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**ALLEVIATION OF THE DISTRESS CAUSED BY  
RING CASTRATION PLUS TAILING OF LAMBS  
AND DEHORNING OF CALVES.**

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fulfilment of the requirements  
for the degree of  
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## Abstract

There is increasing social and economic pressure to improve the welfare of animals in our care. In the present study plasma cortisol concentrations were measured to assess the pain-induced distress caused by ring castration plus tailing in lambs and dehorning in calves and the extent to which the distress caused by these procedures can be reduced by using different alleviation strategies.

Local anaesthetic injected into the neck of the scrotum just before ring application significantly reduced the cortisol response to ring castration plus tailing in lambs, but local anaesthetic injected into the testes just after ring application only marginally reduced this response. A castration clamp was applied across the full width of the scrotum of lambs for 6 or 10 seconds after ring castration plus tailing to disable the innervation from the scrotal area. The application of the castration clamp for 6 seconds after placement of the ring did not reduce the cortisol response to ring castration plus tailing, whereas application for 10 seconds slightly, but significantly, reduced the peak cortisol concentration. Healing of the castration wound was not complicated by the application of the castration clamp after ring castration plus tailing. Therefore injecting local anaesthetic into the neck of the scrotum just before ring castration plus tailing significantly alleviates the pain-induced distress associated with ring castration plus tailing, but applying the castration clamp in combination with ring castration plus tailing has little benefit.

Local anaesthetic given prior to dehorning virtually abolishes the cortisol response to dehorning for the duration of action of the local anaesthetic, but once the local anaesthetic wears off cortisol concentrations increase resulting in a delayed cortisol response equivalent to the overall cortisol response to dehorning when local anaesthetic is not used. This delayed cortisol response is thought to be stimulated by inflammation-related pain. The non-steroidal anti-inflammatory drugs (NSAIDs) ketoprofen and phenylbutazone and an endogenous cortisol surge stimulated by injecting ACTH were used to assess

whether this delayed cortisol response is associated with inflammation-related pain. Local anaesthetic (5 hour duration of action) plus ketoprofen given prior to scoop dehorning marginally reduced the delayed cortisol response observed once the local anaesthetic wore off, but giving local anaesthetic and phenylbutazone prior to dehorning had no significant effect on this delayed cortisol response. The antinociceptive action and a greater anti-inflammatory potency of ketoprofen compared to phenylbutazone may explain why ketoprofen was more effective than phenylbutazone in reducing this delayed cortisol response. ACTH plus local anaesthetic given prior to dehorning only marginally reduced the delayed cortisol response observed once the local anaesthetic wore off, suggesting that the delayed cortisol response seen when the local anaesthetic wears off is not due primarily to inflammation-related pain

Giving local anaesthetic prior to dehorning and cauterising the amputation wounds prevented the delayed cortisol response after the local anaesthetic wore off and significantly reduced the overall cortisol response to dehorning.

Thus, in the present study long acting local anaesthetic (5 hour duration of action) in combination with NSAIDs had minimal alleviating effects on the pain-induced distress caused by dehorning compared to local anaesthetic alone, but local anaesthetic and cautery provided effective pain-relief.

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## **CHAPTER ONE:           General Introduction**

Over the past 200 years society's attitude on what is acceptable treatment of animals has changed (Monamy, 1996). In the nineteenth century in England changes were made to the legislation, to regulate painful experiments on animals and in 1876 legislation, in the form of the Cruelty to Animals Act, gave consideration to the welfare of laboratory animals (Monamy, 1996). The New Zealand Cruelty to Animal Act (1960) was modelled on British law (Bayvel, 1992). Increasing interest in farm animal welfare stimulated by the media, activities of national and international humane societies, veterinary associations and other groups including welfare scientists, has meant that in recent years people involved in the livestock industry have begun to re-evaluate routine husbandry procedures (Stafford and Mellor, 1993).

The way we manage animals and consider animal welfare within a society is based on a number of factors, including religious beliefs, general standards of education, experience and tradition. The responsibility humans have towards animals and their welfare is a philosophical issue related to ethical considerations within a society (Blackmore, 1992). Furthermore the extent to which humans should be concerned about the welfare of animal is constantly being debated. Peter Singer, an Australian philosopher and author of the well-known book 'Animal Liberation' (1975), argued that there is no difference in the moral status of humans and sentient non-humans and therefore the interests of sentient animals should be given no less consideration than that of humans. However, a more common approach to animal use is that it is reasonable to utilise animals for food, research and other purposes provided that their level of welfare is acceptable.

Ethical views, social pressure, scientific knowledge and legal sanctions help to define what is acceptable animal welfare. The knowledge gained from daily experience with animals and the growing field of animal welfare science will help to solve some of the welfare concerns based on scientific knowledge

gained from the study of animal behaviour, health, immunology, production and physiology (Sandoe and Simonsen, 1992). It is important to be able to assess the welfare of an animal objectively, otherwise the welfare laws that emerge may be arbitrary and even fail to improve the welfare of the animals (Dawkins, 1980).

Assessment of welfare is becoming increasingly important due to rising public concern about the welfare of farm animals and the continuing economic demand for increased efficiency of animal production. One component of the large area of farm animal welfare is the assessment of different husbandry procedures. The ability to assess objectively the pain-induced distress caused by husbandry practices is important so that alternative strategies that are less distressful can be evaluated and recommended for use. This is addressed specifically in the experiments described in this thesis which explores facets of the response to castration and tail docking in lambs, and dehorning in calves.

This chapter deals with the following topics: first, the definition of animal welfare used in the present study; second, the physiology of nociception and the innervation of the scrotum and tail of lambs and the horns of calves; third, the consequences of the types of tissue damage that occur due to different methods of castration, tailing and dehorning; fourth, a brief critique of the different indicators of distress used and why cortisol was used in the present study.

## **1.1 Animal Welfare**

Animal welfare has been defined in the literature in terms of biological fitness of the animal (Barnett and Hemsworth, 1990), as an individual's state as regards its attempt to cope with its environment (Broom, 1986) or in terms of the subjective feelings of animals (Dawkins, 1990). The definition of animal welfare will determine the indices used to measure welfare objectively (Mason and Mendl, 1993). Animal welfare can also be defined in terms of the 'five

freedoms' which focus attention on the major needs of animals (FAWC, 1992). Public disquiet about intensive livestock production stimulated the British government in 1979 to set up the Farm Animal Welfare Council, which formulated the 5 'freedoms' (Gonyou 1994). The codes of recommendations and minimum standards for the welfare of animals, published by MAF in New Zealand, also refer to these five freedoms.

The Five Freedoms (from FAWC, 1992) are:

- 1: Freedom from thirst, hunger, or malnutrition
- 2: Freedom from discomfort and exposure
- 3: Freedom from pain, injury and disease
- 4: Freedom to express normal behaviour
- 5: Freedom from fear and distress

However more recently the five freedoms have been reshaped to highlight five 'domains of potential welfare compromise' (Mellor and Reid, 1994).

The five domains of potential welfare compromise are:

- 1: Thirst/hunger/malnutrition
- 2: Environmental challenge
- 3: Disease/injury/functional impairment
- 4: Behavioural/interactive restrictions
- 5: Anxiety/fear/pain/distress

An animal's welfare varies over a range, from good health to extreme illness. Between these two states lies a continuum of states (Mellor and Reid, 1994). Therefore a subjective judgement will need to be made to distinguish between a distress-free and a mildly distressed state along with what is an acceptable level of distress and what is not (Stafford and Mellor, 1993). Animal welfare science helps us to assess the welfare of animals in an objective manner using different disciplines, including physiology and animal behaviour. However public and economic pressures will also contribute to determining what are and what are not acceptable welfare standards for the animals under our care.

## 1.2 Physiology of noxious input

### *Nociception and Pain perception*

Castration, tail docking and dehorning cause tissue damage, resulting in the subsequent release of inflammatory mediators. Noxious input from injury caused by various stimuli (mechanical, thermal or chemical) stimulate nociceptors. Nociceptors are nerve endings attached to afferent nerve fibres, which can be found in the skin, periosteum, subchondral bone, joint capsule, muscles, tendons, blood vessels, and some viscera (Johnston, 1996). Nociceptors are generally defined as high-threshold mechanoreceptors, low-threshold mechanoreceptors or polymodal receptors. A $\delta$  nerve fibres are associated with high and low-threshold mechanoreceptors and respond to pressure and heat. C fibres are associated with polymodal receptors and respond to mechanical, thermal and chemical stimulation (Johnston, 1996). Furthermore A $\delta$  fibres are characterised as producing fast, sharp sensations, whereas C fibres are characterised as producing slow, burning sensations (Johnston, 1996).

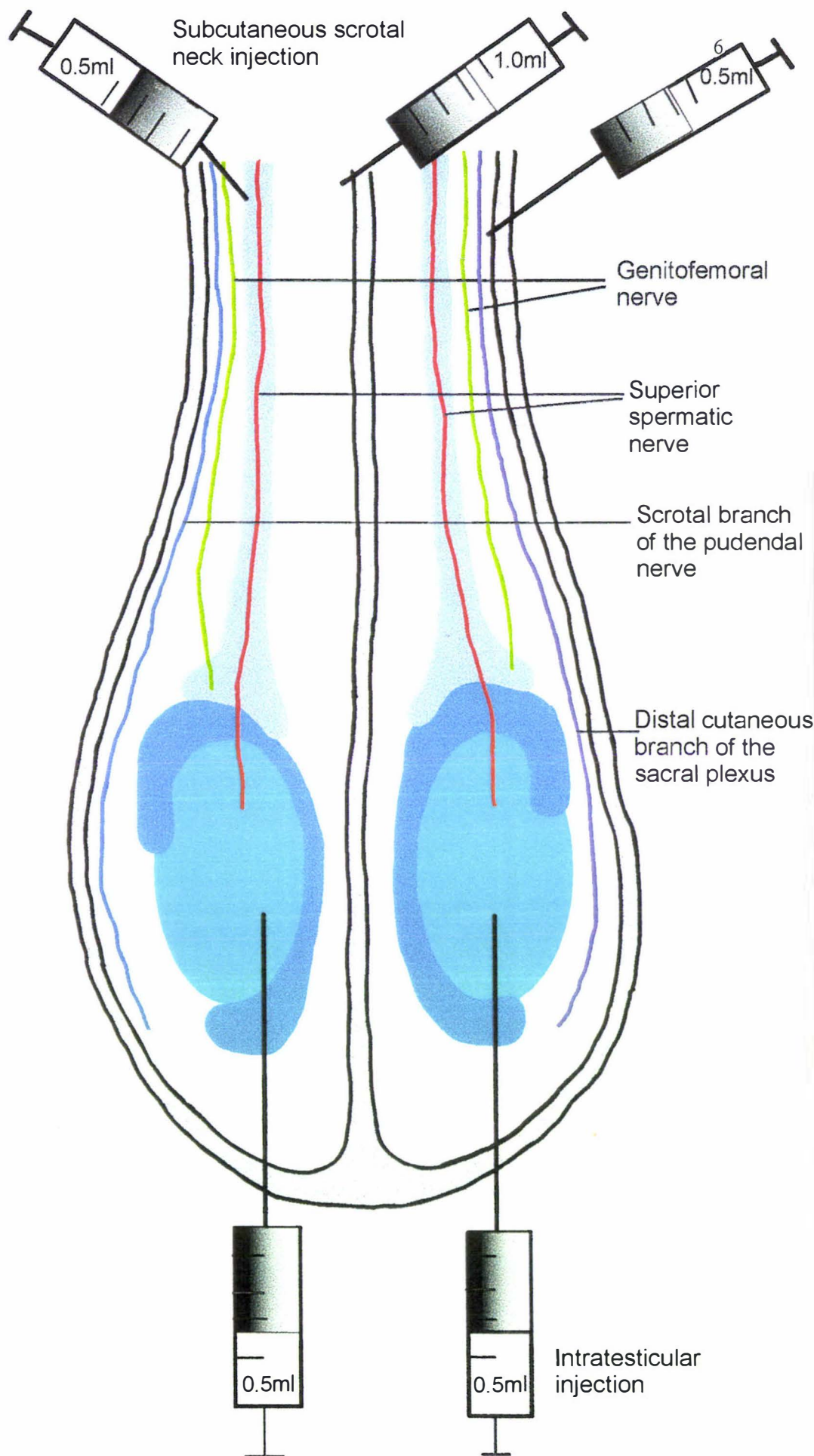
Stimulation of nociceptors occurs when the threshold potential of the receptor is exceeded. The nociceptor threshold is similar for humans and animals (Kitchell, 1987). However, the pain tolerance threshold (the maximum intensity of a stimulus an animal will tolerate voluntarily) varies widely between species and individuals (Mersky, 1979). Once the afferent fibres enter the spinal cord they ascend or descend Lissauer's tract and synapse in various layers of the dorsal spinal horn (Johnston, 1996). Stimulation of nociceptors leads to transmission of neural activity to the spinal cord or the brain stem, thalamus, and the cerebral cortex for sensory processing and perception of the stimulus (Kitchell, 1987). Pain perception varies according to site, duration and intensity of the stimulus and is influenced by previous experience, emotional state, and individual differences (Orlans, 1993).

### ***Innervation of the scrotum and tail of lambs***

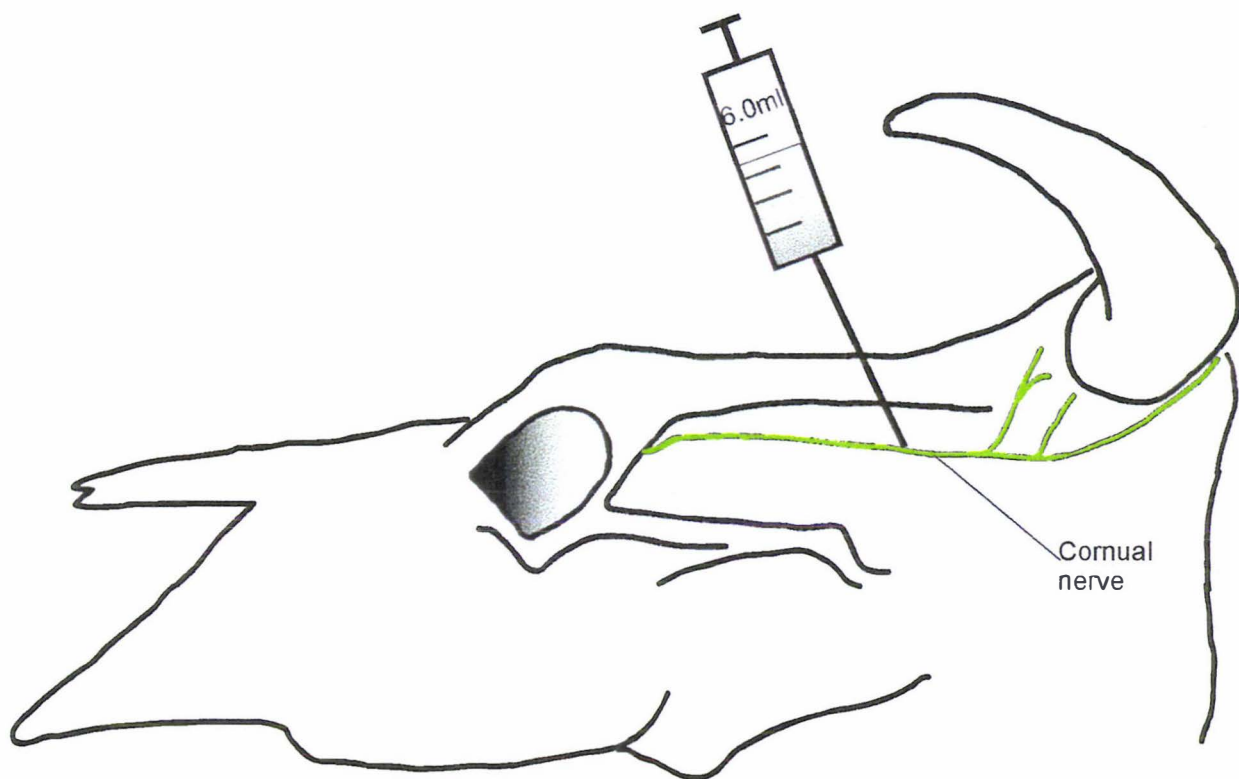
Husbandry procedures such as castration and tail docking of lambs cause stimulation to the nerves of the scrotum and tail resulting in pain-induced distress as measured by plasma cortisol concentrations and behaviour (Mellor and Murray, 1989a,b; Lester *et al.*, 1991a, b; Wood *et al.*, 1991; Kent *et al.*, 1993; Mazzaferro *et al.*, 1993; Molony *et al.*, 1993; Stillwell *et al.*, 1994; St. Louis *et al.*, 1994; Kent *et al.*, 1995; Rhodes III *et al.*, 1995; Lester *et al.*, 1996; Dinniss *et al.*, 1997a, b; Graham *et al.*, 1997; Dinniss *et al.*, 1998; Kent *et al.*, 1998; Molony *et al.*, 1998).

The nerves of the external genitalia of rams are supplied through three routes; the ventral branches of the thoracic and lumbar spinal nerves (cranial and caudal iliohypogastric nerves); the genitofemoral nerve (also known as the inguinal or external spermatic nerve); and the ventral branches of the sacral spinal nerves from which the pudendal nerve originates and then branches into the scrotal nerve and the deep perineal branch (Larson and Kitchell, 1958; Kirk *et al.*, 1987) (Fig. 1.1). The superior spermatic nerves of rams contain afferent fibres from the testes, which are enclosed in the spermatic cords (Cottrell and Molony, 1995). The scrotum is further innervated by the distal cutaneous branch of the sacral plexus and the scrotal nerve (Kirk *et al.*, 1987).

Little is known about the location of nociceptors in the testes, scrotum and related tissues of the ram (Dinniss, 1995). However, polymodal receptors in the testes of dogs, which can function as nociceptors, are located close to the surface of the tunica vaginalis visceralis which surrounds the testes and the epididymis (Kumazawa and Mizumura, 1980a). Nociceptors are also present within the pampiniform plexus in lambs (Cottrell and Molony, 1995). The coccygeal nerves innervate the tail (French and Morgan, 1992).



**Fig. 1.1:** Innervation of the lamb scrotum and the testes and the sites and volumes of local anaesthetic injected.



**Fig. 1.2:** Innervation of the calf horn and the site and volume of local anaesthetic injected.

### ***Innervation of the horns of calves***

Amputation dehorning of calves or disbudding stimulates the nerves supplying the horns or horn buds. The cornual nerve, a branch of the zygomaticotemporal division of the maxillary nerve, innervates the sensitive dermis of the horn. The nerve divides into two or more branches and approaches the horn separately from beneath the thin frontalis muscle (Dyce *et al.*, 1996) (Fig. 1.2).

### **1.2.1 Consequences of tissue damage as a result of different husbandry procedures**

There are three main types of tissue damage caused by husbandry procedures which involve tissue removal: severing through cutting, burning through cautery and hypoxic/anoxic damage or death through ischaemia (Mellor and Stafford, in press).

