentrations during calving than their normally calving cohorts (Derar & Abdel-Rahman, 2012).
Heifers that are aborted in the feedlot may experience adverse welfare from complications including retained fetal membranes. Although a large proportion of aborted heifers do not require antimicrobial treatment, some develop fatal metritis and septicemia if not treated (Booker et al., 1992). Dexamethasone is often given along with the abortifacient in heifers that are pregnant for more than 120-150 days, and this steroid anti-inflammatory agent can suppress the immune system, potentially making aborted heifers more susceptible to respiratory disease (Rademacher et al., 2015). Feedlots therefore may choose to postpone aborting pregnant heifers until 3 weeks after arrival (Rademacher et al., 2015) and 3 weeks later in the pregnancy, but the welfare implications of earlier versus later term abortions are unexplored. One study comparing spontaneously calving dairy cows to those that were induced to calve with prostaglandins reported that the mean birth weights were lower in the induced group (50.5 ± 2.8 and 38.8 ± 3.7 kg respectively) and that the cortisol response was lower in the induced group that suggested the smaller calves were less painful to deliver (Nagel et al., 2016). Perhaps aborted heifers experience less pain than those that deliver full-term calves.

Even when appropriate protocols for abortion are implemented, failure to abort occurs in 5.0% to 7.5% of heifers and adding MGA to the feed to inhibit cycling in a pen of intact heifers can also increase the number of failed abortions (Rademacher et al., 2015). Failure to abort may culminate in calving while heifers are still in the feedlot. Heifers calving in the feedlot have a high body condition score, and are at risk for dystocia, caesarian section, retained fetal membranes, prolapsed uterus, obturator nerve paralysis and calving mortality (Buhman et al., 2003; Rademacher et al., 2015).

1.6 Ovariectomy Techniques

Ovariectomy can be performed via either a flank approach or via a colpotomy (trans-vaginal) approach to access the abdominal cavity.

1.6.1 Flank Approach

Spaying via a left flank laparotomy approach requires minimal surgical expertise and might be preferable for heifers that are too small to be ovariectomized using a colpotomy approach. Having small heifers at spaying time is more likely in Australia where calving often occurs throughout the year. In Canada, where calving generally is restricted to a two to three month “calving season”, heifers are spayed between 11 to 15 months of age and are unlikely to be too small.
Flank spaying was the most commonly used technique for spaying heifers until the mid 1990s but is not commonly done at the present time (Williams & Page, 2014). Flank spaying is time consuming, requiring the heifers to be clipped, prepped, and locally anesthetized with lidocaine infiltration of the skin and muscles of the left flank, so only 13 to 15 animals can be ovariectomized per hour (Jubb et al., 2003). A 12 to 15 cm vertical incision is made through the skin and external abdominal oblique muscle and deeper sharp or blunt dissection used to expose the peritoneum, which is sharply incised. The incision is held open to allow air to enter the abdominal cavity helping to separate the pelvic and abdominal organs (de Witte et al., 2006). The left hand is inserted to hold an ovary while the right hand introduces a curved serrated “spay scissor” that cuts the ovarian pedicle. Alternatively, an umbilical clamp can be used to clamp the ovarian pedicle before cutting off the ovary, which is especially beneficial for controlling hemorrhage in older cows (Youngquist et al., 1995). The amputated ovary is removed through the flank incision and the second ovary is removed in similar fashion. The muscle layer is either left unsutured or sutured with absorbable #3 suture material (Horstman et al., 1982). The skin can be sutured, but using a commercial sack stapler for closure is faster and most or all of the staples fall out once the incision has healed (personal observation).

Cattle show a greater frequency of “standing head down” behaviour for 3 days after flank spaying, an indication of discomfort or pain and 16% of flank spayed heifers are reported to experience abnormal or prolonged healing of the incision (Petherick et al., 2013). Disadvantages of flank spaying include increased risk of infection at the incision site, especially if conditions are dusty or rainy, damage to the hide and losses from carcass trim around the incision area at the packing house (Habermehl, 1993). Especially under Australian conditions, fly strike is a potential problem. An advantage of this method is that physically removing the ovaries provides assurance that the heifer is definitely spayed.

The flank approach is the approach most likely to be used in veterinary surgical facilities where asepsis and good hemostasis is desired. Use of a linear cutter that clamps the ovary and staples the pedicle in one motion was described for dairy cows with large follicular cysts that require unilateral ovariectomy (Rizzo et al., 2016). An chain ecraseur may also be used to tighten around the ovarian pedicle to help prevent hemorrhage (Prado et al., 2016). Laparoscopic ovariectomy has also been described (Bleul et al., 2005). These techniques are not practical for large groups of cattle undergoing ovariectomy under ranch conditions.
“Webbing” is a procedure performed occasionally in Australia on aged beef cows. It is not an ovariectomy procedure but deserves mention as it is done to render cows infertile and is also referred to as “spaying”. The ovaries are not removed; instead a specialized instrument is used to remove only a portion of the uterine tubes (formally called fallopian tubes, or oviducts), preventing future pregnancy but still maintaining the current pregnancy and allowing estrus cycling. The flank approach is primarily used for webbing, however there is mention that it can be performed by the passage colpotomy approach described below (Cattle Standards and Guidelines Writing Group, 2013). Webbing adds value to cull cows as they will be in better body condition than if they were pregnant and lactating when marketed about a year later and the risk of exsanguination is lower than for ovariectomy in mature cows (Luciano Gonzalez PhD, University of Sydney, personal communication).

1.6.2 Colpotomy Approach

Colpotomy is a trans-vaginal approach that involves using an instrument to penetrate the dorsal vaginal fornix to enter the abdominal cavity. No visible incision is present on the heifer. Ovariectomies performed via colpotomy are “blind” surgical techniques that rely on the tactile skills of the veterinarian.

One type of colpotomy approach is “passage spaying” that involves creating a vaginal incision large enough to allow a hand to access the ovaries. It has been performed in Northern Australia (Williams & Page, 2014) but the Australian Cattle Guidelines and Standards do not support the use of the mechanical vaginal spreader used in passage spaying because it is painful especially in smaller heifers (Cattle Standards and Guidelines Writing Group, 2013). In this technique the vagina is incised with a hand-held knife and an ecraseur or spay hook is used to sever the ovarian attachments and remove the ovaries. One reference refers to passage spaying as “ovariectomy by colpotomy” (Pinner, 2006) however, the root “colpo” is “the combining form denoting the vagina” (Martin, 2015), therefore colpotomy refers to any incision into the vagina and does not uniquely refer to passage spaying. With more humane instruments available for ovariectomy via colpotomy, there is currently little justification for passage spaying of heifers.

Three ovariectomy devices are used for the colpotomy approach: the Kimberling-Rupp (KR) spay tool, the Meagher Ovary Flute (MOF) and the Willis ovariotome (WO). Each of these instruments are inserted vaginally and directed through the cranial vaginal fornix, dorsal to the
cervix and on or near the midline with one hand, while the other hand is inserted rectally and guides each ovary in turn into an opening that severs its attachments.

1.6.2.1 Kimberling-Rupp Spay Tool

The KR device has been used since the early 1980s and has a “tube within a tube” design. Once the ovary is in the chamber opening, turning the inner tube severs the ovarian attachments and retains it within the instrument (Rupp & Kimberling, 1982). When the KR device is removed from the heifer, the ovaries contained within it provide visual confirmation that the heifer is ovariectomized. This tool is relatively large and inadvertent visceral trauma is a concern. The KR device is reportedly not used in Australia (Cattle Standards and Guidelines Writing Group, 2013). One study within the past decade used the KR device to spay 290 heifers for a clinical trial (Sharman et al., 2011), but few mentions in the current literature suggests that it is not commonly used.

1.6.2.2. Meagher Ovary Flute

The Meagher Ovary Flute was patented in 1999 and is similar to the KR device in that it also removes the ovaries when withdrawn from the vagina. Its cranial end contains a retractable cutting tip. A detailed description for using the MOF is available (Prado et al., 2016). Currently it is reportedly used by a few veterinarians in the US, being trialed on an Australian station, and used to teach ovariectomy to veterinary students in Panama, but no feedback to the designer has yet been forthcoming about its use (Harry Disney DVM, personal communication).

1.6.2.3 Willis Ovariotome

The most popular tool for ovariectomy is the Willis ovariotome (Jubb et al., 2003), developed by South Dakota veterinarian Charles E. Willis (United States patent no. 5,997,551). It is a simple stainless steel rod about 48 cm in length and 6 millimeters in diameter with a sharp teardrop shaped cranial end containing an eye with a slot (Fig 1.3). The opposite end has a 90º bend that functions as a handle. It is marketed in three sizes in Australia because it is used on larger cows as well as heifers in that country (de Witte et al., 2006).

Ovariectomy using the WO is referred to as the “Willis Dropped Ovary Technique” (WDOT), the “Willis Spay Technique”, or the “Dropped Ovary Technique” (DOT) because the ovaries are not removed from the body but are dropped into the abdominal cavity, remaining “in situ” albeit not in their original position (de Witte et al., 2006). This method has been promoted in Australia since the mid 1990s because it is faster and studies have shown that it is less
stressful for cattle than spaying via the flank approach (Petherick et al., 2013). Approximately 30 heifers or more can be spayed per hour with this technique (Jubb et al., 2003); McCosker et al. (2010) reported that up to 500 – 600 animals can be spayed per day by skilled operators.

A detailed explanation of the procedure has been described in an Australian training manual (de Witte et al., 2006). The operator directs the point of the ovariotome through the dorsal vaginal fornix about one to two centimeters above the cervix on the midline with a controlled thrust to enter the abdominal cavity. An ovary is guided into the eye and a caudally directed pull severs its attachments dropping the ovary into the abdomen and the process is repeated on the remaining ovary. The ovariotome is removed, wiped clean and placed in a 0.05% chlorhexidine solution until the next heifer is ready. A drawback of this technique is that there is no visible evidence that the ovariectomy has been properly completed, although occasionally an ovary is caught in the ovariotome when it is removed. The success of the procedure is dependent upon the skill of the operator, because if an ovary is not properly severed pregnancy could still occur.

The small vaginal incision created with the DOT is similar in size to that created by the Natural Orifice Transluminal Endoscopic Surgery (NOTES) procedure, which is the most recent evolution of minimally invasive surgery in humans and companion animals. NOTES provides laparoscopic visual access through the colpotomy incision, and is recognized to benefit patients by shorter recovery time, negligible incisional complications and less postoperative pain (Pader et al., 2011). The additional time and equipment needed for NOTES makes it unfeasible for performing ovariectomies in heifers.

1.6.3 Identification of Spayed Cattle

In Australia, legislation requires ovariectomized cattle to be identified by punching a 15 mm hole in the left ear at the time of spaying (Petherick et al., 2011) as permanent visible identification signifying the animal was spayed. In Canada, it is not a legal requirement to identify spays with a visible mark, but prudent managers will identify spays with a tamperproof metal ear tag or a unique earmark so that they can be easily differentiated from replacement heifers (personal observation).

1.6.4 Licensing Requirements to Perform Bovine Ovariectomy

In North America, spaying is considered a veterinary surgical procedure and therefore can only be performed by a veterinarian. In some parts of Australia, non-veterinarians are
licensed to perform the DOT procedure after undergoing a training program and can contract the work independently. The Australian Cattle Standards and Guidelines discussion paper (Cattle Standards and Guidelines Writing Group, 2013) on spaying states “a person spaying a cow must be a veterinarian or, if permitted in the jurisdiction, be accredited or be under the direct supervision of a veterinarian or a person who is accredited”. In Australia, contractors perform spaying on 68.7% of cattle stations, station owners or employees on 22.9%, and veterinarians on 8.4% of stations (Williams & Page, 2014).

1.7 Post-Operative Complications of Ovariectomy

Intestinal laceration and exsanguination are the two main surgical complications and usually affected animals die within 24 to 36 hours. What happens to heifers before, during and after ovariectomy influences the risk of complications. Heifers are at lower risk if fasted for 24 hours prior to spaying, if well restrained during the surgery, and afterwards are not be moved long distances for two to three days (Jason McGillivray DVM, personal communication). Food and water should be easily accessible for the first few days after spaying to minimize activity. In hot sunny climates shade should also be made available.

The most common cause of peritonitis is iatrogenic visceral trauma from the ovariotome, causing leakage of gut contents into the abdomen. Holding onto the cervix with the rectally inserted hand while the ovariotome penetrates the vagina increases the risk of rectal penetration. The chance of small intestinal or ruminal penetration occurring increases if the animal is not standing still during the procedure (de Witte et al., 2006), and is more likely when using manual mechanical squeeze chutes on cattle not accustomed to handling. Hydraulic chutes provide superior restraint but are unlikely to be available in remote locations (personal observation). Inadequate fasting prior to ovariectomy and overzealous squeezing in the chute both place the rumen and jejunum in closer proximity to the reproductive tract and therefore increase visceral trauma risk (Habermehl, 1993). Inexperienced operators may also inadvertently sever a portion of the small intestine while attempting to cut the ovarian attachments (de Witte et al., 2006). Jubb et al. (2003) reported mortality from DOT ovariectomy as 1/40 (2.5%) in heifers spayed by an inexperienced operator and 0/44 (0%) in heifers spayed by a more experienced operator. Mortality will occur if injury is extensive enough to cause gross contamination of the abdominal cavity.
Cutting the ovarian pedicles creates some bleeding, but hemorrhage can be severe enough to cause exsanguination. Hot weather, excitable temperament, excessive activity or clotting disorders increase the risk of exsanguination. De Witte et al. (2006) notes that dehorning should not be done at the same time as spaying and animals that carry a blood sucking parasite burden such as ticks or *Haemonchus spp.* should not be spayed. Pregnancy is also a risk factor for exsanguination because there is a greater blood supply to the ovaries during pregnancy (de Witte et al., 2006). Organization of a large blood clot may lead to adhesion formation but this has not been reported to be of any clinical significance (de Witte et al., 2006). If a recently spayed heifer is noticed as lethargic with pale mucous membranes, treatment with intravenous fluids and colloids may prevent death, but are unlikely to be available at the remote sites where ovariectomy is often performed. Quiet confinement of heifers with feed and water for two to three days after spaying and avoiding excitement or moving them soon after surgery, especially in hot weather, helps to minimize this complication.

Up to 5% of heifers may show signs of stiffness or straining after ovariectomy but this is reported to resolve within 48 hours (de Witte et al., 2006). In a group of 384 heifers, 0.5% were observed to walk stiffly a day after spaying but the producer was responsible for observing the heifers after ovariectomy and reporting any complications (Habermehl, 1993).

Peritonitis from surgical contamination is a rare complication from in situ ovariectomy (de Witte et al., 2006). An ovariotome contaminated from either inadequate disinfection or infected vaginal tissue could be a potential but unlikely cause of peritonitis. Uterine contents could leak into the severed uterine tubes and contaminate the abdomen and thus pyometra is a contraindication for ovariectomy.

The WO colpotomy site contracts quickly and is difficult to visualize at necropsy 48 hours later, consisting of only slight hemorrhage and bruising (de Witte et al., 2006). It is theoretically possible for intestinal prolapse to occur through the colpotomy site, but this has not been recorded to date (de Witte et al., 2006). Jubb et al. (2003) necropsied cattle a year after DOT ovariectomy and reported areas of localized fibrosis with occasional strictures producing fluid distended oviducts at the ovarian pedicle site, and found 30/242 (12.4%) of spays with ovarian remnants, resulting in 2 (0.8%) pregnancies. Care needs to be taken to ensure that the entire ovary is within the ovariotome eye before severing the pedicle to avoid leaving ovarian remnants.
1.8 Alternatives to Ovariectomy

Although currently not approved for use in cattle in North America, anti-gonadotrophin-releasing hormone (GnRH) vaccination is the most promising alternative for eliminating estrus and preventing pregnancy in heifers. GnRH is produced and stored in the hypothalamus and enters the portal circulation to the anterior pituitary gland in pulses where it binds to receptors that initiate the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Boer et al., 2011). When GnRH concentration is reduced by neutralizing anti-GnRH antibodies, follicle development and estrus is temporarily suppressed and the ovaries atrophy (Hernandez-Medrano et al., 2013). This immunologically induced anestrus condition varies in effect and duration among individuals and requires repeated booster vaccinations.

Currently an anti-GnRH vaccine is approved for use in cattle in Australia and New Zealand for suppressing testosterone production in bulls and ovarian activity in heifers (Bopriva, Zoetis Australia Limited, 38-42 Wharf Road, West Ryde, NSW 2114, Australia). When 76 heifers and cows were vaccinated with an anti-GnRH vaccine and a booster administered 20 to 49 days afterwards, 17% of the trial animals were observed in heat during their 100 days on pasture (Hirsbrunner et al., 2017). The authors suggest that some of these animals were likely anovulatory in spite of showing signs of estrus, as mares vaccinated with anti-GnRH vaccine can show signs of estrus without ovulating (Imboden et al., 2006).

Under Canadian extensive grazing conditions there is some question whether anti-GnRH vaccine could be an effective and easily implemented solution for eliminating estrus and preventing pregnancy in cull heifers. Gathering extensively managed heifers and putting them through the chute for vaccination requires a large labour expense and is stressful to the cattle. Heifer selection occurs prior to spring turnout and tends to coincide with calving season when human resources are limited, and diverting crew to gather and vaccinate heifers could have a negative impact on the calving cowherd. Vaccinating in early March and administering the booster 4 weeks later around March 28 to April 5 would give a median duration of anestrus of 191 days (Hirsbrunner et al., 2017). The vaccine effect would be expected to wear off in a proportion of the heifers before marketing in the fall, and some could become pregnant or divert resources for gain to estrus activity. Additional booster vaccination administration during the grazing season can be problematic. In areas such as the crown grazing permit lands of British Columbia, it is likely not even possible to gather an entire group during the grazing season, as
cattle are well scattered throughout the mountain forests (personal observation). A good gather is only possible when forage becomes depleted and snow starts to fall, and the cattle are motivated to travel to lower altitudes. Future investigation of anti-GnRH vaccine for cull heifers needs to consider its application under the challenges of extensive management.

The vaccine is not entirely innocuous. GnRH by itself is too small and not “foreign” enough to invoke an immune response by itself so it (or an analog of it) must be attached to a larger carrier molecule to induce an antibody response (Hernandez-Medrano et al., 2013). The carrier and adjuvant can provoke local tissue inflammation at the injection site. In a group of 11 cows, pain at injection site was described as moderate to substantial and the injection site reaction took two to four weeks to resolve (Balet et al., 2014). Rectal temperature, heart rate and respiratory rate also increased for up to two days post-vaccination in these cows. As with any vaccine, anaphylaxis may rarely occur.

Bopriva is currently not licensed for use in cattle in North America, although a product for pigs is licensed for immunological castration and reduction of boar taint (Improvest (Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate, 0.2 mg/mL), Zoetis, Kalamazoo, MI, USA). Human safety when handling the product is a major concern. Women of childbearing age are advised not to handle the vaccine and accidental self-injection in men or women would disqualify them from using the product in future, as fertility can be affected in both sexes (Improvest product insert, Zoetis, Kalamazoo, MI, USA).

Spaying heifers immunologically changes the site and type of pain inflicted from the diffuse visceral pain of DOT ovariectomy lasting a few days to superficial acute injection site pain lasting up to two weeks. Extensively managed heifers are stressed just by going through the chute system, and immunologically spayed animals would need to endure this at least twice. Six studies in Bos taurus beef cattle published cortisol concentrations during head gate restraint two to three times higher than cortisol concentrations of Holstein cattle being restrained in a head gate (Grandin, 1997). The welfare implications for handling extensively managed cattle need also to be considered when investigating vaccination programs.

1.9 Anatomy of the Bovine Female Reproductive System

Heifers have a small reproductive tract until entering puberty at eight to ten months of age when it increases to mature size after a few 21-day cycles. The reference used for the
following description of the anatomy of the bovine female reproductive tract is the Textbook of Veterinary Anatomy, 4th Ed (Dyce & Wensing, 2010).

The paired ovaries (Fig 1.4) containing the developing follicles descend from a cranial position near the kidneys during fetal development to lie within the pelvis. Compared to body size, the mature ovaries are small, measuring approximately four by two centimeters (cm). Each ovary is suspended within the broad ligament by the mesovarium, which attaches laterally to the body wall and medially to the reproductive tract. The mesovarium contains the fibromuscular proper ligament of the ovary that extends from the caudal ovarian pole to the uterine horn tip. The ovary resides in a pouch-like ovarian bursa formed by the mesovarium and the portion of the broad ligament that suspends the uterine tube called the mesosalpinx. The ovary may need to be unfurled from the ovarian bursa before placement in the ovariotome eye.

The uterus consists of two horns in close proximity with shared serosal and muscular coverings, joined by short dorsal and ventral intercornual ligaments where they diverge, taper and coil ventrally. Each horn is about 35 cm long and caudally they join together to form a three cm long uterine body. The cervix is approximately 8 to 10 cm long and projects caudally into the vagina. The vaginal fornix surrounds the cervix creating a ring-like “pocket” called the fornix. The site for the DOT colpotomy incision is one to two cm dorsal to the cervix in the fornix.

The dorsal surface of the cranial vagina is the only portion covered by peritoneum and therefore is the only area through which a colpotomy incision can gain access to the abdomen and ovaries. The remainder of the vagina is extraperitoneal. This dorsal cranial vaginal area also provides good surgical access because it contains no major blood vessels, which are located laterally and ventrally, including a large venous plexus on the ventral uterus and vagina. The wall of the vagina contains low circular and longitudinal folds. The vaginal roof rests on its floor, closing its lumen. Occasionally a freemartin (a heifer twin, generally with an aplastic vagina, masculinized by sharing in utero circulation and cells of a bull twin; currently thought to be a chimera) is discovered on rectal palpation and these heifers are sterile and not spayed. The vestibule lies immediately cranial to the vulva and slopes caudoventrally. Placing an instrument into the vagina therefore requires that it be advanced in a craniodorsal direction before reaching the horizontal vagina.

The blood supply to the ovary, uterine tube and cranial uterine horn (Fig. 1.4) is from the ovarian artery that originates directly from the aorta near the caudal pole of the kidney. The
branch to the uterine horn anastomoses with the uterine artery in the broad ligament. Tearing of this vessel can cause intra-abdominal bleeding and bleeding into the broad ligament. The ovarian vein and artery are in very close proximity to allow countercurrent transfer of hormones between the venous and arterial blood.

The tissues placed within the eye of the Willis ovariotome and subsequently severed include the uterine tube, the mesosalpinx, mesovarium and proper ligament of the ovary. The ovarian artery and branch of the uterine artery with their accompanying veins, a lymphatic duct, sympathetic nerve fibres from the ovarian plexus and parasympathetic fibres from the pelvic plexus are also within the pedicle.

1.10 Pain Induced by Ovariectomy

The animal welfare guiding principle “Five Freedoms” has been used as a tool to help guide decisions for improving livestock management. Among these is “the freedom from pain, injury and disease”. The phrase “freedom from” was initially intended to mean “as free as possible from”, since pain is an occasional inevitable experience in all conscious beings and functions to minimize bodily harm (Mellor, 2016). Modern consumers (and therefore also retailers) are keenly interested in the efforts that the livestock industry and veterinarians are providing to minimize the pain experienced by food animals (Weary et al., 2006). Understanding the physiology of pain is the first step in determining how it might be mitigated.

1.10.1 Pain in Animals

Since all mammals have similar nervous system anatomy, it is unlikely that the neurophysiology of pain perception evolutionarily appeared only with *Homo sapiens*, and reasonable to assume that cattle experience pain in a similar fashion to humans (Serrie & Servière, 2014). However, for humans and likely also cattle, pain is subjective because it is a perception and not experienced in precisely the same way in each individual (Hellyer et al., 2007). Acute pain is unpleasant, but it functions to keep the bearer safe from harm by triggering responses designed to avoid injury or keep further injury from occurring (Molony & Kent, 1997). In humans the inability to experience pain is a rare condition that results in serial serious physical injuries and demonstrates the protective role of pain (Serrie & Servière, 2014). In 2003, the American Animal Hospitals Association (AAHA) recognized the importance of pain in veterinary patients by adding pain as the fourth vital sign, after temperature, pulse and respiration (Hellyer et al., 2007).
The International Association for the Study of Pain (IASP) proposed the official definition of *pain* for humans as “an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage” (Mathews et al., 2014). Serrie and Serviere (2014) instead defined vertebrate animal pain as “the awareness than an animal has of an aversive sensory and emotional experience associated with actual or potential tissue damage” and that the painful experience must cause protective motor responses, physiological responses and learned avoidance responses, including behavioural changes. Potentially tissue-damaging noxious stimuli initiate signals to the central nervous system (CNS) that ultimately result in the experience of pain and since the cerebral cortex is the site of perception, in order for pain to be perceived, the individual must be conscious.

### 1.10.2 Pain Definitions

*Nociception* refers to the detection and transmission of a noxious stimulus that travels as an electrical signal (action potential) along the ascending neuronal pathways that ultimately terminate in the cerebral cortex. It is the physiological process that carries the pain signal to the cerebral cortex, but nociception is not synonymous with pain. *Pain* is the perception that arises from nociceptive inputs to the cerebral cortex (Muir & Woolf, 2001).

The *pain pathway* is the physiological process of pain perception. The first step in the pathway is *transduction*, where the noxious stimulus is transformed to an electrical signal (action potential) at the peripheral terminal of primary afferent nociceptive neurons. Next, *transmission* of this signal occurs along the length of the primary afferent to its cell body in the dorsal root ganglion, and ending at its presynaptic terminal in the spinal cord’s dorsal horn. The spinal cord is the site of *modulation* where the signal can be either amplified or tempered. *Projection* of the signal occurs in secondary neurons that ascend cranially to the brain. Ultimately the signal reaches the cerebral cortex where *perception* of the pain occurs (Muir & Woolf, 2001).

*Nociceptors* are specific non-encapsulated receptors at the peripheral sensory endings of primary afferent neurons. *Silent nociceptors* have high thresholds that prevent “ordinary” noxious stimuli from triggering them. However, in the presence of inflammatory mediators their threshold is reduced, allowing them to respond to thermal or mechanical stimuli. This can result in a zone of *hyperalgesia* around the tissue injury site that produces an exaggerated pain response to normally painful stimuli (Hellyer et al., 2007) and a more peripheral zone of *allodynia*, where normally non-painful stimuli are perceived as painful (Muir & Woolf, 2001).
Reflex reactions occur using the reflex arc, which is a portion of the pain pathway. Reflexes are involuntary responses to stimuli, including those that are potentially painful, and may occur before pain can be perceived. The reflex arc is composed of a receptor or nociceptor, its afferent neuron that synapses in the dorsal horn (or brain stem in the head region) and the lower motor neuron that innervates an effector that could be either skeletal muscle (a somatic reflex) or smooth muscle or glands (a visceral reflex) (Hellyer et al., 2007). The ascending portion of the pain pathway that travels to the brain is not involved in evoking a reflex, which is why a reflex can occur prior to perception of pain, or when there is a spinal cord or brain stem lesion cranial to the reflex arc, or even in a decerebrate individual.

