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Hot-iron disbudding pain in calves
Studies on perception of pain and options to increase pain alleviation

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ACADEMIC DISSERTATION

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To my family

“The great art of life is sensation, to feel that we exist, even in pain.”
Lord Byron
Abstract

Disbudding entails destroying calves’ horn buds, and in dairy farming is most often done with a hot-iron. Disbudding is routinely carried out because hornless cattle are considered to be safer for themselves and for humans. Hot-iron disbudding is very painful and causes severe pain-related distress and behavioural changes in calves. Options for treating disbudding-related pain during the procedure, and for 24 hours subsequently, are well known, but continued pain and its management are not much studied in calves after disbudding. Pain can cause restlessness and thus affect calves’ lying time. Pain in humans and rats also changes sleeping behaviour. Pain connected with disbudding often remains untreated. Reasons for this are unclear. Therefore, more knowledge and research are needed on the recognition of calves’ pain after hot-iron disbudding, on the duration of pain and on options to treat it in an effective, safe and practical way. Research is also needed on producer knowledge and attitudes towards pain in calves and their decision-making in connection with pain alleviation.

The objectives of the work reported in this thesis were all connected with gaining an improved understanding of producer perceptions about pain caused to young calves by hot-iron disbudding, and with options available to increase the use of pain alleviation for this common and painful procedure. Initially we asked dairy producers for their perceptions towards disbudding pain in calves. Then, in order to be able to study the duration of pain after disbudding in the future, we attempted to develop a new device to measure calves’ lying and sleeping time: a small, neck-based, wireless accelerometer system. Because new methods and various options for pain alleviation are needed, we investigated if sublingual detomidine provided sufficient sedation in calves to allow administration of local anaesthetics prior to disbudding. Because the use of pain alleviation is often a choice faced by producers, we wanted to study Finnish dairy producers’ interests and motivation regarding pain alleviation in connection with disbudding. We studied Finnish dairy producers’ perceptions on disbudding-related pain and the need for pain alleviation, and how such perceptions affect the actual practice of pain alleviation.

Finnish dairy producers estimated disbudding pain to be severe and producer estimation of pain severity caused by disbudding was correlated with their sensitivity to pain caused by different cattle diseases in general. We were able to develop an accurate device for measuring calves’ lying and sleeping time. Detomidine oromucosal gel was an effective sedative for calves before infiltration of local anaesthetics and disbudding. Finnish dairy producers who estimated the disbudding-related pain and need for pain alleviation to be high had a veterinarian medicate calves before disbudding more often than producers who ranked disbudding pain and need for pain alleviation lower.

Because more studies on duration and alleviation of disbudding pain are needed, our new device for measuring lying and sleeping time in calves could make these studies easier in the future. A non-invasive and user-friendly oromucosal sedation method for calves could enhance the use of local anaesthetics before disbudding by making sedation easier. Our findings among dairy producers support the idea that persons who have knowledge of pain and who think pain alleviation is beneficial and important are also more prone to administer pain alleviation. Education of producers on disbudding-related pain could increase the use of pain alleviation in the future. It could also increase pain alleviation for other cattle diseases because producer perceptions on disbudding-related pain are likely to be connected with pain in cattle in general.
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This thesis is based on the four following publications:


The publications are referred to in the text by their Roman numerals.

Reprints of the original articles I and IV are published with the kind permission of the Journal of Dairy Science.

A reprint of the original article II is published with the kind permission of Elsevier.

A reprint of the original article III is published with the kind permission of the Veterinary Anaesthesia and Analgesia.
**Abbreviations**

AUC  the area under the time-concentration curve
AUC\textsubscript{sed} the area under the time-sedation score curve
β rate constant of the elimination phase
Cmax maximum plasma concentration
COX cyclo-oxygenase
CSS clinical sedation score
EEG electroencephalogram
F bioavailability
GEL-group sublingual administration group (n=10) in Study III
HR heart rate
im intramuscular
IQR interquartile range
iv intravenous
IV-group intravenous administration group (n=10) in Study III
NREM non-rapid eye movement sleep
NRS numerical rating scale
NSAID non-steroidal anti-inflammatory drug
PCA principal component analysis
po per os
REM rapid eye movement sleep
rs Spearman rank
SD standard deviation
SE standard error
SVM support vector machine
Tmax time to maximum plasma concentration
TST total sleeping time in Study II
VAS visual analogue scale
1 Introduction

Disbudding, the removal of calves’ horn buds, is a common procedure in dairy husbandry because hornless cattle are safer to handle (Duffield, 2008) and are also safer among their herd mates (Prayaga, 2007). Disbudding of young calves is typically done with a caustic paste or a hot-iron (Fulwider et al., 2008; ALCASDE, 2009) and the later is the only legal method used in Finland (Finlex, Cattle Welfare Decree, 592/2010). In older animals, removing of horns (dehorning) is normally done surgically using a number of painful techniques, including scooping, shearing, and sawing (Sylvester et al., 1998a, 2004; ALCASDE, 2009; Stafford & Mellor, 2011; AVMA, 2014).

Disbudding with a hot-iron causes severe acute pain, and is associated with behavioural and physiological responses (Morisse et al., 1995; Grøndahl-Nielsen et al., 1999; Stafford & Mellor, 2011), but there is no EU legislation that covers pain management associated with disbudding. The European Council Directive 98/58/EC (EC, 1976) allow any skilled person to destroy or remove the horn-producing area of calves aged less than four weeks with chemical or heat cauterization. In Finland, and in most other European countries, calves over four weeks of age can be disbudded only by a veterinarian (ALCASDE, 2009; Finlex, Cattle Welfare Decree, 592/2010). Application of appropriate anaesthetics and analgesics is not compulsory in most countries, but it is strongly recommended worldwide by professional organisations (AVA, 2004; AVMA, 2014).

Cornual nerve block and ring block around the horn buds using local anaesthetics effectively alleviates disbudding pain and delays its onset (Graf & Senn, 1999). Application of local anaesthetics to non-sedated animals can be difficult. Sedatives, $\alpha_2$-agonists, mainly xylazine, have been used in disbudding-related studies (Faulkner & Weary, 2000; Stock et al., 2013). Their use is beneficial because sedation delays the acute cortisol response and makes application of local anaesthetics easier (Grøndahl-Nielsen et al., 1999; Stafford et al., 2003; Stilwell et al., 2010).

Disbudding-related postoperative pain has been successfully alleviated with non-steroidal anti-inflammatory drugs such as meloxicam (Heinrich et al., 2009; Stewart et al., 2009; Heinrich et al., 2010; Allen et al., 2013), ketoprofen (McMeekan et al., 1998a; Faulkner & Weary, 2000) and carprofen (Stilwell et al., 2012). Usually disbudding-related pain studies concentrate on pain and its alleviation in the period up to 24 hours after disbudding (Milligan et al., 2004; Heinrich et al., 2009; Duffield et al., 2010), but there is also evidence that disbudding-related pain can last longer than 24 hours (Heinrich et al., 2010; Theurer et al., 2012; Mintline et al., 2013), and more studies are therefore needed on this aspect. The lying and sleeping behaviour of calves could represent a new method for studying the duration of pain as pain can promote restlessness in animals (Morisse et al., 1995; Petrie et al., 1995a; Kent et al., 1998; Tom et al., 2001; Heinrich et al., 2010; Theurer et al., 2012) and pain disrupts sleep in humans (Raymond et al., 2004) and in laboratory animals (Andersen et al., 2006; Leys et al., 2013). Also calves’ total daily resting time is usually very constant and it is not easily affected by environmental stressors (Hänninen et al., 2005). Activity-based automatic measurements could support long surveillance studies of animal behaviour after painful procedures, as reported by Theurer et al. (2012).

Despite the existing knowledge on painfulness, global recommendations for pain alleviation and several common analgesic medicines available, pain and distress related to disbudding and dehorning of calves often remains untreated (Hoe & Ruegg, 2006; Hewson et al., 2007; ALCASDE, 2009; Vasseur et al., 2010; Gottardo et al., 2011). Producers taking care of calves play
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a key role in whether or not disbudding-related pain is alleviated. However, in Finland, as in other European countries, the use of veterinary drugs is highly restricted and legally controlled (Finlex, Act on the medical treatment of animals, 387/2014), and thus producer motivation to use pain alleviation is particularly important because use of sedatives, local anaesthetics and analgesic drugs requires veterinary intervention, which represents extra costs for the producer.

The reasons are unclear as to why some producers provide pain medication to disbudded calves and others do not. Identification and monitoring pain in farm animals is not a simple task according to veterinarians and farmers (Ison & Rutherford, 2014). There are several reasons why the use of pain alleviation is complicated, especially for farm animals, but should be increased in the future (Anil et al., 2005; Hewson et al., 2007). Public concern about pain in production animals is also growing (Spooner et al., 2014). Steps for better understanding and treatment of pain related to disbudding modified from literature reviews by Anil et al. (2005) and Weary et al. (2006) are shown in Figure 1.

Figure 1. Steps for better understanding and alleviation of pain related to disbudding (modified from literature reviews by Anil et al. (2005) and Weary et al. (2006).
2  Review of the literature

2.1  Disbudding

Disbudding is a common husbandry practice in the cattle industry, especially on dairy farms, because hornless cattle are safer (Goonewardene et al., 1999; ALCASDE, 2009) and injuries to cattle and other animals are reduced (Prayaga, 2007). Disbudding destroys the horn buds in young calves up to 8–12 weeks of age (depending on breed) before any horn material is visible (ALCASDE, 2009). Calf age at disbudding varies, but in practice disbudding can be performed when horn buds can be removed with a heated disbudding iron or a caustic paste (horn buds are approximately 5 to 10 mm long at this age).

In the literature, use of the terms ‘disbudding’ and ‘dehorning’ varies and is often confusing, and it is usual that ‘dehorning’ is used also when ‘disbudding’ is meant. Because the aims of this thesis were all connected with hot-iron disbudding-related pain in young calves, this literature review focuses only on hot-iron disbudding. Other disbudding methods and dehorning is described only briefly and then considered only when necessary (some information, particularly on pain management, is only studied in relation to dehorning and in some studies the term ‘dehorning’ refers to ‘disbudding’).

2.1.1  Hot-iron disbudding

Hot-iron disbudding, also termed cautery disbudding (Stafford & Mellor, 2011) or thermal disbudding (Graf & Senn, 1999) means that calf horn bud tissue is destroyed by burning with a heated metal bar with a concave tip (Figure 2). The very hot (approximately 600 °C) metal bar burns the horn bud and surrounding tissue. This leads to the destruction of all the epidermal and dermal skin layers through to the subcutaneous tissue at the burn site. In addition, it causes tissue damage and oedema around the burn, and thus increases the sensitized area around the burned horn bud (Junger et al., 2002). A hot-iron disbudding device is heated electrically or with a gas flame prior disbudding (AVMA, 2014).