#### ***Surgery***

Castration and tailing of lambs and dehorning of calves are husbandry practices that can involve the cutting or amputation of tissues. Tissue injury from cutting results in noxious sensory input due to direct mechanical stimulation of the nociceptors. Furthermore tissue damage, as a result of these husbandry practices, stimulates the inflammatory response leading to the release of chemicals and enzymes, resulting in hyperalgesia generated by these neurogenic and algogenic substances (Dahl and Kehlet, 1991).

Tissue injury starts the arachidonic cascade, which involves free arachidonic acid being metabolised mainly by two enzymatic pathways, producing prostaglandins, thromboxanes and leukotrienes. Prostaglandins and thromboxanes are formed by the cyclo-oxygenase pathway, and leukotrienes are produced by the lipoxygenase pathway (Dart, 1992). Prostaglandins are thought to cause nociception indirectly by reducing the threshold and thus sensitising nociceptors to stimulation (Johnston, 1996). Furthermore, complement, cytokines, leukotrienes, and prostaglandins prolong the

inflammatory response by causing vasodilation and increased vascular permeability (Johnston, 1996). After stimulation, peripheral endings of the nociceptive afferent nerves release substance P which can function as an excitatory neurotransmitter in the dorsal spinal horn and is known as a neurogenic pain mediator. Substance P can also influence the inflammatory response by altering vascular permeability and by inducing the release of algogenic substances (Dart, 1992).

Hyperalgesia is caused by peripheral tissue damage and is characterised by a reduction in the nociception threshold and often by spontaneous pain (Dahl and Kehlet, 1991). Primary hyperalgesia is characterised by changes that occur within the site of injury, due to sensitisation of the nociceptors. Secondary hyperalgesia relates to changes in the surrounding tissues, likely due to changes in the peripheral and central nervous system (Dahl and Kehlet, 1991). Together, primary and secondary hyperalgesia contribute to a hypersensitive state found after injury, which is apparent as an increase in the responsiveness to noxious stimuli along with a decrease in the pain threshold at the site of injury and in the surrounding uninjured tissue (Woolf and Chong, 1993). Thus, the pain of tissue injury caused by cutting or amputation has two main phases, an initial nociceptor barrage due to tissue damage, followed by continued nociception due to inflammation and hyperalgesia.

### ***Ischaemia***

Ring castration and tailing of lambs can produce noxious sensory input in the form of ischaemic pain, which can last up to 3.5 hours as measured by behaviour and cortisol concentrations (Mellor and Murray, 1989a,b; Lester *et al.*, 1991a; Wood *et al.*, 1991; Kent *et al.*, 1993; Molony *et al.*, 1993; Kent *et al.*, 1995; Lester *et al.*, 1996; Dinniss *et al.*, 1997a, b). Ischaemia is caused by occlusion of the blood vessels distal to the rings producing an hypoxic state in the tissues, which progresses to an anoxic state. At sufficiently low oxygen levels in the tissues the nociceptors become disabled. If occlusion continues the nociceptors distal to the ring will die as will all other tissues distal to the ring (Cottrell and Molony, 1995; Dinniss *et al.*, 1997a; Mellor and Stafford, in press).

Ischaemia leads to tissue death, and the damaged tissues release serotonin, histamine and bradykinin which further stimulate nociception (Johnston, 1996). The cell membranes can be stimulated by mechanical and chemical stimuli, so another likely cause of pain due to ischaemia is the accumulation of large amounts of lactic acid in the tissues, formed as a consequence of the anaerobic metabolism that occurs during ischaemia (Dart, 1992). Following limb or tissue ischaemia, changes in venous and tissue lactate, oxygen, glucose, and energy substrates occur. Hypoglycaemia and hypoxia have been found to cause the greatest increase in C-fibre action potential activity (MacIver and Tanelian, 1992).

The standard rubber ring used to castrate lambs does not block the conduction of afferent peripheral C-fibres in the superior spermatic nerve immediately after application (Cottrell and Molony, 1995). The activation of peripheral C-fibres is associated with burning, aching pain in tourniquet-induced limb ischaemia in humans (MacIver and Tanelian, 1992). Furthermore, rubber ring castration initiates afferent activity which persists for more than 90 minutes, which corresponds broadly with the maximum cortisol response observed in ring castrated lambs (Cottrell and Molony, 1995).

### ***Cautery***

Cautery is used for tail docking, disbudding or after scoop dehorning in calves to alleviate the pain-induced distress associated with dehorning. Third degree burns result in the complete destruction of the skin and other tissues, and are associated with little or no pain in humans (Bonica, 1990). Sensory nerve receptors including nociceptors are in the dermis, and are destroyed by third degree burns resulting in a loss of sensation in the affected area (Bonica, 1990). Therefore as cautery is likely to cause third degree burns the acute pain associated with tail docking, disbudding or dehorning involving cautery is likely to be reduced. There is some evidence to support this (Lester *et al.*, 1991a; Petrie *et al.*, 1996a; Sylvester *et al.*, 1998a).

## 1.3 Assessment of Distress

The assessment of pain-induced distress experienced by animals undergoing specific husbandry procedures is necessary in order to compare the noxiousness of the different procedures used. The pain-induced distress caused by different methods of castration and tailing in lambs and dehorning in calves has been assessed using cortisol and behaviour to determine the least distressful method for carrying out these procedures. To be as objective as possible, it is important that the assessment of animal welfare is based on scientific knowledge of an animals' behaviour, health and physiology (Sandoe and Simonsen, 1992).

### 1.3.1 Physiological indices of distress

#### ***Hypothalamic-pituitary-adrenal axis and physiological indices of distress***

Corticotropin-releasing factor (CRF) is synthesised and secreted from the paraventricular nuclei (PVN) and supraoptic nuclei (SON) within the hypothalamus, and controls Adrenocorticotrophic hormone (ACTH) secretion (Minton, 1994). ACTH is secreted from the corticotropes in the anterior pituitary gland. It is the principal regulator of cortisol synthesis and secretion (Minton, 1994). ACTH release controls the basal secretion of cortisol (circadian rhythms) along with its response to stressors, and both CRF and ACTH secretion are inhibited by glucocorticoid negative feedback (Jacobson and Sapolsky, 1991). A wide variety of physiological and psychological stimuli have been used to stimulate the HPA-axis, such as different husbandry procedures (Stafford and Mellor, 1993), novelty (Bassett and Cairncross, 1973; Wiepkema and Koolhaas, 1993) and coitus (Szechtman *et al.*, 1974).

The HPA-axis responds to physiological and psychological stimuli, such as novelty, anticipation of electric shock, and non-appearance of food (Bassett and Cairncross, 1973; Wiepkema and Koolhaas, 1993; Levine, 1985; Rushen, 1986). Therefore, plasma cortisol concentrations have been used to measure the psychological and physiological distress response caused by specific

stressors in animals. Cortisol secretion in response to different stressors (handling, restraint, heat stress, transport) in domestic farm animals has been extensively studied (Mellor and Murray, 1989a; Stafford *et al.*, 1996; Minton, 1994). The frequent use of cortisol concentrations as an index of distress in animals is due to a number of factors, including its presence in readily available biological fluids (blood, urine or saliva) and its ease of measurement via radioimmunoassay techniques. However, circulating levels of cortisol reach an effective maximum relatively rapidly after significant noxious stimulation and once this maximum rate of cortisol secretion is reached any larger stimulation only prolongs the period of maximum secretion (Harbuz and Lightman, 1992). This 'ceiling effect' was also postulated when the cortisol responses to a variety of husbandry procedures of varying noxiousness were compared (Molony and Kent, 1997). The peak cortisol concentrations distinguished between the least, intermediate and most severe procedures, but not between the 3 most severe or the 3 least highly ranked (Molony and Kent, 1997). However, a 'ceiling effect' was also shown when using some behavioural indices to compare the same procedures. There are some concerns about the basis of a 'ceiling effect' as only the peak cortisol concentrations were compared not the integrated cortisol response to each procedure. Corticosterone concentrations in rats becomes attenuated to repeated stressors, returning towards control levels after a week (Harbuz and Lightman, 1992). Therefore cortisol may not be a reliable index to assess chronic stress as habituation or adaptation of the HPA-axis may occur. Alternatively, the animal may in fact become less stressed by increasingly familiar stimuli.

Corticosteroid levels have been shown to increase during coitus in some male rats (Szechtman *et al.*, 1974), just before laying in hens (Beuving, 1983), while nursing in mammals (Walker *et al.*, 1992), and situations where psychological stress is likely to be absent (Rushen, 1986; Mason and Mendl, 1993). Furthermore, corticosteroid levels vary due to diurnal rhythms (Hudson *et al.*, 1975). Hence, corticosteroid levels can fluctuate in the absence of unpleasant stimuli (Mason and Mendl, 1993). Therefore, pretreatment values and appropriate control groups need to be included in the experimental design in order to account for these factors. However where procedures, such as

different methods of castration in lambs, are being assessed, common sense leads to the reasonable conclusion that the cortisol response to these procedures is stimulated by pain-induced distress and not pleasure.

The activity of the sympathetic nervous system can be used to measure distress in animals by means of changes in heart rate, skin resistance, peripheral blood flow, and catecholamine secretion (Mason and Mendl, 1993). Bradycardia, slowing of the heart rate, or tachycardia, speeding up of the heart rate, have also been observed in response to stress. A significant increase in the heart rate of sheep has been shown in response to an approaching dog, introduction into a new flock and visual isolation (Baldock and Sibly, 1986). Furthermore, the heart rate of broiler birds was used to compare the distress caused by two different methods of handling, an automatic broiler harvester or being caught by hand; an automatic broiler harvester caused briefer tachycardia than handling by people (Duncan, 1986). Molony and Kent (1997) have found changes in heart rate to be consistent with predicted pain. However, there were many interfering variables such as eating, exercise, and extraneous noises. Therefore, it may be difficult to use changes in heart rate as a practical index for the assessment of pain (Molony and Kent, 1997).

Catecholamines are released more rapidly in response to a stressor, when compared to the slower release of glucocorticoids. Thus catecholamine levels may give a more immediate indication of an animals' response to a stressor, such as dehorning. Catecholamine levels and corticosterone concentrations when compared in rats given foot shock treatment showed a similar response except that corticosterone concentrations remained elevated for longer (Levine, 1985). Few studies have used catecholamines to measure the distress response in domestic animals, as it is difficult to measure catecholamines compared to the relative ease of measuring plasma cortisol concentrations (Minton, 1994). Progesterone, the secretion of which is stimulated by ACTH, could also be used as an indicator of distress. The progesterone response to dehorning has been shown to be similar to the cortisol response to dehorning (Cooper *et al.*, 1995).

Cortisol was used as an indicator of pain-induced distress in the present study as the cortisol patterns to castration plus tailing and dehorning are well-established. This allowed comparison with other work carried out in this field. Moreover, its advantages as an indicator of both psychological and physiological distress and its relative ease of measurement were attractive.

### 1.3.2 Behavioural indices of distress

Changes in behaviour, particularly the occurrence of abnormal behaviours, have been used as indicators of welfare (Barnett and Hemsworth, 1990). Behavioural changes such as posture, locomotor activity (restlessness), and evoked behaviour can be used to measure distress (Mellor and Murray, 1989a; Molony *et al.*, 1993; Lester *et al.*, 1996). Recording behaviour is a physically non-invasive method compared to blood sampling by venepuncture. However, the nature of the aversive stimulus or situation may influence how an animal reacts behaviourally to the stressor. For example, lambs castrated using a knife stand still with legs out-stretched and lambs ring castrated initially show high levels of restlessness (Shutt *et al.*, 1988; Molony *et al.*, 1993; Lester *et al.*, 1996). The scrotal wound of lambs castrated with a knife is likely to be inflamed and hyperalgesic, so that such animals may stand still to avoid or reduce mechanical stimulation to the sensitive tissues (Wood and Molony, 1992). As the behavioural responses to different husbandry amputations can be procedure-specific, it is difficult to draw conclusions about the relative intensities of these different methods based on behavioural comparisons alone (Lester *et al.*, 1996). Behavioural responses are harder to interpret because they can be influenced by the evolutionary response to stress, for example hiding or herding behaviour (Mason and Mendl, 1993). Furthermore, it is difficult to know which behaviours are most appropriate for assessing welfare.

Animals can respond behaviourally and physiologically in attempts to adapt to environmental change (Barnett and Hemsworth, 1990). The main criterion of useful indices of distress is that they must show signs of change in response to a stressor. However, change *per se* is not an indication of distress, as an animal's behaviour and physiology continually change and adjust to maintain

homeostasis (Barnett and Hemsworth, 1990). Common problems with measuring an animal's welfare are: different indices of distress do not always agree and inconsistencies between studies occur, due to the factors which can confound results such as age, sex, species and individual differences, which can make the interpretation of results difficult (Mason and Mendl, 1993; Stafford and Mellor, 1993). So, no matter what index of distress is used, whether behavioural or physiological, it will have its limitations which will need to be taken into account when designing experiments.

## **1.4 Outline of Thesis**

This thesis consists of four chapters: an introduction, two experimental chapters, and a general discussion. Each experimental chapter includes a brief summary, an introduction, and sections on materials and methods, results, and discussion. The introductions to the experimental chapters provide more detail about the specific husbandry procedures being investigated.

The first experimental study (chapter 2) assessed aspects of the alleviation of pain-induced distress caused by ring castration plus tailing in lambs. The second experimental study (chapter 3) assessed aspects of the alleviation of the pain-induced distress caused by amputation dehorning in calves.

## **CHAPTER TWO: Alleviation of the Distress Caused by Ring Castration and Tailing of Lambs**

### **2.1 Chapter Summary**

Two methods designed to alleviate the pain-induced stress caused by ring castration plus tailing of lambs were investigated. First, local anaesthetic was injected into the neck of the scrotum or the testes just prior or immediately after ring castration plus tailing. Secondly, a castration clamp was applied across the full width of the scrotum for 6 or 10 seconds after ring castration plus tailing. Changes in the plasma cortisol concentrations were observed for up to 4 hours after ring castration plus tailing. Furthermore lambs were examined weekly for up to 6 weeks to observe the healing of the castration wounds caused by the ring method or the ring plus clamp method. Local anaesthetic injected into the neck of the scrotum just before ring application significantly reduced the cortisol response to ring castration plus tailing, but local anaesthetic injected into the testes just after ring application only marginally reduced the response. The application of the castration clamp for 6 seconds after placement of the ring to the scrotal neck did not reduce the cortisol response to ring castration plus tailing, whereas application for 10 seconds after ring placement significantly reduced the peak cortisol concentration. Healing of the castration wound was not complicated by the application of the castration clamp after ring castration plus tailing.

### **2.2 Introduction**

Castration is used to prevent indiscriminate breeding and reduce potentially injurious sexually-based behaviours (Wood and Molony, 1992). Handling and hygiene problems can arise during processing at meat works when the scrotum is too long (Dobbie *et al.*, 1985). In some areas lambs can reach an acceptable carcass size of better quality before they reach puberty and become sexually active (Wood and Molony, 1992). However, in other areas, like the United

Kingdom, some sheep husbandry conditions are such that ram lambs cannot reach market size before puberty, thereby making castration a necessary husbandry practice (Cottrell and Molony, 1995). Tail docking is carried out to reduce the accumulation of faeces and urine on the wool of the tail and the hindquarters, which may result in poor hygiene and an increased risk of blowfly strike (Wood and Molony, 1992). The occurrence of blowfly strike has been found to be five times greater in lambs with tails compared to docked lambs (French *et al.*, 1994). Blowfly strike (ovine myiasis) is the cutaneous infestation of sheep by the larvae of blowflies, caused by blowflies laying their eggs on moist areas on the sheep, such as urine stains and faeces (French *et al.*, 1992). The blowfly larvae hatch and feed on the sheep, and affected animals can die within 3 days of becoming struck (Brightling, 1988). Tail docking is used extensively in tropical and temperate climates where it is considered essential, but it is less common in colder countries where blowfly strike is less prevalent (Stafford and Mellor, 1993). There are several different castration and tail docking methods: surgical, ring, castration clamp, ring plus castration clamp, short scrotum creation, immunocastration and chemical castration (Table 2.1). The castration and tail docking method used depends on farmer preference and guidance from advisory bodies [e.g. Animal Welfare Advisory Committee (N.Z.), Farm Animal Welfare Council (U.K.)].

### **2.2.1 Distress**

The definition of distress used here includes all the physical and/or psychological components of an unpleasant experience (McMeekan, 1997). Assessment of the distress experienced by animals is important so that the relative aversiveness of different treatments can be determined and compared. The main methods of assessing distress in animals are physiological and/or behavioural indices. Physiological indices of distress include adrenal secretion (catecholamines and cortisol), heart rate, ventilation rate, and brain chemistry (for more details see general introduction) (Fraser and Broom, 1990). Behavioural observations can also be used to measure distress, for example behavioural patterns that deviate from the norm (Blackshaw and Allan, 1985). Behavioural characteristics indicative of castration and/or tailing distress are

restlessness, normal and abnormal standing/walking, normal and abnormal lying, and immobility with neck extensions together with hyperventilation (Mellor and Murray, 1989a; Wood *et al.*, 1991; Mazzaferro *et al.*, 1993; Molony *et al.*, 1993; St. Louis *et al.*, 1994; Kent *et al.*, 1995; Rhodes III *et al.*, 1995; Lester *et al.*, 1996; Graham *et al.*, 1997; Molony *et al.*, 1997; Dinniss *et al.*, 1998; Kent *et al.*, 1998). The behavioural response to castration and/or tailing is seemingly procedure specific. For example lambs react to surgical castration and/or tailing by abnormal standing (statue standing), whereas lambs castrated and/or tailed with rings initially show a high level of restlessness. The characteristic abnormal behaviours associated with different methods of castration and/or tailing cannot be used to interpret the relative intensity of the distress caused by these procedures, whereas the presence/absence of abnormal behaviours corresponds well with the duration of the cortisol response (Lester *et al.*, 1996).

Distress responses of lambs to different methods of castration and/or tail docking, using cortisol and behaviour as indices, are now well established (Mellor and Murray, 1989a, b; Lester *et al.*, 1991a, b; Wood *et al.*, 1991; Kent *et al.*, 1993; Mazzaferro *et al.*, 1993; Molony *et al.*, 1993; Stillwell *et al.*, 1994; Rhodes III *et al.*, 1995; ; Kent *et al.*, 1995; Lester *et al.*, 1996; Dinniss *et al.*, 1997a, b; Graham *et al.*, 1997; Kent *et al.*, 1998; Molony *et al.*, 1997). Changes in plasma cortisol concentrations were used as a measure of distress in the present study due to the well-established patterns after castration plus tailing, and for ease of comparison with other work done in this area.

The full range of chronic effects associated with castration and/or tailing are unknown. Productivity and infection are variables that can be measured and observed, but other possible consequences such as hyperalgesia and phantom pain are more difficult to assess. Primary hyperalgesia occurs in the injured area; this may result from the direct effect of inflammatory mediators, released from the damaged tissues, on the nociceptors (Johnston, 1996). Secondary hyperalgesia can occur in the uninjured tissues surrounding the injury. Hyperalgesia allows low level stimuli to produce pain by activating sensitised nociceptors (Johnston, 1996). All methods of castration and/or tail docking

cause a nociceptor barrage, which may result in primary and secondary hyperalgesia making animals more sensitive to otherwise benign stimuli.