The terms pain, stress and distress are often used together in animal welfare discussions. Stress is an inferred internal state that results from a real or perceived threat to an individual’s physiological or psychological wellbeing. Pain can be one of many stressors (causes of stress) that can provoke behavioural changes and physiological responses that include release of catecholamines and glucocorticoids and mobilization of the immune system. However, no single stress response is evoked in all stressful experiences and an experience can be stressful even if one or more responses if not present. The diurnal peak of cortisol is a normal occurrence that can rival the magnitude of some stressors and a cortisol rise can occur after a bout of playful exercise. Different species, breeds within species and individuals within a breed may have variable stress responses (National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals, 2008).

Distress is a maladaptive state that results from an individual’s inability to cope or adapt to a stressor. Duration and intensity of a stressor determines whether stress progresses to distress. Distress may manifest as overt maladaptive behaviours (e.g. aggression) or subclinical pathological conditions (e.g. immunosuppression) (National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals, 2008).

1.10.3 The Physiological Dimensions of Pain

Pain in humans is described as having three physiological dimensions. The sensory-discriminative dimension includes information to the individual about the location, intensity, and duration of a painful stimulus. The motivational-affective dimension causes an individual to change behaviour in response to the disturbance, distress or suffering that pain causes to their well-being. The cognitive-evaluative dimension includes the effects of previous experience,
social influences, anxiety, and conditioning to establish how disruptive the pain is to the individual’s well-being. This last dimension involves primarily cortical activity and because the human cerebral cortex differs in form and function from other mammals, this dimension may differ markedly in animals (Hellyer et al., 2007).

1.10.4 Classification of Pain

Pain can be classified by location, duration, and mechanism. Describing pain can include more than one classification, such as fracture pain that can be described as deep (location) somatic (location) acute (duration) inflammatory (mechanism) pain.

1.10.4.1 Classification by Location

Somatic pain arises from structures other than the viscera and visceral pain arises from internal organs. Superficial pain arises from nociceptors in the skin. Deep pain originates from somatic nociceptors deeper than the skin or from visceral nociceptors.

1.10.4.2 Classification by Duration

Trauma, surgical procedures and infections cause acute pain that is caused by tissue damage. Acute pain is transitory, decreases as healing takes place, and it generally can be mitigated by analgesic drugs (Hellyer et al., 2007). If pain is protracted and persists past the time needed for healing an injury, it becomes chronic pain, serving no beneficial function to the bearer and causing distress (Muir & Woolf, 2001).

1.10.4.3 Classification by Mechanism

Pain that is brief and protective (such as contacting a barbed wire fence) is nociceptive pain, which results in finite localized pain with minimal or no tissue damage and sets in motion a protective sequence of events (moving away from the barbed wire) (Babos et al., 2013). This type of pain is also referred to as physiologic pain and protects the body from harm by stimulating protective reflexes and physiologic responses in addition to conscious movement or avoidance responses (Muir & Woolf, 2001).

Clinical pain occurs when a noxious stimulus is sufficiently intense or prolonged to cause tissue damage and is either inflammatory or neuropathic in origin (Muir & Woolf, 2001). Inflammatory pain results from tissue damage and inflammation and involves release of mediators that can increase nociceptor sensitivity to cause hyperalgesia. Surgical pain is inflammatory pain that can be subdivided into procedural pain that occurs during the surgical procedure and postoperative pain that decreases in intensity with healing.
Neuropathic pain arises from nociceptive neurons that sustain injury caused by endocrine disorders (e.g. diabetes mellitus), infection (e.g. shingles), or trauma including surgical transection (e.g. amputation). Glial cells, which provide structural and nutritional support to neurons and have a role in their repair, are believed to be important in the development of neuropathic pain when damaged and activated (Jha et al., 2012). Humans describe neuropathic pain as burning, or stabbing. It is severe, and can be long lasting and difficult to treat. There are no reports of neuropathic pain in cattle, but logically any surgery that transects nerves could be a source of such pain in that species.

1.10.5 The Pain Pathway

The pain pathway is the anatomical and physiological mechanism that detects a noxious stimulus (transduction) and carries its signal (transmission and projection) to its culmination as pain perception in the cerebral cortex and includes means of innate suppression or amplification of that signal (modulation).

1.10.5.1 Transduction and Transmission

Myelinated A-delta and unmyelinated C fibres are the two main types of nociceptive primary afferents that innervate skin, deep somatic tissues and viscera. Transduction occurs when nociceptors located on the distal ends of A-delta and C fibres change their shape to allow cation influx in response to a noxious stimulus that generates an electrical signal (action potential). Rapidly transmitted signals from A-delta fibres (5 to 30 m/s) are reported as sharp, stabbing or pricking pain in humans and if noxious stimulation continues, C fibres become recruited to more slowly (0.4 to 1.4 m/s) transmit dull burning or aching pain signals in proportional to the severity of tissue injury (Dubin & Patapoutian, 2010; Hellyer et al., 2007). Tissues and organs vary in nociceptor density, from none in the brain and liver to high concentrations in superficial tissues (Serrie and Serviere, 2014). Most nociceptive afferents are polymodal and are triggered by more than one type of noxious stimulus, such as either thermal or mechanical (surgical) stimuli. Some also release substances from their axonal terminals in the periphery that contribute to inflammation, such as substance P (SP) release that contributes to edema (Babos et al., 2013). Large myelinated A-alpha and A-beta fibers generally rapidly transmit non-noxious stimuli such as touch; however during injury or inflammation they can also transmit tactile stimuli as being noxious (Hellyer et al., 2007).
The cell bodies for visceral primary afferent nociceptive neurons are located in the dorsal root ganglia and synapse in the dorsal horn of the spinal cord. When the afferent nociceptive signal reaches the presynaptic membrane, neurotransmitter vesicles are released to carry the nociceptive signal into the synaptic cleft to the postsynaptic membrane of secondary or intermediate nociceptive neurons (Babos et al., 2013).

1.10.5.2 Modulation

Modulation takes place in the spinal cord by two groups of dorsal horn neurons that change nociceptive signals by either amplifying or tempering them. One group is specific for nociception and pain localization and a second group (called wide range dynamic (WDR) neurons) synapses with both nociceptive and non-nociceptive neurons and is responsible for referred pain (Hellyer et al., 2007). It is unknown if cattle experience referred pain.

The WDR neurons are also thought to be most important in development of the spinal facilitation of pain called windup. Windup occurs when C fibers send frequent, continuous signals that cause an increased release of several neurotransmitters, principally glutamate that upregulates the ubiquitous NMDA receptors (NMDAr) on the post-synaptic neurons. This results in an exaggerated response to subsequent nociceptive signals that increases the severity of pain that can last for hours or even days (Hellyer et al., 2007).

Estrogen levels also influence the NMDAr because they are in close proximity to spinal neuron estrogen receptors; so triggering estrogen receptors also stimulates the NMDArs (Sengupta, 2009). This may cause variation in pain response to ovariectomy in a group of heifers on any given day, as those in estrus may perceive more pain than those in diestrus.

1.10.5.3 Projection and Perception

Once the nociceptive signal has reached the dorsal horn, two main ascending nociceptive tracts project the signal cranially to the brain, depending on the signal source.

The spinocervicothalamic tract (Fig. 1.5) carries superficial pain signals, for example those originating from an incised scrotum or a branded hide. Secondary neurons in this pathway extend cranially ipsilaterally and synapse at the level of C1 – C2, decussate and cross to the contralateral side, continue through the brain stem, and extend some connections to its reticular formation (RF) before terminating rostrally in the thalamus. From there, nociceptive signals extend to the cerebral cortex that allow localization of pain to the injury site (Hellyer et al., 2007).

DOT ovariectomy instead engages the spinoreticular tract (Fig.1.5) that carries deep and
visceral pain signals. Poor localization of visceral pain is a consequence of the diffuse and multisynaptic connections in this tract. Connections are sent cranially and caudally for several segments from the dorsal horn entry point, secondary neurons ascend bilaterally with diffuse decussation along the length of the spinal cord, and the majority of fibres terminate in the RF of the brainstem rather than the cerebrum. The cerebral cortex only indirectly receives visceral pain signals via diffuse RF connections to the thalamus (Hellyer et al., 2007).

The spinoreticular tract also activates the limbic system, consisting of the hypothalamus (site of autonomic system regulation affecting blood pressure, heart rate, and hunger), amygdala (fear, anxiety, aggression, emotional memory), cingulate gyrus (attention, emotional processing), and hippocampus (memory) (Rajmohan & Mohandas, 2007) and therefore likely mediates the emotional component of an individual animal’s pain. Memories of a painful event can cause fear manifesting as reluctance to enter the area where pain occurred, as demonstrated by Rushen (1986) when sheep exposed four times to electroimmobilization displayed aversion to entering the chute with increased transit times compared to sheep that were only physically restrained.

Neural connections to the hypothalamus invoke a stress response when noxious signals immediately activate the sympatожно-adreno-medullary (SAM) axis and more slowly activate the hypothalamic-pituitary adrenocortical (HPA) axis. The SAM axis releases epinephrine and norepinephrine (catecholamines) and increases heart rate while the HPA axis secretions include corticotropin-releasing hormone (CRH) that stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH), ultimately increasing cortisol release. The stress response helps heal painful injuries by mobilizing tissue substrates by catabolic hormone release and therefore untreated pain can eventually cause clinical weight loss (Nagel et al., 2016). Fear and anxiety can increase the hypothalamic response, even if no pain stimulus is present (Hellyer et al., 2007). Pain response investigations need to account for this when dealing with extensively managed cattle that are rarely handled.

Epidural or intrathecal (“spinal”) local anesthetics can diminish the stress response but peripheral nerve local anesthesia is not as effective at reducing it. Opioid administration has almost no effect on the stress response, but administering the alpha-2 agonist medetomidine was reported to prevent catecholamine and cortisol concentration increases in dogs undergoing ovariohysterectomy (Hellyer et al., 2007).
1.10.6 Pain Sensitization and Treatment

Tissue damage can result in amplified pain sensitivity at the peripheral signal detection level and/or the central secondary neuron level.

1.10.6.1 Peripheral Sensitization

Amplified pain perception caused by the inflammatory response is called peripheral sensitization. Acute inflammation is part of the healing process provoked by injury, including surgical trauma, with clinical signs of redness, heat, swelling, pain and loss of function of the affected tissues (Ackermann, 2014). Ruptured tissue cells spill their contents containing hydrogen and potassium ions, ATP, and many enzymes including thrombin, cyclooxygenase-2 (COX-2), and nitric oxide synthase (NOS) into the surrounding extracellular fluid. This stimulates adjacent inflammatory cells (including neutrophils, mast cells, macrophages and lymphocytes) to produce many types of inflammatory mediators including prostaglandins, serotonin, histamine, bradykinin, cytokines and growth factors. This “soup” of mediators lowers the threshold and increases the responsiveness of A-delta and C nociceptors. It also recruits silent nociceptors, creating a zone of hyperalgesia around the injury and a zone of allodynia on its periphery that functions to protectively rest or immobilize the injury site to help aid in healing (Muir & Woolf, 2001). Nonsteroidal anti-inflammatory drugs (NSAIDs) such as meloxicam inhibit the COX-2 enzyme necessary for prostaglandin production. They not only decrease the clinical signs of inflammation, but by reducing the inflammatory mediator load on nociceptors, also reduce peripheral sensitization and pain (Anderson & Edmondson, 2013).

1.10.6.2 Central Sensitization

Unchecked pain leads to central sensitization that causes increased pain perception, which can also lead to hyperalgesia and allodynia. Sustained frequent nociceptive signals result in spinal cord windup, decreased signal inhibition and increased neuronal excitability so less intense noxious stimuli results in a more severe perception of pain (Muir & Woolf, 2001) even after the initial noxious signals stop (Babos et al., 2013) and also recruits Aβ afferents to relay nociceptive signals for non-noxious stimuli (Anderson & Edmondson, 2013). Opioids, alpha-2 agonists and ketamine administered preemptively before pain occurs all dampen sensory neuron activity and help prevent central sensitization and are more effective than when administered after pain has been established (Anderson & Edmondson, 2013).
1.10.7 Visceral Pain and Analgesia

Visceral pain is not as well understood as somatic pain (Sengupta, 2009). DOT ovariectomy limits surgical trauma to the cranial vagina and peri-ovarian tissues and thus activates only the spino-reticular pathway producing acute visceral procedural pain and post-surgical visceral inflammatory pain. Visceral hypersensitivity starts at the level of the visceral nociceptors, including the kappa-opioid receptor (KOR) and the NMDAr that can be targeted by analgesics prior to surgery. Systemic administration of KOR agonists such as butorphanol was reported to decrease visceral pain by blocking the sodium channels of afferent sensory nerves (Sengupta, 2009).

Visceral pain is difficult to localize for several reasons in addition to the diffuse multisynaptic nature of its spino-reticular tract connections. Visceral tissues have an A-delta:C fibre ratio of about 1:10 unlike the somatic tissue ratio of approximately 1:2 (Hellyer et al., 2007), so visceral organs have five times as many fibers conveying dull, achy pain signals per fiber carrying sharp pain signals compared to somatic tissue. Visceral sensory afferents connect with somatic sensory afferents in the spinal cord, which can result in referred pain (Hellyer et al., 2007). A large proportion of visceral nociceptors are silent nociceptors (Gaynor & Muir, 2015) and therefore are recruited only during an inflammatory response. Instead of precise exclusive receptor fields, visceral afferents have large receptor fields that overlap, so even a painful stimulus localized to a small area may be conveyed by several neurons covering a large area. Sengupta (2009) reported that estrogen increased the size and sensitivity of pelvic visceral nociceptor fields in female rats, resulting in less severe pain during diestrus which may explain some of the variation in painful behaviour within a group of ovariectomized heifers.

Vagal nerve (Cranial Nerve X) activation can dampen visceral pain signals. The vagus nerve is the longest cranial nerve, contains mostly parasympathetic fibers, and extends caudally to innervate the thoracic and abdominal viscera. Rats that were vagotomized or had their vagus nerves anesthetized with lidocaine had higher visceromotor responses to painful colorectal distention than rats with intact, unanesthetized vagal nerves (Chen et al., 2008). The paired vagus nerves are contained within the carotid artery fascial sheaths in the neck area (Dyce & Wensing, 2010); pressure applied over the carotid sinus area on the ventral neck in other species (humans, dogs, cats) can activate the vagus nerve (personal observation). There are no reports of direct vagal stimulation in cattle, but it may be possible that restraining cattle in a head gate and
squeeze chute during ovariectomy may inadvertently help to mitigate pain.

1.11 Pain Assessment in Cattle

The Gold Standard of pain measurement is human self-reporting on a scale of 0 (no pain) to 10 (worst possible pain). Since animals are non-verbal and are incapable of self-reporting, this gold standard is not relevant and animal pain can only be measured indirectly (Millman, 2013). Pain in cattle is assessed by measuring behavioural and physiological changes, with some researchers considering food and water intake as separate measures of general body functioning (Weary et al., 2006). Weary et al. proposed that in animals the gold standard in behavioural pain response validation is measuring the effectiveness of analgesia on painful conditions by comparing pain assessment measurements before and after analgesic administration. These comparison measurements can also confirm the assumption that an animal was in pain if analgesic administration abolishes or diminishes existing painful behaviour (Gleerup et al., 2015).

Heifer ovariectomy is a surgical procedure that generally takes less than two minutes to perform (J.C. Petherick et al., 2011) but nevertheless fits into the category of “procedural harm” because acute pain is generated (Halteman, 2011). Reference to the pain of ovariectomy via colpotomy is sparse in the literature. A human prospective study of 222 abdominal surgeries using the colpotomy approach with a 93% follow up rate had no reports of vaginal pain mentioned one week and three months after surgery. The surgeries were not ovariectomies and the vaginal incisions were sutured, but there was no mention of providing analgesia, as the colpotomy was not a source of pain (Mofid et al., 2013). A woman whose uterus and uterine tubes were removed via colpotomy reported her pain level upon waking from anesthesia as 2 out of 10 after surgery and required no further analgesia (A.K. Hunter, personal communication).

1.11.1 Behavioural Measurements

Weary et al. (2006) described three categories of behaviours for pain assessment. The first category is an increase in pain-specific behaviours such as high frequency vocalizations in castrated piglets (Weary et al., 1998) and includes defensive behaviours such as kicking when pressure is exerted on the scrotum of recently castrated bulls (Marti et al., 2017b). The second category is a decrease in frequency or magnitude of normal activity behaviours, such as reduced lying times in castrated calves compared to presurgical lying times (White et al., 2008) and includes lethargy and a reluctance to move away from human approach. The third category considers measures of choice or preference, such as lame broiler chickens consuming more feed
containing an analgesic rather than unmedicated feed compared to sound cohorts (Danbury et al., 2000). This third category is possible to explore for research purposes but not useful for producers. Observing for changes in normal activity behaviours is time-consuming and also not practical for producers. The most producer-friendly measures are therefore those that use pain-specific behaviours (Gleerup et al., 2015).

Behaviour can be measured subjectively or objectively (Millman, 2013). Subjective measurements include the Visual Analog Scale (VAS) score, numerical rating scale (NRS) scores, and simple descriptive scores (SDS) to assess pain intensity and can determine a cut-off point for rescue analgesia and evaluate its effectiveness (de Oliveira et al., 2014). Objective measurements include recorded time spent in various postures or traversing a known distance, or measurements between limbs during ambulation, and frequencies of pain-related behaviours (Meléndez et al., 2017b).

1.11.1.1 Subjective Behavioural Measurements

The most subjective behavioural measurement is the visual analog scale (VAS) score. VAS scores noted by trained blinded observers prior to and after administration of a drug and the difference between them is used as a measure of effectiveness of an analgesic drugs in humans (Ludington & Dexter, 1998). A VAS score is marked on a horizontal 10 cm line with the left extremity denoting 0 or no pain and the right extremity denoting the worst possible pain. The mark is measured from the left hand side and assigned a numerical value (cm) with higher values corresponding to increased pain severity. A potential confounder to VAS scoring in cattle restrained in a hydraulic squeeze chute, especially with a neck extender, is that this restraint is more effective at restricting movement in response to a painful procedure than a manual mechanical squeeze chute (personal observation).

Numerical pain scoring scales can be developed for use on a particular species, as some pain behaviours are species-specific, and also for a particular type of painful event. Pain scales for cattle have been developed by the International Veterinary Academy of Pain Management (IVAPM), University of Glasgow, UNESP-Botucatu (Brazil), and University of Copenhagen in conjunction with Aarhus University and Swedish University of Agricultural Sciences. Gleerup et al. (2015) developed their Cow Pain Scale for “fearless” (gentle) Danish Holstein cows experiencing mild to moderate pain. De Oliveira et al. (2014) developed their pain scale for acute postoperative pain in Bos indicus castrated bulls, but their “miscellaneous behaviour”
category lends itself to including behaviours for other surgical procedures.

It is postulated that because cattle are a prey species, they are stoic by nature and do not overtly display pain-related behaviours that might draw the attention of predators (Weary et al., 2006). A “facial grimace scale” has been created for other prey species, such as horses, rats, mice and rabbits and has been proposed for dairy cattle to detect subtle signs of pain (Gleerup et al., 2015). Using 43 Danish Holstein cows, Gleerup et al. (2015) developed a pain scale that included scores for six behaviours: facial expression, attention to surroundings, head position, ear position, response to approach and back position, and scored each on a scale of 0 to 2 for a highest possible score of 12. Using clinical examinations of the cows and response to analgesia, the group validated the scale and assigned a score of 3 or higher as indicative of a cow in pain and requiring analgesia. This pain scale was designed for detecting mild to moderate pain, as behaviours associated with severe pain (vocalizing, teeth grinding, moaning, shivering, straining, and weight shifting/kicking) were not included.

A pain scale can be a composite of subjective and objective scoring. De Oliveira et al. (2014) used blinded observers, viewing video footage of 40 two to three year old castrated 365 ± 51 kg Bos indicus bulls, to validate the UNESP-Botucatu pain scale for assessing acute post-operative pain in cattle. The scale is from 0 to 10, with scores from 0 to 2 assigned in the categories of locomotion (normal/abnormal gait), interactive behaviour (reactivity to approach, isolation from herd mates), activity (restless or immobile), appetite, and miscellaneous behaviours (e.g. tail-flicking, foot-stamping, lying with head on the ground and wound-licking with the highest score assigned if two or more of these are present). The authors demonstrated a significant change in the behaviours of the bulls from 24 hours before castration to four hours post-castration and determined that a score of ≥ 5 indicated that further analgesia was required.

The Bovine Pain Scale developed by the International Veterinary Academy of Pain Management (IVAPM) assigns a numerical score from 0 (no pain) to 4 (severe pain) to observed behaviours (Table 1.1). It is recognized that these intangible and highly subjective measures are poorly defined and inter-observer reliability is likely to be poor (Millman, 2013).

**1.11.1.2 Objective Behavioural Measurements**

Recording video of animals has enabled behaviour frequencies to be counted objectively and in some cases, using only a portion of the video recording provides the same information while reducing the time spent watching the recorded behaviours. A study in 31 lambs
investigating castration pain found that the frequency and duration of postures every two minutes for 96 minutes and every six minutes for the subsequent 84 minutes (scan sampling of video footage) after castration or sham castration, was no different than collecting those values from continuous video footage (Molony et al., 2002). Frequencies of tail-flicking, kicking, falling, and vocalization as well as escape behaviour measured as force exerted on load cells and strain gauges mounted on the head-gate and squeeze chute during branding of weaned steer calves have been measured (Schwartzkopf-Genswein et al., 1997). Video recording of head motion while in a manual squeeze chute to measure distance and velocity of head movement during branding was found to be better than behaviour frequencies or exertion force measures at detecting treatment differences (Schwartzkopf-Genswein et al., 1998). Millman (2013) suggests that vocalization frequency may not be a robust measure for pain in cattle because of their inherently stoic nature compared to other species; for example the prevalence of vocalizing during disbudding was 13% in cattle and 100% in goats. Measuring behavioural response to touch such as using the von Frey filament for stimulating mechanoreceptors (Millman, 2013) is useful for superficial injuries, but would not be useful for ovariectionomy because the tissue trauma site is not accessible.

There are few reports in the literature reporting behavioural changes in DOT ovariectomized heifers. One study of 522 Hereford and Hereford cross yearling heifers weighing an average of 230 kg indicated that two heifers “walked stiffly” the day following ovariectomy for a morbidity rate of 0.4% (Habermehl, 1993), however the author relied on the producer to report morbidity without written methodology. Both Habermehl (1993) and Jubb et al. (2003) reported that the DOT generated no more discomfort than pregnancy palpation or artificial insemination (AI). A study using 430 Brahman and Brahman cross cows and heifers described DOT spayed animals as appearing “largely unaffected”, freely moving and bright and alert compared to 5% to 10% of flank spays appearing dull, reluctant to move and hanging their heads postoperatively (Jubb et al., 2003). McCosker et al. (2010) reported 4/574 DOT spayed heifers were “depressed, slow to rise or walking with restricted gait” between one and four days after surgery, for a morbidity rate of 0.7%; in another group of 200 heifers, 18 displayed tail elevation (9%) for up to two hours post-spaying, with 3 heifers (1.5%) continuing this behaviour intermittently for two days.

Petherick et al. (2013) conducted a welfare assessment using 238 Bos indicus cows and heifers comparing animals ovariectionomized via the flank or DOT approaches to those subjected to
physical restraint (PR) alone, mock AI, or electroimmobilization (EI) with physical restraint. Behaviour frequencies were noted on an ethogram by three trained observers in vehicles using binoculars on the day of treatment and days one through three post-treatment. Flank spaying caused more behavioural differences than DOT spaying compared to the PR, mock AI and EI control groups and the authors concluded that flank spayed heifers experienced pain of longer duration than the DOT spayed animals. The DOT spaying caused few behavioural differences from the control groups on the day of spaying (heifers showed more time in a head-down posture, less time spent eating and increased time in sternal recumbency) and for three days after spaying (more head-down postural observations). No differences in other behaviours on the ethogram (including stiff tail posture, ruminating, licking body, rubbing, vocalizing, teeth-grinding, trembling, butting/pushing herdmates, retreating from herdmates, grooming herdmates or receiving grooming) were recorded between DOT heifers and the controls. The results from this study suggest that behavioural responses might not be sufficiently revealing for measuring the pain of ovariectomy, or that DOT ovariectomy does not provoke much pain.

1.11.1.2.1 Flight Speed

Escape behaviour of cattle is measured by the time that it takes an animal to pass between two consecutively placed sensors after release from the squeeze chute (Millman, 2013). Infrared sensors (Vetters et al., 2013), “electronic” recordings (Burrow & Corbet, 2000), dual laser beams (Coombes et al., 2014), and light beam generators and reflectors (Müller & von Keyserlingk, 2006) have all been employed to measure transit time for calculating flight speed. Flight speed (FS), also called “chute exit velocity” (Baldridge et al., 2011), is derived from converting the transit time to m/s, so longer transit times transform to slower flight speeds. The conversion allows comparisons between studies, as 1.7 m, 2.2 m (Burrow & Corbet, 2000), 1.2 m (Müller & von Keyserlingk, 2006), 1.83 m (Vetters et al., 2013) and between 1.7 and 2.2 m (Coombes et al., 2014) have all been used for recording transit time. Müller and von Keyserlingk (2005) used crossbred Aberdeen Angus heifers weighing 215 ± 24 kg four times in four weeks to confirm that FS is highly repeatable. In cattle with ≥50% Bos indicus ancestry, FS was reported as moderately heritable and considered the best method of selection for temperament (Burrow & Corbet, 2000). Petherick et al. (2011 & 2013) used flight speed to block Bos indicus cattle into treatment groups prior to conducting studies comparing spaying and restraint techniques because of the correlation between faster flight speed and less stress-coping ability in that species.
1.11.1.2.2 Stride Length

Currah et al. (2009) described video recording of cattle traversing an alley after release from a squeeze chute to measure stride length (SL) from two pictures captured with both hind feet on the ground for two strides with alternate limb placement. The SL is the average of distance between these two measurements from the midpoint of each hind foot. Three month old beef calves castrated after having epidural lidocaine regional anesthesia and the NSAID flunixin meglumine intravenously had significantly longer stride lengths at 4 and 8 hours after castration than calves castrated with only epidural lidocaine and calves castrated with no pain medication (Currah et al., 2009). The authors attributed the result to the analgesic anti-inflammatory effect of flunixin during its 8 to 12 hour half-life that allowed the flunixin treated calves to feel more comfortable and express stride lengths closer to pre-castration values. There are no reports of the use of stride length as an indicator for pain of ovariectomy in cattle.