Figure 2. A calf’s horn bud after successful hot-iron disbudding.
Hot-iron cauterization is a popular method used to disbudd calves. In a survey conducted in Canada 88.7% of producers used hot-iron cauterization (Vasseur et al., 2010) and the respective figures were 91% in studies conducted in Italy (Gottardo et al., 2011) and 67.3% in the USA (Fulwider et al., 2008). According to the ALCASDE report (2009) hot-iron is the most used method of disbudding, especially in northern and central Europe. Legislation and practices differ among countries, but in Finland hot-iron cauterization is the only legal method for disbudding calves (Finlex, Cattle Welfare Decree, 592/2010).

### 2.1.2 Other methods for destroying horn buds or horns

Caustic paste disbudding is done by applying a thin layer of sodium or calcium hydroxide over the horn bud of calves under the age of six weeks (Vickers et al., 2005; Stilwell et al., 2008, 2009). Caustic paste disbudding causes pain and distress to the calves (Morisse et al., 1995; Vickers et al., 2005; Stilwell et al., 2009).

Horn buds can also be removed physically by using disbudding scoops, knives, shears, spoons, cups and tubes (Petrie et al., 1995b; McMeekan et al., 1998a, 1998b, 1999; Gibson et al., 2007; ALCASDE, 2009; AVMA, 2014).

Dehorning refers to amputation of the horns once they grow longer and become attached to the underlying frontal sinus. The dehorning of older dairy cattle is mainly performed in Europe using the wire or saw method, but some countries also report use of other instruments, including the guillotine and dehorning shears (ALCASDE, 2009). Amputation dehorning results in open wounds and the methods used are painful (Sylvester 1998b; Sutherland et al., 2002a, 2002b; Sylvester et al., 2004). Subsequent infections can lead to welfare problems.

Tipping is practised as an alternative to dehorning cattle of various ages. Tipping can range from light tipping (2 cm cut off the end of the horn with no bleeding, i.e. blunting the horn) to heavy tipping (reducing the length of the horn to around 10 cm with bleeding and exposed cavities) (Prayaga, 2007).

### 2.1.3 Alternatives to disbudding and dehorning

Because all methods for destroying horns are painful for the animals (as reviewed by Stafford & Mellor, 2011), alternative procedures are needed. One practical alternative to disbudding, and to eliminating disbudding-related pain, is to favour polled (i.e. genetically hornless) sires when breeding cows (Guatteo et al., 2012). Some cattle breeds are polled, but most dairy breeds and many beef breeds, still produce horns. It is possible to breed polled European type cattle (Bos taurus) because there is a simple genetic basis for polledness (Prayaga, 2007; Spurlock, 2014). In Finland, West Finnish Cattle, one of the traditional national dairy breeds, is polled (Tike, 2009).

### 2.2 Hot-iron disbudding-related pain in calves

Pain is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP 1979, P 250 IASP* Subcommittee on taxonomy, Pain 1979, 6, 249). Molony and Kent (1997) further define pain as “an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal’s physiology and behaviour to reduce or avoid damage, to reduce the likelihood of reoccurrence and to promote recovery".
Hot-iron disbudding causes physiological indications of pain in calves, such as cortisol response (Petrie et al., 1995b), increase in heart rate (Grøndahl-Nielsen et al., 1999; Stewart et al., 2008), increase in plasma ACTH and vasopressin concentrations (Graf & Senn, 1999) and also increase in plasma noradrenalin and adrenaline concentrations (Mellor et al., 2002). During hot-iron disbudding, without sedation and pain alleviation, calves struggle and the procedure requires strong restraint of the calves. Calf behaviours during hot-iron disbudding without pain management include ear flicking, tail wagging, head moving, tripping and rearing (Graf & Senn, 1999; Grøndahl-Nielsen et al., 1999).

Several studies address disbudding-related pain over the course of hours (Graf & Senn, 1999; Grøndahl-Nielsen et al., 1999; Stewart et al., 2009), and up to 24 hours (Faulkner & Weary, 2000; Heinrich et al., 2009; Duffield et al., 2010; Stilwell et al., 2010). Studies of longer-lasting pain are fewer. Mintline et al. (2013) reported that disbudding wounds can remain sensitive for at least 75 hours after the procedure and Heinrich et al. (2010) showed that calves can feel pain for at least 44 hours following hot-iron disbudding. Evidence from one study suggests that, based on changes in calves’ lying time, disbudding-related pain may persist for up to 4 days (Theurer et al., 2012). More research is needed to evaluate the duration of pain after hot-iron disbudding.

It is not well-defined when acute pain becomes chronic in animals. Another area where further research is needed is understanding when pain becomes irritation (Stafford & Mellor, 2011). Different classifications have been used to describe pain duration. Usually pain that results from injury or inflammation has survival value and may play a role in the healing process of an animal by promoting behaviours that minimize re-injury. It is termed acute pain. Acute pain can also be prolonged and it is generally accepted that pain persisting beyond the expected healing period is pathologic (Ashburn & Staats, 1999). Molony and Kent (1997) defined non-functional pain as; unnecessary pain occurs when the intensity or duration of the experience is inappropriate for the damage sustained or when the physiological and behavioural responses to it are unsuccessful at alleviating it”. So chronic pain may have no obvious cause and it serves no useful purpose (as reviewed by Ashburn & Staats, 1999 and Viñuela-Fernández et al., 2007).

Evidence of chronic pain exists in cattle after tail docking (Eicher et al., 2006) and in lame cattle (Whay et al., 1998; Laven et al., 2008). Behavioural signs of long-term pain were also reported in calves up to 42 days after rubber-ring castration (Molony et al., 1995). However, as reviewed by Stafford and Mellor (2005), chronic or prolonged pain in cattle after castration needs to be studied further because not all studies show signs of chronic pain, for example, after rubber-ring castration. Prolonged (lasting for days or weeks) and chronic (lasting for months) pain in animals is still poorly understood and needs further research as reviewed by Viñuela-Fernández et al. (2007). It should be also noted that routine painful procedures, such as disbudding and castration, are usually performed on young calves. Young animals could be more vulnerable to pain than older animals because of their immature nervous system (Boucher et al., 1998; Johnston et al., 2011).

Pain can cause restlessness in animals (Petrie et al., 1995a; Kent et al., 1998; Tom et al., 2001; Heinrich et al., 2010). Resting and sleeping behaviours could serve as behavioural indicators for pain. Several authors suggested that sleep patterns (Ruckebusch, 1975; Siegel 2005) or activity rhythms (Ruckebusch, 1975; Veissier et al., 1989; Scheibe et al., 1999) could serve as measures for the adaptation of animals to their environment. Disbudding-related pain could disrupt such adaptation.
2.3 Registering resting and sleeping behaviour in calves automatically

Several factors may affect the resting and sleeping behaviour of calves in production environments. Environmental temperature (Schrama et al., 1995), feeding (Phillips, 2004), space allowance (Wilson et al., 1999), weaning (Veissier et al., 1989), lighting (Weiguo & Phillips, 1991), social company (Babu et al., 2004) and age (Wilson et al., 1999; Panivivat et al., 2004) were all reported to affect the calves’ resting behaviour.

Most newborn calves are hideous by nature: in the wild the dam hides the calf from the herd for 3-4 days to ensure bonding with the calf. During this period, the calves spend their time mainly lying down, except during nursing periods (Lidfors, 1994). Thus, newborn calves spend the most of their day resting. Five to six month old calves have been shown to rest for 60-80% of the day in barns and the overall time spent resting decreased only slightly as the calves grew older (de Wilt, 1985; Wilson et al., 1999; Panivivat et al., 2004).

Automated measurements of animal behaviours represent potentially important tools for enhanced information about the adverse effects of painful procedures on animals. Accelerometers are small devices that continuously measure gravitational force in multiple axes. These values can be processed to determine activity and postural behaviours of animals by using validated algorithms. Accelerometers have been used in several species for automatic monitoring of behaviours, such as grazing in goats (Moreau et al., 2009), postures and stepping in pigs (Ringgenberg et al., 2010), lameness and gait in dairy cows (Pastell et al., 2009; Chapinal et al., 2011), gait patterns in calves (de Passillé et al., 2010), lying behaviour in dairy cows (Martiskainen et al., 2009; Robert et al., 2009; Ledgerwood et al., 2010) and also lying time and activity (Theurer et al., 2012) and play (Rushen & de Passillé, 2012) in calves. Activity-based monitoring with accelerometers has been validated in humans (Lötjönen et al., 2003; review by Morgenthaler et al., 2007; review by Sadeh, 2011) and pain has been reported to cause sleep-disturbances in humans (Raymond et al., 2004; reviewed by Finan et al., 2013) and in laboratory animals (Andersen et al., 2006; Leys et al., 2013).

Little is known about sleep in cattle and only a few studies on calf sleep have been conducted (Hänninen et al., 2008a, 2008b). Zepelin et al. (2005) defined mammalian sleep by saying that a sleeping individual sustains “quiescence in a species-specific posture accompanied by reduced responsiveness to external stimuli, has a quick reversibility to the wakeful condition and characteristic change in the electroencephalogram”. Thus far the sleep of all mammalian farm animals fits this definition. Electrophysiologically sleep is split into two main phases: rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM) (Tobler, 1995). Sleep is commonly measured by registering both sleeping behaviour and brain electrophysiology, EEG (electroencephalogram). Sleep states in animals can be identified through the animals’ behaviour as has been successfully done for zoo animals (Tobler, 1992; Tobler & Schwierin, 1996), mice (Storch et al., 2004) and calves (Hänninen et al., 2008a, 2008b) but less successfully for adult cattle (Ternman et al., 2012, 2014). Unlike in adult cows, sleep can be defined from the behaviour of calves (Hänninen et al., 2008a), but automatic activity-based monitoring for measuring sleep in calves is lacking.

2.4 Options for alleviating hot-iron disbudding-related pain

The options for alleviating hot-iron disbudding-related pain in young calves are discussed here. For information about alleviating the pain caused by other disbudding and dehorning methods,
the reader is referred to an excellent review by Stock et al. (2013). The use of anaesthetics and analgesics in cattle is difficult because cattle are production animals, and knowledge of milk and meat withdrawal times is essential to prevent residues in animal products intended for human consumption. The availability of approved agents for anaesthesia and analgesia in cattle differs among countries and if approved agents are not available, practitioners can use the drugs in an extra-label manner if correct meat and milk withdrawal times can be established. Because hot-iron disbudding is done on young calves, milk withdrawal times are not a problem and also long meat withdrawal times go usually without problems for production. Extra-label use of anaesthetic and analgesic compounds in cattle is discussed in detail in a recent review by Smith (2013).