Neuroma formation is thought to be a result of peripheral nerve transection with removal of the distal portion of a limb (French and Morgan, 1992). Neuromas are electrically active; the amount of activity varies with age, species and the animal's environment. Neuromas are sensitive to a variety of stimuli leading to increased activity and increased pain associated with the neuroma (Bonica, 1990). Tail docking results in the severing of both coccygeal nerves, castration results in the severing of the genitofemoral nerve, pudendal nerve, superior spermatic nerve and the sacral plexus (Fig. 1.1). Neuromas have been demonstrated after husbandry tissue damage in a range of species: tail docking in pigs and dogs (Gross and Carr, 1990; Simonsen *et al.*, 1991), and beak amputation in chickens (Gentle, 1986). French and Morgan, (1992) dissected the tail stump of docked and non-docked lambs, and concluded that the presence of terminal neuromas and irregular innervation in the tail stumps of docked lambs might lead to chronic pain or increased sensitivity long after amputation. Phantom pain usually involves a perceived sensation of pain in a body part that has been amputated. In a survey of American military veterans with amputated limbs it was usual for the pain to be severe enough to cause considerable discomfort (Sherman *et al.*, 1984). These sensations may fade with time or remain indefinitely. Phantom limb pain is often described as crushing or tearing pain (Diadmond and Coniam, 1997). Some post-amputation pain arises from the stump, which may be due to neuroma formation, and is often apparent as acutely tender areas. Neuroma and phantom limb pain are frequently encountered together (Diadmond and Coniam, 1997). Although, the acute cortisol and behavioural responses caused by castration plus tail docking are well characterised during the first 4 hours after treatment animals may suffer from unnoticed chronic pain due to hyperalgesia, neuroma formation and/or phantom pain, which may continue indefinitely.

## 2.2.2 Different methods of castration and tail docking

Different methods for castration and tailing of lambs and how they are conducted, are summarised in Table 2.1.

### ***Surgical castration and tailing***

Surgical castration involves cutting off the distal one third of the scrotum with a knife to expose the testicles and then removing the testicles by drawing them out with serrated tongs applied to the body of each testis without cutting the spermatic cords (Lester *et al.*, 1991a), or removing the testes after scraping and cutting or cauterising the spermatic cords (Molony *et al.*, 1993). In surgical tailing, the tail is cut off using a knife. The cutting of tissues during surgical castration and/or tailing causes an immediate nociceptor barrage. The damaged tissues release bradykinin, histamine and serotonin, which are thought to cause pain by chemical stimulation of the nociceptors. Prostaglandins are also released and are thought to act indirectly by reducing the threshold of nociceptors to such stimuli (Johnston, 1996). Surgical castration and tailing has been shown to cause acute distress for up to 8 hours after treatment, so this technique is not recommended for either procedure (Lester *et al.*, 1991a,b). The distress caused by surgical tail docking does not affect the overall productivity (weight gain) of lambs however (Filmer, 1938; Wohlt *et al.*, 1982; Rhodes *et al.*, 1994).

### ***Cautery tail docking***

Cautery is only used for tail docking; a gas heated anvil-scissor docking iron can be used or an electrocauteriser, where the extreme heat of the jaws severs the tail (Lester *et al.*, 1991a; Mazzaferro *et al.*, 1993; Stillwell *et al.*, 1994; St. Louis *et al.*, 1994), or the tail is cut and the stump is cauterised (Graham *et al.*, 1997). The resulting third degree burns cause complete destruction of the skin, and are associated with little or no pain in humans (Bonica, 1990). Sensory nerve receptors including nociceptors are in the dermis, and are destroyed by third degree burns resulting in a loss of sensation in the affected area (Bonica, 1990). Therefore as cautery is likely to cause third degree burns the acute pain associated with tail docking may be less than that caused by cutting off the tail with a knife. Tail docking using cautery results in a cortisol response similar to

control handled lambs (Lester *et al.*, 1991a; Mazzaferro *et al.*, 1993; Stillwell *et al.*, 1994; Graham *et al.*, 1997). Analgesics (local anaesthetic, diclofenac or an analgesic spray) in combination with cautery tail docking was ineffective at reducing the cortisol response to this treatment as cautery alone resulted in a cortisol response equivalent to control handling (Graham *et al.*, 1997).

### ***Ring castration and tailing***

Ring castration involves a constrictive rubber ring being placed on the neck of the scrotum with a specialised instrument (elastrator), so that both testes are distal to the ring and the two teats are above the ring (Mellor and Murray, 1989a). A ring can be placed on the tail for tail docking. Short scrotum creation involves the ring being applied distal to the testes, so that the testes are retained against the abdominal wall, resulting in infertility by elevated testicular temperature (Lester *et al.*, 1991a). Short scrotum creation produces the growth rate and lean carcass benefits of entire rams, without the hygiene problems at the works associated with dirty scrotums (Probert and Davies, 1986). However the method does not produce infertility in 100% of the lambs (Probert and Davies, 1986). Placement of constrictive rubber rings during ring castration and/or tailing or short scrotum creation causes an initial nociceptor barrage due to the mechanical pressure caused by the rings, which prevents blood flow but initially does not prevent transmission of the nerve impulses (Cottrell and Molony, 1995). Ischaemic pain follows the initial nociceptor barrage, hypoxia progressing to anoxia of the tissues leads to tissue death, and the damaged tissues release serotonin, histamine and bradykinin which further stimulate the nociceptors (Johnston, 1996). The build up of lactic acid due to anaerobic metabolism may also stimulate nociceptors. At sufficiently low oxygen levels in the tissues nociceptors become disabled. If occlusion continues the nociceptors die as well as the other tissues distal to the occlusion. Ring castration and/or tailing results in a rapid rise in the cortisol concentration which peaks approximately 40-60 minutes after treatment and returns to baseline levels within approximately 2-3 hours (Mellor and Murray, 1989a; Lester *et al.*, 1991a; Wood *et al.*, 1991; Kent *et al.*, 1993, 1995, 1998; Mazzaferro *et al.*, 1993; Stillwell *et al.*, 1994; Rhodes III *et al.*, 1994; Kent *et al.*, 1995; Lester *et al.*, 1996; Dinniss *et al.*, 1997a, b; Graham *et al.*, 1997, Kent *et*

*al.*, 1998). It was thought that applying a smaller/tighter rubber ring may disable the innervation from the testes and scrotum more quickly. However, the smaller rubber ring produced a more marked cortisol response over a shorter period of time (Kent *et al.*, 1995). Short scrotum creation reduced the cortisol response compared to ring castration (Dinniss *et al.*, 1997a). However, short scrotum creation plus ring tailing did not reduce the cortisol response to ring castration plus tailing (Lester *et al.*, 1991a). As testicular and pampiniform nociceptors are not excited by the short scrotum technique (Cottrell and Molony, 1995), the difference of these results may be a result of the noxious input from the tail when rings were applied to both the scrotum and tail.

Local anaesthetic has been used in combination with ring castration and/or tailing of lambs to alleviate the pain-induced distress caused by these procedures (Appendix 1). Local anaesthetic injected into the scrotal neck, spermatic cords, each testis, and tail virtually abolished the distress response, as measured by cortisol, associated with ring castration plus tailing (Wood *et al.*, 1991). Local anaesthetic has also been injected into specific sites to assess the most effective area of administration for castration alone: scrotal neck, spermatic cords, scrotal neck and spermatic cords, or testes (Dinniss *et al.*, 1997a). Local anaesthetic injected into the scrotal neck, scrotal neck and spermatic cords or testes 15-20 minutes before ring castration virtually abolished the cortisol response to this procedure (Dinniss *et al.*, 1997a).

The benefits of stress-induced analgesia in reducing the distress experienced by lambs ring castrated and tailed was tested using naloxone (an opioid sensitive antagonist); there seemed to be minimal increment due to the administration of naloxone suggesting that lambs receive little benefit from naloxone-sensitive stress-induced analgesia (Wood *et al.*, 1991).

### ***Castration by applying a castration clamp***

The castration clamp is usually applied to each spermatic cord twice, the second application distal to the first, ensuring that skin and underlying tissues in the medial parts of the scrotum remain uncrushed (Wood and Molony, 1992).

Crushing of the spermatic cords and the associated blood vessels results in hypoxia/anoxia of the testes and consequently infertility, but the cords may slip during crushing or may not be crushed adequately resulting in incomplete sterilisation (Wood and Molony, 1992). The crushing action of the castration clamp causes an initial nociceptor barrage, due to the associated tissue damage, followed by inflammatory pain due to the release of inflammatory mediators from the damaged tissues. Application of the castration clamp causes an initial rise in the cortisol concentration, which peaks approximately 30-40 minutes after treatment before returning to baseline values at 90-180 minutes after treatment (Kent *et al.*, 1995; Dinniss *et al.*, 1997a). Application of the castration clamp, irrespective of clamp application time (1 or 10 seconds), does not result in a smaller cortisol response than ring castration (Kent *et al.*, 1995; Dinniss *et al.*, 1997a), and 1 second application of the clamp can result in 10% failure to castrate (Dinniss *et al.*, 1997a). Administration of local anaesthetic into the testes, spermatic cords or the neck of the scrotum, 20 minutes or 1-2 minutes prior to castration using a castration clamp (applied for 10 seconds), did not significantly reduce the cortisol response compared to ring castration (Dinniss *et al.*, 1997a; Molony *et al.*, 1997). Lambs can be tailed using the castration clamp, but this is not a common procedure (Kent *et al.*, 1995).

### ***Ring plus clamp method***

The ring and castration clamp methods have been combined in an attempt to alleviate the distress caused by ring castration plus tailing (Appendix 1). The ring plus clamp method involves the rings being applied in the same way as for the ring alone method, and the clamp being applied across the full width of the scrotal neck and/or the tail just distal to the rings, for up to 10 seconds (Kent *et al.*, 1993, 1995). The ring method of castration and/or tail docking results in ischaemic pain due to the application of the rubber rings to the neck of the scrotum and the tail. However, the additional application of the castration clamp across the full width of the scrotum and/or tail after ring placement is intended to crush and disable the innervation from all tissues distal to the ring and prevent or reduce the afferent transmission of nociceptor impulses due to

ischaemia. This alleviates the pain-induced distress caused by ring castration plus tailing (Kent *et al.*, 1993, 1995; Dinniss *et al.*, 1997a). Application of the castration clamp for 6 seconds across the full width of the scrotum and tail following ring castration and tailing significantly reduced the magnitude of the cortisol response in 5-day-old lambs compared to ring castration plus tailing (Kent *et al.*, 1993). However, the magnitude of the cortisol response was only marginally reduced by the ring plus clamp method in 21 and 42-day-old lambs (Kent *et al.*, 1993; Molony *et al.*, 1997). Application of the clamp for 10 seconds in combination with ring castration plus tailing significantly reduced the magnitude and duration (by 60 minutes) of the cortisol response to ring castration plus tailing in 5-day-old lambs (Kent *et al.*, 1995). Application of the clamp for 1, 5, or 10 seconds using the traditional double clamping method in combination with ring castration did not reduce the cortisol response to ring castration (Dinniss *et al.*, 1997a). However, applying the castration clamp in the traditional way leaves a gap of uncrushed tissue and intact nerves, so that continued noxious input from the scrotum may account for the difference in results. Local anaesthetic injected into the testes, scrotal neck, or spermatic cords before ring plus clamp castration reduced the magnitude of the cortisol response compared to ring castration (Dinniss *et al.*, 1997a; Molony *et al.*, 1997).

### **Chemical castration**

Chemical castration of calves has been carried out by injecting  $\alpha$ -hydroxypropionic acid into the testes which causes necrosis of the testicular tissue and atrophy of the testes, and a sclerosing agent (88% lactic acid) which causes destruction of the spermatogenic and hormone-producing cells (Fordyce *et al.*, 1989; Cohen *et al.*, 1990). Chemical castration of lambs has also been studied using formaldehyde in ethanol injected into the caudae epididymides (Mercy *et al.*, 1985). Chemical castration requires more care and time than other conventional methods of castration, healing takes longer and secondary sexual characteristics may not be eliminated in some animals, also infertility is not guaranteed (Fordyce *et al.*, 1989, Cohen *et al.*, 1990). Chemical castration involving injection of a corrosive substance, such as lactic acid, into the testes

of the lamb, would result in mechanical stimulation of the nociceptors due to the pressure of the injected fluid and chemical stimulation of the nociceptors. The destruction of the tissues would stimulate nociception until the tissues are destroyed or the chemical is neutralised. Chemical castration in calves resulted in a reduced cortisol response when compared to surgical castration. However, the cortisol response in chemically castrated calves remained elevated compared to control handled animals for up to 6 hours after treatment (Cohen *et al.*, 1990).

### ***Immunocastration***

Immunocastration is less commonly used as a form of castration, being still at an experimental stage. Immunocastration involves neutralisation of an endogenous reproductive hormone, such as luteinising releasing hormone, by active immunisation (Robertson *et al.*, 1982). Immunocastration has been accomplished by actively immunising calves against luteinising hormone-releasing hormone, causing reduced testosterone secretion and testis involution. However this method is not 100% effective, it requires secondary immunisation, with animals reverting to normality after 6 months (Robertson *et al.*, 1982). The distress response of animals to immunocastration has not apparently been studied as yet.

### **2.2.3 Aims of this study**

Local anaesthetic injected into the scrotal neck, spermatic cords, testes and the tail of lambs 20 minutes prior to ring castration plus tailing virtually abolished the cortisol response to this procedure (Wood *et al.*, 1991). Injection of local anaesthetic into the scrotal neck, scrotal neck and spermatic cords, or the testes 15 minutes prior to ring castration also virtually eliminated the cortisol response to this procedure (Dinniss *et al.*, 1997a). However, it is impractical in the field to handle animals twice; once to administer local anaesthetic and again to carry out castration and/or tail docking. Therefore, one of the aims of this study was to determine whether injecting local anaesthetic just before or

just after ring castration plus tailing would reduce the distress response to this procedure.

It is impractical to inject local anaesthetic into the testes, scrotal neck, spermatic cords and tail due to the time it would take to inject local anaesthetic into all these sites and the cost of the local anaesthetic. Therefore, in the present study local anaesthetic was injected into the neck of the scrotum or the testes to determine the most effective site to administer local anaesthetic to alleviate the pain-induced distress caused by ring castration plus tailing and to minimise the time and cost of using local anaesthetic.

Ring castration plus tailing followed by application of a castration clamp across the full width of the scrotal neck and tail for 6 seconds in young lambs was found to reduce the magnitude of the cortisol response compared to that caused by ring castration plus tailing only (Kent *et al.*, 1993). It was further demonstrated that if the clamp was applied for 10 seconds in combination with ring castration plus tailing the magnitude and the duration of the cortisol response was also significantly reduced (Kent *et al.*, 1995). However, both studies involved group sizes of only 6-7 animals. Therefore, in the present study the cortisol distress response of lambs ring castrated plus tailed were compared with lambs ring castrated plus tailed in combination with a clamp being applied across the full width of the scrotum for 6 or 10 seconds using larger groups of lambs (n=19).

Injecting lambs with ACTH has been shown to produce a marked cortisol response compared to different methods of castration and/or tailing. Therefore, in the present study, ACTH was administered in lambs to assess whether the cortisol response to any of the methods of castration plus tailing were limited by the secretory ability of the adrenal cortex.

Method	Technique
Surgical	C: Distal one third of the scrotum is cut off with a knife or an incision is made to expose the testicles. The testicles are then removed using serrated tongs without cutting the spermatic cords or removing the testes after scraping and cutting or cauterising the spermatic cords. T*: The tail is cut with a knife.
Ring	C: Constricting rubber ring is placed on the neck of the scrotum using an elastrator, with the testes distal to the ring. T*: Constrictive rubber ring is placed on the tail, with an elastrator.
Bloodless castrator	C: The castration clamp is usually applied twice to each spermatic cord, the second time distal to the first, leaving uncrushed skin and tissue between the two pairs of crush lines. T*: The clamp is not conventionally used for tail docking.
Ring plus clamp method	C: Constrictive rubber ring is placed on the neck of the scrotum as above. A castration clamp is then applied across the full width of the scrotum just distal to the ring. T*: A ring is placed as above and the castration clamp is applied across the full width of the tail just distal to the ring
Cautery	T*: Cautery is used only for tail docking. Cautery is carried out using a docking iron or by cutting off the tail then cauterising the wound.
Short-scrotum creation	C: A ring is placed using an elastrator so that both testicles are proximal to the ring and are held against the body wall.
Chemical castration	C: A chemical solution, such as lactic acid, is injected into the testes, causing necrosis of the testicular tissue.
Immunocastration	C: Neutralisation of an endogenous reproductive hormones, such as luteinising releasing hormone, by active immunisation.

\*Tails are docked so that the stump will cover the anus in rams and the vulva in ewes.

**Table 2.1:** Different castration and tail docking methods in lambs, and how they are conducted.

The healing rate of the castration wound in calves ring castrated and ring plus clamp castrated have been compared, but this has not been done in lambs (Molony *et al.*, 1995). In the present study the rate of healing of the scrotal injuries after castration using the ring method or the ring plus clamp method were compared during the first six weeks after castration plus tailing to assess whether the ring plus clamp method in lambs had any adverse effects on wound healing.

## **2.3 Materials and Methods**

There were three components to this study, two acute (study 1 and 2) and one chronic (study 3).

### **2.3.1 Animals**

The day before the trial lambs were weighed and their necks clipped to facilitate blood sampling by jugular venepuncture. Lambs were randomly allocated into pens (110cm x 190cm) with their dams, with a maximum of 3 lambs per pen (Fig. 2.1). Twins were put in the same pens, but were allocated to different treatments. Each lamb was colour coded with scourable marker spray for easy identification of treatment. Ewes had free access to water overnight but not food. One hour before the experiment the lambs were placed in pens adjacent to their dams, but they were still able to see, hear and smell their dams. Three studies were conducted.

#### ***Study one***

Fifty one Coopworth lambs 6 weeks of age, weighing between 13 - 30 kg (mean 20 Kg) were used in this study, which was spread over two trial days.

### ***Study two***

Sixty six Coopworth lambs 3 to 6 weeks of age, weighing between 4 - 20 kg (mean 11 Kg) were used in this study, which was spread over two trial days.

### ***Study three***

One hundred and fifty seven Coopworth lambs were used in this study (Table 2.4); 66 of them were those used previously in study two. Lambs were brought in for examination weekly for 4-6 weeks. Lambs were restrained and their castration wound examined by the same person on all examination days. The rate of wound healing was scored from 5.0 – 0.0, or as H for completely healed (Table 2.3).

#### **2.3.2 Blood sampling**

Blood samples were taken during studies 1 and 2. Blood samples (5ml) were taken by venepuncture from either jugular vein immediately before treatment (time 0) then regularly for four hours after treatment. During blood sampling the lambs were picked up by one handler and restrained, while a second person took the blood sample. The whole procedure from retraining the animal to the time the animal was released lasted no more than 15 seconds. Animals were bled in the same order throughout the experiment.