1.11.1.2.3 Feeding Duration and Frequency

The GrowSafe system (GrowSafe Systems Ltd, Airdrie, AB, Canada) uses radiofrequency identification (RFID) ear tag technology to identify individuals to monitor and record feeding intake and frequency data. Since RFID ear tags are required for all Canadian cattle, using GrowSafe for monitoring feeding behaviour is easier to use in Canada than the similar Insentec system (Repelweg, Marknesse, Netherlands) that uses transponder collars for identifying individuals (Theurer et al., 2013).

Feeding duration can be measured from the time a heifer starts to eat until she stops eating at a feed bunk using video recording that allows many heifers to be observed during a time period. No reference could be found measuring feeding duration or frequency for yearling heifers fed hay at a feed bunk. Petherick et al. (2013) reported that when observed directly, DOT spayed heifers spent less time eating than unspayed control heifers on the day of spaying, but not in the three days following spaying.

1.11.1.2.4 Activity Logging

Securing an accelerometer to the hind limb of a heifer with a self-adhesive bandage provides remote measurement of activity in the horizontal axis (heifer is recumbent with legs horizontal to the ground), and vertical axis (heifer is standing with legs vertical to the ground) and demonstrates 98% agreement with video analysis of an animal lying or standing (Theurer et al., 2013). There are several data activity loggers including the Fedometer system (ENGS, Rosh
Pina, Israel) (Wolfger, Mang, Cook, Orsel, & Timsit, 2015), the HOBO Pendant G Acceleration Data Logger (Onset Computer Corporation, Bourne, MA) and several models available from MEMSIC, Inc. (Andover, MA; formerly Crossbow Technology, Inc.) (White et al., 2008). The HOBO model has a Standard Operating Procedure (SOP) developed by the University of British Columbia (UBC) Animal Welfare Program (Lomb et al., 2018). The HOBO records the g-force on the x, y and z axes every minute into its 64 kB memory and HOBOware software converts the downloaded g-force recordings to degrees of tilt that can be translated to standing and lying times (expressed as percent), durations (expressed in minutes) and bouts (expressed as numbers). Standing bouts are the number of movements from lying to standing and lying bouts are the number of movements from standing to lying. Standing and lying times are inverses of one another (both will add up to 100%), but standing and lying bouts are not necessarily equivalent because an unsuccessful attempt to stand from a lying position will still be recorded as a bout. Standing and lying times can be generated hourly or daily.

There are conflicting reports regarding standing and lying times for cattle in pain. One study reported that calves spent increased time standing in the 24 hours post-castration (White et al., 2008) and another reported calves spent more time lying for five days after castration and dehorning (Pauly et al., 2012). Petherick et al. (2013) used direct observation to determine that DOT spayed heifers spent more time lying compared to unspayed but restrained heifers on the day of spaying. No other reports to date have described standing or lying times after ovariectomy in cattle.

1.11.2 Physiological Measurements

Stress and distress can be measured using two physiological responses to pain. The sympathoadrenomedullary (SAM) axis of the autonomic nervous system immediately releases the catecholamines that rapidly increase heart rate after a painful stimulus. The hypothalamic-pituitary-adrenocortical (HPA) axis initiates a cascade of events with slower onset and longer duration that ultimately releases cortisol and functions to promote healing. Even though catecholamine and cortisol concentrations are objective measurements, subjective judgment is made about the causes of the SAM and HPA responses because non-verbal animals cannot communicate the level of pain or stress they are experiencing (Mellor & Stafford, 2000).

Pain as one of the defining components of inflammation and inflammatory markers that can be measured from a blood sample include white blood cell count (WBC), N:L
(neutrophil:lymphocyte) ratio, and serum haptoglobin (Hp) and serum amyloid A (SAA) concentrations (Marti et al., 2017a). Substance P (SP) is a peptide released from neurons that has been investigated as a pain marker (Coetzee et al., 2012) and can also be measured in serum.

1.11.2.1 Catecholamines: Epinephrine and Norepinephrine

A study in bull calves using jugular catheter blood samples harvested every 30 seconds for 20 minutes reported that plasma epinephrine and norepinephrine concentrations peaked at two minutes after surgical castration and returned to baseline by three minutes and 5.5 minutes respectively (Stewart et al., 2010). This study also reported that plasma cortisol concentration peaked at 15 minutes after castration and did not return to baseline by 20 minutes.

1.11.2.2 Cortisol

Cortisol, the primary hormone responsible for the stress response, is not a direct measure of pain, but indicates the degree to which an experience is physically and emotionally noxious (Mellor & Stafford, 2000). Corticotropin Releasing Hormone (CRH; formerly called corticotropin-releasing factor (CRF)) is released from the hypothalamus, which then stimulates corticotropin (Adrenocorticotropic Hormone or ACTH) release from the pituitary gland that causes cortisol production by the paired adrenal cortices. Release of CRH is influenced by physical activity, illness and circadian rhythm that results in highest cortisol concentrations in the morning and lowest concentrations at night (Klein, 2013). Cortisol response is proportional to the severity of the stress so that more cortisol is produced with higher stress levels (Klein, 2013). The half-life of cortisol is about 60 minutes (Klein, 2013). About 90% or more of cortisol released is bound to plasma proteins with 75% bound to transcortin, 15% bound to albumin, and 10% is unbound, or free cortisol. Cortisol must be in its unbound (free) form to penetrate into target cells to exert its effect (Klein, 2013). Cortisol stimulates metabolism, increases blood glucose, catabolizes protein (except in the heart and brain), increases glomerular filtration rate in the kidneys and inhibits the inflammatory response.

A “ceiling effect” for cortisol concentrations is recognized and demonstrated in lambs by Moloney and Kent (1997) when cortisol concentrations for groups undergoing unilateral castration, bilateral castration or bilateral castration with tail docking all had similar cortisol concentrations, and were all higher than groups undergoing short scrotum “castration” or tail docking alone (Molony & Kent, 1997). This may be related to the process of cortisol production in the adrenal cortex becoming overwhelmed, as 11β-hydroxylase used to convert progesterone
to cortisol becomes a limiting enzyme and results in increased progesterone concentrations during HPA axis stimulation (Whitlock et al., 2012).

Total plasma or serum cortisol measures both the unbound and bound forms, but the free form diffuses passively into the saliva to provide an indicator of active cortisol concentration (Peeters et al., 2011). Studies in dogs (Vincent & Michell, 1992) and horses (Peeters et al., 2011) reported salivary cortisol concentrations reached 5–10% of peak plasma or serum concentrations. In a study of 13 three month old Holstein calves following ACTH administration (a stress simulator), salivary cortisol concentrations peaked 40 minutes after administration and gradually decreased to return to baseline by 360 minutes (Negrão et al., 2004). Collecting saliva samples for cortisol analysis is considered less invasive and more welfare-friendly than collecting blood by venipuncture for serum cortisol determination. For cattle that are not accustomed to human contact, the experience may still be stressful in spite of not being painful.

Molony et al. (2002) reported that the timing of the cortisol peak was no different between groups of lambs with varying degrees of tissue injury from castration, with or without tail docking. This study plotted severity of treatment to frequency of painful behaviours and cortisol to find that the 6 treatment groups listed from most severe to least severe: castration with tail docking (CTD), bilateral castration (C2), unilateral castration (C1), scrotal castration (C0), lidocaine anesthetized scrotal castration (LA), and handled only (H); controls were tail docked (TD). The frequency of pain-related behaviours increased with the severity of treatment and lidocaine local anesthesia greatly reduced these behaviours and eliminated the cortisol rise. The area under the curve (AUC) for plasma cortisol (total exposure to cortisol over a period of time) was highly correlated with the peak plasma cortisol concentration and the authors concluded that peak cortisol concentration is therefore a reasonable measure to demonstrate pain reduction (Molony et al., 2002).

Cortisol accumulates in the hair, and hair has been used in humans, rhesus macaques, cats, dogs, dairy cows and heifers and beef bulls (Moya et al., 2013) to measure long term cortisol production. The normal circadian cortisol secretion variations and handling stress to obtain the sample are not reflected in the hair sample concentration. A study using 15 Holstein dairy heifers demonstrated that heifers given IV ACTH injections on days 0, 7 and 14 had higher hair cortisol concentrations than control heifers given IV saline injections in hair sampled on days 14 and 28 (González-de-la-Vara et al., 2011). This study compared age and hair colour, reporting
that 15-day-old Holstein calves had higher hair cortisol concentrations than two year olds and that white hair accumulated more cortisol than black hair. The authors concluded that hair cortisol measurement is useful to determine magnitude of accumulated or long-term stress over a period of up to 28 days.

1.11.2.3 Acute Phase Proteins: Haptoglobin and Serum Amyloid A

Acute phase reaction (APR) is the body’s systemic response to infection, inflammation and injury including surgical trauma (Ceciliani et al., 2012). The APR results in fever, increased white blood cell (WBC) count, and changes in a group of proinflammatory cytokine induced proteins called acute phase proteins (APP). The APR’s purpose is to restore homeostasis by neutralizing infectious agents and controlling and finally resolving inflammation (Ceciliani et al., 2012). APP measurement is more sensitive at detecting inflammation than the leukogram and APP blood concentrations tend to return to baseline levels when inflammation resolves, but can remain high if inflammation becomes chronic (Kirbas et al., 2015). The acute phase biomarkers (APB) serum amyloid A (SAA) and haptoglobin (Hp) have been used to measure disease inflammatory response (Kirbas et al., 2015) and the stress response (Lomborg et al., 2008) in cattle. Unlike other species, SAA and Hp are both considered major positive APPs in cattle because their concentrations can increase 100 -1000 times higher than their normally low baseline concentrations (Cornell University College of Veterinary Medicine, 2013).

SAA peaks 24 to 48 hours after stimulation and its concentration subsequently decreases quickly (Cornell University College of Veterinary Medicine, 2013). It is produced mainly in the liver and acts as a chemoattractant of neutrophils and monocytes, scavenges cholesterol from dying cells, and bovine SAA opsonizes both gram positive and gram negative bacteria for more efficient phagocytosis (Ceciliani et al., 2012).

Hp peaks 48 to 72 hours after inflammation begins and decreases more slowly than SAA (Cornell University College of Veterinary Medicine, 2013). It scavenges free hemoglobin to limit iron availability for bacterial growth and to prevent oxidative damage. Hp also has anti-inflammatory effects and improves angiogenesis (Ceciliani et al., 2012).

1.11.2.4 Hemogram

The hemogram, also called the complete blood count (CBC), can be evaluated with automated cell counters. It consists of an evaluation of erythrocytes (red blood cells (RBC)), leukocytes (white blood cells (WBC)) and platelets.
The RBC count includes the total number of RBCs (per microliter), hematocrit (HCT), hemoglobin (HGB), and the erythrocyte indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Beef breeds tend to have higher RBC counts than dairy breeds (Roland et al., 2014). During acute blood loss a decrease in RBC count and HGB takes place after a few hours when hemodilution occurs, and after two days RBC regeneration begins.

The complete WBC count includes the total number of WBCs (per microliter) and the differential leukocyte count. Leukocytes consist of granulocytes (neutrophils, eosinophils, and basophils), lymphocytes and monocytes. Compared to other species, the normal neutrophil-to-lymphocyte ratio (N:L ratio) in adult cattle (reported for dairy breeds) is low, at about 1:2 (Roland et al., 2014). The bovine granulocyte bone marrow reserve is small compared to other species, and is reflected as a neutropenia in peracute inflammation with neutrophils increasing after three to five days (Roland et al., 2014). The stress response can be exhibited in the leukogram as neutrophilia, lymphocytopenia, eosinopenia, and sometimes monocytosis, and is therefore reflected as an increased N:L ratio (Kulberg et al., 2002). Epinephrine release can cause a short-lived leukocytosis. The most usual causes of neutrophilia in cattle are chronic inflammation (caused by infection or trauma) and chronic stress (Roland et al., 2014).

Platelets (thrombocytes) are megakaryocyte fragments than are important for hemostasis. Bovine platelets are small compared to other species and survive for up to ten days in the blood. Stress-induced epinephrine release triggers splenic contraction, liberating platelets from storage and resulting in thrombocytosis. Severe blood loss can be a reason for thrombocytopenia. Platelets start clumping after four to six hours in EDTA tubes or when in heparinized tubes and be a reason for falsely low platelet counts (Roland et al., 2014).

Because bovine erythrocytes and thrombocytes are small compared to other species, automated cell counters need to be calibrated for bovine blood to avoid spuriously low counts. Compared to other species, changes in the bovine leukogram tend not to be as pronounced, and results should be carefully interpreted (Roland et al., 2014).

1.11.2.5 Substance P

SP is a peptide released from neurons in the periphery and CNS in response to nociceptive signals (Mosher et al., 2014). SP functions in neurotransmission and neuromodulation and plays a role in various physiological processes including apoptosis,
inflammation and sleep, but most research has focused on its role in pain signal transmission to the CNS (Mitchell et al., 2013). One castration study in four to six month old beef calves reported that SP concentrations increased while cortisol did not differ compared to sham castrated controls and the authors suggested that SP can be a biomarker for pain in cattle (Coetzee et al., 2008). A dehorning study by Coetzee et al. (2012) supported this by reporting mean SP concentrations lower ($P = 0.04$) in scoop dehorned dairy calves treated with the NSAID meloxicam (71.4 ± 20.8 pg/ml) compared to dehorned calves without meloxicam (114.7 ± 20.8 pg/ml).

Not every pain investigation using SP in cattle has found it useful as a pain biomarker. A study of acute castration pain in beef calves of three different ages using surgical and banding methods did not report a difference in mean SP concentrations compared to sham castrated calves during the two hours after castration; however, there was a treatment-time interaction reported as surgically castrated calves had higher cortisol levels compared to sham castrated calves at two hours after castration (Meléndez et al., 2017b). Similarly, a study of chronic pain in beef calves at one week and two and four months of age sampled at intervals until healing was complete did not report a difference in SP concentrations between sham castrated and surgically or band castrated calves (Marti et al., 2017a).

1.11.2.6 Rectal Temperature

Normally body temperature is maintained within a narrow range (average 38.3, range 36.7 to 39.1 ºC for beef cattle) even when ambient temperature varies (Klein, 2013). Rectal temperature rises from baseline when responding to pyrogens produced by infectious agents, during exercise, when unable to disperse heat from the body (by sweating, increasing respiratory rate, or if ambient temperature is too high), if abnormal conditions such as dehydration interfere with thermoregulation, or when pharmaceuticals directly influence the thermoregulatory centres. The hypothalamus is the main thermoregulatory centre in the body, as it contains heat-sensitive neurons that monitor brain (core) temperature (Klein, 2013). Above or below a normal “set point”, the hypothalamus triggers mechanisms to lose or retain and produce body heat respectively. The rise in body temperature from infection improves leukocyte activity (Klein, 2013).

Prostaglandins (PG) are one of many fever-inducing pyrogens that access the hypothalamus via the *organa vasculosum of the lamina terminalis* (OVLT) because of its rich
blood supply and negligible blood-brain barrier. This stimulates the hypothalamus to produce metabolites including PGE\(_2\) that cause the body temperature set point to rise until the pyrogen is metabolized. NSAIDs exert their antipyretic action by blocking PGE\(_2\) production (Klein, 2013).

### 1.12 Analgesic Pharmaceuticals

Analgesic drug efficacy is more dependent upon the nature of the surgical procedure (e.g. orthopedic, abdominal, epidermal) than the analgesic properties of the drugs used and therefore should be evidence-based for a specific procedure (Gaynor & Muir, 2015). Analgesics have not been investigated as much in cattle as in companion animal species.

In humans and companion animals, monitoring response to analgesia and altering treatment plans is possible, but in extensively managed cattle, there are few opportunities to administer analgesic treatments, as handling itself is stressful to these animals and therefore should be kept to a minimum. An opportune time to administer analgesics is when heifers are confined in the race (alley) on their way into the squeeze chute where surgery is performed and/or in the squeeze chute itself (personal observation). Treatments therefore need to be easy to administer and rapidly effective. While it may be possible when dealing with small groups of cattle, treating a group of animals in one pass through the chute and then returning them a second time through the chute after a time lapse to ensure effective analgesia before a procedure is not possible when several hundred animals need to be processed during the course of a day.

#### 1.12.1 Pharmaceutical Definitions

Using a combination of drugs together is called *multimodal therapy*, *multimodal analgesia* or *balanced analgesia*. When used together, drugs that exert their analgesic effect via different mechanisms can be expected to act synergistically (also called *supra-additively* as the effect is greater than a simple additive effect) and therefore the dosage of each drug can be reduced from its sole-use dosage (Gaynor & Muir, 2015). This dose reduction provides a greater margin of safety from unwanted adverse drug effects and can reduce costs. When the drug combination is administered prior to surgery or other noxious event, it is called *pre-emptive analgesia* (or *preemptive antihyperalgesia*). The term *preventive analgesia* is used when post-procedural pain reduction compared to a placebo occurs when the drugs are administered during the peri-procedural period (before, during or after the procedure). In humans it has been demonstrated that preoperative NSAID administration is not superior to immediate post-operative administration (Gaynor & Muir, 2015).
1.12.2 Categories of Analgesic Drugs

Drugs for pain treatment fall into six categories: local anesthetics, corticosteroids, opioids, alpha-2 agonists, non-steroidal anti-inflammatory drugs (NSAIDs), and ‘others’, this latter category including cyclohexamines (dissociative anesthetics) such as ketamine (Gaynor & Muir, 2015). The American Pain Society recommended in 2005 that pain needs to be recognized and treated promptly, and multimodal therapy that includes drugs in different categories is desirable (Babos et al., 2013).

Drugs in the local anesthetic and corticosteroid categories are not suitable for formulating analgesic protocols for ovariectomy. Although the local anesthetic lidocaine is probably the most commonly used agent for preventing procedural pain in cattle, it is not possible to locally infuse lidocaine into the tissues traumatized during ovariectomy. Administering lidocaine as an epidural would require a sufficiently high volume to diffuse to the cranial lumbar/caudal thoracic area to anesthetize the ovarian nerves. This “high” epidural would also unacceptably render a heifer ataxic or unable to walk because lidocaine blocks action potential conduction of motor as well as sensory neurons (personal observation). Corticosteroids alleviate pain via their anti-inflammatory effect, but undesirable effects of immune suppression that could facilitate post-surgical infection make NSAIDs more attractive choices for anti-inflammatory pain relief (Gaynor & Muir, 2015). Drugs other than local anesthetics and corticosteroids are therefore most amenable for analgesic use for ovariectomy.

1.12.2.1 Butorphanol

Opioids generally are considered the most effective analgesics but differ in potency and their effect in different species. This category includes natural and synthetic drugs with morphine-like effects exerted on opioid receptors including OP$_3$ or µ (mu or MOP), OP$_2$ or κ (kappa or KOP) and OP$_1$ or δ (delta or DOP) that are found both in the periphery and in the CNS (Gaynor & Muir, 2015). The number, location and density of opioid receptors differ by species. Activation of MOPs and KOPs can cause hyperthermia or hypothermia respectively (Gaynor & Muir, 2015).

Butorphanol (l-N-cyclobutylmethyl-3,14-dihydroxymorphinan) is a mixed opioid agonist-antagonist that stimulates the moderately analgesic KOPs and blocks the more
profoundly analgesic MOPs and is therefore considered a “weak” opioid suitable for less severe pain (Babos et al., 2013). It also provides sedation and augments effects of simultaneously administered analgesics (Gaynor & Muir, 2015).

Butorphanol is available as a 10 mg/mL injectable solution in 10 or 50 mL vials under the trade name Torbugesic (Zoetis Canada Inc., Kirkland, PQ). It is labeled for use in horses and therefore administration to cattle is extra label drug use (ELDU) and requires informed client consent and a written withdrawal period for all food animal use. Canadian gFARAD recommends a 14-day meat withdrawal time (WDT) for dosages up to 0.045 mg/kg in cattle (Enouri, 2017).

In Canada butorphanol tartrate is classified as a narcotic in Schedule I of the Controlled Drugs and Substances Act (s.c. 1996 c.19)(Government of Canada, 2017). Its use must be recorded in a Controlled Substance Drug Log that is subject to audit by an inspector from the Office of Controlled Substances and it must be kept in a locked cabinet when not in use. Non-veterinarians can administer butorphanol under supervision of a veterinarian and since licensed veterinarians are the only people able to perform ovarectomy surgery in Canada, they can oversee the administration of butorphanol to cattle by a trained technician while spaying.

Butorphanol provides a calming effect in ruminants, unlike most other species, and it can be administered via intravenous (IV), intramuscular (IM) or subcutaneous (SQ) routes and at lower dosages when combined with other sedative drugs such as xylazine (Abrahamsen, 2013). It is also absorbed transmucosally and in humans it is available as a spray for intranasal (IN) application (https://www.pharmacomp.com/health-canada-drug-product-database/butorphanol). This route might be useful in cattle as well, however, after administration, a wait time of at least one minute should elapse for onset of clinical effect before performing surgery (Nigel Caulkett DVM, personal communication).

**1.12.2.2 Xylazine**

Xylazine is the most commonly used large animal sedative (Abrahamsen, 2013). It is an alpha-2 adrenergic agonist supplied as a clear 2% or 10% solution, however only the 2% solution is labeled for use in cattle in Canada. Cattle are particularly sensitive to its effects and require one-tenth the dosage that horses require on a mg/kg basis. Xylazine has dose dependent sedative, analgesic and muscle relaxant effects (Abrahamsen, 2013), consequently sedation confounds behavioural assessment of analgesia. Xylazine provides superb visceral analgesia lasting from
20 minutes up to two hours (Gaynor, 2010) and microdoses of <1 µg/kg (<0.001 mg/kg) of xylazine or other alpha-2 agonists act synergistically with opioids to enhance analgesia (Gaynor & Muir, 2015). The meat WDT for xylazine at dosages up to 0.33 mg/kg intramuscularly in cattle is three days (Enouri, 2017).

Analgesic potency varies between species and analgesia is effected in the CNS, although alpha-2 receptors located in the periphery are responsible for a local anesthetic effect. Individual susceptibility can vary, because xylazine administered to excited animals under catecholamine influence have a reduced response to its analgesic and sedation effects and require larger dosages (Abrahamsen, 2013; Hopkins, 1972). Xylazine can be administered by IM, IV, SQ, and epidural routes. It is absorbed transmucosally and captured elk given intranasal xylazine showed onset of sedation in less than a minute, were more relaxed and had lower cortisol concentrations compared to elk given saline intranasally (Cattet et al., 2004).

Xylazine can have dose dependent adverse effects in cattle. Cattle become recumbent when overdosed with xylazine, so cautious dosing is necessary if animals must remain standing. Even when given at dosages of 0.05 mg/kg IV or 0.1 mg/kg IM, half of calm bovine patients will become recumbent (Abrahamsen, 2013). However, unless administered with other analgesics, dosages that do not induce recumbency are unlikely to provide sufficient analgesia (Abrahamsen, 2013). Other adverse effects include decreased gastrointestinal motility (hence fasting for ruminants is important to prevent bloating) and slower heart and respiratory rates. Xylazine sensitizes the myocardium to catecholamine induced arrhythmias, so excited animals are at risk of cardiac arrest. After an initial increase in blood pressure (BP) for five to ten minutes, BP and cardiac output decrease with possible resultant hypoxemia. Xylazine increases uterine tone and may result in abortion in late gestation cows and heifers. It can interfere with thermoregulation via CNS mediated mechanisms and cause hyperthermia, which peripheral vasoconstriction can exacerbate, especially in hot, humid environments (Gaynor & Muir, 2015). The reversal agents yohimbine or tolazoline can be administered to minimize adverse effects, but they also reverse clinical effects including analgesia (Scofield et al., 2010).

Alpha-2 agonists close calcium channels on presynaptic primary nociceptors in the dorsal horn by a different mechanism than opioids, with both causing hyperpolarization of the secondary neurons resulting in profound synergism for analgesia and sedation when drugs of
both categories are used together (Epstein, 2014). In the spinal cord, alpha-2 receptor stimulation by xylazine inhibits SP and norepinephrine release (Anderson & Edmondson, 2013).

Xylazine can provide epidural analgesia without motor blockade, but requires a time interval between administration and surgery, therefore limiting this technique to small groups of cattle. Twenty-nine heifers and cows undergoing caesarian section were given epidural xylazine at a dosage of 0.07 mg/kg diluted to a volume of 7.5 mL to ensure sufficient cranial migration of the drug. Average time to onset of good to adequate surgical analgesia judged by reaction to flank pinprick was 22.6 ± 4.7 min in 24 animals and in the remaining 5 animals (17%), adequate analgesia was not attained within 30 min (Caulkett et al., 1993). The authors reported that 28% of the treated animals were moderately ataxic.

1.12.2.3 Ketamine

Ketamine, a dissociative anesthetic (2-(2-chlorophenyl)-2-(methylamino)cyclohexanone), is the most commonly used anesthetic agent in large animals (Abrahamsen, 2013). Ketamine is a Schedule I narcotic and requires the same controlled drug record keeping and supervision as described for butorphanol. It is approved for veterinary use only in cats, but ELDU is common in other species including horses, dogs, camelids and ruminants (personal observation). The meat WDT for cattle is 3 days for dosages up to 10 mg/kg (Enouri, 2017).

Ketamine is the most potent NMDAr antagonist clinically available, and its ability to block NMDAr ion channels prevents windup and central sensitization by preventing calcium ion influx into neurons (Quibell et al., 2011). Ketamine causes vasodilation, and increases heart rate and blood pressure and therefore antagonizes xylazine induced vasoconstriction, bradycardia and hypotension (Abrahamsen, 2013). Ketamine microdoses alone are not analgesic, but 0.001 to 0.01 mg/kg/min provides analgesia for severe pain if given with opioids or alpha-2 agonists (Gaynor & Muir, 2015). Ketamine blocks NMDA receptors to provide analgesia in two ways: by diminishing central sensitization a hyperalgesic effect occurs, and by activating descending modulation an antinociceptive effect occurs (Gaynor & Muir, 2015).