Efficient pain management in connection with hot-iron disbudding should minimize the pain during the procedure and prevent the development of chronic or neuropathic pain and changes in the calf nervous system associated with hyperalgesia. Multimodal and pre-emptive analgesia can be used to reach this goal, as reviewed by Stock & Coetzee (2015) in cattle. Pre-emptive analgesia means that analgesia is provided before any painful stimuli have occurred. This prevents the development of hypersensitization during the procedure and can result in less postoperative pain (Woolf & Chong, 1993). The goal of multimodal analgesia during disbudding is to provide effective pain control in calves by blocking the pain pathways at multiple sites, using agents with different modes of action. Thus, it is a general recommendation that calves receive sedation, local anaesthesia and NSAID before hot-iron disbudding (Faulkner & Weary, 2000; AVA, 2004; Stafford & Mellor, 2011; AVMA, 2014; Stock & Coetzee, 2015).

2.4.1 Sedatives, \( \alpha_2 \)-adrenoceptor agonists

Sedatives are used prior to hot-iron disbudding, usually to make handling of animals easier and less stressful for the animals, and also for the safety of the operator. The most common sedatives used for cattle and horses are \( \alpha_2 \)-adrenoceptor agonists, xylazine and detomidine. Adrenergic receptors are part of the autonomic nervous system and they mediate their physiological effects via adrenaline and noradrenaline. \( \alpha_2 \)-adrenoceptors are located mostly pre-synaptically and their activation leads to decreased excretion of noradrenaline into the synaptic space (Langer, 1974; Berthelsen & Pettinger, 1977). \( \alpha_2 \)-adrenoceptor agonists also produce analgesia by stimulating receptors in the dorsal horn of the spinal cord and in the brainstem, where modulation of nociceptive signals is initiated (Kuraishi et al., 1985; Hayashi et al., 1995).

\( \alpha_2 \)-adrenoceptor agonists have a number of side effects. The swallowing reflex is lost and regurgitation can result in pulmonary aspiration. Other possible side effects of administration of xylazine include bradycardia, pulmonary side effects and increased peripheral resistance (Campbell et al., 1979; Rioja et al., 2008). Side effects of detomidine include bradycardia, hypotension, diuresis and hyperglycaemia (EMEA, 1996).

Sedation and analgesia induced by \( \alpha_2 \)-adrenoceptor agonists can be reversed with \( \alpha_2 \)-agonist antagonists (Raekallio et al., 1991; Stafford et al., 2003) and also the majority of the side effects of the agonists are reversed. Almost all the \( \alpha_2 \)-agonist antagonists have been used in cattle sedated with xylazine. \( \alpha_2 \)-agonist antagonists used in cattle include yohimbine (with aminopyridine), tolazoline and atipamezole. Atipamezole has been used to reverse the effects of xylazine (Thompson et al., 1991; Arnemo & Solli, 1993; Rioja et al., 2008) as also tolazoline after scoop dehorning in calves (Stafford et al., 2003). Raekallio et al. (1991) showed that atipamezole antagonized medetomidine in calves and recovery from sedation was rapid and smooth.
Xylazine is a $\alpha_2$-adrenoceptor agonist commonly used before disbudding (0.2 mg/kg im 20 min before disbudding) (Faulkner & Weary, 2000; Stilwell et al., 2010), and its use combined with butorphanol (xylazine 0.2 mg/kg and butorphanol 0.1 mg/kg, im, 20 min before disbudding) eliminated calf response to the administration of the local anaesthetic (Grøndahl-Nielsen et al., 1999). It was shown that the combination of local anaesthetic and xylazine as a sedative agent alleviates pain during the hot-iron disbudding procedure (Grøndahl-Nielsen et al., 1999).

Detomidine is a potent $\alpha_2$-adrenoceptor agonist that is commonly used for sedation or premedication in horses (DiMaio Knych & Stanley, 2011) and cattle (Salonen et al., 1989), including calves (Peshin et al., 1991). The pharmacokinetic profile of detomidine is quite similar in adult horses and cows (Salonen et al., 1989). To our knowledge, detomidine has not been used to sedate calves in disbudding-related studies.

In veterinary medicine sedative agents are most commonly administered parenterally to the animals, but detomidine could be also administered sublingually. Sublingual administration of an oromucosal gel formulation of detomidine produced safe and practical sedation in horses (DiMaio Knych & Stanley, 2011; Kaukinen et al., 2011). Oromucosal detomidine is approved in Europe to sedate horses for non-invasive veterinary procedures. In addition, it can be used by the horse owners themselves during husbandry interventions, and the meat withdrawal period is zero days (EMEA, 1996). Sublingually administered oromucosal gel formulation of detomidine can be considered safer for humans and animals than injectable detomidine, especially when administered by the animal owners themselves.

$\alpha_2$-adrenoceptor agonists are only mild analgesics and they are not efficient enough to alleviate hot-iron disbudding-related pain when used alone. It was reported that calves treated with only xylazine showed a strong behavioural response to hot-iron disbudding (Faulkner & Weary, 2000; Stilwell et al., 2010). Therefore, they should not be used without local anaesthetic during hot-iron disbudding.

2.4.2 Local anaesthetics

Local anaesthetics reversibly block the transmission of action potentials along a nerve axon by blocking Na+ channels and stabilizing excitable cell membranes, thus preventing nociception (Butterworth & Strichartz, 1990). Cornual nerve block before disbudding is performed by injecting local anaesthetic under the skin around the cornual nerve, midway between the calf’s horn bud and the lateral canthus of the eye (Faulkner & Weary, 2000; Fierheller et al., 2012). Ring block is performed by injecting local anaesthetic under the skin around the horn bud at several injection sites (Faulkner & Weary, 2000; Fierheller et al., 2012).

Cornual nerve block and ring block around the horn buds with the local anaesthetic lidocaine alleviate hot-iron disbudding pain effectively and delay its onset (Graf & Senn, 1999). Lidocaine (20 mg/mL) is the most popular local anaesthetic used in hot-iron disbudding-related studies as a corneal nerve block (Morisset et al., 1995; Petrie et al., 1995b; Grondahl-Nielsen, 1999; Stilwell et al., 2010) usually given at 5 mL/horn 10 min before disbudding (Milligan et al., 2004; Heinrich et al., 2009; Duffield et al., 2010; Heinrich et al., 2010), or as a corneal nerve block and ring block together (Graf & Senn, 1999; Faulkner & Weary, 2000; Stewart et al., 2009). Lidocaine is effective for 2 to 3 hours after administration (Graf & Senn, 1999). Other options for local anaesthesia before disbudding are bupivacaine, which is effective for approximately 4 hours (studied in connection with scoop dehorning) (McMeekan et al., 1998b), procaine hydrochloride
Concentrated lidocaine (Doherty et al., 2007) and lidocaine combined with epinephrine (Milligan et al., 2004).

Unfortunately, because of the short duration of action of local anaesthetics, their use alone does not provide adequate postoperative pain relief for disbudded calves after a hot-iron is used (Graf & Senn, 1999).

Local anaesthetics generally have poor skin permeability and thus they are of limited effectiveness for topical pre-emptive pain alleviation before disbudding (Lomax et al., 2008; Fierheller et al., 2012). A topical eutectic mixture of a local anaesthetic (EMLA) cream was ineffective for anaesthesia of horn buds in two-month-old calves (Fierheller et al., 2012). A gel-based viscous topical local anaesthetic gel containing lidocaine, bupivacaine, adrenaline (epinephrine) and cetrimide (Tri-Solfen; Bayer Animal Health, Australia) was able to provide short-term wound anaesthesia after scoop dehorning (2-month-old calves, 4 mL on each side), when used as the sole analgesic and administered immediately to the open wounds (Espinoza et al., 2013). The same topical anaesthetic was also reported to produce rapid desensitization of scrotal mucosal tissue, with the effect lasting 24 hours, in castrated beef calves (Lomax & Windsor, 2013a). Furthermore, this topical agent was effective in alleviating pain of mulesing, castration and tail docking in sheep and also improved wound healing (Lomax et al., 2008, 2010 and 2013b). The use of topical anaesthesia may provide a valuable, cost-effective and practical on-farm method for pain alleviation and the options to use it before and especially after hot-iron disbudding needs to be studied further.

2.4.3 Non-steroidal anti-inflammatory drugs, NSAIDs

NSAIDs are commonly used in veterinary medicine, including for cattle. NSAIDs have anti-inflammatory, analgesic and antipyretic activity. They inhibit production of prostaglandins and thromboxane via inhibition of COX enzymes (Vane, 1971). The most common side effects of NSAIDs include damage to the gastrointestinal mucosa (Wallace, 1997) and kidney failure (Lascelles et al., 2005). Using a combination of two different NSAIDs or administration of NSAIDs above the recommended dose increases the risk of side effects (Reed et al., 2006).

Ketoprofen, a propionic acid derivative, is an NSAID with analgesic and antipyretic properties commonly used in veterinary medicine (EMEA, 1995). It is a chiral compound and it exists in two enantiomeric forms, S (+) and R (-). Ketoprofen undergoes unidirectional chiral inversion from the R- to the S-enantiomer in calves (Landoni et al., 1995a). Orally administered ketoprofen (3 mg/kg), used in combination with local anaesthetic administered 10 minutes before disbudding (4.5 mL of 2% lidocaine per horn bud as corneal nerve block and ring block) has been effective for alleviating pain for 24 hours after hot-iron disbudding when administered 2 hours before disbudding and 2 and 7 hours after disbudding (Faulkner & Weary, 2000). Ketoprofen (3 mg/kg im), administered ten minutes before disbudding, in combination with local anaesthetic (5 mL of 2% lidocaine hydrochloride with 0.05 mg/mL epinephrine), alleviated short-term pain (8 to 24 hours) after disbudding (Milligan et al., 2004; Duffield et al., 2010).

Meloxicam (enolic acid) is an NSAID with an elimination half-life of 24–28 hours in calves (EMEA, 1999b). The pharmacokinetic properties of orally administered meloxicam have been studied in calves (Coetzee et al., 2009; Mosher et al., 2012; Fraccaro et al., 2013). Meloxicam (0.5 mg/kg im given 10 min before disbudding), in combination with local anaesthetic (cornual nerve block with 2% lidocaine hydrochloride with 0.05 mg/mL epinephrine 5 mL per horn bud 10 minutes before disbudding) was effective for alleviating pain after hot-iron disbudding.
Review of the Literature

for 24 hours (Heinrich et al., 2009) and for 44 hours (Heinrich et al., 2010). Stewart et al. (2009) found that meloxicam 0.5 mg/kg iv given 55 min before disbudding alleviated the pain for 2–3 hours when given together with local anaesthetic (2% lidocaine hydrochloride, 5 mL around the each cornual nerve and 3-4 mL as a ring block per horn). Meloxicam (0.5 mg/kg po) given immediately after disbudding as the only analgesic increased calves’ lying time during 4 days after disbudding compared with control calves (Theurer et al., 2012).

Flunixin is an NSAID with an elimination half-life of 10.5 hours in calves (EMEA, 1999a). The pharmacokinetic properties of intravenously administered flunixin have been studied in calves (Landoni et al., 1995b; Fraccaro et al., 2013) and flunixin combined with local anaesthetic (2% procaine hydrochloride 10 mL on each side) reduced plasma cortisol levels of calves for 3 hours after hot-iron disbudding (Huber et al., 2013).