### ***Study one***

Blood samples were taken at time 0, and at 20, 40, 60, 90, 120, 150, 180, 210, and 240 minutes after treatment.

Study	Treatment	N	Plasma cortisol concentration (nmol/L)	Integrated cortisol response (nmol/L.min)
One	Control	8	49 ± 6.2	3,194 ± 546
	LASc control	8	47 ± 8.9	2,820 ± 438
	LAT control	9	64 ± 6.7	3,992 ± 830
	Ring CT	9	58 ± 12	21,803 ± 2,767 <sup>abc</sup>
	LASc + ring CT	8	65 ± 8.0	12,401 ± 2,721 <sup>abcd</sup>
	LAT + ring CT	9	55 ± 9.0	16,737 ± 2,643 <sup>abc</sup>
Two	Ring CT	19	46 ± 4.4	26,127 ± 3,401
	Ring CT + clamp 6	19	42 ± 5.1	25,378 ± 2,821
	Ring CT + clamp 10	19	49 ± 4.6	21,755 ± 2,378
	ACTH	9	42 ± 5.6	30,373 ± 5,811
Three	Ring CT	70		
	Ring CT + clamp	87		

**Table 2.2:** Pretreatment (t=0) cortisol concentrations (mean ± SEM) in lambs. There was no significant difference (P>0.05) between mean pretreatment cortisol concentrations, shown by one way analysis of variance (ANOVA). The mean (± SEM) integrated cortisol response of each group over the trial period of 4 hours. [a = significantly different from control; b = significantly different from LASc control; c = significantly different from LAT control; d = significantly different from Ring CT control (P < 0.05)].

Score	Description
5.0	No injury, intact scrotum.
4.0 - 3.5	Crepitus and swelling.
3.0 - 2.5	Swelling decreased, no crepitus.
2.0 - 1.5	Increasing degrees of dry necrosis.
1.0	Dry hard scrotum in varying stages of detachment distal to ring.
0.5	Scrotum easily removed – attached but only hanging by wool fibres.
0.0	Scrotum lost, but lesions not completely healed.
H	Healed, lesion only evident as a small star-shaped scar.

**Table 2.3:** Injury/ healing scale for lamb scrotums after ring castration and ring and clamp castration.

## ***Study two***

Blood samples were taken at time 0, and at 20, 40, 60, 80, 110, 140, 170, 200, 230, and 260 minutes after treatment.

### **2.3.3 Treatments**

Treatments were carried out immediately after the first blood sample was taken. One person restrained the lamb while a second person carried out the treatment. In each trial the same person conducted the treatments.

## ***Study one***

### ***Control handling ('Control')***

The animals were held and the scrotum and tail massaged but left intact.

### ***Local anaesthetic injected into the scrotal neck control ('LASc control')***

Local anaesthetic (2 ml of 2% Lignocaine hydrochloride: Nopaine, Ethical Agents, Auckland) was injected into 3 sites around the scrotal neck, which was then massaged to disperse the anaesthetic, 5-10 seconds prior to treatment: 1ml was injected into the antero-medial surface of the scrotum and 0.5ml into each lateral surface. Lambs were then treated the same as control animals.

### ***Local anaesthetic injected into the testes control ('LAT control')***

Local anaesthetic (1 ml of 2% Lignocaine hydrochloride) was injected into each testis through the caudal pole, 5-10 seconds after treatment. Lambs were then treated the same as control animals.

### ***Ring castration plus tailing ('Ring CT')***

Rubber rings (Allflex New Zealand Ltd., Palmerston North) were applied using an elastrator (Elastrator Ltd., Blenheim) (Fig. 2.2) to the neck of the scrotum, after ensuring that both testes were distal to the ring (Fig. 2.3), and to the tail so that the tail stump would cover the anus of each lamb.

***Local anaesthetic injected into the scrotal neck + ring castration and tailing immediately ('LAsC + ring CT')***

Local anaesthetic was injected into 3 sites around the scrotal neck, 5-10 seconds *before* ring castration and tailing as described for LAsC control and ring CT lambs.

***Local anaesthetic injected into the testes + ring castration and tailing ('LAT + ring CT')***

Local anaesthetic was injected into each testis 5-10 seconds *after* ring castration and tailing as described for LAT control and ring CT lambs.

***Study two***

***Ring castration plus tailing ('Ring CT')***

Lambs were castrated and tailed as described for Ring CT lambs in study one.

***Ring castration plus tailing and clamp 6 seconds ('Ring CT + clamp 6')***

Ring castration and tailing was carried out as described for Ring CT lambs. The castration clamp (Burdizzo castration clamp) (Fig. 2.4) was then applied once for 6 seconds across the full width of the scrotum just distal to the rubber ring (Fig. 2.5).

***Ring castration plus tailing and clamp 10 seconds ('Ring CT + clamp 10')***

Ring castration and tailing was carried out as described for Ring CT lambs. The castration clamp was then applied once for 10 seconds across the full width of the scrotum just distal to the rubber ring.

***ACTH injection ('ACTH')***

Synthetic adrenocorticotropin (Synacthen; Ciba pharmaceuticals, Auckland) was injected into a jugular vein at a dose of 14 µg/kg body weight; this was the same dose as that used by Dinniss *et al.* (1997a) to elicit a large cortisol response.

## **Study three**

### ***Ring castration plus tailing ('Ring CT')***

Lambs were castrated and tailed as described for ring CT lambs in study one.

### ***Ring castration plus tailing and clamp ('Ring CT + clamp')***

This group contained the entire ring CT + clamp 6 and ring CT + clamp 10 lambs from study two, and additional ring CT + clamp 6 lambs.

#### **2.3.4 Plasma cortisol assay**

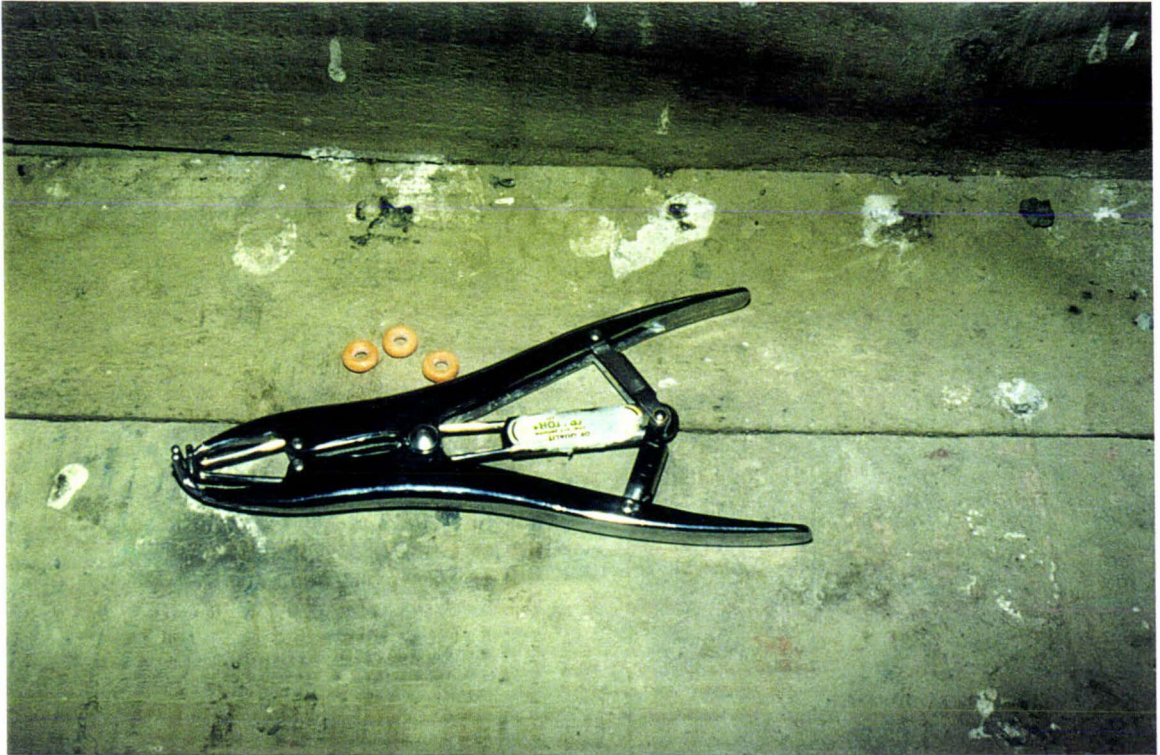
The blood was collected into heparinised vacutainers. After blood samples were taken they were centrifuged and the plasma was separated and stored at -20 °C until required. Cortisol concentration of the plasma was determined using a non-extraction tritium radio-immuno-assay method (Endocrine Sciences, 4301 Lost Hills Rd, CA 91301). The lowest detectable concentration was 1.0 nmol/L. The intra-assay and inter-assay coefficients of variation were 5% and 13% (study 1), and 7% and 12% (study 2), respectively.

#### **2.3.5 Integrated cortisol responses**

The integrated cortisol response was calculated to determine changes in magnitude and duration of the cortisol response after the application of the treatments. The integrated cortisol response is defined as the area between a horizontal line drawn through the pretreatment concentration and the cortisol curve during the period when the concentrations were greater than the pretreatment values (Mellor and Murray, 1989a).



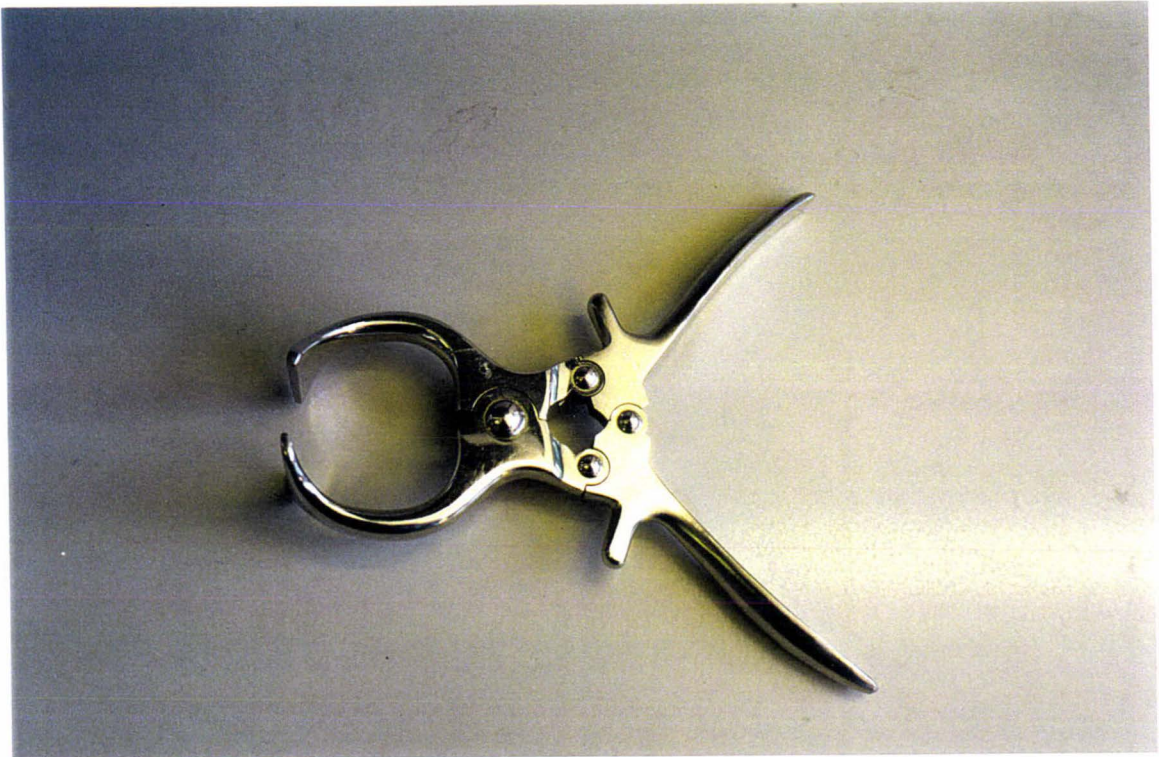
**Figure 2.1:** Pen layout during trials to separate lambs from their mothers.



**Figure 2.2:** Elastrator and rings.



**Fig. 2.3:** Ring castration and method of holding lambs for treatment.



**Fig. 2.4:** Castration clamp.



**Fig. 2.5:** Application of the castration clamp following ring castration.

### 2.3.6 Statistical analysis

The cortisol concentrations are presented as the mean  $\pm$  standard error of the mean (SEM) at each sample time. To compensate for individual variation, the pretreatment value was subtracted from the concentration of all subsequent samples. All graphical presentations represent changes in the mean concentration after time 0. Significant differences between means were determined using unpaired t-test with Welch's correction for unequal variance and one-way analysis of variance (ANOVA) (GraphPad software Prism V 2.01); (any data was considered significantly different when  $P < 0.05$ ). Statistical analysis including repeated measures analysis of variance (SPSS for windows V 8.0) were also conducted on  $\log_{10}$  transformed data. As there were very few differences in the significance of outcomes using untransformed and transformed data, untransformed data have been presented.

## 2.4 Results

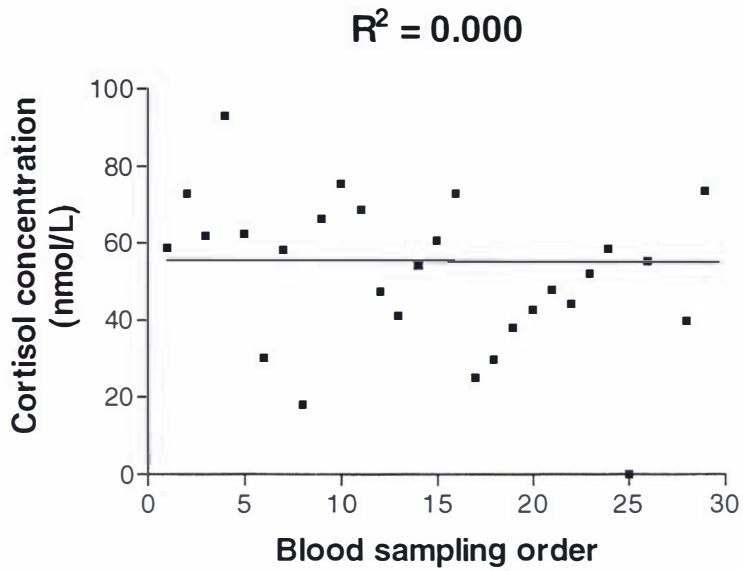
There were no significant differences ( $P > 0.05$ ) between the pretreatment plasma cortisol concentrations in the different groups in studies 1 and 2 (Table 2.2). The order in which the pretreatment blood sample was taken did not significantly affect the pretreatment concentrations (Figure 2.6).

### ***Study one***

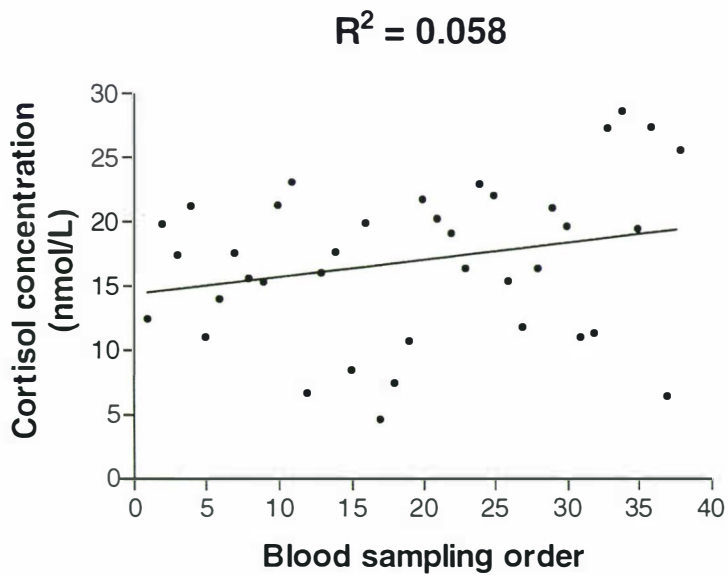
#### ***Control, LASc control, LAT control***

None of the control treatments resulted in a significant change in plasma cortisol concentration throughout the period of study, nor did the cortisol concentrations differ significantly between these groups (Fig. 2.7).

A



B



**Fig. 2.6:** Relationship between plasma cortisol concentration and the order in which the pretreatment blood samples were taken (A – study 1; B – study 2).

### ***Ring CT***

Application of rings to the scrotum and the tail caused a rise in the mean plasma cortisol concentration that was significantly greater ( $P > 0.05$ ) than pretreatment values 20 minutes after treatment (Fig. 2.7). The cortisol concentration increased until it peaked at 187 nmol/L at 60 minutes, returning to pretreatment values approximately 210 minutes after treatment.

### ***LASc + ring CT***

Injection of local anaesthetic into the scrotal neck followed immediately by ring castration plus tailing was associated with a significant increase in the mean cortisol concentration during the first 40 minutes (Figure 2.8). The concentration peaked at 116 nmol/L at 60 minutes after treatment and then decreased, returning to pretreatment values by 180 minutes. The plasma cortisol concentrations of LASc + ring CT lambs were significantly lower ( $P < 0.05$ ) than those of ring CT lambs at 20, 40, 60, and 150 minutes after treatment (Fig. 2.8).

### ***LAT + ring CT***

Injection of local anaesthetic into the testes immediately after ring castration plus tailing was associated with a significant increase in the mean cortisol concentration by 20 minutes after treatment (Fig. 2.9). Cortisol concentrations peaked at 138 nmol/L at 60 minutes and then decreased, reaching pretreatment values by 210 minutes after treatment. There was no significant differences between the concentrations in ring CT and LAT + ring CT lambs at any time.

### **Integrated cortisol responses**

The integrated cortisol response was calculated over the whole study period, time 0 until 4 hours after treatment. The integrated cortisol response for LASc + ring CT lambs was significantly lower than the response for ring CT lambs. The integrated cortisol response for all castrated and tailed groups were significantly greater than the integrated response for the control groups (Table 2.2).

## **Study two**

### ***Ring CT***

Ring castration plus tailing caused mean plasma cortisol concentrations to rise significantly above pretreatment values during the first 20 minutes, which peaked at 232 nmol/L at 60 minutes, returning to pretreatment values by approximately 170 minutes after treatment (Fig. 2.10).