Ketamine can be given by IV, IM SQ and epidural routes and its onset of action via the IM route is five minutes (Quibell et al., 2011). It is also transmucosally absorbed and in a study of 90 people in severe pain (>80 mm on 100 mm VAS scale) IN ketamine had comparable onset time and degree of pain reduction as IV morphine (Shimonovich et al., 2016).
1.12.2.4 Butorphanol, Xylazine and Ketamine in Combination (BXK)

Abrahamsen (2013) describes a “ketamine stun” as a small dose of ketamine added to any chemical restraint protocol, and the use of butorphanol, xylazine and ketamine (BXK) in combination is reported in cattle for restraint and analgesia (Abrahamsen, 2008, 2013; Baldridge et al., 2011; Pauly et al., 2012). The term “stun” comes from the appearance of recumbent BXK treated animals, as they appear awake but are unconcerned about the procedure being performed. Abrahamsen (2013) described the effect of BXK as “semi-anesthesia” or “chemical hypnosis” when used in dosages that result in recumbency, and as “chemical restraint” for the effect in standing patients. If somatic tissues are to be incised when using BXK, local anesthesia is still required to perform surgery. BXK can be used by SQ, IM and IV routes, and time to effect and duration varies with the route administered, SQ being the slowest but longest lasting and IV being the quickest onset but also quickest to wear off. There are two dosage levels described for the ketamine stun, the lower dosage causing a “standing stun” and the higher dosage causing the “recumbent stun” and is more commonly used in small ruminants and camelids, with about a 15 minute duration (Abrahamsen, 2013). CgFARAD recommends a 14 day meat withdrawal time for intramuscular BXK “standing stun” dosages in cattle (Enouri, 2017).

The “standing stun” is more commonly used in cattle than small ruminants (Abrahamsen, 2013). There is no tolerance for recumbency during ovariection, as it would increase the risk of potentially fatal visceral trauma or exsanguination. Removing a recumbent ruminant from the squeeze chute or race is time consuming and a risk for the safety of both heifer and humans. Recumbency also poses a risk for potentially fatal bloating or aspiration pneumonia in the animal that would require care and take resources away from the primary activity of spaying the group. A study using a combination of xylazine (0.05 mg/kg) and ketamine (0.1 mg/kg) without butorphanol administered IV 30 seconds prior to castration of beef calves (260-310 kg) reported lower serum cortisol concentrations in treated calves compared to controls during the first 60 minutes post-castration that corresponded to quantifiable ketamine and xylazine levels in the blood (Coetzee et al., 2010). Although all calves were standing for castration, two calves became recumbent during blood sample collection during that hour, suggesting that the dosages used would be too high for use during ovariection.

1.12.2.5 Meloxicam

Non-steroidal anti-inflammatory drugs (NSAIDs) can be effective analgesics for patients
with mild inflammatory pain (Babos et al., 2013). NSAIDs are anti-inflammatory because they inhibit COX enzymes that form prostaglandins from arachidonic acid. The COX-1 isoenzyme produces prostaglandins necessary for bodily maintenance functions including gastric mucosal protection and the COX-2 isoenzyme is inducible and produced during inflammation. NSAIDS more specific for the COX-2 are therefore less likely to produce gastric erosion. COX-2 specific NSAIDs do cause more thrombotic risk by decreasing production of anti-platelet-aggregating prostacyclin whereas COX-1 specific NSAIDs reduce production of platelet-aggregating thromboxane and may promote bleeding (Babos et al., 2013). NSAIDs inhibit CNS glial cell prostaglandin production that contributes to central sensitization and they also provide a synergistic effect with opioids (Gaynor & Muir, 2015). Systemic administration of NSAIDs allows them to be widely distributed to the tissues, including those that are not accessible to local anesthesia, and their duration is longer than for local anesthetics (Mellor & Stafford, 2000).

In humans, meloxicam is recognized as being more COX-2 selective, but at higher dosages is less selective, and ketoprofen is considered more COX-1 selective (Babos et al., 2013). Both of these NSAIDs are approved for use in cattle. An advantage to using meloxicam in cattle is that it has a longer half-life of 26 hours (Barrier et al., 2014) and its anti-inflammatory effects are reported to last up to three days (Olson et al., 2016) which means that extensively managed animals would avoid the stress of going through the chute system for daily re-treatment.

In a study of meloxicam (0.5 mg/kg) or placebo administered SQ to 35 dairy cows and 25 heifers within 3.4 ± 0.3 hours of parturition for pain mitigation of calving, meloxicam did not affect SAA or Hp concentrations on days 2, 4 and 15 post-calving compared to a placebo injection and there was no difference in rectal temperature between groups (Mainau et al., 2014). This study also reported that meloxicam treated heifers logged more pedometer steps than control heifers in the week after calving, and meloxicam treated cows had more frequent meals and total feeding time for two days after calving. Another project randomly administered pre-emptive injectable meloxicam (0.5 mg/kg) or placebo (2.5 ml/100 kg) to 110 beef cows and heifers 7.7 ± 4.1 minutes before undergoing caesarian section and reported that animals treated with meloxicam spent more time lying in the 16 hours following surgery and had more lying bouts for 24 hours after surgery compared to the control group, although there was no difference in number of steps taken between groups (Barrier et al., 2014).
Meloxicam has been investigated for relief of castration pain in calves (Brown et al., 2015; Meléndez et al., 2017; Olson et al., 2016). Brown et al. (2015) reported that oral meloxicam (1 mg/kg) treated calves spent less time in sternal recumbency and more time standing compared to controls. Meléndez et al. (2017a) administered meloxicam (0.5 mg/kg) SQ at six hours (6H), three hours (3H) and immediately before (0H) surgical castration of 34 Angus bull calves (282 ± 28 kg) and reported that SP concentrations were lower the day after castration in 0H calves than in the 6H and 3H calves. Stride length was longest in 0H calves compared to 6H and 3H calves for up to four hours after castration and the authors concluded that the optimum time to administer SQ meloxicam was immediately prior to castration.

A generic oral meloxicam suspension has recently become available in Canada and has a label claim for reducing castration pain in cattle (Meloxicam Oral Suspension, USP, Alberta Veterinary Laboratories Ltd., Calgary, AB). This product is less expensive and less invasive to administer than the subcutaneous injectable formulation. It is in a formulation that is easier to administer than crushing tablets and administering them as a drench (Brown et al., 2015), via orogastric tube (Theurer et al., 2012) or with a balling gun in capsule form (Van Engen et al., 2014). Its ease of use allows better producer uptake for mitigating painful procedures in cattle. The meat WDT for oral meloxicam is 35 days.

1.13 Research Objectives

Ovariectomy improves overall welfare for extensively managed cull yearling heifers, and merits a pain mitigation protocol that encompasses both the procedural and post-operative pain induced by the surgical procedure. Using behavioural and physiological measurements in heifers, the research objectives were as follows:
1. to document the magnitude of pain heifers experience during and after ovariectomy
2. to determine whether an intramuscular injection of BXK (butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg) and ketamine (0.04 mg/kg)) administered five minutes prior to ovariectomy can mitigate the procedural and immediate post-surgical pain
3. to determine whether oral meloxicam (1 mg/kg) administered at the time of ovariectomy can reduce the post-surgical inflammation and by extension, post-surgical pain of ovariectomy
4. to determine if BXK and meloxicam can be efficiently administered under ranch conditions
Table 1.1: IVAPM Bovine Pain Scale. Adapted from IVAPM. ([https://ivapm.org/professionals/pain-scale-bovines/](https://ivapm.org/professionals/pain-scale-bovines/))

**IVAPM Bovine Pain Scale**

<table>
<thead>
<tr>
<th>Numerical Score</th>
<th>Behaviours</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No pain: Grazing or eating at feeder or bunk, Interest in surrounding and herd mates, Moves away when approached</td>
</tr>
<tr>
<td>1</td>
<td>Mild pain: Mild postural change such as stiffness, subtle lameness, Reduced interest in surroundings, May warn off herd mates by shaking head or bunting</td>
</tr>
<tr>
<td>2</td>
<td>Mild to moderate pain: Orbital fissures not fully open; dull eyes, Abnormal posture: stiff, not moving, arched back, lame, Decreased appetite</td>
</tr>
<tr>
<td>3</td>
<td>Moderate pain: Standing away from the herd, Stiff and unwilling to move, Not eating and may have visible weight loss, Abnormal posture: head down, tucked tail, ears down, arched back</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain: In addition to above: Rapid shallow respirations or open mouth breathing, Grunting, teeth grinding, Rigid posture or down</td>
</tr>
</tbody>
</table>
Figure 1.1: Spaying Heifers, Portland Ranch Douglas Lake and Guichons, 1923. Heifers were roped by their head and hind feet and tethered between two posts on their right side. A short log was placed under their flank to elevate the left flank area where the incision was made for ovariectomy.

Figure 1.2: Inscription on photo shown in Fig 1.1 written by the late Joseph William Lauder.
Figure 1.3: Willis Ovarirotome
Figure 1.4: Bovine female reproductive tract, ventral schematic view showing blood vessels. A, ovaries; B, uterine tubes; C, uterine horns; D, vagina; E, vestibule. Blood vessels shown: 1, ovarian artery; 1’ uterine branch of ovarian artery; 2, uterine artery; 3, vaginal artery; 4, ovarian vein; 5, accessory vaginal vein; 6, vaginal vein. (Adapted from Dyce, K. M., & Wensing, C. J. . (2010). *Textbook of Veterinary Anatomy* (4th ed.). St. Louis, Mo.USA: Saunders Elsevier.)
Figure 1.5: Diagram representing differences in nociceptive transmission (Pain Pathway) for superficial somatic pain (Spinocervicothalamic Tract on left) and visceral and deep pain (Spinoreticular Tract on right).
CHAPTER 2
MEASURING BEHAVIOURAL RESPONSES TO PAIN MITIGATION FOR OVARIECTOMY IN YEARLING BEEF HEIFERS

2.1 Introduction

Under extensively managed conditions, yearling beef heifers that are not chosen to be herd replacements may undergo ovariectomy (spaying) prior to the summer grazing season to prevent pregnancy. The Dropped Ovary Technique (DOT) using the Willis ovariotome is currently the most common ovariectomy procedure used, although it requires a high degree of technical expertise (Jubb et al., 2003) and data suggest that analgesia should be provided (Petherick et al., 2013). In Canada, the Code of Practice for the Care and Handling of Beef Cattle (National Farm Animal Care Council, 2013) recommends that a veterinarian be consulted for pain mitigation for ovariectomy, which to date has not be described in the literature.

Behavioural changes are indicators of pain in cattle (Weary et al, 2006), but they can be difficult to measure (Pauly et al., 2012). Only one study measured behavioural changes after ovariectomy, reporting that DOT spayed heifers spent more time in sternal recumbency the day after ovariectomy compared to unspayed controls (Petherick et al., 2013). Measuring changes in behaviour after administration of analgesic medications can demonstrate that the drugs are effective at diminishing pain (Gleerup et al., 2015).

An effective analgesic protocol would mitigate the acute procedural pain of ovariectomy as well as the post-procedural inflammatory pain. Multimodal drug therapy employs classes of analgesic drugs that work by different mechanisms to provide improved analgesia and allows use of lower dosages of each drug (Gaynor & Muir, 2015). The combination of butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), and ketamine (0.04 mg/kg) (BXK) administered prior to ovariectomy for procedural pain and an oral formulation of the non-steroidal anti-inflammatory drug meloxicam (1 mg/kg) administered at the time of ovariectomy was selected for providing multimodal analgesia for heifers undergoing DOT ovariectomy.

This trial was a pilot study for a second trial of intensive sampling for both behavioural and physiological changes in indicators of stress, pain and inflammation, comparing heifers ovariectomized with and without pain mitigation. The objectives for this study were to use
behavioural indicators of pain to determine if 1) BXK administered intramuscularly (IM) 5 min prior to ovariectomy would mitigate procedural and immediate postoperative pain, 2) oral meloxicam administered at the time of spaying would reduce inflammatory pain in the days following ovariectomy, and 3) the administration of the drugs could be accomplished in a typical ranch setting. We hypothesized that we would see differences during ovariectomy in Visual Analog Scale (VAS) scores and in frequency of pain–related behaviours during ovariectomy, as well as differences after ovariectomy in stride length, gait, standing/lying behaviour, and feeding duration between spayed heifers receiving analgesic treatment compared to untreated spayed heifers.

2.2 Materials and Methods

2.2.1 Ethics

The University Animal Care Committee (UACC) Animal Research Ethics Board (AREB), University of Saskatchewan (AREB 20160108) and the Lethbridge Research Centre (LRC) Animal Care Committee (ACC #1643) approved this protocol. Heifers used in this study received care according to the Canadian Council of Animal Care guidelines (Canadian Council on Animal Care, 2009).

2.2.2 Animals, Animal Management, and Handling Facility

One hundred and twenty commercial, ranch-owned, Hereford and Angus crossbred 10-12 month old beef heifers (302 ± 27 kg) scheduled for ovariectomy on ranch premises at the end of March 2017 were studied over the course of 7 d in a randomized controlled clinical trial. Heifers were housed in a rail fenced feeding pen about 1 acre in size with approximately 75 m of feeding space and an ad libitum water source on the opposite side to the fence line feeder. Heifers were fed round-baled alfalfa mix hay by feed truck in the morning and grain around mid-morning, and leftover hay was turned over towards the fence line in the afternoon. Softwood shavings were available in the centre of the pen for bedding. Heifers were on a veterinarian designed immunization program that included Clostridial 7-way vaccination (Ultrabac/Somubac, Zoetis Canada Inc., Kirkland, QC).

The handling facility consisted of a metal panel race with a catwalk leading up to a hydraulic squeeze chute with a neck extender (Cattlelac Cattle, Reg Cox Feedmixers Ltd., Lethbridge, AB, Canada), where spaying took place. A digital scale (B.C. Scale Co. Ltd., Langley, B.C. Canada) was placed under the squeeze chute prior to the study for recording
weights. The day before spaying (d -1) the heifers were put through the race and caught individually in the squeeze chute to record body weight and attach accelerometer activity data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Bourne, MA, USA). The accelerometers were covered by protective waterproof padding, wrapped with self-adhesive bandaging material (Flexible Wrap, Professional Preference, McCarthy and Sons Service, Calgary, AB, Canada) and numbered in sequence, then affixed vertically to the lateral side of the right metatarsus according to the protocol developed by the UBC Animal Welfare Program (UBC-AWP, 2013) using assorted colours of 10 cm wide by 4.6 m long self-adhesive bandaging material. A white plastic sequentially numbered dangle ear tag (11.4 cm × 7.6 cm) (Z Tag-Feedlot, Datamars, Kane Veterinary Supplies, Edmonton, AB, Canada) and a metal spay tag (3.5 cm × 0.8 cm) (Ketchum Tamperproof Metal Ear Tags, Ketchum Manufacturing Inc., Brockville, ON, Canada) were placed in each heifer’s right ear. The HOBO number, leg wrap colour, dangle tag number, radio frequency identification (RFID) tag number, and description of each heifer was recorded, along with the time that each heifer entered and exited the squeeze chute. Heifers were fasted in a holding pen after they left the chute on d -1 to minimize potential surgical complications, particularly iatrogenic visceral trauma, during ovariectomy. After spaying, heifers each received 20 mL of 200 mg/mL oxytetracycline (Oxyvet 200 LA, Vetoquinol NA Inc., Lavaltrie, PQ, Canada) as two 10 mL IM injections in the left neck to help prevent iatrogenic infection and an implant (Compudose Implants, Elanco Animal Health, Guelph, ON, Canada) was placed subcutaneously (SQ) in the left ear to improve weight gain on pasture.

2.2.3 Experimental Design and Treatments

Body weight was used to block the heifers into four treatment groups in a 2 × 2 factorial design to evaluate the effect of BXK, MEL and the interaction between BXK, MEL and time. Group allocation was done using a randomization table (https://www.randomlists.com). Treatments were assigned randomly as follows: DIL/DIS - sterile diluent IM and distilled water orally ($n=30$); BXK/DIS - BXK IM and distilled water orally ($n=30$); DIL/MEL - sterile diluent IM and meloxicam orally ($n=30$); and BXK/MEL - BXK IM and meloxicam orally ($n=30$). For ease of group identification during filming of feeding behaviour, treatment groups were identified by stapling coloured tags onto the white identification tags using blue tags for DIL/DIS, orange tags for BXK/DIS, purple tags for DIL/MEL; and green tags for BXK/MEL on the day of spaying (d 0). The technician tasked with directing the treatments on d 0 printed out the group
assignments and drug dosages to ensure the treatment administrators would be blinded to
treatment group the following day.

2.2.4 Analgesic Pharmaceuticals

The BXK was formulated so that a 2 mL volume administered to a 300 kg heifer
delivered 3 mg of butorphanol (0.01 mg/kg), 6 mg of xylazine (0.02 mg/kg) and 12 mg of
ketamine (0.04 mg/kg), and dosage administered to each heifer was calculated using body weight
from d -1. On the morning of d 0, a 120 mL mixture of BXK was made by adding 18 mL of 10
mg/mL butorphanol (Torbugesic, Zoetis Canada Inc., Kirkland, QC), 18 mL of 20 mg/mL
xylazine (Rompun 20 mg/ml Injectable, Bayer Inc., Mississauga, ON, Canada), 7.2 mL of 100
mg/mL ketamine (Narketan, Vetoquinol N.A. Inc., Lavaltrie, QC, Canada) and 76.8 mL of
sterile diluent (Sterile Water USP, Bimeda Canada, Cambridge, ON) into a 250 mL vial which
had its sterile water content discarded. Using 10% of each of the ingredients, 12 mL of BXK
was mixed in a 30 mL sterile empty vial (Sterile Empty Glass Vials Clear, Partnar Animal
Health, Ilderton, ON, Canada) as a reserve supply in the event of any loss. A second 250 mL
vial of sterile diluent was used for the placebo injections.

A draw-off dose syringe (Meloxicam Dosing Gun, Solvet, Alberta Veterinary
Laboratories Ltd., Calgary, AB, Canada) was used to administer the meloxicam 15 mg/mL
suspension from its one litre plastic bottle (Meloxicam Oral Suspension, USP, Solvet, Alberta
Veterinary Laboratories Ltd., Calgary, AB, Canada) and to administer the oral placebo, an
identical bottle and dose syringe was labeled and filled with distilled water. The oral volume
calculated for each heifer was based on a 20 mL per 300 kg dosage.

Each heifer received an IM injection in the right neck with either BXK or DIL, using an
18G 2.5 cm stainless steel needle (Ideal D3 Detectable Needles, Animal Safety Division, Neogen,
Lexington, KY, USA) while she was standing in the race prior to spaying. A cowboy on
horseback in the pen behind the race continually directed heifers into the system, which
facilitated injection by minimizing space for movement between heifers. Tag number was called
out to the coordinating technician who relayed treatment and dosage instructions to the
veterinarian (JL) performing the injections. Time of injection was noted and a digital timer was
used to ensure that no less than 5 min elapsed from injection until each heifer entered the
squeeze chute for spaying. Once in the squeeze chute, body weight was recorded for each heifer
before spaying began. When ovariectomy was completed, a coloured tag was affixed to the
dangle tag according to group assignment as directed by the coordinating technician and either MEL or DIS was administered orally by an experienced ranch hand.

2.2.5 Ovariectomy Technique

One experienced veterinarian blinded to treatment group performed the ovariectomies using the DOT. Briefly, an assistant held each heifer’s tail vertically over her back to keep it away from the vulvar area and provide some restraint. The veterinarian’s non-dominant gloved arm was inserted rectally, feces were evacuated and the reproductive tract palpated to ensure the heifer’s non-pregnant status and absence of tract abnormalities such as freemartinism. With the other hand, a disposable paper towel was used to clean the vulvar area, the ovariotome was retrieved from immersion in a 20 L lidded pail of 0.05% chlorhexidine solution and guided into the vagina and through the dorsal fornix near midline into the abdominal cavity. The rectal hand guided one ovary into the eye of the ovariotome, a caudal pull severed the ovarian attachments and the ovary was dropped into the abdomen; the procedure was repeated for the remaining ovary. The ovariotome was wiped clean with a paper towel and re-immersed in the chlorhexidine solution. Time at the start and end of spaying was recorded to calculate spay times.

2.2.6 Behavioural Measurements

2.2.6.1 Visual Analog Scale (VAS) Score and Pain–Related Behaviour Measurement

Two observers experienced in scoring pain in cattle and blinded to treatment group but not the nature of the procedure stood approximately 1 m from the squeeze chute and used a pre-printed form for each heifer to record Visual Analog Scale (VAS) score and pain-related behavioural responses during ovariectomy (Fig. 2.1). The VAS score was their subjective interpretation of the level of each heifer’s pain during ovariectomy, which they indicated with a mark on a 10 cm horizontal line, where the left extremity (0 cm) indicated no pain and the right extremity (10 cm) indicated extreme pain, as previously described in studies assessing analgesic efficacy (Ludington & Dexter, 1998; Moya et al., 2014). The distance from 0 cm to the mark, to the nearest 0.5 cm from the left extremity, was the measurement used as the VAS score, a higher score reflecting more perceived pain. Pain-related behaviours recorded by the two observers were vocalization, urination/defecation, falling attempts, kicking/leg lifting, body flinching, intense breathing (hyperventilation) and head movements. Tail flicking frequency was reported by the tail-holding assistant as the number of times the heifer attempted tail movement during tail restraint.
2.2.6.2 Stride Length Measurement and Gait Score

After leaving the squeeze chute, the heifers navigated two 90-degree right turns to enter an alleyway and travel past a video camera (Panasonic WVCP474; Panasonic Canada, Inc., Mississauga, ON, Canada) on d-1, d 0 and d 5 for recording video of their stride length on their way back to their home pen, as described in a study investigating response to analgesia in castrated calves (Currah et al., 2009) with some modifications. A 134.6 cm gate panel visible in the video was used as a measurement reference. Two pictures were taken from each heifer’s video recording when her right and left hind feet were both on the ground, one with the left foot ahead and another with the right foot ahead, using GOM Player software (GOM Lab, Gretech Corporation, Seoul, South Korea). Distance between the centre of each hind foot was measured using ImageJ software (Bethesda, MD), and the average of the two strides was recorded as the stride length (SL). Heifers that ran past the camera were treated as missing data points, as measurement was not possible. Tag colour and number were not visible for most heifers because the video camera was set up on the opposite (left) side, so measurements were performed blindly as to treatment group. A list of the order that the heifers were spayed with descriptions (coat colour, leg wrap colour) with their times leaving the squeeze chute was used to confirm correct identity of each heifer.

The video footage used to record SL was used to assign each heifer to a gait category. Each heifer’s gait when traversing past the camera was scored as follows: 1 (walk), 2 (trot), and 3 (run).

2.2.6.3 Standing and Lying Behaviour

Data of the vertical (X) and horizontal (Z) axis orientation of the HOBO accelerometers were recorded every minute when affixed to the heifers to determine standing/lying time budgets (daily percentages), mean standing and lying duration (min/d) and standing and lying bouts (number/d) as validated by the University of British Columbia Animal Welfare Program (UBC AWP, 2013). Standing and lying time budgets were also retrieved on an hourly basis (hourly percentages) for 10 h after spaying on d 0. The accelerometers began logging h 1 of the 24 h day at midnight, and by using the time of spaying of each heifer as a guide, it was possible to compare hourly post-spay data between heifers. “Post-spay h 1” was designated as the h in
which the heifer was spayed; for example, a heifer spayed at 11:30 am would have accelerometer h 12 recorded as “post-spay h 1”, and each subsequent post-spay hour recorded up to accelerometer h 21 (“post-spay h 10”). Accelerometers were removed on d 5 and the contained data downloaded into a computer.

2.2.6.4 Feeding Behaviour and Average Daily Gain (ADG)

Ten rechargeable battery-operated video cameras were set up across the feed alley to view the 75 m length of the fence line feeder in the heifers’ home pen. Numbers were placed on fence posts using duct tape to ensure that cameras were correctly oriented. Plastic bags were secured over the cameras during inclement weather. Batteries were replaced at approximately 1.5 h intervals. On d 0, recording began at the start of spaying, as heifers had access to the home pen as they were ovariectomized to have immediate access to feed and water after their 24 h fast. Recording on d 1 to d 3 began at the time of the morning feeding. Video from the cameras was downloaded daily onto a computer for further analysis. Weather conditions were occasionally snowy, rainy and windy during recording, which rendered some video unusable. Total video time recorded from all cameras therefore varied by day (60.6 h on d 0; 42.3 h on d 1; 13.2 h on d 2 and 6.3 h on d 3). The unit of analysis was the treatment group noted by tag colour while at the fence line feeder. Continuous observation of video was used to record feeding duration with feeding duration time (min) defined as the time a heifer started eating until she stopped eating with brief interruptions from jostling by cohorts of less than 30 seconds considered part of feeding time if she actively continued to seek space at the feeder and resumed feeding.

Body weight was recorded when heifers went through the handling system on d -1, d 0, and d 5 for calculation of ADG for the trial period.

2.2.7 Statistical Analysis

Two heifers were discovered pregnant upon palpation and were not spayed, leaving a total of 118 heifers in the study groups: DIL/DIS (n=29); BXK/DIS (n=29); DIL/MEL (n=30); and BXK/MEL (n=30).

Results were considered significant when \( P \leq 0.05 \) and tendencies were considered to be present when \( 0.05 < P \leq 0.10 \). The individual heifer was the experimental unit for all analyses except feeding duration, where treatment group was the experimental unit. Data were tested for normal distribution with histograms and the Shapiro-Wilk normality test using Stata statistical software (version 14.2, StataCorp, College Station, TX, USA) and outliers were identified using
box plots. VAS score data had 3 missing points for observer 1 and 17 missing points for observer 2; these heifers were removed from the analysis; two outliers with ≥ 1.5 cm difference between observers were also removed from analysis, leaving 96 scored heifers for both observers. Data were log transformed to attain normal distribution for analysis. HOBO accelerometer data generated per day were analyzed from d 0 to d 4, as d-1 and d 5 were not complete 24 h periods. Square root transformation of data for standing and lying duration, and standing and lying bouts, was performed to attain normal data distribution for analysis. Stride length data were analyzed with SAS statistical software (version 9.4, SAS Inst. Inc., Cary, NC) using a repeated measures mixed effects model with d -1 values as covariates to test for treatment effect and interactions. VAS scores and accelerometer data (standing/lying percentages, standing/lying bouts and standing lying durations) were analyzed using the same procedure, but without repeated measures. Fixed effects were BXK, MEL and time and the individual heifer was the random effect. Time was regarded a repeated factor, and for each variable, 3 variance-covariance structures were applied (compound symmetry, autoregressive order 1, and unstructured) to select a covariance structure for the individual heifer (the error term). The most appropriate analysis was the covariance structure that minimized Schwarz’s Bayesian information criterion.