Carprofen is an NSAID with half-life of over 34 hours in 17-week-old calves (Delatour et al., 1996; EMEA, 2004). Carprofen (together with cornual nerve anaesthesia with 5 mL 2% lidocaine hydrochloride per horn bud 15 minutes before disbudding) is found effective for alleviating hot-iron disbudding-related pain for 24 hours in calves (Stilwell et al., 2012).

Previous research thus suggests that NSAIDs, in addition to local anaesthesia, are effective in alleviating pain during hot-iron disbudding and for several hours after it. Many NSAIDs can also be administered orally to the calves but long absorption times can be a problem, for example with oral meloxicam (Coetzee et al., 2009). The buccal meloxicam formulation (meloxicam formulated with vehicle designed to aid transmucosal absorption within the buccal cavity) has been reported to provide analgesia to lambs after castration and tail docking (Small et al., 2014a) and there are preliminary results showing that buccal meloxicam could alleviate castration-related pain also in calves (Small et al., 2014b).

2.5 Producer perceptions on disbudding pain and the use of pain alleviation

What producers think about pain and the importance of pain management is important. The producers play a key role in whether or not pain in calves is treated. Producers’ attitudes can be studied with questionnaire-based surveys. Studies aimed at getting information about farmers attitudes and practices in dairy cattle husbandry have been done, for example, for animal welfare (Kirchner et al., 2014), udder health (Jansen et al., 2009, 2010; Lind et al., 2012), lameness (Leach et al., 2010; Bruijnis et al., 2013; Bennett et al., 2014), herd health management (Derks et al., 2012, 2014; Pothmann et al., 2014), motivation to vaccinate cattle (Elbers et al., 2010), reproductive performance (Caraviello et al., 2006), tail docking (Barnett et al., 1999), castration (Stafford et al., 2000) and disease preventing programmes (Delgado et al., 2012, 2014). The perceptions regarding cattle pain among producers (Gottardo et al., 2011; Thomsen et al., 2012; Becker et al., 2013, 2014) and among veterinarians (Raekallio et al., 2003; Hewson et al., 2007; Laven et al., 2009; Thomsen et al., 2012; Becker et al., 2014; Norring et al., 2014) have also been studied, as has use of pain alleviation for cattle in different painful situations (Raekallio et al., 2003; Hewson et al., 2007; Norring et al., 2014), but to a lesser extent than other management practices.

One option to study perceptions on the severity of pain in cattle among those working with cattle is using pain scales. The visual analogue scale (VAS) is a 10 cm rule from 0 representing no pain to 10 cm, the distance representing the worst pain imaginable. The pain evaluator marks the line according to estimation of pain and the distance from 0 to the mark is measured (Kielland et al., 2010). VAS has been used to study veterinary students’ attitudes to pain in cattle (Kielland
et al., 2009). The numerical rating scale (NRS) from 0 to 10 (Huxley & Whay, 2006) or 1 to 10 (Raekallio et al., 2003; Kielland et al., 2009; Thomsen et al., 2012), has also been used to measure an individual’s perception on the degree of pain. Also using the NRS scale 0 (or 1) represents no pain and 10 the worst pain imaginable.

Information about producers’ attitudes and perceptions on disbudding and disbudding-related pain is quite scarce. Studies on producers’ attitudes and practices about disbudding and dehorning have focused mainly on asking about disbudding-related practices and the prevalence of pain alleviation (Gottardo et al., 2011) and have been usually performed in connection with inquiring about other management practices (Fulwider et al., 2008; Vasseur et al., 2010; Staněk et al., 2014). Information about producers’ perceptions on disbudding-related pain and management is important for better understanding as to why disbudding-related pain often remains untreated (Fulwider et al., 2008; ALCASDE, 2009; Vasseur et al., 2010; Staněk et al., 2014). Persons working with animals have significant influence on animal welfare (Coleman et al., 2003; Hemsworth et al., 2011) and it is known that attitude and behaviour of the producer affect animal welfare, health and production (Rushen et al., 1999; Waiblinger et al., 2002; Boivin et al., 2003, Kauppinen et al., 2012).

Despite recommendations to use sedatives, local anaesthetics and non-steroidal anti-inflammatory drugs to alleviate disbudding-related pain (AVA, 2004; New Zealand Government, 2005; AVMA, 2014), pain alleviation is not common globally. Medical treatment is administered prior to or after calf disbudding only on 20% of European farms (ALCASDE, 2009). In Italy, producers reported that 10% of their disbudded calves received local anaesthetics, 4% received a sedative and 5% received analgesics prior to disbudding, and the majority of respondents were not willing to pay for the veterinary services to treat disbudding-related pain (Gottardo et al., 2011). In a recent study conducted in the Czech Republic, only 7.6% of disbudding procedures were done with pain alleviation (Staněk et al., 2014). In Canada, use of sedatives or local anaesthetics prior to disbudding was reported for 45% of herds, but apparently no NSAIDs were used (Vasseur et al., 2010). In the United States, sedatives or local anaesthetics were used by 12% and NSAIDs by 2% of dairy farmers (Fulwider et al., 2008).

Currently, calling veterinarian is the only legal method for Finnish producers to get pain alleviation to their disbudded calves, as the use of veterinary drugs is highly restricted and legally controlled. A veterinarian can prescribe injectable NSAIDs to producers under certain circumstances, but never injectable sedatives or local anaesthetics. Preventive prescription of sedatives, local anaesthetic agents and injectable NSAIDs is not allowed in Finland, and the veterinarian has to examine the animals before administration of these medicines. The only exception to this rule is that NSAIDs can be prescribed for treating post-operative disbudding-related pain to those herd owners whose herds belong to the national herd surveillance system (Finlex, Act on the medical treatment of animals, 387/2014).

Research has shown that not all veterinarians alleviate pain before disbudding or dehorning (Huxley & Whay, 2006; Hewson et al., 2007; Misch et al., 2007; Fajt et al., 2011). Results of a recent study conducted in Finland indicate a change in the attitude regarding pain alleviation among veterinarians; production animal practice oriented or young veterinarians treat disbudding pain in calves according to Finland’s national recommendations, with sedatives, local anaesthetics and NSAIDs (Norring et al., 2014). It has been shown previously that veterinarians who rank pain high were more likely to use analgesics for calves than veterinarians who rank pain lower (Hewson et al., 2007). Currently we do not know enough about the factors that motivate dairy producers to use pain alleviation in connection with disbudding.


Aims of the Study

3 Aims of the study

The aims of this study were all related to steps towards better understanding and treatment of pain related to hot-iron disbudding in young calves (as shown in Figure 1).

The specific aims of this study were:

1. To study how Finnish dairy producers perceive disbudding-related pain and if producers' perceptions about disbudding-related pain and cattle pain in general are linked.
2. To develop and validate a small wireless accelerometer device for measuring the lying time and sleeping behaviour of calves.
3. To study if oromucosal detomidine produces sufficient sedation in calves to allow administration of local anaesthetics prior to disbudding.
4. To study if Finnish dairy producers' perceptions on disbudding-related pain and need for pain alleviation affect actual provision of pain alleviation.
4 Materials and Methods

This thesis consists of four original scientific peer reviewed articles (I-IV) based on three experiments. In this section, I provide a summary of the main methods of data collection, animals and their care. For more detailed descriptions, the reader may refer to the original articles included at the end of the thesis.

4.1 Study design

We conducted a questionnaire-based survey among Finnish dairy producers to study how they perceive disbudding-related pain (Article I) and if their perception of disbudding-related pain affects the actual provision of pain alleviation (Article IV). Article II is based on a study to develop and validate a small wireless accelerometer device for measuring the lying time and sleeping behaviour of calves. Bearing in mind that pain can alter lying time in calves, this new measuring method could serve as a tool for us in the future work for studying, for example, alleviating pain. Article III is based on a randomised prospective clinical study, during which we examined if oromucosal detomidine produced sufficient sedation in calves to allow administration of local anaesthetics prior to disbudding.

4.2 Subjects and questionnaire (I, IV)

We sent a four-page, postage-paid questionnaire to 1000 Finnish dairy producers. The producers were randomly selected from a geographically balanced list of all 11,244 dairy producers in Finland (Tike, Information Center of the Ministry of Agriculture and Forestry, 2009). A total of 451 questionnaires (45%) were returned. We managed and analysed all data without identifying the respondents or their farms.

We used a questionnaire that consisted of five sections and included 70 questions (Appendix 1). The first, second and third sections comprised background information and questions about disbudding practices. We asked about the prevalence of disbudding (yes or no) and the use of veterinarian to medicate calves before disbudding (always, sometimes, never). The questions in the fourth section (25 disbudding-related statements) were intended for all producers, regardless of whether disbudding took place on the farm or not. In this section respondent agreements with common disbudding-related statements were asked on a 5-point Likert scale (Raekallio et al., 2003), in which 1 corresponded to complete disagreement and 5 to complete agreement. In the fifth section (14 statements) respondent opinion about the severity of disbudding pain without any medication were sought using an 11-point numerical rating scale (NRS), with 0 representing no pain and 10 the worst pain imaginable (Huxley and Whay, 2006; Kielland et al., 2009). The questions reported in this thesis are shown in Table 1.
**Materials and Methods**

**Table 1.** The questions asked from dairy producers (I, IV).

**Statements about disbudding (Likert-scale)**

Disbudding without medication causes the calf pain

The calf requires no pain medication for disbudding

The calf may feel pain for as long as 3 d after the disbudding procedure

Veterinarians take administration of pain medication to the calf seriously

It is too expensive to have a veterinarian medicate the calf for disbudding

Sedation causes more problems for the calf than disbudding without medication

Painless disbudding increases calf’s welfare

I could never disbud calves without any pain alleviation

Medication eliminates pain during disbudding

If I could inject the calf with pain medication myself before disbudding, I would

If I could inject the calf with anaesthetics myself before the disbudding procedure (inject an anaesthetic compound around the horn buds), I would

If I could tranquilize (anaesthetize) the calf myself, I would

**Statements about cattle pain (NRS 0-10)**

Disbudding without pain medication (pain during the burning)

Navel infection in a calf (navel is thick and moist, animal is feverish)

Acute mastitis

Uterine prolapse in cattle

Umbilical hernias the size of a large apple in a calf

Abomasal displacement in cattle

Severe tympania in cattle

Teat trampling in cows (teat broken at the root)

**4.3 Animals, housing and feeding (II, III)**

The animals, housing conditions and feeding in studies II and III are shown in Table 2. All calves included in experiments were healthy and were housed, fed and treated like all other calves in the farms before each study.