### ***Ring CT + clamp 6***

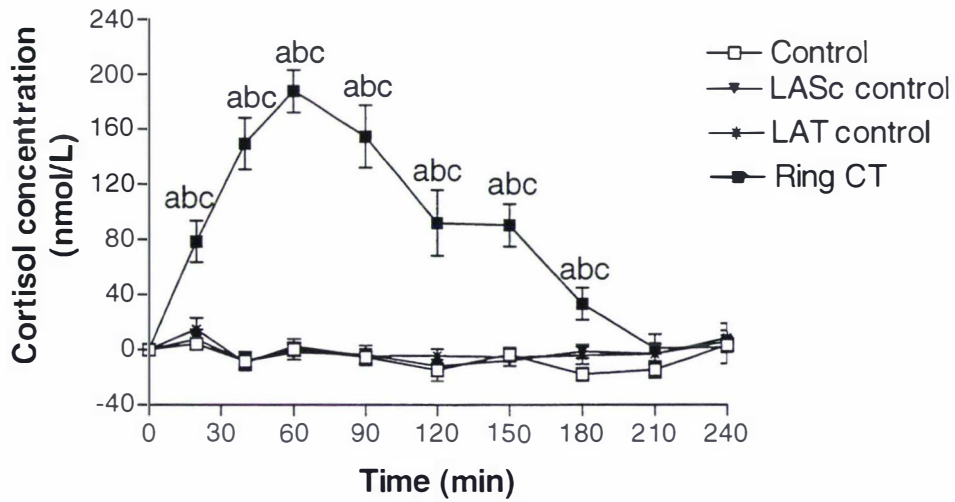
The combined method employing a 6 second clamp application caused a significant increase in the cortisol concentration during the first 20 minutes, which peaked at 200 nmol/L 40 minutes after treatment (Fig. 2.11). The cortisol concentration plateaued between 60 - 110 minutes before returning to pretreatment values at 270 minutes. There were no significant differences between the concentrations in ring CT and ring CT + clamp 6 lambs at any stage ( $P > 0.05$ ).

### ***Ring CT + clamp 10***

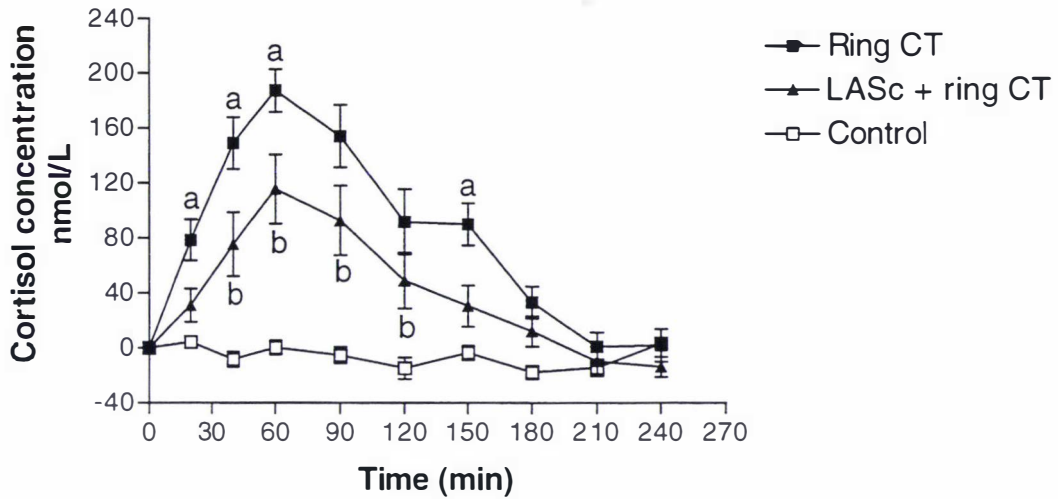
The combined method applying the clamp for 10 seconds caused a significant increase in the cortisol concentration by 20 minutes after treatment. The cortisol concentration peaked at 158 nmol/L at 40 minutes, plateaued until 110 minutes, and returned to pretreatment values by 270 minutes. Cortisol concentrations of ring CT + clamp 10 lambs were significantly lower ( $P < 0.05$ ) than those of ring CT lambs only at 60 minutes after treatment (Fig. 2.12).

### ***ACTH***

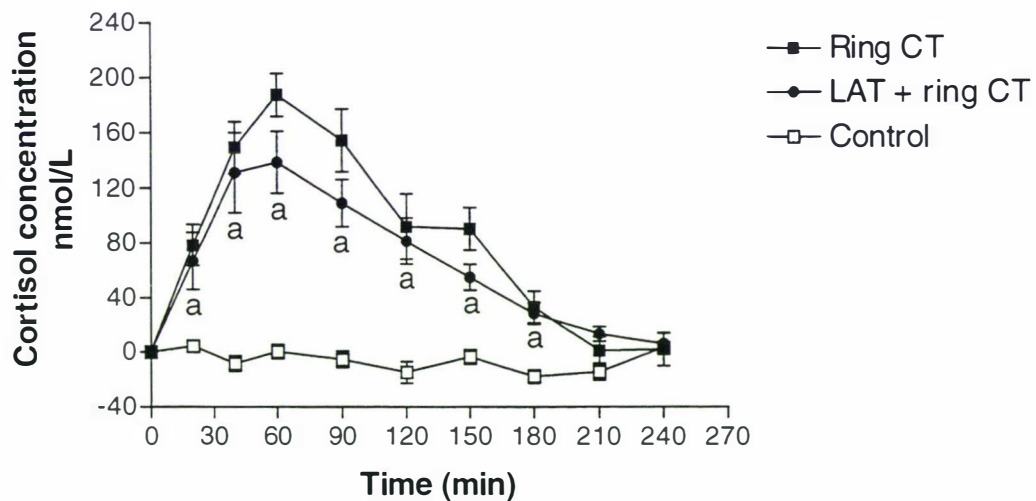
Injection of ACTH caused a significant increase in the cortisol concentration within 20 minutes after treatment (Figure 2.10). The cortisol concentration peaked at 239 nmol/L at 40 minutes, plateaued for 40 minutes before returning to pretreatment values approximately 270 minutes after injection. There were



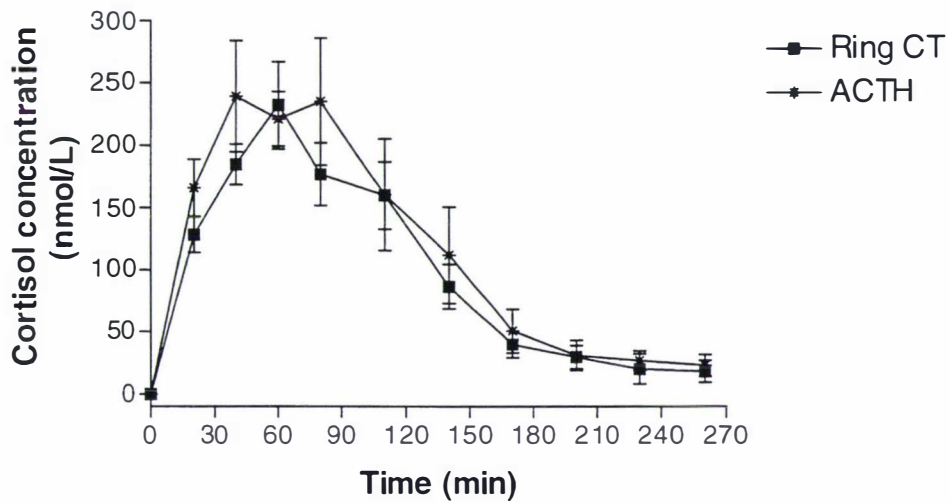
**Fig. 2.7:** Changes in plasma cortisol concentration in lambs in response to control handling, the administration of local anaesthetic, and ring castration plus tailing. [a = significantly different from control; b = significantly different from LAsc control; c = significantly different from LAT control].



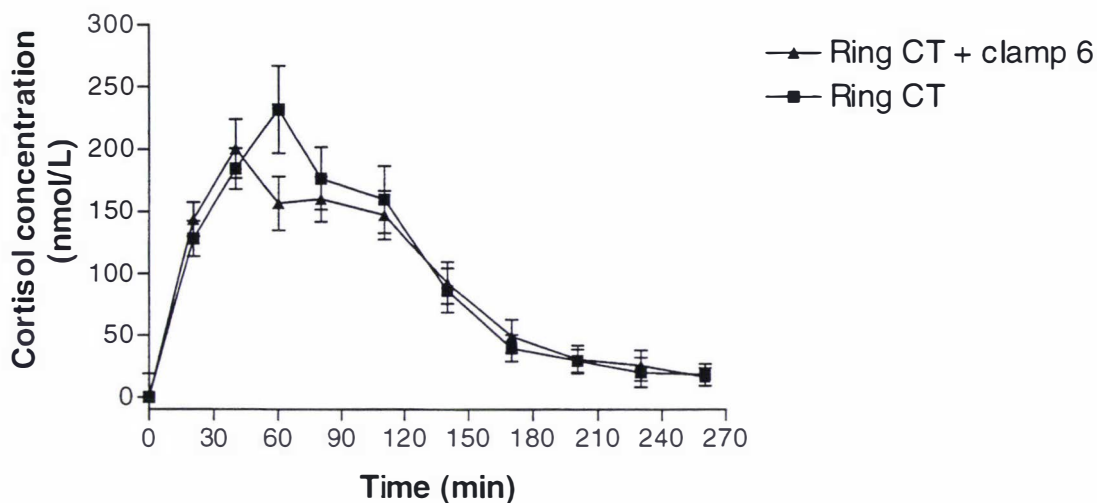
**Fig. 2.8:** Changes in plasma cortisol concentration in lambs in response to control handling (control), ring castration plus tailing (Ring CT) and injection of local anaesthetic (lignocaine) into the scrotal neck immediately before ring castration plus tailing (LAsC + ring CT). [a = Ring CT significantly different from LAsC + ring CT; b = LAsC + ring CT significantly different from control].



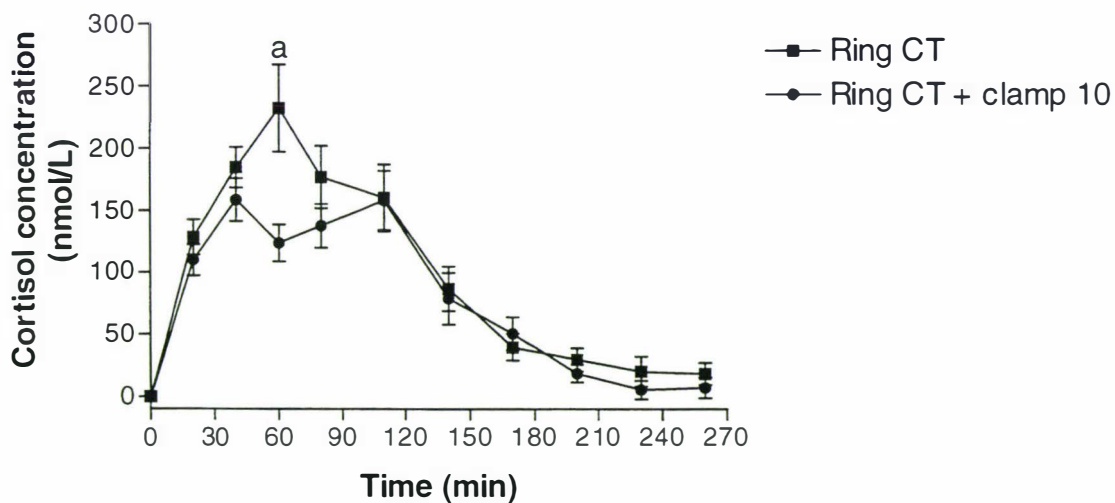
**Fig. 2.9:** Changes in plasma cortisol concentration in lambs in response to control handling (control), ring castration plus tailing (Ring CT) and injection of local anaesthetic (lignocaine) into each testis immediately after ring castration plus tailing (LAT + ring CT). There were no significant differences between the concentrations in ring CT and LAT + ring CT lambs at any time. [a = LAT + ring CT significantly different from control].



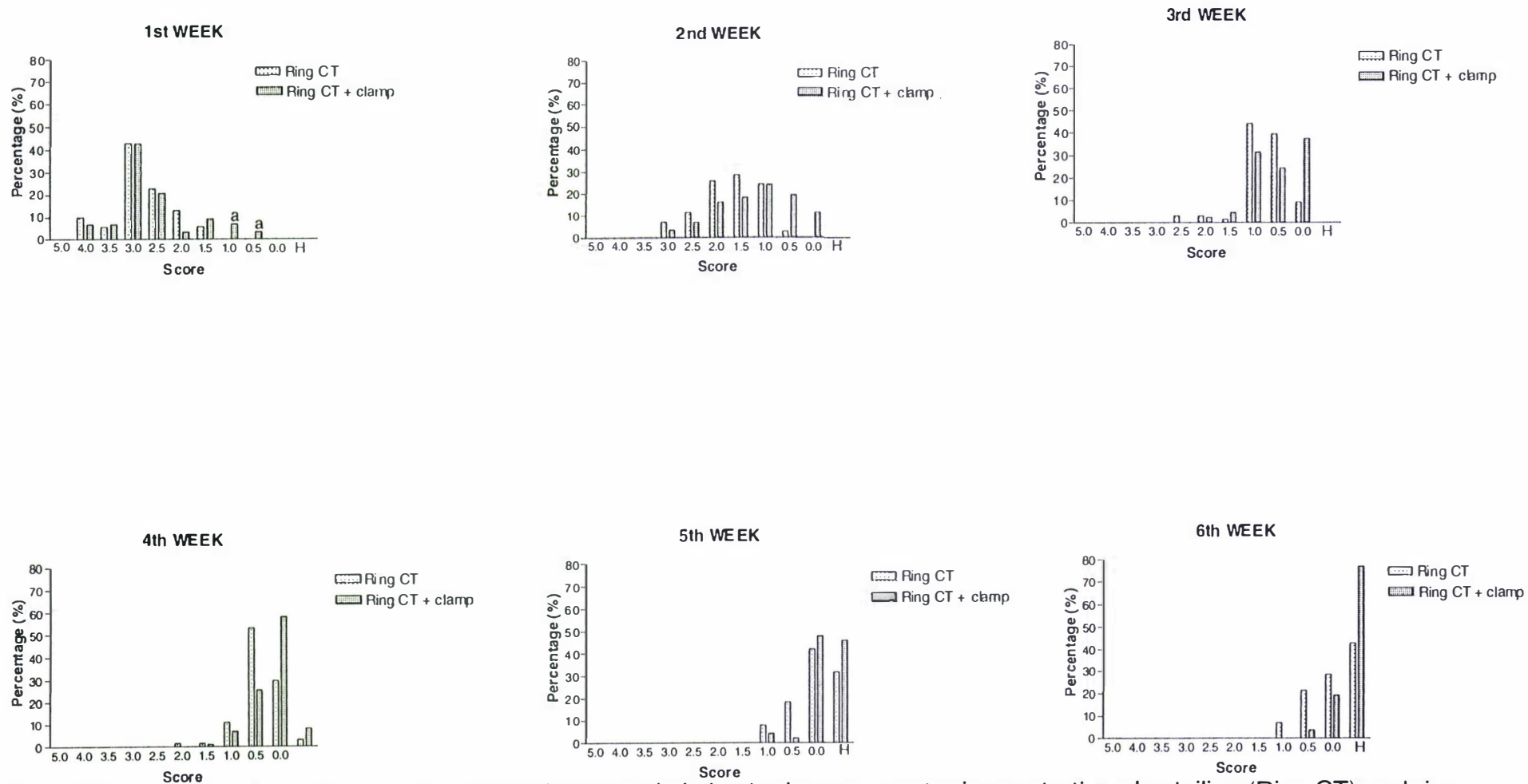
**Fig. 2.10:** Changes in plasma cortisol concentration in lambs in response to injection of ACTH and to ring castration plus tailing (Ring CT). There were no significant differences between the concentrations in ring CT and ACTH lambs.



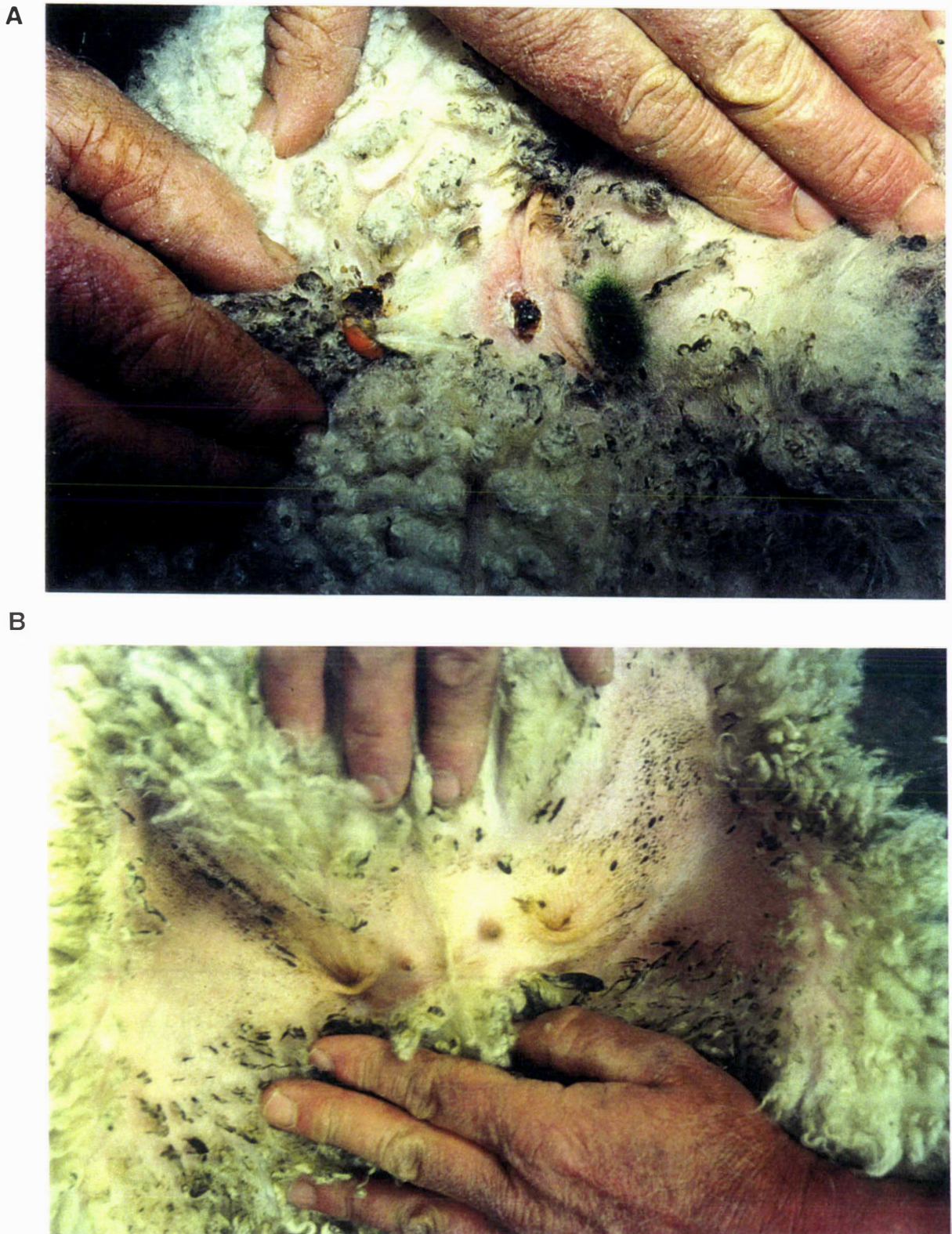
**Fig. 2.11:** Changes in plasma cortisol concentration in lambs in response to ring castration plus tailing (Ring CT) and ring castration plus tailing and clamp 6 seconds (Ring CT + clamp 6). There were no significant differences between the concentrations in ring CT and ring CT + clamp 6 lambs.