Pain-related behaviours during ovariectomy and gait scores after ovariectomy were analyzed with Pearson’s chi-squared two-way tables using Stata for detecting differences between heifers after either DIL or BXK injection. Gait scores for d-1 were analyzed for heifers that were assigned to receive BXK or DIL on d 0 to provide a baseline comparison for d 0 analysis of gait. Mean spay time ± SD (seconds) were determined using Stata and a two-sample t-test performed to compare spay time for heifers that received BXK or DIL prior to ovariectomy.

To analyze feeding duration, the treatment group was used as the unit of analysis. Number of feeding events and duration of each feeding event was counted for each day, and total video recording time was counted for each day. Descriptive statistics were generated using Stata software, data distribution was similar between groups and not normal, therefore the nonparametric Kruskal-Wallis equality of populations rank test was performed and when \( P \leq 0.05 \), post-hoc Dunn’s test of multiple rank sum comparisons with the Bonferroni correction was used to compare groups. ADG to d 5 was analyzed using one-way ANOVA in Stata, with averages of d -1 and d 0 weights used as initial weights because heifers were fasted prior to ovariectomy.
2.3 Results

2.3.1 VAS Score, Pain-Related Behaviours During Ovariectomy, and Spay Times

There was no difference in VAS scores between BXK and DIL treated heifers (Table 2.1) and no interaction effects; however, the oral treatments of MEL or DIS were administered after VAS scoring immediately before the heifers were released from the squeeze chute. There was no difference between observer scores for treatment ($P = 0.80$). Observer 1 scores (mean ± SD) for BXK treated heifers were $1.4 \pm 0.97$ cm, (range 0.1 – 3.7 cm), and for DIL treated heifers were $1.7 \pm 1.07$ cm (0.3 – 4.3 cm); observer 2 scores for BXK treated heifers were $1.6 \pm 1.04$ cm (0.2 – 4.0 cm), and for DIL treated heifers were $1.4 \pm 1.06$ cm (0.1 - 4.7 cm). Pain-related behaviours (defecation, tail flicking, body flinching, head movement, vocalization, and intense breathing) were not different between heifers receiving BXK or DIL although there was a tendency for BXK treated heifers to kick/leg lift more than DIL heifers ($P = 0.09$).

Mean spay times were no different when comparing heifers that received BXK to those that received DIL ($P = 0.80$). BXK treated heifers ($n=57$) had a mean spay time of $31.6 \pm 8.96$ s (range 17 – 56 s) and DIL treated heifers ($n=56$) had a mean spay time of $30.2 \pm 9.61$ s (range 15 – 84 s).

2.3.2 Stride Length and Gait Score

On d 0 stride length was measured for 110 heifers, as camera malfunction resulted in loss of video capture of 10 heifers. Heifers that ran (4 heifers on d 0; 2 heifers on d 5) were unmeasurable and treated as missing data points in the analysis. Repeated measures analysis showed no differences between treatments and no interactions (BXK x time, MEL x time, BXK x MEL x time) (Table 2.4). Numerically, stride lengths for d 5 were shorter than for d 0, but the difference was not significant (Table 2.5).

Gait travelled past the stride length video camera on d -1 showed a tendency for heifers that were randomly selected to receive BXK on d 0 to trot or run instead of walk compared to the heifers selected to receive DIL ($n = 110; P = 0.08$). After spaying on d 0, more BXK heifers walked compared to the DIL heifers ($n = 118; P = 0.002$). Four DIL heifers and no BXK heifers ran past the video camera after ovariectomy on d 0.

2.3.3 Standing and Lying Behaviour
For the first 10 h after spaying, the hourly standing/lying time percentage accelerometer data was available for 118 heifers. Hourly repeated measures analysis revealed a BXK x time effect ($P = 0.03$; Fig. 2.2) as BXK treated heifers stood for a smaller percentage of time during h 4 post-spaying ($P < 0.01$), and stood for a larger percentage of time during h 10 post-spaying ($P < 0.01$). There was also a MEL x time effect ($P < 0.001$), as meloxicam treated heifers stood for a smaller percentage of time during h 5 ($P = 0.03$) and h 10 ($P = 0.02$) and stood for a longer percentage of time in h 7 ($P = 0.02$), h 8 (P= 0.001), and h 9 ($P = 0.02$) post-spaying (Fig. 2.3).

From d 0 to d 4, there was a MEL x time effect ($P = 0.02$) for standing percentage times, with MEL heifers standing for $62.3 \pm 0.75\%$ of the day on d 1 compared to DIS heifers that stood for $66.1 \pm 0.75\%$ of that 24 h period ($P = 0.01$; Fig. 2.4). There was also a MEL x time effect for standing duration, with MEL heifers standing for shorter periods of time ($P = 0.03$) compared to DIS heifers (Fig. 2.5). There were no differences in lying duration, standing bouts and lying bouts between treatments (Table 2.4).

2.3.4 Feeding Behaviour and ADG

There was a difference in feeding duration between groups on d 0 (Table 2.6), with pairwise comparisons of the sum of ranks showing the BXK/DIS and BXK/MEL groups had longer feeding durations compared to the DIL/DIS group ($P = 0.03$ and $P = 0.002$ respectively), and the BXK/MEL group had longer feeding durations than the DIL/MEL group ($P = 0.02$). On d 2, the BXK/DIS group had longer feeding durations than the DIL/DIS group ($P = 0.02$). There was no difference between groups in feeding duration on d 1 and d 3. Overall mean for ADG was $1.9 \pm 2.68$ kg/d and 3 outliers were removed from the analysis. There were no differences in ADG between treatment groups to d 5 after ovariectomy (Table 2.7).

On d 1, 2 and 3 after spaying, heifers were observed to display riding behaviour that was not present on d 0. The cameras set up for filming feeding behaviour did not cover the entire pen so it was not possible to accurately determine riding frequency, but in a five minute period on d 1 18 riding events could be counted.

2.4 Discussion

This study was done under typical ranch conditions to determine the feasibility of using the protocol without disrupting the flow of events during the spaying process. Morbidity and mortality rates for this study were 0%, as no heifers died nor reached any of the endpoints for intervention during the observation period (uninterested in eating/drinking, separated from herd.
and disinterested in surroundings, remained in lateral recumbency or displayed reluctance to move away when approached, stood with continuously arched back, displayed teeth grinding, grunted when walking or lying down, restlessness or pacing displayed, exhibited shallow rapid respiration). Measurement of stress and pain of spaying was limited to behavioural indicators, with the realization that cattle are generally considered stoic with respect to pain, as a herd animal showing painful behaviour may identify itself as being vulnerable to predators (Weary et al., 2006). Pharmaceuticals used were selected based on the ease of administration, rapid time to effect after administration, and cost. Combining butorphanol (an opioid), xylazine (an alpha-2 agonist) and ketamine (a cyclohexamine) together exploits pharmaceutical synergism, as each uses a different analgesic mechanism and allows use of a lower dosage of each drug (Gaynor & Muir, 2015). Because heifers need to be standing for the DOT ovariectomy, the BXK dosage used in this study resulted in a “standing stun” as described by Abrahamsen (2013) that should not cause recumbency. The anti-inflammatory used was an oral meloxicam suspension with a label claim for alleviation of pain and inflammation after castration in cattle and is less costly than the injectable formulation and therefore is more attractive to producers.

2.4.1 VAS Scores and Pain-Related Behaviours During Ovariectomy

There has been no previous account of VAS scoring and pain-related behaviour recording during ovariectomy, but VAS scoring has been used in castration pain studies. VAS scores in 4 month old calves were higher ($P < 0.01$) in those castrated using a knife (2.8 ± 0.06 cm) than those castrated by banding (1.5 ± 0.06 cm) and both scored higher ($P < 0.01$) than sham castrated calves (1.2 cm ± 0.06 cm) (Meléndez et al., 2017b) which supports the perception that VAS scores increase with increasing trauma and pain. In this study, no differences in VAS scores were detected between heifers administered BXK and those administered DIL prior to ovariectomy, but the heifers were effectively restrained in a hydraulic squeeze chute and their tails were held vertically during the procedure to further reduce animal movement within the chute. It is possible that heifers restrained in a mechanical squeeze chute with no “tail-jack” may have opportunity to express behaviour more freely.

There are also physiological differences between visceral and somatic pain that may explain the minimal behavioural changes observed during ovariectomy. Visceral nociceptive afferent receptor fields are larger and overlapping compared to those for somatic nociception, so a noxious visceral stimulus originating in a confined area may be conveyed by several fibres
covering a large area, making it difficult for an individual to localize the site of the resulting pain (Hellyer et al., 2007). Silent nociceptors that are engaged only during inflammation constitute a large proportion of visceral nociceptors (Gaynor & Muir, 2015) and would not yet be triggered during the short time taken to perform an ovariectomy. Visceral tissues have only one-fifth as many “sharp pain” Aδ fibres per “dull pain” C fibres compared to somatic tissues (Hellyer et al., 2007) and it seems more likely that a noticeable behavioural response would occur in response to “sharp pain”. The difference in behavioural response to superficial somatic pain compared to visceral pain can be reflected in VAS scores for castration, which evokes both superficial and visceral pain compared to DOT ovariectomy that evokes only visceral pain. VAS scores for bull calves surgically castrated without analgesia were reported higher ($P < 0.01$) than for sham castrated calves (3.9 cm and 1.3 cm respectively) (Moya et al., 2014), and the heifers ovariectomized in this study had VAS scores similar to those of the sham castrated calves (Table 2.1).

2.4.2 Stride Length and Gait Score

Stride length (SL) has not been previously measured in ovariectomized heifers. It has been used for assessing the pain of castration in calves, as band-castrated calves had longer stride lengths than knife-castrated calves immediately after castration, which corresponded with higher cortisol concentrations in knife castrated calves (Meléndez et al., 2017b). Since the ovariectomized heifers exhibited no differences in stride length between treatments, SL might not be a useful measure of pain associated with ovariectomy. However, in this study, the SL video camera could only be situated by the return alleyway to the home pen, approximately 30 m from the squeeze chute, and required the heifers to negotiate 2 right hand 90° turns to pass by the camera and SL measurements have not been previously described for cattle travelling a meandering route when released from the squeeze chute.

Gait scores provided evidence that BXK was providing a clinical effect in the treated heifers. Heifers administered BXK walked more and trotted/ran less after ovariectomy than heifers not administered BXK (Table 2.3b), but the sedative effects of butorphanol and xylazine (Abrahamsen, 2013), rather than the analgesic effect of BXK, may be responsible for this result.

2.4.3 Standing and Lying Behaviour

Standing and lying behaviour following ovariectomy has been assessed by trained observers in *Bos indicus* cattle using an ethogram for 3 - 4 min scans at 5 or 10 min intervals.
from a vehicle using binoculars, and an increased time spent in sternal recumbency was reported on the day of spaying compared to unspayed controls (Petherick et al., 2013). Accelerometers on the heifers in this study provided a continuous objective measure of standing and lying times. BXK treatment resulted in a longer time in recumbency during the fourth hour and shorter time in recumbency during the tenth hour after ovariectomy. This result is curious, as each constituent drug of BXK at the dosage used probably did not persist longer than 2 hours. Abrahamsen (2013) reports onset of effect after subcutaneous (SQ) administration of this “standing stun” dosage of BXK of 5 to 10 min, with duration of effect between 60 and 90 min. In our trial, administering the BXK IM should have provided as quick an onset with a duration no longer than for SQ administration (Abrahamsen, 2013). A lower cortisol response was reported in calves that were administered pre-surgical xylazine (0.05 mg/kg) with ketamine (0.1 mg/kg) for 10 h after castration compared to those that received xylazine alone (Coetzee et al., 2010). The authors of that study measured plasma concentrations of xylazine and ketamine and suggested that analgesic effects from the combination are extended beyond the time each drug is measurable in plasma. It is possible that the BXK has a lingering analgesic effect, as ketamine is the most potent NMDA blocker clinically available (Quibell et al., 2011) and may have minimized central sensitization, thus preventing more intense pain.

The meloxicam treatment had a larger impact on hourly standing and lying times on d 0, with 3 consecutive hours (7, 8, 9) of longer standing times and the fifth and tenth hours with shorter standing times. Meloxicam reduces peripheral sensitization by inhibiting COX mediated prostaglandin production and therefore reduces post-surgical inflammatory pain (Anderson & Edmondson, 2013). Oral meloxicam was detectable in bovine serum after 30 min post-administration (Coetzee et al., 2012) and a pharmacokinetic study in 145-170 kg Holstein calves reported peak levels of 3.1 µg/mL (range 2.64 - 3.79 µg/mL) occurred at 11.64 h (range 10 - 12 h) after administration (Coetzee et al., 2009), but no reports were found describing the time that therapeutic blood levels were achieved. Since analgesic protocol effectiveness depends upon the procedure being performed (Gaynor & Muir, 2015), from our hourly activity data results, it seems reasonable to suggest that for the pain of ovariectomy, oral meloxicam provides clinical relief beginning at 5 to 7 hours post-procedure. However, accelerometer data is limited to reporting limb inclination, and cannot distinguish between a heifer standing still from discomfort
or standing while contently eating at the feeder, therefore direct observation would be useful for proper data interpretation.

The accelerometer activity data during the 5 d following ovariectomy showed only a meloxicam effect. Nonsteroidal anti-inflammatory drugs (NSAIDs) including meloxicam can be expected to reduce the inflammation at the surgical site, and by extension, the post-operative inflammatory pain. Coetzee et al. (2009) determined that the half-life of oral meloxicam was 27.54 h (range 19.97 - 43.29 h) in Holstein calves approximately 4 months of age. Elimination of a drug from the body is expected after 5 half-lives (Goel et al., 2014), so some meloxicam was likely still present in the MEL heifers, although perhaps at declining effective concentrations, until the end of the trial. Even though the difference in standing time percentage was small between the meloxicam treated heifers and those administered distilled water on d 1, it was statistically significant and may reflect pain tolerance individuality. Female rats under estrogen influence undergo the physiological changes of increased visceral afferent sensitivity and larger receptor field size that heighten visceral pain intensity compared to cohorts in diestrus (Sengupta, 2009). A small difference between heifers with or without meloxicam treatment may not fully reveal the possibility that some individuals experienced a greater degree of visceral pain during their estrus cycle than others. Perhaps the confounding effect of estrogen in a trial could be minimized if heifers were synchronized prior to spaying.

2.4.4 Feeding Behaviour and ADG

Changes in feeding behaviour and feed intake can be used to assess pain (Weary et al., 2006). In the ranch setting for this trial, feeding duration times were the only possible method of measuring feeding behaviour, but wind and precipitation made video capture challenging and affected the length of time heifers could be filmed each day. Nevertheless, all groups had equal opportunities to appear on video, and although the individual numbers of each heifer could not be determined, the group colour tag was visible on the video recordings. Because heifers were fasted prior to spaying, their motivation to eat on d 0 was likely stronger than on subsequent days. As they were spayed, heifers had free access to travel to their home pen where feed was available *ad libitum*, so video recording on d 0 covered the longest period of time to allow all heifers at least 2 h of opportunity to be recorded while eating. Length of video recording from d 1 to 3 varied by day and was determined by weather conditions, but always started at the time of the morning feeding; this was reflected in the higher number of observations/h on d 2 and d 3 with
the shortest video times recorded immediately after feeding. The heifers in both groups that received BXK had longer feeding durations on d 0 which may reflect less discomfort. Visceral analgesia from xylazine has been reported to last up to two hours (Gaynor, 2010) and by adding butorphanol and ketamine its effects are augmented (Gaynor & Muir, 2015) possibly also enhancing its duration of effect. Petherick et al. (2013) reported that DOT spayed heifers that received no analgesia spent less time feeding than restrained but unspayed heifers on the day of spaying but not for three days post-spaying. Our results would support the inference that analgesia provided by BXK changes heifer feeding behaviour on the day of spaying, but this was clinically inconsequential, as the ADG over the observation period was the same between treatments. The lack of difference in feeding duration on d 1 may indicate that the heifers ate to satiety on d 0 after fasting and on d 1 may not have had the same feeding motivation. The BXK group unexpectedly showed longer feeding durations during the filmed observation period on d 2 compared to the control group but differences or lack thereof on d 1 to d 3 were likely confounded by the riding behaviour that we were not able to quantify. Implants are recognized to cause riding behaviour in spayed heifers without any reported resulting problems (Rupp & Hamilton, 1995). It is possible but not verifiable from this study that meloxicam treatment may have affected riding behaviour. Implanting spayed heifers has been shown to improve productivity (Rupp & Hamilton, 1995; Zobell et al., 1993) and is a routine procedure when spaying heifers in British Columbia.

There were no differences between treatments and no interaction effects for ADG in these ovariectomized heifers over the 5 d period. ADG may not be a good pain indicator for ovariectomized heifers, and the lack of treatment effect supports the overall pattern that DOT ovariectomy is an acutely painful trauma that resolves quickly and is minimally disruptive to heifer behaviour including feeding behaviour.

2.5 Conclusions

This pilot study provided insights for our main study evaluating the pain of DOT ovariectomy and its alleviation by measuring both physiological and behavioural pain indicators. Changes to the main study were made based on the pilot study; heifers were not implanted to reduce the confounding effect of riding on behaviour, and the oral treatment delivery method was changed from the draw-off dose syringe to a 35 cc catheter tip syringe to improve dosing accuracy. The $2 \times 2$ factorial experimental design in this study revealed no interactions between
BXK and meloxicam, suggesting that the two treatments could be combined to better align with the principles of the 3 Rs in the main study.

This pilot study also demonstrated that it is possible to implement a pain mitigation protocol in a ranch setting for ovariectomy. Although we were unable to document behavioural differences between heifers treated pre-surgically with BXK or a placebo during the DOT procedure, and minimal behavioural differences after ovariectomy between heifers treated with and without BXK and meloxicam, this may be attributable to the exclusively visceral tissue trauma that occurs with the DOT, which is unique among surgical management procedures performed on cattle. Having physiological measurements of stress and pain would be useful to quantify the magnitude of noxiousness associated with ovariectomy in heifers and a decrease in these measurements after treatment would provide better evidence that this protocol is effective.
Table 2.1: Visual Analog Scale (VAS) scores recorded during DOT ovariectomy of yearling heifers. Scores are averages for those obtained from two experienced observers. BXK or DIL was administered IM a minimum of 5 minutes prior to ovariectomy. Oral treatment with MEL or DIS was administered after ovariectomy was completed.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment LSM$^1$</th>
<th>$P$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BXK DIS</td>
<td>MEL DIS</td>
</tr>
<tr>
<td>VAS Score (cm)</td>
<td>1.50 1.50</td>
<td>1.40 1.70</td>
</tr>
</tbody>
</table>

$^1$LSM indicates values displayed are least squares means.

Table 2.2: Behavioural pain indicators recorded by either of two trained observers during DOT ovariectomy of yearling heifers. Number and percentage of heifers displaying each behaviour is listed for those administered either IM BXK (butorphanol 0.01 mg/kg, xylazine 0.02 mg/kg, and ketamine 0.04 mg/kg; $n=59$) or DIL (sterile diluent; $n=59$) prior to ovariectomy.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>$\chi^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defecation</td>
<td>BXK 4 (6.8%)</td>
<td>DIL 5 (8.5%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Kick/Leg Lift</td>
<td>BXK 5 (8.5%)</td>
<td>DIL 1 (1.7%)</td>
<td>2.81</td>
</tr>
<tr>
<td>Tail Flicks</td>
<td>BXK 52 (88.1%)</td>
<td>DIL 53 (89.8%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Body Flinches</td>
<td>BXK 52 (88.1%)</td>
<td>DIL 49 (83.1%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Head Movement</td>
<td>BXK 19 (32.2%)</td>
<td>DIL 16 (27.1%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Intense Breathing</td>
<td>BXK 6 (10.2%)</td>
<td>DIL 9 (15.3%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Vocalization</td>
<td>BXK 25 (42.4%)</td>
<td>DIL 33 (55.9%)</td>
<td>2.17</td>
</tr>
</tbody>
</table>
Table 2.3a: Cross-tabulation of gait scores of yearling heifers when released from squeeze chute on day before ovariectomy \((n=110)\) by IM treatment they were selected to received prior to ovariectomy on d 0 \((\text{BXK} = \text{butorphanol } 0.01 \text{ mg/kg}, \text{xylazine } 0.02 \text{ mg/kg}, \text{and ketamine } 0.04 \text{ mg/kg}; \text{DIL} = \text{sterile diluent})\).

<table>
<thead>
<tr>
<th>GAIT (d -1)</th>
<th>TREATMENT (d 0)</th>
<th>$P$-value</th>
<th>$\chi^2$ (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walk ((n=59))</td>
<td>BXK 25 (44.6%)</td>
<td>DIL 34 (63.0%)</td>
<td>4.99 (2)</td>
</tr>
<tr>
<td>Trot ((n=49))</td>
<td>BXK 29 (51.8%)</td>
<td>DIL 20 (37.0%)</td>
<td>4.99 (2)</td>
</tr>
<tr>
<td>Run ((n=2))</td>
<td>BXK 2 (3.6%)</td>
<td>DIL 0 (0.0%)</td>
<td>4.99 (2)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>56 (100%)</td>
<td>54 (100%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2.3b: Cross-tabulation of gait scores of yearling heifers when released from squeeze chute after ovariectomy \((n=118)\) by IM treatment of BXK or DIL prior to ovariectomy.

<table>
<thead>
<tr>
<th>GAIT (d 0)</th>
<th>TREATMENT (d 0)</th>
<th>$P$-value</th>
<th>$\chi^2$ (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walk ((n=64))</td>
<td>BXK 41 (69.5%)</td>
<td>DIL 23 (39.0%)</td>
<td>12.98 (2)</td>
</tr>
<tr>
<td>Trot ((n=50))</td>
<td>BXK 18 (30.5%)</td>
<td>DIL 32 (54.2%)</td>
<td>12.98 (2)</td>
</tr>
<tr>
<td>Run ((n=4))</td>
<td>BXK 0 (0.0%)</td>
<td>DIL 4 (6.8%)</td>
<td>12.98 (2)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>56 (100%)</td>
<td>54 (100%)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2.4: Least squares means (LSM) ± SEM for repeated measures analysis of stride length (SL) and accelerometer data by hour on d 0 (1 to 10 h post-ovariectomy) and by day (d 0 to d 4 post-ovariectomy) for yearling heifers administered either IM BXK (butorphanol 0.01 mg/kg, xylazine 0.02 mg/kg, and ketamine 0.04 mg/kg) or DIL (sterile diluent) prior to DOT ovariectomy, and either MEL (meloxicam 1 mg/kg) or DIS (distilled water) administered orally upon completion of ovariectomy.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
<th>SEM</th>
<th>BXK</th>
<th>MEL</th>
<th>BXK × MEL</th>
<th>time</th>
<th>BXK × time</th>
<th>MEL × time</th>
<th>BXK × MEL × time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stride Length (cm)</td>
<td>BXK</td>
<td>DIL</td>
<td>DIS</td>
<td>MEL</td>
<td>DIS</td>
<td>MEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accelerometer Data by hour (d 0):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing time (%)</td>
<td>54.3</td>
<td>54.4</td>
<td>54.2</td>
<td>53.7</td>
<td>0.88</td>
<td></td>
<td>0.66</td>
<td>0.86</td>
<td>0.76</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Accelerometer Data by day:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying Duration (min)</td>
<td>47.7</td>
<td>48.3</td>
<td>47.0</td>
<td>46.5</td>
<td>0.17</td>
<td></td>
<td>0.54</td>
<td>0.99</td>
<td>0.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Standing Bouts (n)</td>
<td>9.5</td>
<td>9.1</td>
<td>9.4</td>
<td>9.3</td>
<td>0.04</td>
<td></td>
<td>0.90</td>
<td>0.37</td>
<td>0.63</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lying Bouts (n)</td>
<td>16.4</td>
<td>16.6</td>
<td>17.2</td>
<td>16.9</td>
<td>0.09</td>
<td></td>
<td>0.57</td>
<td>0.98</td>
<td>0.86</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 2.5: Least Squares Means (LSM) ± SEM for Stride Length (SL) on d 0 and d 5 after DOT ovariectomy of yearling heifers, after treatment on d 0 of IM BXK (butorphanol 0.01 mg/kg, xylazine 0.02 mg/kg, and ketamine 0.04 mg/kg) or DIL (sterile diluent) prior to ovariectomy and either oral MEL (meloxicam 1 mg/kg) or DIS (distilled water) administered upon completion of ovariectomy.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BXK DIS</td>
<td>MEL DIS</td>
</tr>
<tr>
<td>SL d 0 (cm)</td>
<td>56.3</td>
<td>57.2</td>
</tr>
<tr>
<td>SL d 5 (cm)</td>
<td>52.3</td>
<td>51.6</td>
</tr>
</tbody>
</table>
Table 2.6: Number and duration of feeding events (min) observed on video recordings in yearling heifers from day of spaying (d 0) to day 3 (d 3) post-ovariectomy by treatment group (DIL/DIS: control group, IM sterile diluent and oral distilled water; BXK/DIS: IM BXK and oral distilled water; DIL/MEL: IM sterile diluent and oral meloxicam; and BXK/MEL: IM BXK and oral meloxicam).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>d 0 MEAN±SD (OBS)</th>
<th>P value</th>
<th>d 1 MEAN±SD (OBS)</th>
<th>P value</th>
<th>d 2 MEAN±SD (OBS)</th>
<th>P value</th>
<th>d 3 MEAN±SD (OBS)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(OBS)</td>
<td>X²(df)</td>
<td>(OBS)</td>
<td>X²(df)</td>
<td>(OBS)</td>
<td>X²(df)</td>
<td>(OBS)</td>
<td>X²(df)</td>
</tr>
<tr>
<td>DIL/DIS</td>
<td>9.9±11.0 (249)</td>
<td>0.02</td>
<td>12.2±12.1 (185)</td>
<td>0.71</td>
<td>7.6±7.8 (136)</td>
<td>0.04</td>
<td>5.7±6.8 (71)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>[4.11]</td>
<td></td>
<td>[4.37]</td>
<td></td>
<td>[10.30]</td>
<td></td>
<td>[11.27]</td>
<td></td>
</tr>
<tr>
<td>BXK/DIS</td>
<td>13.1±15.3 (200)</td>
<td>0.02</td>
<td>12.9±11.9 (184)</td>
<td>0.71</td>
<td>10.3±9.3 (131)</td>
<td>0.04</td>
<td>7.4±6.8 (85)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>[3.30]</td>
<td></td>
<td>[4.35]</td>
<td></td>
<td>[9.92]</td>
<td></td>
<td>[13.49]</td>
<td></td>
</tr>
<tr>
<td>DIL/MEL</td>
<td>11.1±13.1 (246)</td>
<td>0.02</td>
<td>12.0±12.5 (195)</td>
<td>0.71</td>
<td>9.0±9.25 (146)</td>
<td>0.04</td>
<td>7.0±8.9 (73)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>[4.06]</td>
<td></td>
<td>[4.60]</td>
<td></td>
<td>[11.06]</td>
<td></td>
<td>[11.59]</td>
<td></td>
</tr>
<tr>
<td>BXK/MEL</td>
<td>13.3±14.1 (216)</td>
<td>0.02</td>
<td>13.3±14.7 (194)</td>
<td>0.71</td>
<td>9.9±10.0 (144)</td>
<td>0.04</td>
<td>7.9±8.5 (75)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>[3.56]</td>
<td></td>
<td>[4.59]</td>
<td></td>
<td>[10.91]</td>
<td></td>
<td>[11.91]</td>
<td></td>
</tr>
</tbody>
</table>

1 OBS is number of observations of feeding events.
2 OBS/h is number of feeding events per hour.
Table 2.7: Average Daily Gain (ADG; kg/d) of yearling heifers over 5 days after DOT ovariectomy.