**Table 2.** Housing conditions and feeding for calves included in the experiments.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animals</th>
<th>Housing</th>
<th>Lighting</th>
<th>Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Ten West Finland Cattle dairy calves, 31.7 ± 5.4 days of age.</td>
<td>Private farm, straw-bedded group pen (5.3 m x 3.6 m).</td>
<td>Artificial lighting, manual control. Some natural light from windows. Lights were on from 05:30 to 22:00 and a dim night-light was provided.</td>
<td>Silage, hay, concentrate, water and milk replacer ad libitum.</td>
</tr>
<tr>
<td>III</td>
<td>20 dairy calves, aged 12.4 ± 4.4 days. Weight 50.5 ± 9.0 kg.</td>
<td>Experimental farm, individual pens with straw bedding or group pen with saw dust bedding.</td>
<td>Artificial lighting, manual control. Some natural light from windows. Lights were on during the study.</td>
<td>Hay, concentrate and water ad libitum and milk or milk replacer.</td>
</tr>
</tbody>
</table>
4.4 The automatic measurement system for lying and sleeping behaviours (II)

We designed, constructed and programmed a wireless acceleration measurement device attached to the calves' collars (Figure 3). The dimensions of the device, with one half AA battery attached, were 15 mm × 36 mm × 34 mm and it weighed 19 g. The device used an MMA7260Q (Freescale, Austin, USA) acceleration sensor combined with an nRF9E5 (Nordic Semiconductor, Oslo, Norway) micro controller and transceiver for radio transmission to the computer. We used an 869 MHz radio, which ensured reliable signal transmission in barn conditions (line-of-sight range up to 1 km). The radio channel permitted the transmission of 330 messages per second (four 8-bit bytes: x, y and z acceleration as well as sensor ID) from a single transmitter. We programmed the devices to measure and transmit within the range of ±2 g at 25 Hz and the devices used a simple listen-before-transmit protocol. The sensor is described in more detail in Pastell et al. (2009).

![Image of the device](image)

Figure 3. A wireless 3-dimensional acceleration measurement device that registered movement and inclination in three axes (b) to predict sleep and lying time in calves. The device was attached to the middle of a calf's collar (a).

4.5 Clinical experiment (III)

Prior to drug administrations, we weighed the calves and performed a clinical examination to confirm that they were healthy. We randomly allocated the calves to one of two groups of 10 animals each. We administered detomidine gel (Domosedan Gel 7.6 mg/mL oromucosal gel, Orion Pharma Ltd., Turku, Finland) 80 μg/kg to one group (n=10) sublingually (GEL-group), and to the other group (n=10) we administered intravenously (IV-group) 30 μg/kg detomidine (Domosedan 10 mg/mL solution for injection, Orion Pharma Ltd., Espoo, Finland). In this study we did not use a separate control group as we focused on the differences between the GEL and IV-groups.

We drew the sublingual detomidine into a 1 mL syringe to get an accurate dose and then administered it under the tongue of the calf. Intravenous detomidine was administered as a bolus via the jugular catheter, and then the catheter was flushed with 10 mL of isotonic saline. To alleviate postoperative pain we administered a meloxicam dose of 0.5 mg/kg (Melovem 5 mg/
mL, Orion Pharma Ltd., Turku, Finland) subcutaneously. When clinical sedation was evident we applied the local anaesthetic (Lidocain 20 mg/mL, 1.5 mg/kg per horn bud, Orion Pharma, Finland) subcutaneously around the cornual nerve, and as a ring block around each horn bud (Faulkner & Weary, 2000) and recorded the animal’s response to administration of the local anaesthetic. We confirmed the nerve block with a needle prick, clipped the hair around the horn buds and performed disbudding with hot-iron butane disbudding device (Express Gas Portable Dehorner with a 20 mm head).

**4.6 Behaviour observations (II, III)**

In study II we filmed calf behaviour continuously for 24 hours simultaneously with the accelerometer registering. From the videos calf resting body and head postures (Table 3) were scored by a single experienced observer using CowLog software (Hänninen & Pastell, 2009). In study III, the depth of sedation was observed directly from the behaviour during 240 minutes after drug administration at 14 fixed time points using a 17-point behaviour-based clinical sedation score (CSS, Table 4).
Table 3. Definitions of the behaviours registered (II).

<table>
<thead>
<tr>
<th>Behaviour registered</th>
<th>Example of calves’ behaviour registered</th>
<th>Definition of behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying behaviour</td>
<td><img src="image1.png" alt="Image" /></td>
<td>The calf is lying either on its sternum or on its side when the side of the trunk is positioned against the ground.</td>
</tr>
<tr>
<td>NREM behaviour</td>
<td><img src="image2.png" alt="Image" /></td>
<td>The calf is resting head up, being still (Hänninen et al., 2008a).</td>
</tr>
<tr>
<td>REM behaviour</td>
<td><img src="image3.png" alt="Image" /></td>
<td>The calf is resting with the neck relaxed, and the head against the floor or flank (Hänninen et al., 2008a).</td>
</tr>
<tr>
<td>Total sleeping behaviour</td>
<td><img src="image4.png" alt="Image" /></td>
<td>NREM + REM behaviours</td>
</tr>
</tbody>
</table>
Table 4. The 17-point (0-16 where 0 is no sedation) behaviour-based clinical sedation score (CSS) modified from Kuusela et al. (2001) (III).

| Palpebral reflex (0-3) | 0 Normal  
1 Slightly reduced  
2 Weak  
3 Absent |
| Position of the eye (0-2) | 0 Middle  
2 Turned down |
| Jaw and tongue relaxation (0-4) | 0 Normal, opens the jaws but resists manipulation of the tongue  
1 Bites jaws together  
2 Opens the jaws but strong resistance when tongue is pulled  
3 Slight resistance when tongue is pulled  
4 No resistance |
| Resistance to positioning in lateral recumbency (0-3) | 0 Normal  
1 Turns back to sternal position  
2 Some resistance but stays in lateral recumbency  
3 No resistance or the position is already lateral |
| General appearance (0-4) | 0 Normal  
1 Slightly tired, head drooping  
2 Mild sedation, reacts clearly to handling/injection  
3 Moderate sedation, reacts slightly to handling/injection  
4 Deep sedation, no reaction to handling/injection |

4.7 Detomidine plasma concentrations, heart rate and body temperature (III)

Prior to drug administration we cannulated the v. cephalica (16 G Intraflon) under local anaesthesia for blood sampling. We collected blood (3 mL) in EDTA tubes for analysis of concentrations of detomidine in plasma at 5, 10, 20, 30, 60, 120, 180, 240 minutes after IV and at 30, 60, 90, 120, 150, 180, 210, 240 minutes after GEL. After each sampling we flushed the cannula with 5 mL of isotonic saline with heparin (10 IU/mL) to keep it open. Plasma was separated by centrifugation (3000 g for 15 minutes), frozen and stored at -20 °C until quantitative analysis of detomidine concentrations was performed.

Quantitative analysis of detomidine concentrations in plasma was performed with reversed phase liquid chromatography (Shimadzu Prominence HPLC instrument; Shimadzu Corporation, Japan) combined with mass spectrometric detection (AB Sciex API4000 triple quadrupole mass spectrometer, Framingham, MA, USA) using an internal standard method.

We measured calf heart rate by auscultation at 14 time points 240 minutes after drug administration and the rectal temperature every 30 minute with a digital thermometer. The handling pen had a soft insulated floor with a mattress and adequate sawdust. We covered the calves with blankets when necessary to prevent hypothermia. The range of the room temperature in the pens was 10.0 to 19.4 °C during the study.

4.8 Statistical analysis

Here I summarize the statistical methods used in studies I-IV. For more detailed information, the reader may refer to the original articles included at the end of this thesis.
The statistical analyses were performed with PASW 18.0.1 (SPSS Inc., 2009, Chicago, Illinois, USA) for studies I–IV. In addition we used R 2.12 (R Development Core Team, 2011) together with wmtsa (Constantine & Percival, 2010) and e1071 (Dimitriadou et al., 2010) packages for modelling and statistical analysis of the accelerometer data in study II. In study III, pharmacokinetic variables were calculated from the plasma detomidine concentration-time data with the WinNonlin Professional software package, version 5.3 (Pharsight Corporation, Mountain View, CA, USA) using non-compartmental methods. p<0.05 was considered statistically significant throughout the thesis.

4.8.1 Data from questionnaires (I, IV)

A total of 451 of 1000 questionnaires (Appendix 1) (45%) were returned. Of all 451 respondents, we included 439 responses in the final analysis for study I and 438 responses for study IV. We excluded from the analysis twelve and thirteen responses that systemically lacked answers to section five and four, respectively.

We used factor analysis with principal components analysis (PCA) to establish summary variables in the data to be used in further analyses (I). The 25 different statements concerning disbudding and 14 statements concerning overall cattle pain in promax rotation were used in the factor analysis with PCA. We extracted eigenvalues over 1 and omitted variables with communalities below 0.3 (Zhan & Shen, 1994; Knapp & Brown, 1995; Vaartio et al., 2009). We replaced the missing values with means.

The loadings of the factors, or the correlation coefficients of rows and columns, in the PCA factor analysis gave a total of 11 different components for the 24 different statements. We converted negative loadings into positive ones. We omitted the component if the Cronbach’s α value was under 0.7 (Knapp & Brown, 1995). We analysed the correlations between the factor loadings with Spearman rank tests and only correlations with coefficients over 0.25 are reported here (I).

We further divided the 11-point Likert-scale for evaluating calf pain during disbudding without any pain medication into three classes to describe respondents’ overall perceptions about pain and to help make comparisons with other similar studies (Hoe & Ruegg, 2006; Gottardo et al., 2011): mild pain 0–3, moderate pain 4–7, and severe pain 8–10 (IV).

To describe respondents’ overall perception about disbudding-related pain, and the perceived need for pain alleviation, two sum variables were created. The sum variables were generated in a way that the maximum score of 20 represented a very high perception of pain and a very great need for pain alleviation. Minimum scores of 2 and 4 represented a very low perception of pain and need for pain alleviation. First, to measure respondents’ perception of disbudding-related pain, the respondents’ opinions about the severity of the disbudding pain without any medication (0–10) and the statements “Disbudding without medication causes the calf pain” (1–5), and “The calf may feel pain for as long as 3 d after the disbudding procedure” (1–5) were summed. Second, to describe respondents’ perception of how important it is to treat the pain (need for pain alleviation), a sum variable including the statements “I could never disbud calves without any pain alleviation” (1–5), “Medication eliminates pain during disbudding” (1–5), “Painless disbudding increases calf welfare” (1–5) and “The calf requires no pain medication for disbudding”, revised as “The calf requires pain medication for disbudding” (1–5), were created. Random missing values were replaced with a group-mean before sum variable formation.
We tested the effects on pain sum estimates of having a veterinarian medicate calves prior to disbudding (always, sometimes or never) with a Chi-square test. Results are presented as proportions of respondents and medians (IQR) (IV).