**Fig. 2.12:** Changes in plasma cortisol concentration in lambs in response to ring castration plus tailing (Ring CT) and ring castration plus tailing and clamp 10 seconds (Ring CT + clamp 10). [a = significantly different from Ring CT].



**Fig. 2.13:** Change in healing score of castration wounds in lambs in response to ring castration plus tailing (Ring CT) and ring castration plus tailing and clamp (Ring CT + clamp) over 6 weeks. [a = Ring CT is significantly different from ring CT + clamp].



**Fig. 2.14:** Healing of scrotal wounds: (A) scrotum lost, but lesion is not completely healed (0.0); (B) lesion is completely healed (H).

WEEKS OBSERVED						
	1	2	3	4	5	6
Study 2	70	70	70	69	40	40
Study 3	87	87	82	81	46	
Total	157	157	152*	150**	86	40

\*5 lambs not accounted for \*\*7 lambs not accounted for

**Table 2.4:** The total number of lambs in each week of observation after castration.

no significant differences between the concentrations in ring CT and ACTH lambs at any stage.

### **Integrated cortisol responses**

There were no significant differences between the integrated cortisol responses of any of these groups.

### ***Study three***

Healing scores for castration wounds (Table 2.3) are provided in Figure 2.13, and the number of lambs observed per week are in Table 2.4. The castration wounds one week after castration using the ring or ring plus clamp method showed signs of swelling and a little crepitus in 43% of lambs from both treatments. Furthermore, one week after castration 7% of lambs castrated by the ring plus clamp method had a dry hard scrotum in varying stages of detachment, and in another 3% of these lambs the scrotum could be easily removed. This was significantly greater than in ring castrated lambs, which had not reached stage 1.0 or 0.5 within the first week of observation. By the second week of observation the scrotums were mainly distributed between stages 3.0 and 0.0 (Fig. 2.14) with a high percentage of lambs castrated using the ring plus clamp method being at stages 0.5 and 0.0. By week three most of the scrotums, from both treatments, were dry, hard and at varying stages of detachment or could be easily removed. The scrotal wounds of 8% of lambs castrated using the ring plus clamp method had healed (Fig. 2.14), so that only a small star-shaped scar was evident compared to 3 % of ring castrated lambs, by the fourth week of observations.

The castration wounds of ring plus clamp castrated lambs healed faster with 77% of lambs being completely healed by week 6, compared to 43% of ring only lambs (Fig. 2.13). The scrotal wounds of ring castrated and ring plus clamp castrated lambs all healed without complications. The observations of the castration wounds were only carried out for 4-6 weeks because at this time most of the scrotal wounds had healed or were in the final stages of healing. It was evident within 4-6 weeks that there were no complications caused by the

ring plus clamp method. Therefore it was thought unnecessary to continue observation until all the scrotal wounds had healed completely (Fig. 2.13).

## 2.5 Discussion

Ring castration and/or tail docking of lambs causes acute distress as measured by cortisol, which lasts for up to 3 hours. The cortisol response to ring castration plus tailing in this study was similar to the responses reported in the literature (Mellor and Murray, 1989a, b; Wood *et al.*, 1991; Lester *et al.*, 1991a; Mellor *et al.*, 1991; Kent *et al.*, 1993; Mazzaferro *et al.*, 1993; Stillwell *et al.*, 1994; Kent *et al.*, 1995; Rhodes III *et al.*, 1995; Dinniss *et al.*, 1997a; Kent *et al.*, 1998). Kent *et al.* (1998), Graham *et al.* (1997), and Molony *et al.* (1997) published their work after the experimental work in this study had been completed, hence we were unaware of their recent work during the planning of the present study.

Local anaesthetic has been used to alleviate the pain-induced distress caused by ring castration and/or tail docking (Appendix 1). Local anaesthetic injected into the tail, each spermatic cord, scrotal neck, and each testis 15 minutes prior to ring castration plus tailing virtually abolished the cortisol response to this procedure (Wood *et al.*, 1991). However injecting local anaesthetic into many sites is not cost or time effective. Local anaesthetic was injected into the scrotal neck, each spermatic cord, spermatic cords and scrotal neck, or testes to determine the most effective sites to administer local anaesthetic (Dinniss *et al.*, 1997a, Kent *et al.*, 1998). Local anaesthetic injected into the scrotal neck, spermatic cords and scrotal neck, or testes 20 minutes before ring castration abolished the cortisol response to this procedure. In the present study, local anaesthetic injected into the neck of the scrotum 5-10 seconds prior to ring castration plus tailing significantly reduced the cortisol response (Fig. 2.8) and the integrated cortisol response to this procedure (Table 2.2). Local anaesthetic injected into each testis 5-10 seconds after ring castration plus tailing marginally reduced the cortisol response to this procedure, but not significantly (Fig. 2.9). Therefore, in the present study injecting local

anaesthetic into the scrotal neck was more effective in reducing the distress response caused by ring castration plus tailing.

In the present study, administration of local anaesthetic into the neck of the scrotum significantly reduced the peak plasma cortisol concentration by 36% compared to ring castration plus tailing and significantly reduced the integrated cortisol response by approximately 43% (Fig. 2.8; Table 2.2). Administration of local anaesthetic into the scrotal neck would deliver local anaesthetic into the vaginal cavity, which may block nociception from the cutaneous areas of the scrotum as well as the surface of the testes (Dinniss, 1995). It has been shown in dogs that the polymodal receptors (which can function as nociceptors) are predominantly located close to the surface of the testes within the tunica vaginalis visceralis (Kumazawa and Mizumura, 1980). Therefore, local anaesthetic injected into the scrotal neck may block afferent innervation from the scrotum as well as the testes. Local anaesthetic injected into the scrotal neck may further diffuse into the spermatic cords thereby blocking transmission from the spermatic nerves (Dinniss, 1995). It is therefore reasonable to expect that local anaesthetic injected into the scrotal neck would block nociceptor input from both the scrotum and testes .

Administration of local anaesthetic into each testis after ring application only marginally reduced the peak plasma cortisol concentration by 24% compared to ring castration plus tailing and only reduced the integrated cortisol response by approximately 23%, in the present study (Fig.2.9; Table 2.2). Injecting local anaesthetic into the testes has been found to abolish all spontaneous and evoked impulse activity in the superior spermatic nerve caused by the application of a constrictive rubber ring during ring castration within 2 minutes (Cottrell and Molony, 1995). However, other nerves are also involved in the afferent activity from the scrotal area, such as nerves that originate from the scrotal plexus and other afferent visceral nerves, which may not be effectively blocked by injecting local anaesthetic into the testes (Cottrell and Molony, 1995). Local anaesthetic mixed with dye injected into the testes 15 minutes prior to dissection and visceral examination was shown to have leaked out of the puncture wounds caused by the needle and diffused into the surrounding

scrotal fascia and tunica dartos (Dinniss *et al.*, 1997a). Therefore, injecting local anaesthetic into the testes may block the innervation from the testes and the lower portion of the scrotum.

Previously, lambs have been injected with local anaesthetic 15-20 minutes prior to castration and/or tailing to ensure that the local anaesthetic has taken full effect before proceeding (Wood *et al.*, 1991; Dinniss *et al.*, 1997a). However, it is impractical to handle animals twice, once to inject local anaesthetic and a second time to carry out castration and/or tailing. In the present study, injecting local anaesthetic just before or just after ring castration plus tailing resulted in relatively small (LAsC + ring CT) or non-significant (LAT + ring CT) reduction in the cortisol response caused by these procedures (Fig. 2.8; Fig. 2.9). Kent *et al.* (1998) reduced the magnitude and the duration of the cortisol response to ring castration by administering local anaesthetic into the scrotal neck or the testes just after or before application of the ring. However, local anaesthetic administered 15 minutes prior to ring castration abolished the cortisol response to ring castration (Dinniss *et al.*, 1997a). Thus, administration of local anaesthetic 15 minutes prior to ring castration may have allowed more time for the local anaesthetic to diffuse through the tissues of the scrotum and effectively block the conduction of afferent nerve impulses to cause optimum nerve block.

In the present study, local anaesthetic was administered into the scrotal neck or the testes but not into the tail just before or just after ring castration plus tailing. Dinniss *et al.* (1997a) virtually eliminated the cortisol response caused by ring castration by injecting local anaesthetic into the scrotum or testes, but there was no added complication of noxious input from the tail. Therefore, the continued noxious input from the tail may explain why local anaesthetic injected into the scrotum or the testes before or after ring castration and tailing did not completely alleviate the pain-induced distress caused by this procedure. Re-evaluation of the data of Kent *et al.* (1998) showed that local anaesthetic injected into the scrotum or the testes just before or after ring castration reduced the integrated cortisol response to ring castration only by 77%. However, in the present study local anaesthetic injected into the scrotal neck or

the testes just before or after ring castration *plus* tailing only reduced the integrated cortisol response to this procedure by 43% and 23%, respectively. Therefore, in the present study it is likely that administration the local anaesthetic did not reduce the cortisol response to ring castration plus tailing further because of the continued noxious input from the tail.

The ring method and the castration clamp method have been combined to alleviate the pain-induced distress caused by ring castration and/or tailing (Kent *et al.*, 1993, 1995; Dinniss *et al.*, 1997a,b; Graham *et al.*, 1997; Molony *et al.*, 1997; Kent *et al.*, 1998) (Appendix 1). Application of the castration clamp across the full width of the neck of the scrotum and/or the tail is thought to disable the innervation from the scrotum plus testes and tail thereby alleviating the pain-induced distress caused by the ring method alone. The castration clamp applied in the traditional manner (Table 2.1) in combination with ring tailing and/or castration did not reduce the cortisol response to this procedure (Dinniss *et al.*, 1997a,b). However, the castration clamp applied for 6 or 10 seconds across the full width of the scrotum and/or tail in combination with ring castration and/or tailing reduced the cortisol response compared to the ring method in young lambs (Kent *et al.*, 1993, 1995; Graham *et al.*, 1997; Kent *et al.*, 1998). In the present study, applying a castration clamp for 6 seconds across the full width of the scrotum after ring castration plus tailing did not reduce the cortisol response to this method, whereas applying the clamp for 10 seconds did, but only at 60 minutes after treatment.

The ring plus clamp method was designed to alleviate the pain-induced distress caused by ring castration plus tailing by disabling the scrotal plus testicular innervation and thereby preventing the transmission of nociceptive impulses from the ischaemic tissues distal to the ring (Kent *et al.*, 1993; Dinniss *et al.*, 1997a, b). The castration clamp was initially applied for 6 seconds across the full width of the neck of the scrotum and the tail distal to the ring after ring castration plus tailing (Molony *et al.*, 1993). The magnitude of the cortisol distress response of 5-day-old lambs castrated and tailed using the ring plus clamp method was significantly reduced compared to the response caused by ring castration plus tailing alone (Kent *et al.*, 1993). In the present study, in

lambs aged between 21 and 42 days, applying the castration clamp across the full width of the scrotum for 6 seconds in combination with ring castration plus tailing only marginally reduced the peak cortisol concentration (Fig. 2.11).

In further studies the castration clamp was applied for 10 seconds across the full width of the scrotal neck and tail in combination with ring castration plus tailing to further alleviate the pain-induced distress caused by ring castration plus tailing (Kent *et al.*, 1995). Applying the castration clamp for 10 seconds in combination with ring castration plus tailing reduced the magnitude and the duration of the cortisol response to the ring method in young lambs (Kent *et al.*, 1995, 1998; Graham *et al.*, 1997). However, in the present study, when the castration clamp was applied for 10 seconds across the full width of the scrotum in combination with ring castration plus tailing in 21 to 42 day old lambs, there was a small significant effect on the peak cortisol concentration, but there was no significant difference in the duration or the integrated cortisol response compared to the ring method (Fig. 2.12).

Changes in the cortisol response to ring castration plus tailing and the ring plus clamp method were initially compared in lambs of three different ages (5, 21, and 42 days-old) (Kent *et al.*, 1993). The magnitude of the cortisol response of 5-day-old lambs castrated plus tailed by the ring plus clamp method was significantly reduced compared to lambs ring castrated plus tailed only (Kent *et al.*, 1993). However, there was only a marginal reduction in the cortisol response between treatments in 42-day-old lambs (Kent *et al.*, 1993), these results are similar to those found in the present study using lambs of a similar age (Fig. 2.11; Fig. 2.12). The age difference observed in the cortisol response may be due to maturational changes of the hypothalamic-pituitary-adrenal axis (HPA axis). The 5-day-old lambs studied by Kent *et al.* (1993) were found to have higher pre- and post treatment cortisol concentrations compared to 21 and 42-day-old lambs, which may be explained by the output of adrenal glands being greater per litre of blood plasma in younger and smaller lambs (Collu *et al.*, 1983). Also the responsiveness of the adrenal axis in lambs is not

comparable to mature ewes until they are over two weeks old (Moberg *et al.*, 1980).

Mellor and Murray (1989b) compared the cortisol and behavioural responses in two breeds of lambs to control handling, ring castration and/or tailing and an injection of ACTH at six ages between birth and seven days. In both breeds of lambs studied a marked increase in the cortisol response to noxious stimuli during the first one to three days after birth was observed. However, there was no corresponding age differences in the behavioural response to ACTH (Mellor and Murray, 1989a). The cortisol and behavioural response to different methods of castration plus tailing were compared in lambs of three ages, 5, 21, 42 days old (Kent *et al.*, 1993, Molony *et al.*, 1993). Five-day-old lambs showed higher pre- and post treatment cortisol values (Kent *et al.*, 1993), but the behavioural response showed no corresponding difference between age (Molony *et al.*, 1993). Therefore, the age difference in cortisol response to castration plus tail docking is likely to be the effect of age on the HPA axis rather than an indication of increased distress in younger animals. It is therefore possible to compare treatments using lambs of the same age, but comparison of treatments using lambs of different ages may lead to incorrect conclusions.

The castration clamp may not be as effective at completely disabling the innervation of the scrotum in older and larger lambs, due to the pressure of the jaws of the clamp being distributed over a larger area, thereby not effectively alleviating the pain-induced distress in older lambs.

In the present study, the castration clamp was only applied to the neck of the scrotum after ring castration plus tailing, but not to the tail. Kent *et al.* (1993, 1995, 1998) applied the castration clamp across the full width of the tail as well as the scrotal neck in combination with ring castration plus tailing. Therefore, by not including the application of the castration clamp across the tail in this study any benefits from applying the combined method to the scrotum may have been negated by the continued noxious input from the tail. The cortisol response to ring castration plus tailing in combination with the castration clamp applied for 10 seconds to each spermatic cord and the tail was compared with

not crushing the tail (Dinniss et al 1997b). There was no significant difference between the integrated cortisol response of the two methods (Dinniss et al 1997b). However, Dinniss *et al.* (1997b) applied the castration clamp using the traditional method, so that the spermatic cords would have been crushed, thereby preventing nociceptor transmission from ischaemic testes, but intact nerves between the crush lines could have carried nociceptor impulses from the ischaemic scrotal tissue. Therefore, the noxious input from the scrotal tissues may have negated any benefits from disabling the innervation from the tail. Applying the castration clamp across the width of the tail for 10 seconds in combination with ring tailing significantly reduced the magnitude and the duration of the cortisol response compared to ring tailing alone (Graham *et al.*, 1997). However, tail docking alone using a rubber ring causes relatively low levels of distress (Mellor and Murray, 1989a,b; Lester *et al.*, 1991a). Therefore, the noxious input from the tail after ring castration plus tailing in combination with applying the clamp across the neck of the scrotum may not significantly reduce the alleviation benefits of disabling the innervation from the scrotum.

Lambs were injected with ACTH to elicit a large cortisol response to assess whether the cortisol response to the different methods of castration plus tailing used in this study were limited by the secretory ability of the adrenal gland. The magnitude and the duration of the cortisol response to an injection ACTH was similar to the cortisol response evoked by ring castration plus tailing and castration plus tailing using the combined method (Fig. 2.10). However, the cortisol response elicited by an injection of ACTH in other studies has been significantly greater in magnitude than the cortisol response produced here by ring castration plus tailing (Mellor and Murray, 1989a; Lester *et al.*, 1991a; Dinniss *et al.*, 1997a). Therefore, it is unlikely that the cortisol response to different methods of castration plus tailing in the present study were limited by the secretory ability of the adrenal gland.

Previous studies comparing the cortisol response of lambs to ring castration plus tailing with castration plus tailing using the ring and clamp method have only used small numbers of lambs (n=6 or 7) (Kent *et al.*, 1993, 1995). Therefore, the results obtained from these studies may be the consequence of

a small sample size rather than a true statistical result. In study two 19 lambs were used per treatment group, except for lambs injected with ACTH (n=9). Therefore, the results from the present study may be a fairer representation of an average population. Variation in the castration clamps or the technique used to apply the clamps may also account for the differences in results (Hosie *et al.*, 1996). The difference in the breed of lambs used in these studies, Coopworths compared to Suffolk cross greyface lambs, may have also influenced these results.

In practice applying a castration clamp for 6 or 10 seconds after ring castration plus tailing is not very time efficient. Therefore, Dinniss *et al.* (1997a,b) applied the castration clamp for 1, 5, or 10 seconds in combination with ring castration to assess how effective applying the castration clamp for a shorter length of time would be effective in reducing the cortisol response to the ring method. Dinniss *et al.* (1997a, b) found no benefits of applying the castration clamp in combination with the ring method irrespective of the time the clamp was applied. However, Dinniss *et al.* (1997a, b) applied the castration clamp in the traditional manner. The traditional method of applying the castration clamp leaves a gap of uncrushed tissue between the crush lines caused by the application of the clamp. The application of the castration clamp in this way would disable the spermatic nerves, but noxious input from ischaemic scrotal tissues would continue. Therefore, the continued noxious input from the scrotum may have counteracted any benefits of applying the castration clamp.

The castration clamp is traditionally not applied across the full width of the scrotum to ensure collateral circulation remains intact and prevent tissue death of the scrotum which may lead to infection (Wood and Molony, 1992). By combining the ring method with the clamp method it is acceptable to apply the clamp across the full width of the scrotum as the ring will usually prevent inflammation and infection from ascending into the body. However, few studies have investigated the chronic effects of the different methods of castration and/or tailing in lambs.

In the present study, the healing rate of the castration wound caused by ring castration and ring plus clamp castration was compared. Three weeks after castration the scrotal sacs of lambs from both treatments were dry, hard and at varying stages of detachment. The scrotal sac of lambs castrated using the combined method necrosed, dried up, and eventually sloughed off faster than the scrotal sacs of ring castrated lambs (Fig. 2.13). The crushing action of the castration clamp, especially when applied for 10 seconds, would be likely to reduce the integrity of the tissues of the neck of the scrotum resulting in faster detachment of the scrotal sac in lambs castrated using the combined method compared to ring castrated lambs. The time for the scrotal sac of calves to slough off was found to be significantly reduced when calves were castrated using the combined method compared to the ring only method (Molony *et al.*, 1995). Neither method of castration resulted in death or complications during healing.