<table>
<thead>
<tr>
<th>Tx Group</th>
<th>ADG (kg/d)</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIL/DIS (n=25)</td>
<td>1.50</td>
<td>2.65</td>
<td>1.43 (3) 0.24</td>
</tr>
<tr>
<td>BXK/DIS (n=27)</td>
<td>2.65</td>
<td>2.70</td>
<td>1.43 (3) 0.24</td>
</tr>
<tr>
<td>DIL/MEL (n=27)</td>
<td>1.98</td>
<td>2.68</td>
<td>1.43 (3) 0.24</td>
</tr>
<tr>
<td>BXK/MEL (n=28)</td>
<td>1.24</td>
<td>2.61</td>
<td>1.43 (3) 0.24</td>
</tr>
</tbody>
</table>

1Tx Group is Treatment Group

Figure 2.1: Visual Analog Scale (VAS) score sheet with boxes to mark pain-related behaviours noticed during observation of yearling heifers undergoing DOT ovariectomy.

Heifer #________________

<table>
<thead>
<tr>
<th>Urinate Defecate</th>
<th>Attempt to fall</th>
<th>Kick/ Leg lifting</th>
<th>Tail flicks</th>
<th>Body flinch</th>
<th>Head movement</th>
<th>Vocal</th>
<th>Intense breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pain</td>
<td>Severe pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.2: Least squares means (LSM) ± SEM for BXK x time effect on standing time (as percentage of time spent standing in one hour) for yearling heifers during the 10 hours following ovariectomy for heifers treated with IM BXK (butorphanol 0.01 mg/kg, xylazine 0.02 mg/kg, and ketamine 0.04 mg/kg) or DIL (sterile diluent) prior to spaying. LSMs with differing letters differ ($P \leq 0.05$).

Figure 2.3: Least squares means (LSM) ± SEM for MEL (meloxicam) x time effect on standing time (as percentage of time spent standing in one hour) for yearling heifers during the 10 hours following ovariectomy for heifers treated with oral MEL (meloxicam 1 mg/kg) or DIS (distilled water) immediately after spaying. LSMs with differing letters differ ($P \leq 0.05$).
Figure 2.4: Least squares means (LSM) ± SEM for MEL (meloxicam) x time effect on standing time (as percentage of time spent standing in one day) for yearling heifers during the 4 days following ovariectomy for heifers treated with oral MEL (meloxicam 1 mg/kg) or DIS (distilled water) immediately after spaying. LSMs with differing letters differ \( (P \leq 0.05) \).

Figure 2.5: Least squares means (LSM) ± SEM for MEL (meloxicam) x time effect on standing duration (as consecutive minutes spent standing) for yearling heifers during the 4 days following ovariectomy for heifers treated with oral MEL (meloxicam 1 mg/kg) or DIS (distilled water) immediately after spaying. LSMs with differing letters differ \( (P \leq 0.05) \).
CHAPTER 3
MEASURING PHYSIOLOGICAL AND BEHAVIOURAL RESPONSES TO PAIN MITIGATION FOR OVARIECTOMY IN YEARLING BEEF HEIFERS

3.1 Introduction

Ovariectomy (spaying) prevents pregnancy and improves marketing of cull heifers destined for finishing rather than breeding. The trans-vaginal dropped ovary technique (DOT) is considered the least noxious spaying method, but still causes stress and pain that should be managed (Petherick et al., 2013). The beef Code of Practice guides producers to seek advice for analgesia for spaying (National Farm Animal Care Council, 2013), but no pain mitigation strategy for ovariectomy has been published to date.

Non-verbal species present challenges in measuring pain. Physiological pain indicators may be confounded by stress, but may be more useful than behavioural pain indicators in species such as cattle that are observed by potential predators (Weary et al., 2006). Two studies have reported on the physiological response to spaying (Petherick et al., 2011 & 2013) and reported higher cortisol in DOT spayed heifers than in unspayed controls.

Ovariectomy pain potentially arises from visceral tissue trauma (procedural pain) and the inflammation it provokes (post-surgical pain); both should be addressed for effective pain mitigation. Pre-emptive multimodal therapy using a combination of analgesics delivers synergistically superior analgesia (Gaynor & Muir, 2015). BXK (0.01 mg/kg Butorphanol, 0.02 mg/kg Xylazine and 0.04 mg/kg Ketamine) has been described for chemical restraint in cattle (Abrahamsen, 2013) but each of these drugs also provides analgesia. Meloxicam given at the time of castration was reported to reduce inflammatory pain (Meléndez et al., 2017b). We hypothesized that peri-procedural administration of BXK and meloxicam should provide preventative analgesia for ovariectomy.

The objectives of this study were to determine if 1) BXK administered preemptively (5 min prior to spaying) mitigates the procedural and immediate post-surgical pain of ovariectomy and 2) oral meloxicam administered at the time of spaying mitigates the inflammatory pain in the week following ovariectomy.

3.2 Materials and Methods

3.2.1 Ethics

The Lethbridge Research Centre (LRC) Animal Care Committee (ACC Protocol # 1643) and the University Animal Care Committee Animal Research Ethics Board, University of
Saskatchewan (UACC AUP # 20160108) approved this study protocol. The Canadian Council of Animal Care guidelines (Canadian Council on Animal Care, 2009) were followed for the care of the study heifers.

3.2.2 Animals, Animal Management and Handling Facility

Forty-five commercial red Angus and Angus crossbred yearling heifers (322 ± 27 kg) purchased from two sources were kept at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre (LRC, Lethbridge, Alberta, Canada) for four weeks prior to the start of the study to familiarize them with the facility and handling. Heifers were housed in groups of 9 in 5 feedlot pens (21 m × 27 m). Each pen had an *ad libitum* water supply and a 2.4 m × 24 m concrete apron in front of a feed bunk with a GrowSafe System installed to measure individual heifer feed intake and feeding behaviour. Heifers were fed a ration of 89.2% barley silage, 8.2% dry rolled barley and 2.6% Standard Feedlot Supplement to meet nutritional requirements (National Academies of Sciences Engineering and Medicine, 2016). Heifers were moved from their home pens to a covered handling facility for sampling where they entered a crowding tub leading into a curved metal paneled race with a catwalk along its inside curvature and terminating in a hydraulic squeeze chute (Cattlelac Cattle Handling Systems, Reg Cox Feedmixers Ltd., Lethbridge, Alberta, Canada).

On d -1, heifers were tagged with individual sequentially numbered ear tags and back tags numbered 1 to 9 (Regular Back Tags, Wheel’n’Tree Enterprises, Sherwood Park, AB, Canada) were affixed over the heifers’ backs using a hair adhesive (Livestock cement, Wheel’n’Tree Enterprises, Sherwood Park, AB, Canada) so that each of 5 pens contained 9 identifiable animals for video. One heifer (ear tag #6) presented at the squeeze chute with epistaxis and was excluded from the trial. Accelerometers (HOBO Pendant G Data Logger, Onset Computer Corporation, Bourne, MA, USA) were protected with waterproof padding and covered with self-adhesive bandaging material (Flexible Wrap, Professional Preference, McCarthy and Sons Service, Calgary, AB, Canada) in assorted colours and numbered in sequence. HOBOs were cross-referenced by number and wrap colour to each heifer’s ear tag number and secured vertically to the lateral right hind metatarsus of each animal using a roll of 10 cm wide by 4.6 m long self-adhesive bandaging material. Feed was withheld after sampling on d -1 to fast heifers a minimum of 20 h prior to ovariectomy to reduce the risk of iatrogenic visceral injury from the surgery. On d 0, all heifers were administered a prophylactic treatment.
of 20 mL of 200 mg/mL oxytetracycline (Oxyvet 200 LA, Vetoquinol NA Inc. Lavaltrie, PQ, Canada) intramuscularly in each of 2 sites (maximum 10 mL/site).

3.2.3 Experimental Design and Treatments

Heifers were blocked by body weight and randomly assigned by computer to 1 of 3 treatment groups so there were 3 heifers from each treatment group in each pen: 1) PALP (Positive control (sham ovarietomy) group; n=14): reproductive tract palpated rectally for one min and ovariotome introduced vaginally without creating a colpotomy incision, sterile diluent injection administered IM 5 min prior to palpation and oral water drench administered immediately prior to palpation; 2) SPAY (Negative control group; n=15): ovarietomized without analgesia, sterile diluent injection administered IM 5 min prior to ovarietomy and oral water drench administered immediately prior to ovarietomy; 3) BXKM (Treatment group; n=15): ovarietomized and administered a combination of butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg) and ketamine (0.04 mg/kg) injection administered IM 5 min prior to ovarietomy and oral meloxicam (1 mg/kg) administered immediately prior to ovarietomy. A technician directed the treatment administration from a printed assignment sheet to ensure that treatment administrators were blinded to treatment group. Heifers were brought to the handling facility in 5 groups of 9 during the day that allowed sampling of each group at 1, 2 and 4 h after ovarietomy.

3.2.4 Analgesic Pharmaceuticals

BXK was mixed on the morning of d 0 to create a 2 mL volume that would provide a 300 kg heifer with 3 mg of butorphanol (0.01 mg/kg), 6 mg of xylazine (0.02 mg/kg) and 12 mg of ketamine (0.04 mg/kg). A 40 mL mixture was made by adding 6 mL of 10 mg/mL butorphanol (Torbugesic, Zoetis Canada Inc., Kirkland, QC), 6 mL of 20 mg/mL xylazine (Rompun 20 mg/mL Injectable, Bayer Inc., Mississauga, ON, Canada), 2.4 mL of 100 mg/mL ketamine (Narketan, Vetoquinol N.A. Inc., Lavaltrie, QC, Canada) and 25.6 mL of sterile diluent (Sterile Water USP, Bimeda Canada, Cambridge, ON) into a 100 mL rubber stoppered glass vial. A second 100 mL vial of sterile diluent was used for the placebo injections.

A plastic dose syringe (Monoject 35 cc catheter tip, Covidien, Associated Veterinary Purchasing Co. Ltd., Langley, B.C.) was used to administer the meloxicam (15 mg/mL) suspension (Meloxicam Oral Suspension, USP, Solvet, Alberta Veterinary Laboratories Ltd., Calgary, AB, Canada) and a water drench via dose syringe was used for the oral placebo.
treatment. The volume administered for each heifer was calculated as a 1 mg/kg meloxicam dose based on bodyweight, so that a 300 kg heifer received 20 mL orally of either meloxicam or water.

On d 0, either BXK or DIL was injected IM in the left neck of each heifer using an 18G 2.5 cm stainless steel needle (Ideal D3 Detectable Needles, Animal Safety Division, Neogen, Lexington, KY, USA) while she was standing in the race prior to spaying. Injection was facilitated by an assistant on the catwalk who prevented the last heifer in the race from backing up to minimize movement between heifers. A digital timer was used to record time of injection and verify that a minimum of 5 min had elapsed before palpation or ovariectomy took place. Blood and saliva samples were obtained and an oral drench of either meloxicam or water was administered prior to palpation or spaying. The veterinarian was instructed to palpate heifers in the PALP group for 1 min with similar effort as in the SPAY and BXKM groups.

3.2.5 Ovariectomy Technique

A bovine veterinarian experienced in palpating dairy cattle ovariectomized the heifers with a Willis ovariotome using the dropped ovary technique (DOT). Briefly, while an assistant held the heifer’s tail vertically, the veterinarian’s non-dominant arm was placed rectally to evacuate feces and palpate for pregnancy and reproductive tract abnormalities such as freemartinism. The vulvar area was wiped clean with a paper towel, and his opposite hand retrieved the stainless steel ovariotome from a 0.05% chlorhexidine solution, advanced its pointed teardrop shaped end containing a hole with a sharpened slot (“eye”) through the dorsal vaginal fornix into the abdomen. Each ovary in turn was placed in the ovariotome eye and a backwards pull severed the ovarian attachments and allowed the ovary to drop into the abdominal cavity. The ovariotome was then wiped clean and replaced in the disinfectant solution. Time was recorded at the start and completion of each ovariectomy performed.

3.2.6 Sample Collection Schedule

Heifers had rectal temperatures recorded using a digital electronic thermometer (GLA M750 Livestock Thermometer, San Luis Obispo, CA), and saliva and blood samples were harvested on the day before spaying (d -1), at the time of spaying (d 0), and 1, 2, 4, 24 h and then on d 2, d 4 and d 7 post-spaying. Visual Analog Scale (VAS) scores and pain-related behaviours during ovariectomy were recorded at the time of spaying (d 0). Body weight was recorded with a digital scale under the squeeze chute on d -1, d 0, d 7 and d 29. GrowSafe System feeding
data and accelerometer activity data were recorded continuously from d -1 to d 7. Videos of heifers in their home pens were recorded starting 4 h after ovariectomy for 5 h on d 0 and for 4 h 24 hours later on d 1. Hair samples were gathered on d -1 and d 29, and final body weight and blood samples for estradiol concentration were taken on d 29.

3.2.7 Procedures for Behavioural Measurements of Pain

3.2.7.1 Visual Analog Scale Score (VAS) and Pain-Related Behaviours During Ovariectomy

While in the squeeze chute for ovariectomy or palpation, two trained observers blinded to treatment group marked their subjective assessment of the degree of pain experienced by the heifers during the procedure on a 10 cm horizontal line where the left end represented 0 (no pain) and the right end represented 10 (maximum pain) as described previously (Ludington & Dexter, 1998; Moya et al., 2014). Both observers were experienced in scoring bovine pain on the VAS; however, only one observer had previously observed spaying. The distance from the left end of the line to the observer’s mark, to the nearest 0.5 cm, was the VAS score, with a larger score indicating more severe pain. During the observation period, pain-related behaviours (urination/defecation, falling attempts, kicking/leg lifts, tail flicks, body flinches, head movements and vocalization) were recorded as they occurred by each observer.

3.2.7.2 Flight Speed

Flight speed (FS) was measured using the time taken for a heifer released from the squeeze chute to travel between two pairs of light beam generators and reflectors (electric eyes) (Polaris Multi-Event Timer, FarmTek, Inc., Wylie, TX, USA) situated approximately one m in front of the head gate, at approximately 0.9 m in height, and spaced 2.5 m apart. Transit time between beams was converted from seconds to speed (m/s).

3.2.7.3 Stride Length and Gait Score

After exiting the squeeze chute, heifers travelled in a straight line on their way to their home pen past a video camera set up beside the alleyway (Panasonic WVCP474; Panasonic Canada, Inc., Mississauga, ON, Canada) to record video used for measuring stride length (SL) on d -1, d 0 (immediately and 1, 2 and 4 h after spaying/palpating) and on d 1, 2, 4 and 7 after spaying/palpating, as previously described for measuring response to analgesia in castrated calves (Currah et al., 2009), with some modifications. GOM player software (GOMlab, Gretech Corporation, Seoul, South Korea) was used to take two sequential pictures of each heifer while her hind feet were in contact with the ground and alternate hind limbs placed forward. A 1.003
l m long fence panel visible in the pictures was used as the measuring reference. The stride distance from the midpoint of each hind foot was measured using ImageJ software (Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA) and the average of the two stride distances was recorded as the SL measurement. Heifers that ran past the camera, or those whose feet could not be seen clearly could not be measured for SL, and were treated as missing data points.

Video images used for stride length were used to categorize the gait used by heifers leaving the squeeze chute. Gait was scored as walk, trot or run.

3.2.7.4 Feeding Behaviour and Average Daily Gain (ADG)

Each pen feed bunk was outfitted with a GrowSafe System (GrowSafe Systems, Calgary, AB, Canada) for validated individual continuous heifer feeding behaviour monitoring (Schwartzkopf-Genswein et al., 1999). Two antennae at the feed bunk simultaneously detected individual radio frequency identification (RFID) ear tags when heifers were 50 cm or closer to the load cell suspended feed bunks to log data used to determine mean daily feed intake (kg/d), feeding time (min/d), feeding rate (g/min), meal frequency (number/d), meal duration (min/meal), and meal size (kg/meal). A meal was defined as 300 sec or more that a heifer was detected at the feed bunk. Body weight was recorded for individual heifers while in the squeeze chute on d -1, d 0, d 7 and d 29 for sampling to determine average daily gain (ADG) from d 0 to d 7 and from d 0 to d 29.

3.2.7.5 Standing/Lying Activity and Pain-Related Behaviours After Ovariectomy

Vertical (X) and horizontal (Z) axis orientation data were stored inside the HOBO accelerometers for 24 h periods and reported standing/lying time budgets (daily percentage), mean standing and lying duration (min/d) and standing and lying bouts (number/d) as validated by the University of British Columbia Animal Welfare Program (UBC AWP, 2013). HOBOs were removed on d 7 and contained data was downloaded to a computer.

Video cameras were mounted on light poles to give a full view of each pen. Heifers were videoed for 5 h on d 0 starting after the 4 h post-ovariectomy samples were collected and for 4 h on d 1 to record behaviours (walking, standing, lying in sternal recumbency, lying in lateral recumbency, tail flicking, grooming, foot stamping, back arching, and tail lifting).
3.2.8 Procedures for Physiological Measurements of Stress and Pain

3.2.8.1 Complete Blood Count (CBC)

Jugular venous blood samples were taken using a 2.5 cm 18G double-ended needle secured in a holder (BD Vacutainer Needle and Holder, Franklin Lakes, NJ, USA); two 6 mL tubes containing EDTA anticoagulant and a non-additive 10 mL serum tube were filled. One EDTA tube was used for CBC analysis using a HemaTrue Hematology Analyzer (Heska, Loveland, CO, USA) to yield total white blood cell (WBC) count, lymphocyte, monocyte and granulocyte concentration, hematocrit (HCT), total red blood cell (RBC) count, and total platelet (PLT) count. Neutrophil:lymphocyte ratio estimates were calculated using the granulocyte and lymphocyte concentration data.

3.2.8.2 Cortisol

3.2.8.2.1 Salivary Cortisol

Saliva samples were obtained from the oral cavity using cotton swabs, which were placed in plastic tubes and stored on ice, then frozen within two hours at -20°C until assayed for cortisol concentration using a commercial competitive immunoassay kit according to the manufacturer’s instructions (Salimetrics Cortisol Enzyme Immunoassay Kit, Salimetrics, LLC., State College, PA, USA). Samples were assayed in duplicate, and cortisol concentration was determined from a linear standard curve generated from kit standards ranging from 0.12 to 30 ng/mL. Inter-assay and intra-assay coefficients of variation (CV) were 29.64% and 7.36% respectively.

3.2.8.2.2 Hair Cortisol

Hair from the forehead of each heifer was clipped using electric clippers, placed in a plastic bag and stored for cortisol analysis on d -1 and d 29. From each sample, 250 mg of hair was washed with isopropanol, dried and placed in a 10 mL metal cylinder containing a 12 mm metal ball and ground with a mixer mill (MM 200, Retsch Inc., Newtown, PA, USA) for 5 min at 22 Hz. A measure of 20 mg of minced hair in a 5 mL glass vial was used for cortisol extraction and assayed according to the method described by Moya et al. (2013). Briefly, after adding 1 mL of methanol, sonicating for 30 min and incubating on a shaker at 50° C and 100 rpm for 18 h, 0.8 mL of supernatant was pipetted into a tube and evaporated at 45° C under a nitrogen stream in a fume hood. One hundred µL of phosphate buffered saline was used to reconstitute the
samples and the same commercial kit used for salivary cortisol was used for hair cortisol determination. Inter-assay CV was 15.92% and intra-assay CV was 7.82%.

3.2.8.3 Substance P

Benzimidine hydrochloride (1mM/mL of blood) was added to the second EDTA tube to minimize substance P (SP) degradation, centrifuged at 1.5 x g for 15 min at 0º C and the plasma decanted and frozen at -80º C. These samples were shipped by courier to Ames Iowa for SP analysis at Iowa State University College of Veterinary Medicine by immunoassay as described by Coetzee et al. (2008). Inter-assay and intra-assay CVs were 17.07% and 8.84% respectively.

3.2.8.4 Acute Phase Proteins: Haptoglobin and Serum Amyloid A

Serum was harvested from the 10 mL Vacutainer tubes by centrifuging at 1,600 x g and 4º C for 15 min, transferred to labeled tubes and frozen at -20º C in triplicate. Samples for haptoglobin (0.5 mL) were sent to the Ontario Veterinary College (University of Guelph, Guelph, ON, Canada) for analysis with a Roche Cobas c501 biochemistry analyzer (Roche Diagnostics, Laval, QC, Canada) using Tridelta bovine haptoglobin calibrator reagents (TP-801-CAL, Tridelta Development Limited, Maynooth, County Kildare, Ireland) and two levels of pooled bovine serum controls. Inter-assay CV for haptoglobin analysis was 3.57%; intra-assay CV was not calculated as duplicate samples were not analyzed.

Duplicate serum samples were assayed for serum amyloid A (SAA) using a commercial solid phase sandwich ELISA kit (Tridelta Phase Range Multispecies SAA ELISA kit, Tridelta Development Ltd., Maynooth, County Kildare, Ireland). Inter-assay CV for SAA was 15.50% and intra-assay CV was 8.46%.

3.2.9 Hormonal Assay: Estradiol

The triplicate serum samples decanted from the jugular venous blood draw from each heifer on d 29 were sent to the Western College of Veterinary Medicine (University of Saskatchewan, Saskatoon, SK, Canada) for estradiol (E2) concentration determination using an extracted radioimmunoassay technique performed using prepared standards in charcoal stripped serum (Joseph, Currie, & Rawlings, 1992).

3.2.10 Statistical Analysis

The individual heifer was the experimental unit. One heifer with epistaxis was removed from the trial on d -1 and left 14 heifers in the PALP group and 15 heifers in the SPAY and BXKM groups. Normal data distribution was tested prior to analysis using the PROC
UNIVARIATE procedure (SAS, Version 9.4, SAS Institute Inc., Cary, NC, USA) and outliers were identified with box plots. Normally distributed data were analyzed using the PROC MIXED repeated measures procedure in SAS where d -1 values were used as covariates, except for the bodyweight covariate for calculating average daily gain (ADG), where d -1 and d 0 bodyweights were averaged because heifers were fasted after d -1 sampling. Flight speed from d -1 was also used as a covariate to compare results obtained to determine if heifer temperament may have been a confounder for SL, accelerometer data, feeding behaviour and physiological indicators. Time and treatment were fixed effects and tested for interactions and heifer within pen was a random effect. Time was considered a repeated factor, and for each variable, 3 variance-covariance structures were applied (compound symmetry, autoregressive order 1, and unstructured) to select a covariance structure for the heifer within pen (the error term). The analysis used was the most appropriate covariance structure that minimized Schwarz’s Bayesian information criterion. When tendencies or significant interactions were present, post-hoc PDIF test was used to generate P-values for LSM differences. Physiological data were natural log transformed and pen behavioural data were square root transformed to achieve normal distribution prior to analysis. Non-transformed least squares means (LSM) with transformed standard error of the mean (SEM) were reported. When transformation was unable to normalize data, VAS scores were analyzed with the non-parametric Wilcoxon matched-pairs signed-ranks test using Stata statistical software (version 14.2, StataCorp, College Station, TX, USA) to assess inter-observer agreement. The Kruskal-Wallis rank sum test for nonparametric data using Stata was used to determine whether mean VAS ranks were the same between treatment groups, and to compare mean estradiol ranks between groups on d 29. Two-way tables with Fisher’s exact test in Stata were used for treatment group comparisons of pain-related behaviours during ovariectomy and gait scores. Three outliers from the BXKM group and 1 outlier from the SPAY group were removed to compare spay times. Spay time data were analyzed only for the SPAY and BXKM groups using a two-sample t-test with unequal variances in Stata to compare group mean spay times, as the PALP group was timed for a palpation duration of 1 min. Results were considered significant when \( P \leq 0.05 \) and tendencies were declared when \( 0.05 < P \leq 0.10 \).
3.3 Results

3.3.1 Behavioural Measurement Results

3.3.1.1 VAS Scores, Pain-related Behaviours During Ovariectomy, and Spay Times

There were no differences in VAS scores for each of the two observers ($P = 0.33$, observer 1; $P = 0.556$, observer 2) between treatment groups while heifers were either palpated or spayed (Table 3.1). There was a difference in agreement between observers ($P = 0.005$); observer 1 was inclined to assign scores that were higher than observer 2. There were no differences between groups for falling attempts, kicking/leg lifting, tail flicks, body flinches, head movement or vocalization (Table 3.2). No heifers exhibited urination or spontaneous defecation behaviour while in the squeeze chute. There was no difference ($P = 0.31$) in spay times between groups; mean spay times ± SEM for SPAY and BXKM heifers were 55 ± 11.3 s and 43 ± 4 s respectively.

3.3.1.2 Flight Speed

A treatment x time interaction was identified for FS ($P = 0.03$). At 24 h after ovariectomy/palpation, heifers in the BXKM group exhibited faster FS (2.99 m/s; $P = 0.02$) compared to PALP heifers (2.05 m/s) and tended to be faster than SPAY heifers (1.97 m/s; $P = 0.08$) (Figure 3.1).

3.3.1.3 Stride Length and Gait Score

There were no treatment nor treatment x time interaction effects for heifer stride length and no difference in LSMs between groups (PALP 59.46 ± 1.51 cm; SPAY 59.71 ± 1.51 cm; BXKM 63.20 ± 1.47 cm; Table 3.3). There were no differences in gait scores between treatment groups at any of the sampling times (Table 3.4).

3.3.1.4 Feeding Behaviour and ADG

No differences were observed between groups for the 7 d feeding behaviour and ADG calculated over 29 d (Tables 3.5 and 3.6). However, over the 29 d interval, SPAY heifers tended to have lower ADG ($P = 0.10$) than PALP heifers (Table 3.7).