4.8.2 Collecting behavioural data and developing the model to predict sleep and lying time of calves (II)

In order to develop a model to predict sleep and lying time of calves based on the generated data, we extracted several acceleration features from the raw measurements. Mean, variance and wavelet variance (5 levels) of the horizontal axis of the accelerometer were extracted from the data in 20 s epochs. After extracting the features we used a support vector machine classifier (SVM) with a radial basis kernel to predict different sleep stages and lying behaviour based on our behavioural observations of calf behaviour. We used leave-one-out cross-validation (Hastie et al., 2011) to teach and validate the model. The data from 9 calves were used to teach the model and the data from the remaining calf to validate it. This teaching and validation procedure was then repeated 10 times so that each calf was used to validate the model instructed with the other calves. Model development is described in detail in Pastell et al. (2009). We calculated the average prediction error and its standard error across all 10 folds of cross-validation. After developing the model we scored from the behavioural variables the calf as being in NREM or REM sleep if it had been in the respective posture for at least for 30 s (Hänninen et al., 2008a). We calculated total sleep as the sum of the REM and NREM. We calculated the daily time calves spent lying or asleep as in NREM and REM first from the observed (behavioural data recorded from visual observations) and predicted (from accelerometer data based on the model) behaviours, and compared these using a paired Mann-Whitney U test. This test was chosen because the data were not normally distributed.

4.8.3 Plasma concentrations, CSS and HR (III)

After pharmacokinetic variables were calculated from the plasma detomidine concentration-time data we compared the pharmacokinetic parameters between administration routes using Student’s t-test. We calculated the area under the time sedation score curve (AUC$_{sed}$) using the trapezoidal method. We detected the AUCs and the maximum sedation scores for each animal and compared them between administration routes using Student’s t-test. The differences between administration routes in CSS and HR we compared with linear mixed models, taking repeated samplings into account. Fixed factors were the sampling time, administration route and interaction between them and calf was used as a random factor.
5 Results

The most important results appear in this section, which summarizes the findings from original articles I-IV. For more detailed results, the reader may refer to the original articles included at the end of the thesis.

5.1 Dairy producer perceptions on disbudding-related pain (I, IV)

We established a positive significant correlation for the factor that described how seriously Finnish dairy producers perceived disbudding-related pain and the factor that described how sensitive producers were to pain caused by cattle diseases ($r_s=0.31$, $p=0.001$). Results for the PCA for generated factors and dairy producers' median attitude (IQR) to the disbudding-related statements are shown in Table 5 (I).

Finnish dairy producers estimated disbudding-related pain to be quite severe. Of the $(n=438)$ respondents, 5% estimated that disbudding without pain medication caused only mild pain, 25% moderate pain, and 70% severe pain. Of all respondents 72% $(n=316)$ used disbudding on their farms. From respondents who used disbudding, 69% estimated disbudding related pain as severe, 63% agreed with the statement “The calf requires pain medication for disbudding” and 45% stated that they always called a veterinarian to medicate calves before disbudding. The median (IQR) of all 438 responses for “perception about disbudding-related pain” sum variable was 16.0 (5.0). Respondents' agreements to disbudding-related statements are shown in Table 6 (IV).

Finnish dairy producers were quite willing to medicate calves themselves before disbudding if it were possible according to Finnish legislation. The medians (IQR) of scales 1 to 5 for statements inquiring about producers' willingness to medicate calves without veterinary intervention were 5 (1) to administration of pain medication, 5 (2) for giving local anaesthetics and 4 (3) for sedative administration (I).
### Results

**Table 5.** Table for principal components analysis for Factors I and II and dairy producers’ median attitude (IQR) to the disbudding-related statements (I).

<table>
<thead>
<tr>
<th>Item</th>
<th>Median attitude (IQR)</th>
<th>Factor I: “Taking disbudding pain seriously”</th>
<th>Factor II: “Sensitivity to pain caused by cattle diseases”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disbudding without medication causes the calf pain</td>
<td>5 (1)</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>The calf requires no pain medication for disbudding</td>
<td>1 (2)</td>
<td>-0.69</td>
<td></td>
</tr>
<tr>
<td>The calf may feel pain for as long as 3 d after the disbudding procedure</td>
<td>3 (2)</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Veterinarians take administration of pain medication to the calf seriously</td>
<td>4 (2)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>It is too expensive to have a veterinarian medicate the calf for disbudding</td>
<td>4 (3)</td>
<td>-0.60</td>
<td></td>
</tr>
<tr>
<td>Sedation causes more problems for the calf than disbudding without medication</td>
<td>2 (2)</td>
<td>-0.69</td>
<td></td>
</tr>
<tr>
<td>Painless disbudding increases calf’s welfare</td>
<td>5 (1)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>I could never disbud calves without any pain alleviation</td>
<td>3 (4)</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Statements about cattle pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disbudding without pain medication (pain during the burning)</td>
<td>9 (3)</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Navel infection in a calf (navel is thick and moist, animal is feverish)</td>
<td>8 (3)</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Acute mastitis</td>
<td>8 (3)</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Uterine prolapse in cattle</td>
<td>8 (3)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Umbilical hernia the size of a large apple in a calf</td>
<td>6 (3)</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Abomasal displacement in cattle</td>
<td>8 (3)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Severe tympania in cattle</td>
<td>9 (3)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Teat tramping in cows (teat broken at the root)</td>
<td>8 (3)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Eigenvalues of the factors</td>
<td></td>
<td>5.73</td>
<td>3.31</td>
</tr>
<tr>
<td>Variance explained % (total 36.9 %)</td>
<td></td>
<td>14.68</td>
<td>8.48</td>
</tr>
<tr>
<td>Cronbach’s α</td>
<td></td>
<td>0.81</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Table 6. Opinions of Finnish dairy producers on the statements concerning disbudding and disbudding-related pain management (IV).

<table>
<thead>
<tr>
<th>Statement about disbudding</th>
<th>n</th>
<th>Agree (%)</th>
<th>Somewhat agree (%)</th>
<th>Cannot say (%)</th>
<th>Somewhat disagree (%)</th>
<th>Disagree (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disbudding without medication causes the calf pain</td>
<td>435</td>
<td>57</td>
<td>18</td>
<td>15</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>The calf requires no pain medication for disbudding</td>
<td>427</td>
<td>5</td>
<td>9</td>
<td>17</td>
<td>19</td>
<td>50</td>
</tr>
<tr>
<td>The calf may feel pain for as long as 3 d after the disbudding procedure</td>
<td>418</td>
<td>18</td>
<td>13</td>
<td>37</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>It is too expensive to have a veterinarian medicate the calf for disbudding</td>
<td>435</td>
<td>42</td>
<td>16</td>
<td>10</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Painless disbudding increases calf welfare</td>
<td>435</td>
<td>65</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>I could never disbud calves without any pain alleviation</td>
<td>429</td>
<td>34</td>
<td>10</td>
<td>18</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Medication eliminates pain during disbudding</td>
<td>425</td>
<td>30</td>
<td>33</td>
<td>26</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

5.2 Automatic measurement of calves lying and sleeping behaviour (II)

We obtained successful recordings of acceleration from all ten calves in the study. The 3D-accelerometer recorded acceleration properly, even when calves were free to move around the pen and to interact with their companions. The calves did not destroy or try to manipulate equipment during the study. Different types of movements can be seen from the raw acceleration data. The head movement during walking can be seen clearly from the curve, the movement during lying awake is more settled, and during REM sleep the head is completely still (Figure 4).
Results

Figure 4. An example of raw acceleration data of the horizontal axis during 40 s epoch of walking, lying awake and REM sleep measured from one calf with a three-dimensional accelerometer placed on collar (II).

Our device, together with the developed model, was best able to discriminate standing and NREM sleep from other behaviours, but the predictions for lying awake and REM sleep were less specific (Table 7).

From the total sleeping time our model was able to distinguish (mean ± SE) 90 ± 3%, and 85 ± 4% of the sleeping bout durations and 82 ± 2% of the occurrence of sleep (Figure 5). The model distinguished 66 ± 8% and 70 ± 6% of the total time the calves were in NREM and REM.
sleep, respectively. The model overestimated the daily duration calves slept NREM sleep, and respectively, underestimated the daily REM duration (Figure 5). The model also overestimated the length of NREM bouts. 70 ± 6% of the NREM sleep bout lengths and 80 ± 5% of those for REM sleep were predicted. The numbers of NREM and REM bouts predicted were 77 ± 5%, and 79 ± 4%, respectively (Figure 5).

The model correctly predicted 96 ± 1% of total lying time, 79 ± 6% of the lying bouts durations, and 77 ± 7% of the occurrence of lying bouts. The model underestimated the lying time consistently by an average of 28 min per calf (p<0.05) (Figure 5).

Table 7. A contingency table of the visually observed (true) versus behaviours predicted from the accelerometer as percentage of occurrence of each observed behaviour from 10 calves during 24 h. The true values are calculated as the mode of behaviour during the 20 s epoch used for the prediction.

<table>
<thead>
<tr>
<th>Observed behaviour (%)</th>
<th>Predicted behaviour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standing</td>
</tr>
<tr>
<td>Standing</td>
<td>89</td>
</tr>
<tr>
<td>Lying awake</td>
<td>8</td>
</tr>
<tr>
<td>NREM</td>
<td>1</td>
</tr>
<tr>
<td>REM</td>
<td>4</td>
</tr>
</tbody>
</table>
Results

Figure 5. Comparisons between overall mean durations, bout lengths and behavioural frequencies for lying, total sleep (TST), REM and NREM from 10 calves during 24 h predicted from accelerometer data and recorded from visual observation (II).
5.3 Oromucosal detomidine to calves prior to disbudding (III)

The mean (±SD) maximum plasma concentration of detomidine ($C_{\text{max}}$) (Figure 6) in the GEL-group was 2.1(1.2) ng/mL, and the time of maximum concentration ($T_{\text{max}}$) was 66.0(36.9) minutes. The bioavailability of detomidine was approximately 34% in the GEL-group, calculated as 100% × the dose-adjusted $\text{AUC}_{0-240\text{ min}}$ ratio of the GEL and IV-groups. Pharmacokinetic parameters are shown in Table 8.

The maximum sedation scores for each animal and the overall levels of sedation ($\text{AUC}_{\text{sed}}$) did not differ between the two treatments (Table 8). For the first 30 min after administration, CSS was higher with the intravenous administration (IV) than with the oromucosal administration (GEL). Thereafter, CSS was higher in the GEL than in the IV-group up to 210 min post administration (Figure 7). Heart rate (HR) decreased more in the IV than in the GEL-group (Figure 8).