The possibility of infection from reusing needles and the time and cost of administering local anaesthetic prevents local anaesthetic from being used widely by farmers in the field for castration and/or tailing of lambs. However, a needleless high-pressure injection has been developed for administering local anaesthetic for castration and tailing of lambs (Kent *et al.*, 1998). The alleviation of the pain-induced distress using the needleless injection was compared with a conventional needle and syringe in lambs ring castrated or ring tailed. The needleless injection was as effective in administering local anaesthetic as the needle and syringe (Kent *et al.*, 1998). Therefore, the use of a needleless injection would reduce the potential risk for infection and make the use of local anaesthetic more practical in the field. Furthermore, having to handle animals twice, once to administer local anaesthetic and a second time to castrate and /or tail the lambs is impractical in the field. Previously, local anaesthetic has been administered 15-20 minutes prior to castration and/or tailing to allow enough time for the local anaesthetic to take full effect (Wood *et al.*, 1991; Dinniss *et al.*, 1997a). However, in the present study and others, local anaesthetic has been administered just before or just after castration and/or tailing (Graham *et al.*, 1997; Molony *et al.*, 1997; Kent *et al.*, 1998). Local anaesthetic injected into the tail only minutes prior to tailing virtually

eliminates the cortisol response elicited by ring tailing (Graham *et al.*, 1997). Local anaesthetic administered into the testes or scrotal neck just before or after castration reduces the cortisol response to castration (Molony *et al.*, 1997; Kent *et al.*, 1998). Administration of local anaesthetic into the neck of the scrotum is more effective than an injection into the testes (Kent *et al.*, 1998) (Fig. 2.8; Fig 2.9). Therefore, local anaesthetic administered just before or after ring castration and/or tailing can help to alleviate the pain-induced distress caused by these procedures.

The variation in castration clamp size, the technique used to apply the castration clamp and the variation in the size of animals could make the alleviation benefits of the combined method inconsistent. Evaluation of castration clamps showed that the crushing pressure applied by castration clamps even those of the same size could vary by over 100% (Hosie *et al.*, 1996). For the ring plus clamp method to be effective in the field, an optimum time to apply the castration clamp at an optimum pressure for different ages of lambs must be calculated. A certain amount of skill and awareness of the underlying anatomy are also required for effective application of the castration clamp (Wood and Molony, 1992).

Study one, alleviation of the pain-induced distress caused by ring castration plus tailing using local anaesthetic, is in press with the Australian Veterinary Journal.

## CHAPTER THREE: Alleviation of the Distress Caused by Dehorning in Calves

### 3.1 Chapter Summary

Changes in plasma cortisol concentrations were used in the present study as an index of pain-induced distress. Scoop dehorning in calves stimulates an increase in cortisol concentrations which peak after 30 minutes and then decline to a plateau before returning to pretreatment values about 7 hours after dehorning. Local anaesthetic given prior to dehorning virtually abolishes the cortisol response for the duration of action of the local anaesthetic. Once the local anaesthetic wears off cortisol concentrations rise resulting in an overall cortisol response equivalent to that caused by dehorning alone. It has been suggested that this delayed cortisol response is stimulated by inflammation-related pain which remains unresolved due to the local anaesthetic preventing the initial surge of cortisol, which is a potent anti-inflammatory agent (McMeekan *et al.*, 1998b). Therefore, in the present study the non-steroidal anti-inflammatory drugs (NSAIDs) ketoprofen and phenylbutazone or an endogenous cortisol surge stimulated by injecting ACTH were used to assess whether the delayed cortisol response observed in calves given local anaesthetic before dehorning is stimulated by unresolved inflammation-related pain. Furthermore, giving local anaesthetic prior to dehorning then cauterising amputation wounds was assessed over the 24 hour study period.

Injecting the local anaesthetic lignocaine (duration of action 2 hours) and after 2 hours giving bupivacaine (duration of action 3-4 hours) provided calves with analgesia for up to 5 hours after treatment. Local anaesthetic administered in this way plus ketoprofen given prior to scoop dehorning marginally reduced the delayed cortisol response observed once the local anaesthetic wore off, but giving local anaesthetic and phenylbutazone in a similar manner had no significant effect on the delayed cortisol response. An antinociceptive action and a greater anti-inflammatory potency of ketoprofen compared to phenylbutazone may explain why ketoprofen was more effective than

phenylbutazone at reducing this delayed cortisol response. However, in a previous study ketoprofen plus local anaesthetic (duration of action 2 hours) virtually abolished the cortisol response to dehorning (McMeekan *et al.*, 1998b). This suggests that the duration of action of the local anaesthetic may have some influence on the effectiveness of ketoprofen. ACTH was injected to approximately simulate the cortisol response to dehorning during the 5 hour period of action of the local anaesthetic. ACTH plus local anaesthetic administered prior to dehorning stimulated a cortisol surge that only marginally reduced the delayed cortisol response observed once the local anaesthetic wore off. Finally, injecting local anaesthetic prior to dehorning then cauterising the amputation wounds significantly reduced the integrated cortisol response to dehorning. Thus, in the present study, long acting local anaesthetic (5 hour duration of action) in combination with NSAIDs had minimal alleviating effects on the pain-induced distress caused by dehorning compared to local anaesthetic alone, but local anaesthetic and cautery provided effective pain-relief.

### **3.2 Introduction**

Dehorning of calves is conducted to reduce the risk of injury to stockhandlers and other animals, and to decrease fighting between horned animals (Stafford and Mellor, 1993). Dehorning of calves is also essential to reduce bruising and injury during transportation before slaughter and thereby reduce the pain-induced distress these injuries would cause. Bruising of cattle carcasses causes considerable economic losses to the meat industries of many countries (Shaw, 1976). The amount of bruised tissue trimmed from carcasses is significantly greater during transportation of horned or mixed (horned and dehorned) cattle compared to dehorned/polled animals (Meischke *et al.*, 1974; Shaw, 1976). The alternative to dehorning cattle is to breed the polled characteristic into cattle or introduce polled breeds (Armstrong, 1985). Dehorning, when it is conducted, is usually carried out on older calves or cattle and involves amputation of the horns. However, scoop dehorning can also be carried out on calves as young as 6 weeks old (Petrie *et al.*, 1996a). The main methods of

dehorning are scoop (Petrie *et al.*, 1996a; McMeekan *et al.*, 1997, 1998a, b; Sylvester *et al.*, 1998b), saw (Sylvester *et al.*, 1998b), guillotine (Loxton *et al.*, 1982; Carter *et al.*, 1983; Sylvester *et al.*, 1998b), and embryotomy wire (Sylvester *et al.*, 1998b) (Table 3.1). Prevention of horn growth by non-amputation methods in young calves with minimal horn development is known as disbudding. All methods of disbudding are designed to damage the germinal tissue in the horn bud. The main methods of disbudding include cautery (Laden *et al.*, 1985; Boandl *et al.*, 1989; Wohlt *et al.*, 1994; Petrie *et al.*, 1996a), caustic paste (Morisse *et al.*, 1995), chemical injection (Koger, 1976) or cryosurgery (Bengtsson *et al.*, 1996) (Table 3.1).

### 3.2.1 Distress

The definition of distress used in the present study includes all the physical and/or psychological components of an unpleasant experience (McMeekan, 1997). Distress is usually assessed in animals using physiological and/or behavioural indices. Changes in plasma cortisol concentrations has been used extensively to assess the distress response of calves in response to dehorning (Carter *et al.*, 1983; Laden *et al.*, 1985; Boandl *et al.*, 1989; Wohlt *et al.*, 1994; Morisse *et al.*, 1995; Petrie *et al.*, 1996a; McMeekan *et al.*, 1997, 1998a, b; Sylvester *et al.*, 1998a, b). Cortisol was used in the present study because of its well-established pattern following dehorning and to facilitate comparison of the present results with those from other work done in this area. Behavioural characteristics used to measure distress in calves due to dehorning and disbudding are standing, lying, grazing/ruminating, tail shaking and ear flicking (Morisse *et al.*, 1995; McMeekan, 1997). These behaviours were found to be distinctly different for at least the first 4-6 hours after treatment in dehorned and disbudded calves compared to control handled calves. Therefore, these behaviours are useful indicators of acute dehorning distress in calves (McMeekan, 1997). The acute cortisol and behavioural responses caused by dehorning or disbudding in calves continue for one and a half to 7-8 hours after treatment depending on the method of removal and the age of the calves (Laden *et al.*, 1985; Wohlt *et al.*, 1994; Morisse *et al.*, 1995; Petrie *et al.*, 1996a).

While the acute distress response caused by different dehorning methods can be measured using cortisol concentrations and/or behaviour, animals may also suffer from chronic pain due to hyperalgesia or neuroma formation which are more difficult to measure. All methods of dehorning without analgesia cause a nociceptor barrage, which may result in primary and secondary hyperalgesia. Hyperalgesia of the dehorning wounds and surrounding tissues can make animals more sensitive to otherwise benign stimuli, as hyperalgesia allows low level stimuli to produce pain by activating sensitised nociceptors (Johnston, 1996). Primary hyperalgesia occurs in the injured tissues due to direct effects of inflammatory mediators, released from the damaged tissues, on the nociceptors (Johnston, 1996). Secondary hyperalgesia can also occur in the uninjured tissues surrounding the injury probably due to changes both in the peripheral and central nervous system (Dahl and Kehlet, 1991). The formation of neuromas, unorganised bulbous or nodular masses of nerve fibres, is thought to be the inevitable result of peripheral nerve transection (French and Morgan, 1992). Therefore, as dehorning results in the severing of branches of the cornual nerve, neuroma formation is possible and may result in chronic pain. Neuromas are sensitive to a variety of stimuli, which can stimulate increased abnormal electrical activity and consequently increased pain can be associated with the neuromas (Bonica, 1990). The amount of this abnormal electrical activity varies with age, species and the animals' environment. Neuromas have been demonstrated after husbandry tissue damage in a range of species, including tail docking in pigs (Simonsen *et al.*, 1991) and dogs (Gross and Carr, 1990), and beak amputation in chickens (Gentle, 1986).

The productivity (weight gain) of calves after dehorning or disbudding has been measured to determine if different ages of calves or different methods of dehorning/disbudding result in chronic effects (Loxton *et al.*, 1982; Laden *et al.*, 1985). The productivity of calves and adults dehorned using a guillotine at four ages (4, 7, 19, and 30 months) was compared to polled cattle to establish the effect of age on recovery (Loxton *et al.*, 1982). There was no effect of age or of dehorning itself on the weight of calves or adults (Loxton *et al.*, 1982). Productivity (weight gain) of animals disbudded by cautery has also been compared with that of non-disbudded calves, to determine whether disbudding

results in any chronic effects (Laden *et al.*, 1985). Dehorning or disbudding did not reduce the weight of calves irrespective of the method used or age.

### **3.2.2 Different methods of dehorning and disbudding**

Different methods for dehorning and disbudding of calves and how they are conducted, are summarised in Table 3.1.

#### ***Amputation dehorning***

There are four main methods of amputation dehorning (scoop, saw, guillotine, and embryotomy wire). Scoop dehorning is the method dealt with most in the literature. Scoop dehorning is conducted using a device which has two interlocking semicircular blades attached to handles (Fig. 3.2). The act of closing the blades, by separating the handles and pushing down towards the calf's head, amputates the horn, adjacent skin and some underlying bone (Petrie *et al.*, 1996). Amputation of horn buds involves the cutting of tissues causing an immediate nociceptor barrage. The damaged tissues probably release inflammatory mediators, such as prostaglandins, bradykinin, histamine and serotonin, and substance P is released from nerve endings possibly resulting in pain caused by chemical stimulation of the nociceptors (Dahl and Kehlet, 1991), or in the case of prostaglandins, reducing pain threshold levels (Johnston, 1996). Amputation dehorning results in a similar cortisol response, irrespective of the method of amputation or the depth of the wound (McMeekan *et al.*, 1997; Sylvester *et al.*, 1998b).

#### ***Cautery disbudding***

Cautery disbudding involves cauterisation of the skin and dermis (including germinal tissue) around the horn buds with a heated disbudding iron which is applied over each horn bud for 3-5 seconds (Petrie *et al.*, 1996a). Disbudding using cautery arrests the growth of the horn buds due to damage to the germinal tissue and cessation of blood flow (Wohlt *et al.*, 1994). Cautery disbudding may cause third degree burns. Third degree burns result in the complete destruction of the skin, and are associated with little or no pain in humans (Bonica, 1990). Sensory nerve receptors including nociceptors in the

dermis are destroyed by third degree burns resulting in a loss of sensation. Therefore, as cautery causes third degree burns the acute pain associated with disbudding may be reduced. The plasma cortisol concentrations elicited by cautery disbudding first increase significantly above and then return to pretreatment values between 1.5 and 4 hours after treatment (Laden *et al.*, 1985; Wohlt *et al.*, 1994; Morisse *et al.*, 1995; Petrie *et al.*, 1996a). Cautery disbudding caused the least pain-induced distress, as measured by cortisol, compared to other methods of disbudding and dehorning (Morisse *et al.*, 1995; Petrie *et al.*, 1996a).

### ***Chemical and caustic disbudding***

Chemical disbudding involves injecting a chemical into or under the horn buds. One example of chemical disbudding is an injection of calcium chloride solution under the centre of the horn buds (Koger, 1976). This causes necrosis of the horn bud, thereby preventing horn growth or causing the horn buds to slough off. However, if the concentration of solution is not adequate or the injection is given incorrectly, the result can be incomplete disbudding (Koger, 1976). Disbudding can be conducted by rubbing a caustic preparation, such as potassium hydroxide, on the horn buds for 2 minutes, which results in necrosis of the horn buds (Morisse *et al.*, 1995). Chemical disbudding will cause mechanical stimulation of the nociceptors due to the pressure of the fluid being injected, also in both chemical and caustic disbudding the applied chemicals may stimulate the nociceptors. Nociception would continue until the tissues are destroyed or the corrosive chemical is neutralised. Chemical disbudding caused a larger cortisol distress response than cautery disbudding (Morisse *et al.*, 1995).

### ***Cryosurgical disbudding***

Cryosurgical disbudding of calves involves the tissues of the horn buds being frozen to below  $-20^{\circ}\text{C}$ . Freezing causes formation of intracellular ice crystals leading to cell destruction and eventually, after some weeks, the dead tissue will slough off (Bengtsson *et al.*, 1996). Equipment intended for cryosurgery in humans, such as for the removal of warts, is used for disbudding: a metal

cryoprobe cooled by nitrous oxide is placed against the horn buds or liquid nitrogen is applied directly onto the tissue. Although the cryoprobe method only caused damage to the superficial layers of the horn bud, the liquid nitrogen method caused total necrosis of the horn bud (Bengtsson *et al.*, 1996). Cryosurgical disbudding takes 10 minutes for each calf, hence making it impractical for on-farm use with large numbers of animals.

### **3.2.3 Alleviation strategies for dehorning and disbudding in calves**

Strategies for minimising the pain-induced distress caused by dehorning or disbudding of calves include cauterisation of the horn amputation wounds with or without local anaesthetic and the administration of analgesics such as local anaesthetic and NSAIDs.

#### ***Analgesia***

Administration of local anaesthetic, NSAIDs or local anaesthetic plus NSAIDs have been used to alleviate the pain-induced distress caused by dehorning or disbudding (Boandl *et al.*, 1989; Morisse *et al.*, 1995; Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a, b; Sylvester *et al.*, 1998a). Administration of local anaesthetic 15-20 minutes prior to dehorning virtually eliminates the cortisol response to dehorning throughout the duration of action of the local anaesthetic, but once the local anaesthetic wears off the cortisol concentrations increase, irrespective of whether the duration of action of the local anaesthetic is 2, 4 or 8 hours (Petrie *et al.*, 1996a; ; McMeekan *et al.*, 1998a; Sylvester *et al.*, 1998a). The integrated cortisol responses of calves aged 1.5 to 4 months dehorned with and without local anaesthetic are often similar (Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a), but there is one report in 6 month-old calves where the overall cortisol response was reduced by about half using local anaesthetic with a 2 hour duration of action (Sylvester *et al.*, 1998a).

It is usually impractical to handle calves twice, once to inject local anaesthetic and secondly to carry out dehorning, so the effects of giving local anaesthetic immediately prior to dehorning have been evaluated (McMeekan *et al.*, 1998a). There was no difference in the cortisol response observed between calves

injected with local anaesthetic just prior to dehorning and calves given local anaesthetic 20 minutes prior to dehorning (McMeekan *et al.*, 1998a). However, calves injected with local anaesthetic just prior to dehorning displayed behavioural responses to the act of horn amputation whereas calves injected with local anaesthetic 20 minutes prior to dehorning did not (McMeekan *et al.*, 1997). The immediate behavioural response of the former group suggests that although there was no difference in plasma cortisol concentrations at this time, the local anaesthetic had not had enough time to take full effect. Therefore, measuring catecholamine concentrations or heart rate may be more effective in detecting an immediate distress response to dehorning as the response time for cortisol release is slower (McMeekan *et al.*, 1998a).

Calves scoop dehorned in combination with ketoprofen exhibited an initial cortisol response similar to that of calves scoop dehorned without pain-relief, but the cortisol concentrations returned to pretreatment values 2 hours after dehorning (McMeekan *et al.*, 1998b). In the same study local anaesthetic (duration of action 2 hours) plus ketoprofen administered 20 minutes prior to dehorning virtually abolished the cortisol response to dehorning. Furthermore, local anaesthetic (duration of action 4 hours) in combination with ketoprofen given prior to dehorning virtually abolished the cortisol response to dehorning for the duration of action of the local anaesthetic. Once the local anaesthetic began to wear off, after 4 hours, the cortisol concentration gradually increased, but was only significantly greater than control values at 9.33 hours after dehorning (McMeekan *et al.*, 1998b).

### **Cautery**

Cauterising the wounds caused by amputation dehorning of calves has been used to alleviate the pain-induced distress caused by dehorning. Such cautery marginally reduced the magnitude of the cortisol response to scoop dehorning, but not the duration (Sylvester *et al.*, 1998a). Local anaesthetic in combination with cauterising the dehorning wounds has been used to further alleviate the pain-induced distress caused by dehorning, where it virtually abolished the cortisol response to scoop dehorning (Sylvester *et al.*, 1998a).

### 3.2.4 Aims of this Study

Scoop dehorning causes pain-induced distress in calves for up to 7 hours as inferred by changes in their plasma cortisol concentrations. Local anaesthetic given prior to dehorning virtually abolishes the cortisol response to dehorning throughout the duration of action of the local anaesthetic, but when the local anaesthetic begins to wear off the cortisol concentrations begin to rise returning to pretreatment values after calves dehorned without analgesia (Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a, b ; Sylvester *et al.*, 1998b). It has been postulated that the cortisol response to dehorning consists of two phases, an initial peak due primarily to the nociceptor barrage caused by amputating the horns, followed by a plateau phase due mainly to inflammation (McMeekan *et al.*, 1998b). Consequently, the delayed cortisol response observed in calves dehorned with local anaesthetic may be stimulated by inflammation-related pain that remains unresolved due to the prevention of the usual dehorning induced cortisol response by the prior injection of local anaesthetic, as cortisol is a potent anti-inflammatory agent.