3.3.1.5 Standing/Lying Activity and Pain-Related Behaviours After Ovariectomy

There was a treatment tendency for standing duration ($P = 0.10$; Table 3.8), as the BXKM heifers stood for longer durations ($P = 0.03$) than PALP heifers. All other accelerometer generated data (standing/lying time %, lying duration and standing and lying bouts) were not different between treatment groups. Similarly, there were no differences between groups for
pain-related behaviours of tail flicking, grooming, foot stamping, back arching and tail lifting on the video recordings taken on d 0. However, on d 1 after palpation/ovariectomy a treatment effect ($P = 0.01$) was observed as BXKM heifers spent less time lying and in sternal recumbency ($P = 0.003$) than the PALP heifers and SPAY heifers spent less time lying and in sternal recumbency ($P = 0.04$) than PALP heifers (Table 3.8).

### 3.3.2 Physiological Measurement Results

#### 3.3.2.1 CBC

A treatment x time effect ($P < 0.004$) was observed in the RBC counts between treatment groups (Table 3.8). BXKM heifers had lower RBC counts than PALP heifers post-spaying at 24 h ($P < 0.001$) and 48 h ($P = 0.04$) and SPAY heifers tended to have lower RBC counts than PALP heifers at 24 h post-spaying ($P = 0.09$; Figure 3.3). Platelet counts were not different between treatment groups ($P = 0.79$). WBC counts showed a treatment x time effect ($P < 0.001$; Table 3.8); at 2 h post-spaying SPAY heifers had higher WBC counts than BXKM heifers ($P = 0.02$) and 4 h post-spaying both SPAY and BXKM heifers had higher WBC counts than PALP heifers ($P< 0.001$; Figure 3.4). No treatment x time effect differences were observed for N:L ratios between groups ($P = 0.19$; Table 3.9).

#### 3.3.2.2 Salivary and Hair Cortisol

A treatment effect tendency ($P = 0.08$; Table 3.8) and a treatment x time interaction tendency ($P = 0.09$) were observed for salivary cortisol concentration, as SPAY and PALP heifers had higher concentrations than BXKM heifers ($P = 0.01$ and $P = 0.03$ respectively; Figure 3.2) at 1 h after spaying/palpation. At 2 h after ovariectomy, SPAY heifers had higher salivary cortisol concentrations than BXKM heifers ($P = 0.005$) and at 4 h after ovariectomy, both SPAY and BXKM heifers had higher concentrations ($P = 0.02$ and $P = 0.03$ respectively) than PALP heifers. There were no differences between treatment groups on d 1, 2, 4 and 7 after spaying/palpation ($P > 0.10$).

There were no differences between groups for hair cortisol concentrations after 29 d ($P = 0.90$).

#### 3.3.2.3 Substance P

No treatment ($P = 0.87$), nor treatment x time interaction ($P = 0.93$) effects, were detected between groups for SP (Table 3.9).
3.3.2.4 Acute Phase Proteins

Haptoglobin (Hp) concentrations showed a treatment ($P = 0.02$) and treatment x time interaction effect ($P < 0.001$), as SPAY heifers had higher Hp concentrations than both PALP and BXKM heifers (Table 3.9; Figure 3.5). SPAY heifers had higher Hp concentrations than BXKM heifers at all sampling times after ovariectomy on d 1, 2, 4 and 7 ($P = 0.03$, $P = 0.01$, $P < 0.001$ and $P = 0.01$ respectively). SPAY heifers had higher Hp concentrations than PALP heifers on d 1 ($P < 0.001$) and tended to have higher concentrations than PALP heifers on d 2 ($P = 0.08$) and d 7 ($P = 0.08$). PALP heifers had a tendency to have lower Hp concentrations than BXKM heifers on d 1 ($P = 0.10$) but had higher concentrations than BXKM heifers on d 4 ($P = 0.001$).

SAA concentration analysis showed a treatment x time interaction effect, as both SPAY and BXKM heifers had higher SAA concentrations than PALP heifers ($P = 0.02$; Table 3.9). At the time of spaying (d 0), there was a tendency for BXKM heifers to have higher ($P = 0.06$) SAA concentrations than PALP heifers and at 1 h after ovariectomy, the BXKM heifers had higher SAA concentrations than PALP heifers ($P = 0.04$; Figure 3.6). SPAY heifers tended towards higher SAA concentrations at 4 h post-spaying than BXKM heifers ($P = 0.08$). The day following ovariectomy (d 1), PALP heifers had lower SAA concentrations than SPAY ($P = 0.05$) and BXKM heifers ($P = 0.02$). A tendency for PALP heifers to have lower concentrations than SPAY heifers was observed on d 4 ($P = 0.06$) and d 7 ($P = 0.10$).

3.3.2.5 Rectal Temperature

A treatment x time effect between groups was detected for rectal temperature (RT) ($P < 0.001$; Table 3.9). At the time of spaying (d 0) SPAY heifers tended to have higher RT than BXKM heifers ($P = 0.06$; Figure 3.7). BXKM heifers displayed higher RT than both PALP and SPAY heifers at 1 h ($P = 0.003$) and 2 h ($P = 0.013$ and $P = 0.005$ respectively) after ovariectomy. At 24 h after ovariectomy, PALP heifers had higher RT than BXKM heifers ($P = 0.02$) and SPAY heifers ($P = 0.04$).

3.3.2.6 Estradiol

Differences in estradiol (E2) concentrations between treatment groups on d 29 were not statistically significant ($\chi^2 = 1.9(2); P = 0.40$; Table 3.10). The SPAY and BXKM groups both had individuals with no detectable E2 concentrations.
3.4 Discussion

Among the management procedures performed on cattle, little has been published regarding the degree of stress or distress caused by ovariectomy. In response to a plea for a welfare assessment of bovine ovariectomy (Pinner, 2006), a preliminary study using physiological measures on *Bos indicus* cows and heifers ovariecotomized without analgesia concluded that DOT ovariectomy induced stress and pain; this was confirmed in a second study in which the authors concluded that DOT spaying should not be done without managing the stress and pain of the procedure (Petherick et al., 2011; Petherick et al., 2013). Numerous pain mitigation studies have been described for bovine castration (Coetzee, 2011) but no studies to date have provided evidence of an effective analgesic plan for the pain of bovine ovariectomy.

The heifers spayed in this study had a 0% morbidity rate and no mortalities occurred. McCosker et al. (2010) cited morbidity rates for DOT ovariectomy of 0.7% (4/574) with affected heifers being “depressed, slow to rise or walking with restricted gait” for 1 – 4 d. In that study, 9% (18/200) heifers in a second group demonstrated elevated tail carriage for up to 2 h post-spaying, with 1.5% (3/200) showing this behaviour intermittently for 2 d. Mortality rates for the 2 groups of heifers was 0.5% (3/574) occurring within 4 d of spaying (including 1 pregnant heifer) and 1.5% (3/200) occurring 10 - 21 d after spaying but these deaths were presumed to be infectious in origin as another animal was found showing clinical signs of tetanus. No antibiotics had been administered at the time of spaying to either group of heifers.

3.4.1 Analgesic Pharmaceuticals

To design an analgesic protocol for ovariectomy, the injectable combination of butorphanol, xylazine and ketamine (BXK) was chosen to target the acute surgical procedural pain because this combination acts synergistically for better analgesic effect than is possible when each drug is used alone (Coetzee, 2011). BXK is also rapidly clinically effective within 5 min (Abrahamsen, 2013) and therefore could be administered while the heifers were waiting in the race, avoiding the stress of additional handling of extensively managed heifers with large flight zones. A review of analgesia for castration in calves cited numerous studies using analgesics given 20 min prior to the procedure and noted that the external validity of this procedure was poor as it was unlikely to be adopted in practice, while other studies that administered analgesics within seconds of the procedure being performed did not find differences, likely because the drugs were not given enough time to achieve therapeutic blood
levels (Coetzee, 2011). Squeeze chute capture of the heifers allowed administration of oral meloxicam to reduce post-operative inflammatory pain, and with BXK provided a multimodal approach that extended into the post-surgical period previously suggested to provide superior pain mitigation (Coetzee, 2011). The pharmaceuticals used in this study, with the exception of xylazine, are subject to Extra-Label Drug Use (ELDU) regulations and therefore their use requires informed client consent and written withdrawal periods for food animal use. Meat withdrawal time for BXK at the dosage used is 14 d (Enouri, 2017) and because butorphanol and ketamine are regulated by the Controlled Drugs and Substances Act, BXK must be administered under veterinary supervision with appropriate storage and record keeping. Meat withdrawal time for oral meloxicam is 35 d. Treatment cost per heifer was approximately $3 for BXK and $4 for oral meloxicam.

3.4.2 Behavioural Pain-Related Measurements

3.4.2.1 VAS, Pain-Related Behaviours and Spay Times

The time needed to complete DOT ovariectomy averaged less than a minute per heifer; although the BXKM heifers required slightly less time for the procedure, the difference was not significant. The veterinarian performing the ovariectomies was very experienced in reproductive tract palpation, but had previously performed few DOT ovariectomies. Jubb et al. (2003) reported an average of 68 s to complete DOT ovariectomy on 44 Bos indicus cross heifers and indicated that operator hand and arm fatigue and restraint problems for heifers with “poor temperament” were time delay issues; these were not problems in our study because the heifers were spayed/palpated in 5 small groups throughout d 0 and were effectively restrained in a hydraulic squeeze. The butorphanol and xylazine in BXK also produces a mild sedative effect, which helps to minimize movement during ovariectomy, and may be especially useful when dealing with heifers with “poor temperament” in a non-hydraulic squeeze chute. DOT ovariectomy time becomes faster with operator experience; an experienced veterinarian performing the procedure in the pilot study for this trial spayed 118 heifers at an average of approximately 30 s per heifer (Lauder et al, 2018; unpublished data).

Behaviour of DOT spayed heifers has been described as “largely unaffected” by the procedure in the days following ovariectomy (Habermehl, 1993; Jubb et al., 2003). It has been proposed that as prey animals, cattle are more inclined to conceal behavioural indicators of pain to avoid attracting predator attention (Coetzee, 2011; Weary et al., 2006). To our knowledge this
study, and the pilot study that preceded it, were the first attempts to measure behaviours during ovariectomy by using VAS scoring and recording of behaviours occurring during the procedure, and the first to measure behaviours using flight speed, stride length, gait score, continuous standing/lying activities with an accelerometer, continuous feeding behaviour using the GrowSafe System, and video recording analysis in the days following ovariectomy.

Although there was a difference in agreement between observers in VAS scoring, only one observer had witnessed DOT spaying previously, and this difference in prior exposure may have influenced the magnitude of the scoring, as one observer assigned scores generally between 0.5 to 1 cm higher than their counterpart. VAS scores were lower than reported for castration; for example, reported mean VAS scores of 2.7, 2.5, and 3.3 cm for 282 ± 28 kg Angus bull calves treated with meloxicam at 0, 3 and 6 h pre-castration (Meléndez et al., 2017a) were all higher than the mean VAS scores for the SPAY group of 0.95 ± 1.13 (observer 1) and 0.53 ± 0.62 (observer 2), supporting the notion that spaying was not as noxious as castration, even when bull calves were previously treated with meloxicam. Melendez et al. (2017a) used a hydraulic squeeze chute for restraint during castration, but there was no mention of tail restraint used; the heifers in our study were restrained in a hydraulic chute with their tails held vertically during palpation/ovariectomy. Perhaps heifers would more freely display pain-related behaviours if a non-hydraulic squeeze chute was used with no tail restraint. Small group size may have been a factor in the inability to detect behavioural differences, however in an acute pain castration study groups of 10 to 12 calves were sufficiently large to detect differences in VAS scores and stride length, as well as leg movement, vocalization, tail flicking, lying, standing, walking and eating behaviours (Meléndez et al., 2017).

The lack of difference between treatment groups for pain-related behaviours recorded during ovariectomy (Table 3.2) may reflect the nature of the tissue trauma caused by DOT ovariectomy. The surgical injury of the DOT is limited only to visceral tissue, unlike the tissues traumatized from castration that includes both superficial and visceral tissues. Superficial nociceptive signals are relayed by the spinocervicothalamic tract in the central nervous system (CNS) to the somatosensory cerebral cortex, however visceral nociceptive signals are relayed by the bilaterally ascending and multisynaptic spinoreticular tract in the CNS that has limited connections to the thalamus and only indirectly reaches the cerebral cortex where pain is perceived (Hellyer et al., 2007). The diffuse connections in this tract and large size of
overlapping visceral nociceptor fields cause difficulty in the ability to pinpoint visceral pain localized to a small area. Compared to superficial tissues, visceral tissues have a larger proportion of C fibres that convey dull, aching pain signals compared to Aδ fibres that convey sharp pain signals (Hellyer et al., 2007); painful behaviour may be more likely to manifest with more Aδ fibre recruitment such as that resulting from scrotal incision. A large component of “silent” nociceptors are present in visceral tissue that are recruited only during inflammation (Gaynor & Muir, 2015) and would not yet have been provoked during the short time required for ovariectomy. Estrogen also affects visceral pain by increasing the size of pelvic visceral nociceptor fields and their sensitivity, and because estrogen receptors are in close proximity to NMDA receptors that are involved with central pain sensitization, estrogen receptor activation can also increase stimulation of NMDA receptors (Sengupta, 2009). Therefore it is likely that individual heifers in a group undergoing ovariectomy would experience more varying degrees of pain than if they were synchronized prior to spaying.

3.4.2.2 Flight Speed, Stride Length and Gait Score

Flight speeds for the heifers appeared to reflect individual animal temperament, as there were no differences between groups for treatment effect. A previous study with Bos indicus cattle used flight speed along with body weight for blocked randomization into treatment groups because of its negative correlation with weight gain (Petherick et al., 2013). The treatment × time effect on d 1 showing BXKM heifers with faster flight speeds than PALP heifers and a tendency to be faster than SPAY heifers does suggest that the BXKM heifers may have felt more comfortable when the oral meloxicam effect was at its approximate peak (Coetzee et al., 2009).

Stride length has been used to measure the pain of castration in calves, as castration pain can cause shortened stride length (Currah et al., 2009). The absence of differences in stride length and gait scores between treatment groups in our study may demonstrate that they may not be suitable indicators for the visceral pain of DOT ovariectomy.

3.4.2.3 Activity, Feeding Behaviour and ADG

Pain in animals is recognized as a reason for reduced activity and feed intake (Weary et al., 2006). Petherick et al. (2013) reported that more \( P < 0.05 \) DOT spayed heifers stood head down and in lay in sternal recumbency \( P < 0.05 \) and fewer were observed feeding \( P < 0.05 \) than restrained unspayed controls on the day of ovariectomy, but not in the 3 d following ovariectomy. These results differ from our video observations that did not reveal any
behavioural differences on the day of ovariectomy, but on the day following ovariectomy both BXKM and SPAY heifers spent less time lying and in sternal recumbency than the unspayed controls. Continuous GrowSafe System feed intake monitoring revealed no differences between treatment groups in feeding behaviour and accelerometer data found minimal differences in activity; this may be because the heifers were confined to small pens and perhaps had less motivation to exhibit differences in activity and feeding, as feed and water were both close at hand. The only difference in accelerometer logged activity over the course of the trial was that BXKM heifers had longer standing durations than PALP heifers, which may support the efficacy of the analgesic protocol. The tendency for the SPAY heifers to have lower ADG than PALP heifers over a 29 d interval is consistent with the findings from Jubb et al. (2003) who reported that heifers ovariectomized using the DOT technique (without analgesia) had lower rates of gain than unspayed controls over a 2 month period and Petherick et al. (2011) who reported lower bodyweights in DOT spayed heifers (without analgesia) compared to unspayed controls at 21 and 42 d after the procedure. The heifers in this trial were not implanted in order to avoid confounding by riding activity that accompanies implanting, although it is a common procedure to improve performance after ovariectomy in North America (Rupp & Hamilton, 1995). Overall, behavioural differences in activity including feed intake in DOT ovariectomized heifers seem to be unsuitable for pain measurement purposes, or the pain from ovariectomy is either not severe enough or of long enough duration to cause a change in behaviour.

3.4.3 Physiological Stress and Pain-Related Measurements

Physiological stress and pain indicators provided further insight into the magnitude of stress and pain caused by ovariectomy and the efficacy of the analgesic protocol. Previously two Australian studies used the physiological measures of plasma bound and unbound cortisol, non-esterified fatty acids (NEFA), creatine kinase (CK), aspartate aminotransferase (AST) and serum Hp to investigate the stress of flank and DOT ovariectomy, with differences for DOT spayed animals reported in both studies as higher plasma bound cortisol concentrations for yearling and 2 y old *Bos indicus* heifers compared to unspayed controls between 0 and 8 h post procedure (Petherick et al., 2013; Petherick et al., 2011).

3.4.3.1 CBC

The CBC results included lower RBC counts in BXKM heifers than PALP heifers at 24 and 48 h post-ovariectomy that likely reflects the internal blood loss involved with the
procedure, while the SPAY heifers showed a tendency to have lower RBC counts compared to the PALP heifers at 24 h post-ovariectomy; however, the lower RBC counts were clinically insignificant. Unchanged platelet counts suggests that the RBC count was not adversely affected by meloxicam, as it is considered to be more specific to blocking the COX-2 enzyme and therefore is less antithrombogenic than NSAIDs than are more COX-1 specific, although it is recognized that it potentially can block COX-1 enzyme production at higher dosages (Babos et al., 2013). However, splenic contraction from stress induced epinephrine release may also have played a role in maintaining the platelet count within normal limits (Roland et al., 2014). The lower WBC counts in the PALP heifers compared to the SPAY heifers at 2 h and both the SPAY and BXKM heifers at 4 h may reflect a stress neutrophilia from the increased cortisol release in the SPAY heifers and a smaller delayed cortisol release in the BXKM heifers. An increased N:L ratio can accompany the stress response, but the estimated N:L ratio was only numerically higher in the SPAY heifers and not significantly higher, although the N:L ratio for all groups was higher than the normal ratio of 0.5 reported by Roland et al. (2014) for health adult dairy cows, which may reflect handling stress. Chronic inflammation from infection or trauma and stress are the most common causes of increased WBC counts in cattle (Roland et al., 2014), and the short lived leukocytosis in the SPAY heifers suggests that DOT ovariectomy does not induce chronic inflammation or stress and the shorter leukocytosis in the BXKM heifers suggests that the medications may have reduced the inflammation and stress of ovariectomy.

3.4.3.2 Cortisol

Cortisol is the primary hormone responsible for the stress response, and although it does not directly measure pain, cortisol concentration has been recognized as corresponding to degree of noxiousness of a procedure but is also affected by handling stress (Coetzee, 2011; Coetzee et al., 2010; Mellor & Stafford, 2000). Its release is the culmination of events occurring when the hypothalamic-pituitary-adrenal (HPA) axis is stimulated and is influenced by physical activity and a circadian rhythm that results in lowest concentrations at night (Klein, 2013). Unbound (free) cortisol is the active form of the hormone, constitutes approximately 10% of plasma cortisol, and diffuses freely into the saliva (Klein, 2013). Stress simulation by adrenocorticotropin hormone (ACTH) administration in 3 month old Holstein calves caused peak salivary cortisol concentrations at 40 min with a gradual return to baseline by 360 min (Negrão et al., 2004). In our study, the SPAY heifers developed a typical cortisol response to surgery, the
PALP heifers had a response of lower magnitude from vigorous rectal palpation, but the BXKM heifers did not show any increase in salivary cortisol at the 1 h and 2 h sampling times which corresponds to the time that the butorphanol-xylazine-ketamine combination (BXK) would be providing a clinical effect (Abrahamsen, 2013). All 3 drugs act via different mechanisms to synergistically provide superior analgesia than each can provide separately; this allows lowering the dosage of each drug that minimizes unwanted effects and lowers cost. Butorphanol and xylazine also provide a mild sedative effect at the dosages used in this study, so it is unknown how much the analgesia or sedation effects of the BXK contributed to moderating the cortisol response. The small rise in cortisol that appeared at the 4 h sampling period suggests the effects of the BXK had worn off by that sampling time.

Hair cortisol has been used to measure long-term cortisol secretion in several species including cattle because it accumulates in the hair and is considered a measure of the magnitude of long-term stress for up to 28 d (Moya et al., 2013). Mean hair cortisol concentrations from 7-8 month old beef calves (304 ± 40.5 kg) tended (P = 0.09) to be higher in unmedicated castrated calves (3.4 ± 0.10 nmol/L) than in calves castrated with the local anesthetic lidocaine (3.4 ± 0.10 nmol/L) 28 days after castration (Melendez, 2018). The absence of an increase in hair cortisol in the study heifers suggests that the acute noxiousness of DOT ovariectomy resolves without creating long-term stress or pain.

3.4.3.3 Substance P

SP is a neuropeptide that functions in neurotransmission, neuromodulation, inflammation and sleep, but most interest has focused on its CNS role in nociceptive signal transmission (Mitchell et al., 2013). Its use as a pain biomarker has yielded conflicting results, such as a study reporting lower SP concentrations in dehorned calves treated with meloxicam compared to untreated dehorned calves (Coetzee et al., 2012), yet another reporting no difference in SP concentrations between sham, surgical, and band castrated calves (Marti et al., 2017a). SP may not be a useful pain biomarker for DOT ovariectomy, as there were no differences between groups detected in our study.

3.4.3.4 Acute Phase Proteins

Positive acute phase proteins (APP) are manufactured and released as part of the acute phase reaction (APR) in response to infection, inflammation and injury including surgical trauma, with some evidence indicating their usefulness as markers of stress (Ceciliani et al.,
SAA and Hp are both major positive APPs in cattle produced mainly in the liver with blood concentrations increasing within 8 h and peaking in 24-48 h after stimulation, with SAA peaking before Hp (Kirbas et al., 2015). Measuring SAA and Hp provides more sensitive detection of inflammation than CBC, and concentrations follow the course of inflammation, dropping to baseline levels upon resolution of inflammation and remaining high if inflammation becomes chronic (Kirbas et al., 2015).

Baseline concentration of SAA in healthy mature cattle was reported as 1.3 ± 0.4 mg/L (1 µg/mL) (Ceciliani et al., 2012), but all groups in our study had mean concentrations higher than 50 µg/mL at 0, 24, 48, 96 and 168 h sampling times, which may have been associated with the handling stress of sampling and the juvenile age of the heifers. In a dissertation, reported mean concentrations of SAA post-castration were 122 to 153 ± 0.09 mg/L (µg/mL) in a study investigating meloxicam and lidocaine for analgesia in 7-8 month old beef calves (304 ± 40.5 kg); SAA concentrations began rising from a baseline of approximately 50 mg/L at the 24 h sampling time (Melendez, 2018). It is possible that normal baseline concentrations for peri-pubertal beef cattle under typical handling conditions are higher than for mature cattle. An increase in SAA occurred at the 1 h sampling time in all groups, when BXKM heifers had higher concentrations than PALP heifers, which was the time the SAA peak concentration was achieved in the BXKM heifers, as their SAA concentrations declined before SPAY and PALP heifers; by the 4 h sampling time BXKM heifers tended to have lower concentrations than SPAY heifers which may have been a response to the anti-inflammatory effect of meloxicam. Sheep have been reported to have increased SAA concentrations after injection of vaccine (Ceciliani et al., 2012) but no reports have described an increase after injectable analgesic administration. SAA plays a role in the immune response and SAA concentrations increase in the 10 h after bacterial infection (Kirbas et al., 2015); the rapid response in our heifers may have resulted from infectious organisms introduced during ovariectomy. Some extrahepatic production of SAA occurs in tissues including the uterus; xylazine increases uterine tone (Abrahamsen, 2013) and may also have affected the SAA concentration in the BXKM heifers. SPAY and PALP heifers achieved peak SAA concentrations at 2 h post palpation/ovariectomy and by 24 h both BXKM and SPAY heifers had higher SAA concentrations than PALP heifers, which may reflect the acute inflammation of the ovariectomy procedure. SAA concentrations returned to baseline levels by
24 h in PALP heifers and by 48 h in BXKM and SPAY heifers, suggesting faster recovery from the acute inflammation resulting from palpation alone.

Hp concentrations peaked on d 2 for all treatment groups and SPAY heifers had higher concentrations on d1 compared to BXKM and PALP heifers, and on d 2, 4 and 7 compared to BXKM heifers, suggesting that the heifers spayed without analgesic medication developed the most inflammation. By d 7, Hp concentrations in BXKM and PALP heifers had almost returned to baseline levels, but SPAY heifers were still approximately twice as high as baseline. Hp concentrations declined most sharply for BXKM heifers after its d 2 peak to levels lower than both SPAY and PALP heifers by d 4, suggesting that meloxicam provided an effective anti-inflammatory effect for DOT ovariectomy.

3.4.3.5 Rectal Temperature

Normal range for rectal temperature (RT) in cattle is 36.7 – 39.1º C, averaging 38.3º C, and rises in response to pyrogens, exercise, inability to disperse heat, interference with thermoregulation from dehydration or when pharmaceuticals affect the thermoregulatory centres (Klein, 2013). The increased RT that the BXKM heifers displayed at 1 and 2 h post-ovariectomy compared to the SPAY and PALP heifers occurred likely in response to one of the drugs administered. BXK used at the dosages in this study has a reported clinical effect duration of up to 1.5 h (Abrahamsen, 2013), so component of BXK was most likely responsible for the hyperthermia, as it occurred during the time the heifers were expected to experience its clinical effect. Xylazine has been reported to cause hyperthermia by interference with thermoregulation in the CNS, that may be exacerbated by peripheral vasoconstriction (Gaynor & Muir, 2015). The transient hyperthermia in the BXKM heifers may have been a result of the xylazine CNS effect, as the vasodilatory effect of ketamine antagonizes the vasoconstrictive effect of xylazine (Abrahamsen, 2013).

3.4.3.6 Estradiol

Although there were no statistical differences, the unspayed heifers had numerically higher E2 concentrations, and only the spayed heifers had individuals with no detectable E2 concentrations. Mean estradiol concentrations in non-pregnant crossbred Angus heifers were reported as 42.4 ± 9.0 pg/mL during the late luteal phase on d 16 of the estrus cycle (Ellig et al., 2016), much higher than the concentrations in the heifers in this study. The heifers in this trial were in good body condition and they should have been post-pubertal, but their implant history
was unknown. Estradiol concentrations fluctuate during the estrus cycle of intact heifers, with the majority of the cycle at baseline concentrations, and in a group of 14 intact heifers, very few individuals might have had high enough concentrations to trigger a statistically significant difference between groups. Estradiol is mainly produced in ovarian follicles, but there is some production in other tissues such as the adrenal glands, so it is perhaps not unexpected that most of the ovariectomized heifers had detectable E2 concentrations. Jubb et al. (2003) examined DOT ovariectomized heifers a year later at slaughter and found 12.4% (30/242) had ovarian remnants with 67% of remnants consisting of a 1-2 mm nodule with up to a 15 mm fluid-filled cyst and no revascularized ovaries within the abdominal cavity were mentioned. Rapid revascularization within 1 month of ovarian amputation would be necessary for follicular function to return (Terazono et al., 2012). Jubb noted that care must be taken to ensure that the entire ovary is within the eye of the ovariotome so only the pedicle is cut and not a portion of the ovary, because remaining ovarian remnants could still result in pregnancy. E2 concentrations may not to be a useful determinant of ovarian remnants or autografts.