Table 8. Pharmacokinetic parameters for detomidine and sedation scores after sublingual (80 µg/kg) and intravenous (30 µg/kg) administration (III).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Detomidine 80 µg/kg sublingually (n=10)</th>
<th>Detomidine 30 µg/kg intravenously (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>2.1 ± 1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>66.0 ± 36.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{tot}}$</td>
<td>226 ± 110</td>
<td>252 ± 127</td>
<td>0.62</td>
</tr>
<tr>
<td>Maximum sedation score</td>
<td>9.9 ± 2.1</td>
<td>10.7 ± 2.1</td>
<td>0.40</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{sed}}$</td>
<td>1026 ± 477</td>
<td>784 ± 209</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Figure 6. Mean (±SD) plasma detomidine concentrations after sublingual (80 µg/kg, solid line) and intravenous (30 µg/kg, broken line) administration of detomidine to calves. n = 10 per group (III).
Results

Figure 7. Mean (±SD) composite sedation scores (0-16) after sublingual (80 µg/kg, solid line) and intravenous (30 µg/kg, broken line) administration of detomidine to calves. n = 10 per group. *Statistically significant difference (p<0.05) between the groups. (II)

Figure 8. Mean (±SD) heart rate after sublingual (80 µg/kg, solid line) and intravenous (30 µg/kg, broken line) administration of detomidine to calves. n = 10 per group. Statistically significant difference (p<0.05) between the groups is marked with an asterisk (III).

Time from sedation to local anaesthetic administration was 11 minutes (range 5 to 20 minutes) in the IV-group and 38 minutes (range 25 to 55 minutes) in the GEL-group. None of the calves substantially resisted infiltration of the local anaesthetic nor reacted to hot cauterization of horn buds. All recoveries were eventless, and no adverse reactions were noted in any of the animals. All calves were able to stand up and walk at the end of the observation period.
5.4 Dairy producer perceptions and the use of pain alleviation (IV)

The median (IQR) of all 438 responses for “perceived need for pain alleviation” sum variable was 15.0 (5.0). Respondents who always had a veterinarian medicate their calves before disbudding estimated pain and the need for pain alleviation to be higher than producers who used a veterinarian sometimes or never (p<0.01 for all, Table 9) (IV).

Table 9. Differences in the frequency of dairy producers (n = 294) having a veterinarian medicate their calves before disbudding on the producers’ median (Interquartile range, IQR) perceptions of pain estimation and need for pain alleviation. Different letters in a row indicate statistically significant differences (p<0.01 for all) (IV).

<table>
<thead>
<tr>
<th>Frequency of having a veterinarian medicate calves before disbudding</th>
<th>Pain sum estimates (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always (n = 141)</td>
<td>Sometimes (n = 42)</td>
</tr>
<tr>
<td>Median (IQR) pain estimation¹ (2-20)</td>
<td>18.0 (2.0) a</td>
</tr>
<tr>
<td>Median (IQR) need for pain alleviation² (4-20)</td>
<td>18.0 (3.0) a</td>
</tr>
</tbody>
</table>

¹ Pain estimation sum variable includes the following statements (min-max): respondents’ opinions about the severity of the disbudding pain without any medication (0–10) and the statements "Disbudding without medication causes the calf pain" (1–5), and "The calf may feel pain for as long as 3 d after the disbudding procedure" (1–5).

² Need for pain alleviation sum variable includes the following statements (min-max): "I could never disbudd calves without any pain alleviation" (1–5), "Medication eliminates pain during disbudding" (1–5), "Painless disbudding increases the calf welfare" (1–5) and "The calf requires pain medication for disbudding" (1–5).
Discussion

6 Discussion

This thesis focuses on gaining an improved understanding of producer perceptions about pain caused to young calves by hot-iron disbudding, and on options to increase the use of pain alleviation in connection with this very common and painful procedure. As suggested in the reviews by Anil et al. (2005) and Weary et al. (2006), there are multiple steps for better pain alleviation in animals. This issue can be thought to apply also to pain caused by hot-iron disbudding of calves. We need to be able to recognize and understand the disbudding-related pain as well as to measure the duration of pain after the procedure. To be able to alleviate the pain, we need to have effective, safe and practical methods and analgesic agents for pain alleviation in different situations. And finally, and maybe most importantly, veterinarians and herd owners, those people who take care of the animals, need to have the interest and motivation to use pain alleviation for calves. Unfortunately, pain management in calves is far from unproblematic or common, and there are numerous problems in every step towards better pain alleviation in calves.

6.1 Finnish dairy producers’ estimation of hot-iron disbudding-related pain

Finnish dairy producers are generally well aware that hot-iron disbudding without any medication is very painful for calves. This finding is important because the first obstacle to better pain alleviation is recognizing the pain. Overall Finnish dairy producers estimated hot-iron disbudding-related pain to be severe. Almost 70% of producers who used disbudding estimated pain to be severe (IV) and the median estimation of pain among all respondents were 9 from 10 (I). One third of the respondents stated that disbudding pain could last for at least 3 days in calves (IV). Producers’ estimation of pain is a very important field of study as producers often make decisions whether pain in cattle is to be alleviated or not, and thus they need to be able to recognize the pain. The study method has been developed and used in medicine to study the role of nurses as the main caregivers in recognizing patient pain. The idea is simple; ask those who are responsible for pain recognition and pain assessment (especially when a patient cannot express pain with words), what they think about pain (Salanterä et al., 1999; Brown & Timmins, 2005; Akuma & Jordan, 2012; Kiekkas et al., 2014). Because pain recognition in animals in production environments often relies on deviation from normal behaviour, producers are often the best people to evaluate the pain in animals in their care. While our results show that producers estimated disbudding-related pain as being severe, we cannot conclude how different people evaluate and recognize pain in calves because we did not ask about producers’ knowledge concerning calf pain behaviours. This needs to be taken into consideration when planning future studies.

Previous knowledge about producers’ estimations of cattle pain and attitudes towards pain are quite scarce, and it is difficult to compare different studies with different questionnaires. Still producers’ pain estimation seems to be higher in our study compared with the few studies conducted in other countries. In the United States, 50% of dairy producers believed that disbudding caused “moderate” or “a lot” of pain (Hoe & Ruegg, 2006) and in Italy, over 40% of the farmers regarded post disbudding pain as moderate, lasting up to 6 h (Gottardo et al., 2011). Producers’ estimation of pain duration after disbudding needs further study. As we only asked
their opinions about the statement if pain lasts for at least 3 days, we do not know for how long producers actually think pain lasts after disbudding.

Dairy producers who took disbudding pain seriously were also more sensitive to pain in cattle in general (I). This finding is supported by the idea that producers with positive attitudes to animal welfare scored higher for empathy and for perception of animal pain questions than those producers with negative attitudes (Kielland et al., 2010). We used NRS to measure dairy producers’ estimations of hot-iron disbudding-related pain and cattle pain in general (I, IV). NRS have been used previously to estimate pain (Thomsen et al., 2012; Ison & Rutherford, 2014) and in our study the scale worked well. Response rate was good when compared with similar studies conducted previously among producers and veterinarians (Hewson et al., 2007; Thomsen et al., 2012; Ison & Rutherford, 2014) and respondents answered our questions about pain with good frequency. The mean pain scores on NRS among producers in our study (I) and Finnish veterinarians (Norring et al., 2014) were similar concerning that pain caused by hot-iron disbudding without pain medication; the mean pain score was 9 for both groups. Ison and Rutherford (2014) found also that, overall, pain scores did not differ between pig producers and veterinarians, although there were some differences for pain scores in individual diseases. In Denmark dairy producers and veterinarians tended to agree which diseases were painful to cows, although producers generally considered diseases slightly more painful than veterinarians (Thomsen et al., 2012). It can be concluded that the use of NRS represents a good tool for measuring and comparing estimations of pain for different groups of people working with cattle.

6.2 Automatic measurement of calf lying and sleeping behaviour

We succeeded well in measuring lying and sleeping time in calves (II). Our device, together with the developed model, represents a promising tool for automated and continuous measurements of calf resting behaviour as it was able to predict over 95% of the total lying time. The model underestimated only 3% of the total lying time per calf during each 24 hour observation period. Also the durations and occurrences of lying bouts were recorded well, although not so accurately than the total lying time. Furthermore, our device was also able to measure well total sleeping time in calves, although different sleep stages need more validation. Our device and the developed model worked well in the group pen at the production environment. One extra benefit is that accelerometers with a wireless data acquisition system have no memory limitations. Our registering method is also non-invasive and does not disturb the animals as the small device is attached to the calves’ collars. Compared with recording behaviours of calves from videos, automatic measurement systems are time saving and can be used for observation over several days, as reviewed by Theurer et al., 2013. Long surveillance of calves’ resting behaviour after disbudding with accelerometers can improve our understanding on disbudding-related pain as studied by Theurer et al. (2012).

Before this study, activity-based monitoring with accelerometers had not been used for measuring sleep and various sleep stages in calves. There is evidence in the literature that pain alters sleep in humans (Raymond et al., 2004) and in laboratory animals (Andersen & Tufik, 2003; Andersen et al., 2006; Leys et al., 2013) and thus sleep might be worth measuring after disbudding. Our model was able to distinguish, on average, 90% of the total sleeping time of calves. Also the durations of sleeping bouts and the occurrence of sleep were predicted quite well, although more validation is needed. Our model’s good prediction of the total sleeping time
in calves is in line with studies conducted with healthy humans (as reviewed by Sadeh, 2011). Our device was not so successful in distinguishing the total time the calves slept NREM and REM sleep during the observation period, and had problems in distinguishing the occurrence of different sleep phases and estimating the length of NREM and REM bouts. The same problems were also reported in sleep studies with human infants (as reviewed by Sadeh, 2011). It is possible that the accelerometer was not sensitive enough to all the subtle movements during wakefulness that the calves performed. The fact that young calves rest and sleep polyphasically throughout the day could also decrease the accuracy of actigraphy measurements for lying and sleeping bouts and occurrence of sleep in calves because the 20 s epoch used was unable to detect exact transition times between vigilance states. This is similar to findings for humans in which increased wakefulness during the night generally decreased the accuracy of actigraphy measurements (De Souza et al., 2003; Paquet et al., 2007). More research is needed on distinguishing the total time calves spent in different stages of sleep.

6.3 Oromucosal detomidine to sedate calves before disbudding

We established that oromucosal detomidine can be used successfully on calves to sedate them before administration of local anaesthetics and disbudding (III). The detomidine-induced sedation was the same with both intravenous and sublingual administration routes. However, maximum sedation was reached earlier in the IV-group than in GEL-group. Our findings suggest that 80 μg/kg of detomidine administered sublingually had equally potent sedative effects as 30 μg/kg of intravenously administered detomidine.

We did not use a control group in this study because we wanted to compare the effects of the two administration routes for the application of local anaesthetics. However, for this reason, further studies are needed about the overall effects of oromucosal detomidine anaesthesia in calves.

According to the literature, xylazine is the most commonly used sedative before hot-iron disbudding (as reviewed by Stock et al., 2013) and, to our knowledge, there are no previous studies on disbudding and using detomidine as a sedative, but detomidine has been studied in calves (Peshin et al., 1991). Detomidine can be administered intravenously (Salonen et al., 1989; Mama et al., 2009), intramuscularly (Salonen et al., 1989; Mama et al., 2009) or sublingually (Kaukinen et al., 2011) in horses. Studies on horses support that detomidine effects are both dose and route dependent (Salonen et al., 1989; Mama et al., 2009). Kaukinen et al. 2011 found that sedation started sooner after im administration than after the oromucosal gel administration in horses. Studies conducted in horses using the same dose of detomidine show that the plasma concentrations following intravenous administration reached their maxima earlier and returned to baseline levels more rapidly compared with intramuscular administration (Salonen et al., 1989; Mama et al., 2009).

As there was a risk that a fraction of the gel dose could be swallowed, we carefully administered the gel under the tongue. The volume of the gel was small (<1 mL per calf) and it seemed to remain easily in the oral cavity. Detomidine might undergo extensive first-pass metabolism if swallowed, but the amount of this metabolism is not known in calves. Thus further studies are still needed about the pharmacokinetics of detomidine in calves with this administration route.

In a previous study (Salonen et al., 1989), the pharmacokinetic profile of parenterally administered detomidine in milking cows was close to that of adult horses. In our study, the
approximated bioavailability of sublingually administered detomidine in calves (34%) seemed to be relatively similar to the previously reported bioavailability in horses (22%) (Kaukinen et al., 2011). Our study was not designed to be a pharmacokinetic study, as our primary aim was to evaluate clinical sedation. We collected plasma samples up to 4 hours after drug administration, which probably affected the approximated bioavailability. The elimination phase (β-phase) was not attained within the follow up time after sublingual administration, and thus we could not extrapolate the plasma concentration versus time curve to infinity. Further studies are needed to follow detomidine-sedated calves for longer than 4 hours.

The decrease in heart rate in calves was less intense with sublingual than with intravenous administration, similar to earlier reports for horses (Malone & Clarke, 1993; Kaukinen et al., 2011). We suggest that the haemodynamic effects of detomidine might be less severe with the sublingual than with the intravenous route. However, further studies are needed on the safety of sublingually administered detomidine gel in young calves.

In our study we measured calf body temperature and used blankets during sedation to prevent hypothermia. Also the floor in the study pen was well insulated. Thus, hypothermia was not an issue in our study. Hypothermia is one adverse effect of the use of sedatives because they turn down an animal’s own thermoregulatory system. Hypothermia can be especially problematic in young animals that may have difficulties in maintaining their body temperature (Piccione et al., 2003; Borderas et al., 2009). Vasseur et al. (2014) found that the body temperature of 5-d-old calves decreased immediately after injection of xylazine. In practice, it is important to monitor the body temperature and prevent hypothermia (or hyperthermia in very hot environments). Hypothermia in sedated animals can affect the drug metabolism and prolong recovery. This has to be considered when using sedatives in young calves, especially in cold production environments.

Although sublingual detomidine administration results in a less intense decrease in calf heart rate, it also resulted in more prolonged sedation compared with intravenous administration. It might thus be of further interest to study a third option of administration, namely intramuscular. Prolonged sedation can be a problem because the longer the duration of sedation, the longer time calves are susceptible to hypothermia. The use of an antidote could represent a solution to this problem, but although such agents exist and studies show that they can be used for calves (Raekallio et al., 1991; Rioja et al., 2008), to our knowledge there are no studies on using them in connection with disbudding to young calves. Stafford et al., (2003) studied tolazoline in connection to amputation dehorning in 3-month-old calves and found that xylazine and tolazoline should not be used without local anaesthetics in connection to dehorning. The pre-emptive use of local anaesthetic and NSAID is needed with sedatives before hot-iron disbudding or dehorning and it is even more crucial when an antidote is administered, because also the mild analgesic effects of sedatives are reversed. If the sedation could be reversed after hot-iron disbudding, calf exposure to hypothermia would be reduced and it would also provide benefits to the producer because the extra observation time required for sedated animals would be much shorter. The use of sedatives and antidotes, and especially differences in doses, efficacies and safety between different administration routes, needs more research in calves.

We used behavioural observations to study differences in sedation between the two administration routes as the accelerometer developed in study II was not validated to measure the depth of anaesthesia, but sleeping behaviour of non-sedated calves. Further work is needed to study if an accelerometer could be used in such pharmacological studies.
6.4 Dairy producer perceptions on disbudding-related pain and need for pain alleviation affect the actual use of pain alleviation

We show here that dairy producers who rank disbudding-related pain and the need for pain alleviation high also value and use pain alleviation more before disbudding than producers who rank pain and the need for pain alleviation lower (IV). One has to keep in mind the limitation of comparing results from different surveys, but it appears that the producers in our study ranked disbudding pain higher and used pain alleviation more often than the producers in previous studies (Fulwider et al., 2008; Gottardo et al., 2011). The positive relationship between producers’ perceptions of disbudding pain and their willingness to use pain alleviation for calves is in line with a study conducted among Canadian veterinarians: those veterinarians who perceived dehorning without analgesia to be painful were more likely to use analgesics (Hewson et al., 2007). Knowledge about pain and positive attitudes towards pain management are important in decision-making regarding use of pain alleviation, as shown also among medical nurses (Clarke et al., 1996; de Rond et al., 2000; Abdalrahim et al., 2011) and knowledge about pain can motivate to use pain alleviation. Also information about what prohibits the use of pain alleviation is important. Veterinarians have been reported to mention many obstacles to the use of analgesics in cattle (Hewson et al., 2007; Thomsen et al., 2010; Fajt et al., 2011).

Although attitudes and knowledge about pain may have a strong influence on producer decision-making, whether or not pain is alleviated before disbudding, there might be also other factors involved. Not all producers who estimated pain as being high and pain alleviation as being important used a veterinarian to medicate calves in our study. In Finland, as in other European countries, the use of veterinary drugs is highly restricted and legally controlled (Finlex, Act on the medical treatment of animals, 387/2014). Thus the producer’s motivation to use pain alleviation is particularly important: as the use of sedatives, local anaesthetics and analgesic drugs requires veterinary intervention, it represents extra costs for the producer. It is also possible that some farms in remote areas face difficulties to access a non-emergency veterinary service. Many producers stated that it is too expensive to call a veterinarian to medicate calves and thus the costs might limit the use of pain medication, especially for farms with economic problems. Because of this, more information about farm economics and decision making on using pain alleviation is needed. Reasons for the producer not providing adequate pain medication despite recognizing the disbudding pain remained unclear, and this issue needs further investigation. It is also possible that the person answering our questionnaire was not the same person who decided if disbudded calves were medicated on the farm or not. Unfortunately, our survey did thus not provide reasons for why producers choose not to call veterinary services before disbudding.

We also found that producers were quite willing to administer pain alleviation (NSAID, local anaesthesia and sedation) themselves if it were legal (I). This topic should be discussed more when planning and updating the legislation. Finding safe, effective, convenient and practical non-injectable ways of administering sedatives (such as oromucosal), local anaesthetics (such as topical anaesthetics both for pre- and postoperative use) and NSAIDs (such as buccal meloxicam) and training producers to use them, might ensure the use of pain alleviation during disbudding also when veterinary service is not a practical or economic option.
6.5 Practical relevance and future studies

1) Recognizing and understanding the disbudding-related pain:

The NRS scale worked well for estimating pain, but it also has its limitations (Wood et al., 2010) and we only asked respondents to assess pain. It is possible to validate and use a more detailed questionnaire with the NRS scale connected to questions on calf-pain-related behaviour and respondent knowledge about pain and pain alleviation. Developing such questionnaires about pain in cattle for producers might result in getting more information about producer decision-making regarding pain alleviation among cattle. Also such a validated questionnaire could possibly be used to gauge the effect of education provided to producers on their attitudes and practices regarding pain alleviation. Validated questionnaires to evaluate the knowledge and attitudes of health care personnel on pain have been used previously (Zanolin et al., 2007; Nimbalkar et al., 2014).

2) Being able to measure the duration of pain after disbudding:

Currently, we do not know for how long calves feel pain after hot-iron disbudding. Measuring calf lying time and sleeping behaviour is possible with our device in production environments. Studying the changes in calf resting behaviours may provide us new information about the duration of pain and effectiveness of different NSAIDs on pain alleviation after disbudding. Because according to the literature, meloxicam is the only NSAID for which efficacy on treating postoperative pain after disbudding over 24 hours is being studied, more research on pain alleviation is needed.

3) Having methods and analgesic agents for practical, efficient and safe alleviation of disbudding-related pain:

Sublingual administration of detomidine could be used to sedate calves, especially in situations where parenteral administration is not possible, for example, in older extensively managed beef calves for dehorning or beef calves with their mothers close for disbudding. Sedation of these animals is needed to administer local anaesthesia because administration of local anaesthetics to non-sedated animals requires very strong physical restraint and is very stressful for the animals and dangerous for the operator. With sublingual administration calves could be sedated before any needles are needed with minimal animal handling and stress. Also, if it were possible to reverse sedation with an antidote after disbudding, sublingual detomidine might be a safer and more practical method to sedate calves. This needs to be studied further.

4) Having the interest and motivation to alleviate the disbudding-related pain:

We found evidence that if a producer perceives pain and the need for pain alleviation to be high he or she is more willing to use pain alleviation before disbudding than a person who ranks pain and the need for pain alleviation lower. Increased knowledge about pain, calf-pain-related behaviours and the benefits of pain alleviation among producers could change the application of pain alleviation in the future. Education about disbudding (as a very common procedure in cattle management) related pain could also lead to increased use of pain alleviation in cattle in general
because we found evidence that producers who perceive disbudding-related pain as being severe, were also more aware of pain in cattle in general. This possibility needs to be studied further.

Disbudding is not only painful procedure for calves that is often done without pain alleviation. Pain management is still rarely used also in connection with other procedures known to be very painful for calves, such as castration (Stafford et al., 2000, 2002; Huxley & Whay, 2006), dehorning (Hewson et al., 2007) and tail docking (Fulwider et al., 2008). Also, little is known about the benefits and options to treat pain in calves in connection with various diseases. For example, NSAID treatment is an effective supportive therapy for calves with neonatal diarrhoea (Barnett et al., 2003; Todd et al., 2010). So, studying calf pain, options to measure pain and effectiveness of different analgesic agents in pain alleviation as also options to increase pain alleviation is very important. Disbudding-related pain can serve as an example of calf pain. The idea of improving pain management for calves by researching disbudding-related pain is represented in Figure 9.

![Figure 9. A diagram representing the issues associated with studies of disbudding-related pain and improved pain management for calves in the future.](image-url)
7 Conclusions

1. The majority of Finnish dairy producers perceived disbudding-related pain as being severe and those producers who considered disbudding-related pain to be serious were also more sensitive to pain in cattle in general.
2. We developed a device that provides a good method to measure lying and sleeping time continuously in calves in a production environment without disturbing the animals. Our model predicted the total lying and sleeping time of calves accurately, but differentiation of different sleep stages needs further development.
3. Oromucosal detomidine produced sufficient sedation in calves for administration of local anaesthetics before disbudding.
4. Finnish dairy producers who estimated disbudding-related pain and the need for pain alleviation to be high actually used pain alleviation in connection with disbudding more than producers who estimated pain and the need for pain alleviation to be lower.
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