3.5 Conclusions

Behavioural measurements provided minimal evidence that DOT ovariectomy was stressful or painful to yearling heifers, which may be because the surgical trauma is limited to visceral tissues and perhaps because cattle do not tend to overtly display painful behaviour to potential predators. The physiological measurements performed, particularly salivary cortisol, SAA and Hp, provided evidence that DOT ovariectomy provokes acute stress and pain that resolves within several days. Differences in these physiological indicators demonstrated that administering an IM injection of BXK 5 minutes prior to ovariectomy and oral meloxicam at the time of ovariectomy reduced the procedural stress and pain as well as the post-surgical inflammatory pain caused by the procedure. Clinically insignificant hyperthermia developed for 2 h after ovariectomy, but precautions such as providing shade should be considered under high ambient temperature conditions. This combination of BXK and meloxicam can be used by veterinary practitioners to provide evidence-based analgesia for ovariectomy. It is a protocol easily implemented in clinical practice, and may also provide a measure of chemical restraint in locations where hydraulic squeeze chutes are unavailable.
Table 3.1: Visual Analog Scale (VAS) scores by two observers (OBS 1 and OBS 2) recorded while yearling beef heifers were undergoing palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM). Observers were blinded to treatment group.

<table>
<thead>
<tr>
<th>VAS Score (cm)</th>
<th>Mean ± SD</th>
<th>Min-Max</th>
<th>(X^2(\text{df})); (P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OBS 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PALP ((n=14))</td>
<td>0.57±0.18</td>
<td>(0.0-2.1)</td>
<td></td>
</tr>
<tr>
<td>SPAY ((n=15))</td>
<td>0.95±1.13</td>
<td>(0.0-3.3)</td>
<td></td>
</tr>
<tr>
<td>BXKM ((n=15))</td>
<td>0.79±0.54</td>
<td>(0.0-1.7)</td>
<td>2.20(2); (P = 0.33)</td>
</tr>
<tr>
<td><strong>OBS 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PALP ((n=14))</td>
<td>0.46±0.63</td>
<td>(0.0-2.0)</td>
<td></td>
</tr>
<tr>
<td>SPAY ((n=15))</td>
<td>0.53±0.62</td>
<td>(0.1-2.2)</td>
<td></td>
</tr>
<tr>
<td>BXKM ((n=15))</td>
<td>0.55±0.56</td>
<td>(0.1-2.1)</td>
<td>1.18(2); (P = 0.56)</td>
</tr>
</tbody>
</table>

Table 3.2: Pain-related behaviours observed during palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM) of yearling beef heifers, recorded by two observers blinded to treatment group.

<table>
<thead>
<tr>
<th>Behaviour (obs/n)</th>
<th>PALP</th>
<th>SPAY</th>
<th>BXKM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falling Attempt</td>
<td>1/14</td>
<td>0/15</td>
<td>1/15</td>
<td>0.76</td>
</tr>
<tr>
<td>Kick/Leg Lift</td>
<td>1/14</td>
<td>2/15</td>
<td>2/14</td>
<td>1.00</td>
</tr>
<tr>
<td>Tail Flick</td>
<td>11/14</td>
<td>13/14</td>
<td>11/13</td>
<td>0.67</td>
</tr>
<tr>
<td>Body Flinch</td>
<td>11/14</td>
<td>11/15</td>
<td>10/14</td>
<td>1.00</td>
</tr>
<tr>
<td>Head Movement</td>
<td>8/14</td>
<td>7/15</td>
<td>5/14</td>
<td>0.55</td>
</tr>
<tr>
<td>Vocalization</td>
<td>1/14</td>
<td>6/15</td>
<td>5/14</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\(^1\)Denotes observed number of heifers exhibiting behaviour/total number of heifers recorded for that behaviour in the group
Table 3.3: Treatment x time effect ($P = 0.72$) on stride length (LSM ± SEM) for yearling heifers palpated (PALP), DOT ovariectomized without analgesia (SPAY) and DOT ovariectomized with analgesia (BXKM) measured in the hours following palpation/ovariectomy using a video camera to capture images of heifers while their hind feet were in contact with the ground.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>96 h</th>
<th>168 h</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALP (n=14)</td>
<td>57.2</td>
<td>57.1</td>
<td>60.7</td>
<td>63.4</td>
<td>54.4</td>
<td>60.9</td>
<td>58.1</td>
<td>63.9</td>
<td>2.31</td>
</tr>
<tr>
<td>SPAY (n=15)</td>
<td>58.5</td>
<td>59.6</td>
<td>59.1</td>
<td>60.2</td>
<td>57.3</td>
<td>58.7</td>
<td>61.0</td>
<td>63.2</td>
<td>2.34</td>
</tr>
<tr>
<td>BXKM (n=15)</td>
<td>61.6</td>
<td>60.6</td>
<td>60.8</td>
<td>63.2</td>
<td>61.5</td>
<td>65.0</td>
<td>63.8</td>
<td>69.1</td>
<td>2.31</td>
</tr>
</tbody>
</table>

Table 3.4: Gait scores for yearling heifers palpated (PALP), DOT ovariectomized without analgesia (SPAY) and DOT ovariectomized with analgesia (BXKM) on d 0. Heifers were video recorded after leaving the squeeze chute at each sampling time before palpation/ovariectomy (d - 1), immediately after palpation/ovariectomy (d 0) and in the hours following ovariectomy (h 1 to h 168).

<table>
<thead>
<tr>
<th>Gait Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
</tr>
<tr>
<td>d -1</td>
</tr>
<tr>
<td>d 0</td>
</tr>
<tr>
<td>h 2</td>
</tr>
<tr>
<td>h 4</td>
</tr>
<tr>
<td>h 24</td>
</tr>
<tr>
<td>h 48</td>
</tr>
<tr>
<td>h 96</td>
</tr>
<tr>
<td>h 168</td>
</tr>
</tbody>
</table>

* Indicates that there was one or more missing data points when an image was not available for determination of gait.
Table 3.5: Feeding Behaviour using GrowSafe data for yearling beef heifers palpated (PALP; \(n=14\)), DOT ovariectomized without analgesia (SPAY; \(n=15\)) and DOT ovariectomized with analgesia (BXKM; \(n=15\)) on d 0. Data was gathered from d 0 to d 7.

<table>
<thead>
<tr>
<th>Item</th>
<th>PALP</th>
<th>SPAY</th>
<th>BXKM</th>
<th>SEM</th>
<th>T</th>
<th>Time</th>
<th>T × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake, kg</td>
<td>12.3</td>
<td>12.2</td>
<td>11.5</td>
<td>0.47</td>
<td>0.12</td>
<td>&lt; 0.001</td>
<td>0.92</td>
</tr>
<tr>
<td>Feeding time, min</td>
<td>220.8</td>
<td>225.5</td>
<td>212.1</td>
<td>8.69</td>
<td>0.28</td>
<td>&lt; 0.001</td>
<td>0.98</td>
</tr>
<tr>
<td>Feeding rate, g/min</td>
<td>56.8</td>
<td>56.3</td>
<td>54.4</td>
<td>1.27</td>
<td>0.32</td>
<td>&lt; 0.001</td>
<td>0.64</td>
</tr>
<tr>
<td>Meal frequency, n</td>
<td>14.3</td>
<td>14.8</td>
<td>14.1</td>
<td>0.47</td>
<td>0.60</td>
<td>&lt; 0.001</td>
<td>0.86</td>
</tr>
<tr>
<td>Meal duration, min</td>
<td>16.4</td>
<td>17.0</td>
<td>16.5</td>
<td>0.87</td>
<td>0.80</td>
<td>&lt; 0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>Meal size, kg/meal</td>
<td>0.92</td>
<td>0.93</td>
<td>0.86</td>
<td>0.005</td>
<td>0.27</td>
<td>&lt; 0.001</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Table 3.6: Least Squares Means (LSM ± SEM) for Bodyweight and Average Daily Gain (ADG) of yearling beef heifers over 7 days following palpation (PALP; n=14), DOT ovariectomy without analgesia (SPAY; n=15) and DOT ovariectomy with analgesia (BXKM; n=15).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>PALP</th>
<th>SPAY</th>
<th>BXKM</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, kg</td>
<td></td>
<td>315</td>
<td>315</td>
<td>314</td>
<td>4.3</td>
<td>0.99</td>
</tr>
<tr>
<td>Final BW (d 7), kg</td>
<td></td>
<td>328</td>
<td>326</td>
<td>325</td>
<td>3.2</td>
<td>0.60</td>
</tr>
<tr>
<td>ADG, kg/day</td>
<td></td>
<td>1.94</td>
<td>1.58</td>
<td>1.51</td>
<td>0.440</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table 3.7: LSM ± SEM for Bodyweight and Average Daily Gain (ADG) from d 0 to d 29 for yearling beef heifers palpated (PALP), DOT ovariectomized without analgesia (SPAY) and DOT ovariectomized with analgesia (BXKM) on d 0.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>PALP (n=13)¹</td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>315</td>
<td>0.99</td>
</tr>
<tr>
<td>Final BW (d 29), kg</td>
<td>374a</td>
<td>0.03</td>
</tr>
<tr>
<td>ADG, kg/day</td>
<td>1.77c</td>
<td>0.03</td>
</tr>
</tbody>
</table>

¹One outlier was removed from the PALP group.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, kg</td>
<td>315</td>
<td></td>
</tr>
<tr>
<td>Final BW (d 29), kg</td>
<td>362b</td>
<td></td>
</tr>
<tr>
<td>ADG, kg/day</td>
<td>1.34d</td>
<td></td>
</tr>
</tbody>
</table>

a,b,c,dLSM with differing superscripts within a line differ (P ≤ 0.05).
Table 3.8: Standing/lying behaviour and pain-related behaviour of yearling heifers after palpation (PALP; \( n = 14 \)), DOT ovariectomy without analgesia (SPAY; \( n = 15 \)) and DOT ovariectomy with analgesia (BXKM; \( n = 15 \)). Accelerometer data was analyzed from day of palpation/ovariectomy (d 0) to d 7 after palpation/ovariectomy to generate least squares means (LSM) \( \pm \) SEM for each group. Pain-related behaviours were tallied using recorded video after sampling at hour 4 on the day of ovariectomy (5 hours of video observed) and on the day following ovariectomy (4 hours video observed).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>( P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PALP</td>
<td>SPAY</td>
<td>BXKM</td>
</tr>
<tr>
<td>Accelerometer data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing, %</td>
<td>46.5</td>
<td>48.7</td>
<td>49.3</td>
</tr>
<tr>
<td>Standing duration, min</td>
<td>52.5(^b)</td>
<td>55.7(^ab)</td>
<td>59.7(^a)</td>
</tr>
<tr>
<td>Lying duration, min</td>
<td>49.3</td>
<td>50.8</td>
<td>48.3</td>
</tr>
<tr>
<td>Standing bouts, n</td>
<td>13.3</td>
<td>13.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Lying bouts, n</td>
<td>16.2</td>
<td>15.2</td>
<td>15.7</td>
</tr>
<tr>
<td>Behavior 4 h after treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking, min</td>
<td>11.4</td>
<td>10.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Standing, min</td>
<td>98.4</td>
<td>73.7</td>
<td>89.9</td>
</tr>
<tr>
<td>Lying, min</td>
<td>187.4</td>
<td>191.1</td>
<td>158.7</td>
</tr>
<tr>
<td>Lying (sternal), min</td>
<td>187.4</td>
<td>187.7</td>
<td>156.4</td>
</tr>
<tr>
<td>Lying (lateral), min</td>
<td>0.0</td>
<td>3.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Tail flicking, n</td>
<td>162</td>
<td>70</td>
<td>117</td>
</tr>
<tr>
<td>Grooming, n</td>
<td>6.8</td>
<td>11.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Foot stamping, n</td>
<td>4.6</td>
<td>5.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Back arching, n</td>
<td>0.4</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Tail lifting, n</td>
<td>4.6</td>
<td>6.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Behavior 24 h after treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking, min</td>
<td>10.4</td>
<td>11.0</td>
<td>15.2</td>
</tr>
<tr>
<td>Standing, min</td>
<td>20.9</td>
<td>22.0</td>
<td>30.5</td>
</tr>
<tr>
<td>Lying, min</td>
<td>128.8(^c)</td>
<td>84.5(^d)</td>
<td>60.3(^d)</td>
</tr>
<tr>
<td>Lying (sternal), min</td>
<td>128.8(^c)</td>
<td>84.3(^c)</td>
<td>60.3(^c)</td>
</tr>
<tr>
<td>Lying (lateral), min</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Tail flicking, n</td>
<td>183</td>
<td>324</td>
<td>229</td>
</tr>
<tr>
<td>Grooming, n</td>
<td>2.9</td>
<td>4.7</td>
<td>9.7</td>
</tr>
<tr>
<td>Foot stamping, n</td>
<td>3.6</td>
<td>5.8</td>
<td>9.0</td>
</tr>
<tr>
<td>Back arching, n</td>
<td>0.4</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Tail lifting, n</td>
<td>7.4</td>
<td>5.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\(^a,b,c,d,e,f\) LSMs within the rows with dissimilar superscripts differed \((P \leq 0.05)\).
Table 3.9: Physiological data (LSM ± SEM) for yearling beef heifers after palpation (PALP; \( n=14 \)), DOT ovariectomy without analgesia (SPAY; \( n=15 \)) and DOT ovariectomy with analgesia (BXKM; \( n=15 \)). Heifers had saliva and blood samples collected at the time of palpation/ovariectomy (d 0) and at 1, 2, 4, 24, 48, 96 and 168 hours after palpation/ovariectomy and hair samples were harvested on d 0 and d 29. LSMs with differing superscripts within a row differ \((P \leq 0.05)\).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>( P)-value</th>
<th>( P)-value</th>
<th>( P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperature, °C</td>
<td>PALP</td>
<td>0.08</td>
<td>0.32</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>SPAY</td>
<td>0.12</td>
<td>0.08</td>
<td>&lt; 0.001</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.04</td>
<td>0.87</td>
<td>&lt; 0.001</td>
<td>0.93</td>
</tr>
<tr>
<td>Salivary cortisol, nmol/L</td>
<td>PALP</td>
<td>0.11</td>
<td>0.02</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>SPAY</td>
<td>0.35</td>
<td>0.21</td>
<td>&lt; 0.001</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.07</td>
<td>0.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Substance P, pg/mL</td>
<td>PALP</td>
<td>0.12</td>
<td>0.28</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>SPAY</td>
<td>0.52</td>
<td>0.50</td>
<td>&lt; 0.001</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.29</td>
<td>0.35</td>
<td>&lt; 0.001</td>
<td>0.19</td>
</tr>
<tr>
<td>Haptoglobin, g/L</td>
<td>PALP</td>
<td>0.01</td>
<td>0.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SPAY</td>
<td>0.11</td>
<td>0.21</td>
<td>&lt; 0.001</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.10</td>
<td>0.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum Amyloid A, µg/mL</td>
<td>PALP</td>
<td>0.10</td>
<td>0.28</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>SPAY</td>
<td>0.52</td>
<td>0.50</td>
<td>&lt; 0.001</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.29</td>
<td>0.35</td>
<td>&lt; 0.001</td>
<td>0.19</td>
</tr>
<tr>
<td>Hair cortisol, pg/mL</td>
<td>PALP</td>
<td>0.12</td>
<td>0.28</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>SPAY</td>
<td>0.52</td>
<td>0.50</td>
<td>&lt; 0.001</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.29</td>
<td>0.35</td>
<td>&lt; 0.001</td>
<td>0.19</td>
</tr>
<tr>
<td>Complete blood cell count</td>
<td>PALP</td>
<td>0.10</td>
<td>0.28</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Red blood cells, (10^6/µl)</td>
<td>SPAY</td>
<td>0.52</td>
<td>0.50</td>
<td>&lt; 0.001</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>BXKM</td>
<td>0.29</td>
<td>0.35</td>
<td>&lt; 0.001</td>
<td>0.19</td>
</tr>
<tr>
<td>Platelet count, (10^3/µl)</td>
<td>PALP</td>
<td>9.20</td>
<td>0.52</td>
<td>&lt; 0.001</td>
<td>0.79</td>
</tr>
<tr>
<td>White blood cells, (10^3/µl)</td>
<td>SPAY</td>
<td>0.50</td>
<td>0.50</td>
<td>&lt; 0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>N:L ratio</td>
<td>PALP</td>
<td>0.07</td>
<td>0.35</td>
<td>&lt; 0.001</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Table 3.10: Mean ± SD serum estradiol concentrations for non-implanted yearling beef heifers on d 29 after being palpated (PALP), DOT ovariectomized without analgesia (SPAY) and DOT ovariectomized with analgesia (BXKM) on d 0.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN</th>
<th>SD</th>
<th>MIN-MAX</th>
<th>$\chi^2$ (df)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALP (n=14)</td>
<td>1.42</td>
<td>0.93</td>
<td>0.19-3.30</td>
<td>1.9 (2)</td>
<td>0.40</td>
</tr>
<tr>
<td>SPAY (n=15)</td>
<td>1.00</td>
<td>0.93</td>
<td>0.00-2.89</td>
<td>1.9 (2)</td>
<td>0.40</td>
</tr>
<tr>
<td>BXKM (n=14)</td>
<td>1.05</td>
<td>0.80</td>
<td>0.00-2.36</td>
<td>1.9 (2)</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Figure 3.1: Flight Speed of yearling heifers after palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM) on d 0. Differing letters over sampling time point indicates differences between groups ($P \leq 0.05$).

Figure 3.2: Salivary cortisol concentrations in yearling beef heifers palpated (PALP), DOT ovariectomized without analgesia (SPAY) and DOT ovariectomized with analgesia (BXKM) on d 0. Differing letters over sampling time points indicate differences between groups ($P \leq 0.05$).
Figure 3.3: RBC Counts for yearling beef heifers after palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM) on d 0. Differing letters over sampling time point indicates differences between groups ($P \leq 0.05$).

Figure 3.4: WBC count for yearling beef heifers after palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM) on d 0. Differing letters over sampling time point indicates differences between groups ($P \leq 0.05$).
Figure 3.5: Haptoglobin concentrations in yearling beef heifers after palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM) on d 0. Differing letters over sampling time point indicates differences between groups ($P \leq 0.05$).

Figure 3.6: Serum Amyloid A (SAA) concentrations in yearling beef heifers after palpation (PALP), DOT ovariectomy without analgesia (SPAY), and DOT ovariectomy with analgesia (BXKM) on d 0. Differing letters over sampling time point indicates differences between groups ($P \leq 0.05$).
Figure 3.7: Rectal temperature in yearling beef heifers after palpation (PALP), DOT ovariectomy without analgesia (SPAY) and DOT ovariectomy with analgesia (BXKM) on d 0. Differing letters over sampling time point indicates differences between groups ($P \leq 0.05$).
4.1 Conclusions

The objectives of this thesis were to 1) determine the magnitude of pain that heifers experience during ovariectomy and in the days following ovariectomy, 2) to determine whether an intramuscular injection of butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg) and ketamine (0.04 mg/kg) administered 5 minutes prior to ovariectomy can mitigate the acute procedural and immediate post-surgical pain of ovariectomy, 3) to determine whether oral meloxicam (1 mg/kg) administered at the time of ovariectomy can reduce the post-surgical inflammatory pain of ovariectomy and 4) to determine if this pain mitigation protocol could be easily implemented under ranch conditions. Very little has been published regarding the stress and pain of ovariectomy in heifers and no publications have yet described an effective pain mitigation plan for the procedure.

Chapter 1 reviewed the relevant literature for bovine ovariectomy, including the indications, animal welfare issues and techniques, as well as analgesic pharmaceuticals and animal pain and its measurement using behavioural and physiological indicators. The welfare issue regarding ovariectomy in the literature has been confined to that surrounding the surgery itself, without consideration to the future welfare implications of pregnancy prevention in the months after the surgery. It is hoped that this thesis will help enlighten readers to consider the welfare of spayed heifers once they are past the “farm gate”.

Chapter 2 described a pilot project under ranch conditions on 120 heifers scheduled for ovariectomy to investigate behavioural measures of pain and whether evidence for analgesic effectiveness could be gathered with behavioural data from a $2 \times 2$ factorial design. The procedural pain of DOT ovariectomy was difficult to determine from behavioural measurement, as VAS scores and pain related behaviours did not differ between treatments, but heifers were very effectively restrained in a hydraulic squeeze chute with tail restraint. The difference in gait scores immediately after ovariectomy did provide evidence that injecting BXK to heifers waiting in the race was rapidly effective. Environmental conditions made video recording of feeding behaviour challenging, but the longer feeding durations displayed by the heifers in the groups that had received BXK suggested that it may have provided some relief from the visceral
discomfort provoked by the surgery. The meloxicam effect on activity starting 5 h after ovariectomy and in the day following ovariectomy provided evidence of its efficacy for postsurgical inflammatory pain relief, however the common management practice of implanting spayed heifers likely confounded the activity measurements when riding activity started on d 1. It was worthwhile to establish that administering the analgesics did not slow down the process of spaying, an important consideration for adoption by producers who often need to spay hundreds of animals in a day.

Chapter 3 described an experiment on 45 heifers under research facility conditions to generate measurements of physiological as well as behavioural indicators of stress and pain for ovariectomy using an unspayed but palpated control group and spay groups treated and untreated with the BXK and meloxicam analgesic protocol. Limited resources prevented adding a control group that received only BXK and meloxicam with no other manipulation; creating 4 smaller groups would have reduced the power of the experiment to detect differences. Behavioural measurements again yielded few indications of pain and relief of pain; flight speed and standing/lying activity differences on the day following ovariectomy providing some evidence that BXK and meloxicam treated heifers felt more comfortable. The physiological biomarkers provided more insight into the stress and pain of ovariectomy, and how much it was reduced by BXK and meloxicam. The salivary cortisol and Hp response curves were highest and of longest duration in the heifers spayed without analgesia; the salivary cortisol concentrations suggest that the surgery is acutely stressful and painful, and the Hp concentrations suggest inflammation from ovariectomy lasts longer than 7 days. In the spayed heifers treated with BXK and meloxicam, the salivary cortisol response was eliminated during the time that BXK was delivering its expected clinical effect, and the Hp response was reduced to levels even lower than the palpated control group by d 4, suggesting that meloxicam was effective in reducing the post-surgical inflammation and pain that accompanies it. The SAA response curve gave some insight into the acute nature of the DOT ovariectomy surgical trauma for the ovariectomized heifers, as the heifers that were only palpated had the lowest response curve of the 3 groups. Whether there was a synergism between the BXK and meloxicam cannot be determined from this experiment, but there is good evidence that this drug combination mitigated both the procedural and postsurgical inflammatory pain of DOT ovariectomy.
Overall, this project confirmed that DOT ovariectomy provokes a relatively rapidly resolving acute stress and pain, that can be mitigated using BXK IM 5 minutes prior to the procedure and oral meloxicam at the time of the procedure.

4.2 Future Research

The welfare issues of cull beef heifers have not yet been addressed in the literature. Although outnumbered by steers in the feedlot, heifers fed for market deserve some investigation into their welfare. It seems intuitive that the welfare of a cull heifer is better if she is not pregnant when she arrives in the feedlot, but the magnitude of stress provoked by induced abortion has not been researched. It would be informative to explore whether repeated estrus cycling is stressful for a grazing heifer and if she might be in distress if she cannot find a bull. How does imposing a state of anestrus by spaying impact her Quality of Life? Perhaps the most important question that needs answering is whether it is more stressful/distressing for a heifer to be aborted than it is to never experience estrus. Answering this question may restrict the merits of ovariectomy to its production and management benefits and limit its animal welfare indications to preventing heifer calving mortality under extensive unsupervised grazing conditions.

Extensively managed beef herds only have a few opportunities to be handled, and each trip through the chute is both stressful to the cattle and expensive in labour costs for the producer. Ovariectomy is a permanent solution for pregnancy prevention that requires only one handling event. Minimizing the number of times beef heifers are handled should be an important consideration for any future research projects. Anti-GnRH vaccine in its present form is not a suitable alternative for ovariectomy in extensively grazed cattle in terms of cost and animal welfare, as it requires repeated handling events, causes injection site pain, and its effects may not persist for the duration of the grazing period. Future research efforts may be better directed to investigation into other analgesics that are rapidly effective, easily administered, and have a duration lasting several days.

The visceral pain provoked by DOT ovariectomy did not provide much behavioural evidence of pain in our experiments. Although it was not possible to closely monitor behaviour of the heifers in our experiments in the first 4 hours after ovariectomy because of location (experiment 1) and physiological sampling (experiment 2), future attempts to measure behavioural responses during that time frame may be valuable. Resources for future welfare
research on bovine ovariectomy may be better utilized on physiological biomarkers, particularly SAA, Hp and cortisol. An additional control group that is administered drugs without surgery would be useful to determine the response of the animals to the drugs themselves when they are not experiencing pain.

4.3 Implications

A limiting factor for more widespread implementation of DOT ovariectomy is that the technique is technically challenging to learn and physically challenging to perform in large numbers. It is hoped that sufficient evidence has been presented to show that the pain of the procedure can be mitigated, and that this can provide future support to those who wish to pursue training to perform DOT ovariectomy.

The Code of Practice for the Care and Handling of Beef Cattle recommends that producers seek veterinary advice for alleviating the pain of ovariectomy; documenting the efficacy of multimodal administration of injectable BXK and oral meloxicam for this purpose provides practitioners with an evidence-based information resource. This analgesic protocol can be used in a ranch setting under the supervision of a veterinarian, and because DOT ovariectomy is a surgical procedure in Canada, a licensed veterinarian must perform the procedure and therefore can also oversee the administration of BXK in most jurisdictions. BXK administration also can help to prevent potentially fatal iatrogenic visceral trauma by its mild sedative effect that provides a degree of chemical restraint to limit movement while ovariectomy is being performed, particularly if a non-hydraulic squeeze chute is used. The BXK and meloxicam protocol also has the potential to be modified and used for other surgical procedures requiring analgesia when groups of cattle are processed through a handling facility, such as for castration, dehorning or branding.
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