Local anaesthetic (duration of action 2 hours) in combination with the NSAID ketoprofen given prior to dehorning virtually abolishes the cortisol response to dehorning (McMeekan *et al.*, 1998b). However, local anaesthetic (duration of action 4 hours) plus ketoprofen virtually abolished the cortisol response to dehorning throughout the period of local anaesthetic action, once the local anaesthetic began to wear off the cortisol concentrations gradually increased (McMeekan *et al.*, 1998b). Therefore, in the present study the effectiveness of ketoprofen in combination with long acting local anaesthetic on the dehorning distress response over a 24-hour period was assessed.

NSAIDs have two different analgesic actions, a peripheral anti-inflammatory action and a central antinociceptive action (McCormack and Brune, 1991). The

Method	Technique
Scoop dehorning	Scoop dehorning is carried out using a device which has two interlocking semicircular blades attached to handles. The act of closing the blades, by separating the handles and pushing down towards the calf's head, amputates the horn, adjacent skin and some underlying bone (Petrie <i>et al.</i> , 1996a).
Saw	Horns are amputated using a butcher's saw (Sylvester <i>et al.</i> , 1998b).
Guillotine shears	Horns are amputated using small shears (Sylvester <i>et al.</i> , 1998b).
Embryotomy wire	Horns are amputated using embryotomy wire (Sylvester <i>et al.</i> , 1998b).
Cautery disbudding	This involves cauterising the skin and dermis (including germinal tissue) around the horn buds with a heated disbudding iron applied over each horn bud for 3-5 seconds (Petrie <i>et al.</i> , 1996a).
Caustic disbudding	This involves spreading potassium hydroxide on the horn buds for 2 minutes, which results in necrosis of the horn buds (Morisse <i>et al.</i> , 1995).
Chemical disbudding	This involves injecting a chemical into or under the horn buds, i.e. calcium chloride solution, resulting in necrosis of the horn buds, thereby preventing horn growth (Koger, 1976).
Cryosurgery	Cryosurgical disbudding involves freezing the horn buds to below $-20^{\circ}\text{C}$ , causing formation of intracellular ice crystals leading to cell destruction of the horn bud (Bengtsson <i>et al.</i> , 1996).

**Table 3.1:** Description of different methods of dehorning and disbudding in calves.

proportions of these actions differ between NSAIDs. Ketoprofen apparently has both actions, whereas phenylbutazone may have a predominantly anti-inflammatory action and very little direct antinociceptive action. Therefore, in the present study phenylbutazone was administered in combination with local anaesthetic prior to dehorning to assess whether the rise in cortisol concentrations observed once the local anaesthetic wears off is due mainly to inflammation-related pain.

Cortisol is a natural and potent anti-inflammatory agent, the secretion of which is reduced in calves given local anaesthetic prior to dehorning. In the present study, local anaesthetic and ACTH were given prior to dehorning to approximately simulate the cortisol response to dehorning during the 5 hour period of local anaesthetic action. Previous studies have shown that an exogenous injection of ACTH results in an integrated cortisol response equivalent in magnitude but not in pattern to that of scoop dehorning (Petrie *et al.*, 1996a; Sylvester *et al.*, 1998b). The purpose here was to assess whether the cortisol response stimulated by dehorning contributes to the resolution of inflammation-related pain associated with the amputation wounds.

Cauterising the scoop wounds in combination with giving local anaesthetic (duration of action 2 hours) abolished the cortisol response to scoop dehorning (Sylvester *et al.*, 1998a). This was an unexpected finding. Therefore, in the present study the amputation wounds of calves were cauterised in combination with giving a long acting local anaesthetic and blood samples were taken for up to 24 hours after treatment to confirm the findings of Sylvester *et al.* (1998a).

The plasma cortisol concentrations were observed throughout the 24 hour period after treatment in all animals to assess changes in the cortisol response to these treatments, especially changes between 10 and 24 hours after dehorning, a period which has not been previously studied.

### 3.3 Materials and Methods

#### 3.3.1 Animals

One hundred and three Friesian calves, 3-4 months of age, weighing between 56 and 169 Kg (mean 96 Kg) were used in this study, carried out over 4 trial days. The day before the trial calves, were brought into an open-sided shed with sawdust bedding where they were weighed and divided into 2 pens, 15 animals per pen. Each calf was numbered with a scorable marker for easy identification. Calves had access to food and water over night, but not during the trial period. One hour before commencement of the trial, calves were grouped together in smaller pens (10.4m<sup>2</sup>) located in the same shed (Fig. 3.1).

#### 3.3.2 Blood sampling

Blood samples (10ml) were taken by venepuncture from either jugular vein at 30 minutes before treatment, just prior to treatment, then every 30 minutes for 16 hours, then hourly for another 8 hours until the end of the trial, 24 hours after treatment. During blood sampling the calves were restrained against the side of the pen by two handlers, while a third person took the blood sample. The whole procedure of capture to the time the animal was released usually lasted no more than 20 seconds. Animals were sampled in the same order throughout the experiment.

#### 3.3.3 Treatments

The treatments were carried out immediately after the second blood sample (time 0) was taken. Two people restrained the calf while a third person (a veterinarian) carried out the treatment. The treatments were carried out by the same person for each trial. No animals were re-used in this study.



**Fig. 3.1:** Pen layout during trials.



Fig. 3.2: Scoop dehorner.



Fig. 3.3: Horn amputation using the scoop.



**Fig. 3.4:** Injection of local anaesthetic.



**Fig. 3.5:** Cauterising iron.

### ***Control handling ('control')***

The animals were restrained and the horns were massaged but left intact.

### ***Local anaesthetic (5 hours) plus Ketoprofen ('LA5:K control')***

Local anaesthetic (6 ml of 2% Lignocaine hydrochloride; Nopaine, Ethical Agents, Auckland) was injected around each cornual nerve, midway along the lateral edge of the frontal bone crest (McMeekan *et al.*, 1998a, b) (Fig. 3.4), and at the same time ketoprofen (3 ml of 10% Ketofen, Rhone Merieux, France) was injected intravenously into a jugular vein of calves 30 minutes prior to horn buds being massaged but not amputated. Two hours after treatment local anaesthetic (6 ml of 0.25% bupivacaine hydrochloride; Marcain; Astra Pharmaceutical Pty Ltd, Australia) was injected in the same way as for lignocaine. The duration of local anaesthetic action was designed to be approximately 5 hours.

### ***Local anaesthetic (5 hours) plus Phenylbutazone ('LA5:P control')***

Local anaesthetic was administered as described for LA5:K control calves. At the same time as the first injection of local anaesthetic was administered phenylbutazone (3 ml of phenylbutazone 200 mg/ml, Techvet laboratories L.t.d.) was injected intravenously into a jugular vein of calves 30 minutes prior to horn buds being massaged but not amputated.

### ***Local anaesthetic (5 hours) plus Adrenocorticotrophic hormone ('LA5:ACTH')***

Local anaesthetic was administered as described for LA5:K control calves. At time 0 synthetic adrenocorticotrophic hormone (Synacthen; Ciba pharmaceutical's, Auckland) was injected intravenously into a jugular vein at a dose of 0.28 µg/kg to elicit a marked cortisol response (Petrie *et al.*, 1996a, b; Sylvester *et al.*, 1998b).

### ***Dehorning by scoop ('DH')***

The horns were removed with a standard dehorning scoop (Barnes Dehorner, Stones, USA) consisting of two interlocking semicircular blades each of

diameter 56 mm x 38 mm attached to handles (Fig. 3.2). The act of closing the blades, by separating the handles and pushing down towards the calf's head, amputated the horn, adjacent skin and some underlying bone (Petrie *et al.*, 1996b) (Fig. 3.3).

***Local anaesthetic (5 hours) plus dehorning ('LA5: DH')***

Local anaesthetic was administered as described for LA5:K control calves. Dehorning was carried out as described for the DH calves 30 minutes after the first injection of local anaesthetic.

***Local anaesthetic (5 hours) plus Ketoprofen plus dehorning ('LA5:K DH')***

Local anaesthetic and ketoprofen were administered as described for LA5:K control calves. Dehorning was carried out as described for the DH calves 30 minutes after the first injection of local anaesthetic.

***Local anaesthetic (5 hours) plus Phenylbutazone plus dehorning ('LA5:P DH')***

Local anaesthetic and phenylbutazone were administered as described for LA5:P control calves. Dehorning was carried out as described for the DH calves 30 minutes after the first injection of local anaesthetic.

***Local anaesthetic (5 hours) plus Adrenocorticotrophic hormone plus dehorning ('LA5:ACTH DH')***

Local anaesthetic and ACTH were administered as described for LA5: ACTH calves. Dehorning was carried out as described for the DH calves 30 minutes after the first injection of local anaesthetic.

***Adrenocorticotrophic hormone plus dehorning (at 6 hours) ('ACTH / DH')***

Synthetic adrenocorticotrophic hormone was injected intravenously into the jugular vein of calves at time 0 and then dehorning conducted 6 hours later.

### ***Adrenocorticotrophic hormone plus adrenocorticotrophic hormone (at 6 hours) ('ACTH / ACTH')***

Synthetic adrenocorticotrophic hormone (0.28 µg/kg) was injected intravenously into a jugular vein of calves at time 0 and then again 6 hours later.

### ***Local anaesthetic (5 hours) plus dehorning plus Cautery ('LA5: DH + cautery')***

Local anaesthetic was administered as described for LA5:K control calves. Dehorning was carried out as described for the DH calves 30 minutes after the first injection of local anaesthetic. The scoop wounds were then cauterised using a custom built cautery iron (Fig. 3.5). The iron was shaped as a half sphere with a diameter of 30 mm. When sufficiently hot to burn wood it was applied firmly over the wound to cauterise the tissues. This usually took about 6 seconds per wound (Sylvester *et al.*, 1998a).

#### **3.3.4 Plasma cortisol assay**

The blood was collected into heparinised vacutainers, centrifuged and the plasma separated and stored at  $-20^{\circ}\text{C}$  until required. Cortisol concentrations of plasma were determined using a non-extraction tritium radio-immuno-assay method (Endocrine Sciences, 4301 Lost Hills Rd, CA 91301). The lowest detectable concentration was 1.0 nmol/L. The intra-assay and inter-assay coefficients of variation were 7% and 13%, respectively.

#### **3.3.5 Integrated cortisol responses**

The integrated cortisol response was calculated to determine changes in the magnitude and duration of the cortisol response after the application of the treatments. The integrated cortisol response is defined as the area between a horizontal line drawn through the pretreatment concentration and the cortisol curve during the period when the concentrations were greater than the pretreatment values (Mellor and Murray, 1989a).

### 3.3.6 Statistical analysis

The cortisol concentrations are presented as the mean  $\pm$  standard error of the mean (SEM) at each sample time. To compensate for individual variation, the pretreatment value was subtracted from the concentration of all subsequent samples. All graphical presentations represent changes in the mean concentration after –30 minutes. Significant differences between means were determined using unpaired t-test with Welch's correction for unequal variance and one-way analysis of variance (ANOVA) (GraphPad software Prism V 2.01); (any data was considered significantly different when  $P < 0.05$ ). Statistical analysis including repeated measures analysis of variance (SPSS for windows V 8.0) were also conducted on  $\log_{10}$  transformed data. As there were very few differences in the significance of outcomes using untransformed and transformed data, untransformed data have been presented.

## 3.4 Results

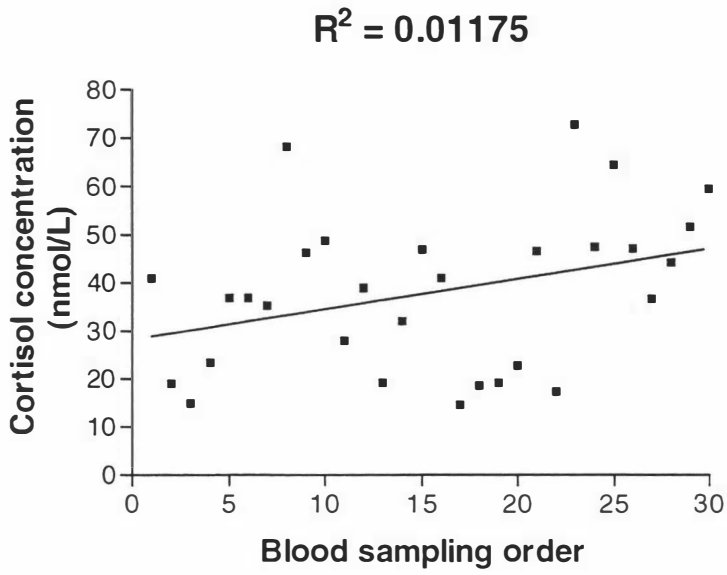
The mean pretreatment cortisol concentrations were significantly different between some groups ( $p < 0.05$ ) (Table 3.2). These differences were small in comparison with the responses of calves to dehorning without local anaesthetic or an anti-inflammatory drug. Blood sampling order was found to have no significant effect on cortisol pretreatment values (Fig. 3.6.).

### ***Control***

Control handling of calves did not cause a significant change in plasma cortisol concentration, throughout the period of study (Fig. 3.7).

### ***LA5:K control***

A significant elevation of 33 nmol/L above the mean pretreatment concentration ( $p < 0.05$ ) occurred at 0 hour, and 30 minutes after administration of lignocaine and ketoprofen. Thereafter, the mean cortisol concentration did not differ significantly from pretreatment levels (Fig. 3.7).



**Fig. 3.6:** Relationship between plasma cortisol concentration and the order in which the pretreatment blood samples were taken.

Treatment	n	Plasma cortisol concentration (nmol/L)
Control	8	38 ± 5.3
LA5:K control	9	46 ± 5.8
LA5:P control	8	27 ± 7.8
LA5: ACTH	8	62 ± 10 <sup>cd</sup>
DH	8	20 ± 4.6 <sup>ab</sup>
LA5: DH	8	22 ± 6.4 <sup>bd</sup>
LA5:ACTH DH	9	46 ± 9.4 <sup>e</sup>
LA5:K DH	8	27 ± 9.0 <sup>d</sup>
LA5:P DH	10	39 ± 8.5
ACTH / DH	9	35 ± 6.4 <sup>de</sup>
ACTH / ACTH	8	50 ± 7.0 <sup>cf</sup>
LA5:DH + Cautery	10	42 ± 4.6 <sup>ef</sup>

**Table 3.2:** Pretreatment (t=-30min) cortisol concentrations (mean ± SEM) in calves. [a - significantly different from control; b - significantly different from LA5:K control; c - significantly different from LA5:P control; d - significantly different from LA5:ACTH control; e – significantly different from DH; and f – significantly different from LA5: DH (p < 0.05)].

There were no significant differences between the mean cortisol concentrations of control and LA5:K control calves, except at 7.5 hours (Fig. 3.7).

#### ***LA5:P control***

A significant elevation of 64 nmol/L above the mean pretreatment concentration ( $p<0.05$ ) occurred at 0 hours, and 30 minutes after administration of lignocaine and phenylbutazone. Thereafter, the mean cortisol concentration did not differ significantly from pretreatment levels (Fig. 3.7).

There were no significant differences between the mean cortisol concentrations of control and LA5:P control calves, except at 0.0 and 13 hours (Fig. 3.7).

#### ***LA5: ACTH***

Injection of ACTH in LA5: ACTH calves caused a marked significant increase in the mean cortisol concentration within the first 30 minutes after injection ( $p<0.05$ ). The mean concentration peaked at 179 nmol/L at 1.5 hours and had returned to pretreatment values by 3 hours after injection (Fig. 3.7). Thereafter, the mean cortisol concentration deviated significantly from the pretreatment level between 5-8 hours and between 11-15 hours when it fell to 30–40 nmol/L below it ( $p<0.05$ ).

The mean cortisol concentrations of control and LA5: ACTH control calves differed significantly between 0 and 2.5 hours, between 5.5 and 8 hours, and at 18 hours after ACTH injection (Fig. 3.7).

#### ***DH***

During the first 30 minutes after dehorning there was a marked rise ( $p<0.05$ ) in cortisol concentration to a peak of 137 nmol/L above the pretreatment level (Fig. 3.8). This was followed by a fall to plateau values, which were maintained between 1.5 and 5 hours. The mean cortisol concentration then returned to pretreatment levels by 7 hours after dehorning. Thereafter, the mean cortisol concentration did not deviate significantly from the pretreatment level throughout the rest of the study period (Fig. 3.8).

The mean cortisol concentration of dehorned calves was significantly different from those of control calves between 0 and 6 hours after dehorning, then again between 9 and 9.5, and at 13 and at 15 hours (Fig. 3.8).

#### ***LA5: DH***

In calves which were dehorned 30 minutes after administration of local anaesthetic, a significant rise in mean cortisol concentrations began at 5 hours and returned to pretreatment values by 11 hours after treatment (Fig. 3.8). Between 5.5 and 10 hours the mean cortisol concentrations were significantly higher than pretreatment values, peaking at 128 nmol/L. Thereafter, the mean cortisol concentration did not deviate significantly from the pretreatment level throughout the subsequent period of study.

The mean cortisol concentration of LA5: DH calves was not significantly different from those of control calves during the first 5 hours and from 9 hours after dehorning (Fig. 3.8). Between 6 and 7 hours after treatment the mean cortisol concentrations were significantly greater than values from DH calves ( $p < 0.05$ ) (Fig. 3.8). Also, the mean cortisol concentration of LA5: DH calves was significantly elevated compared to those of LA5: ACTH DH calves between 5.5 and 6 hours after dehorning ( $p < 0.05$ ) (Figure 3.11), and was significantly elevated compared to that of LA5: K DH calves at 6 hours ( $p < 0.05$ ) (Fig. 3.9).

#### ***LA5:K DH***

The mean cortisol concentration was 26 nmol/L above the pretreatment value 30 minutes after administration of lignocaine and ketoprofen (time 0); subsequently the mean cortisol concentration was significantly ( $p < 0.05$ ) above pretreatment values between 5 and 11 hours only (Fig. 3.9). The cortisol concentration peaked at 65 nmol/L at 6.5 hours after treatment.

The mean cortisol concentration of LA5:K DH calves was significantly elevated compared to that in LA5:K control calves between 6.5 and 12 hours, and at 16 and 18 hours after treatment ( $p < 0.05$ ). The mean cortisol concentration of LA5:K DH calves was significantly elevated compared to DH calves at 6.5 and 7 hours and between 8 and 9 hours ( $p < 0.05$ ). The mean cortisol concentration

was of LA5:K DH calves was significantly lower than that in LA5: DH calves at 6 hours after treatment ( $p<0.05$ ) (Fig. 3.9).

### **LA5:P DH**

The mean cortisol concentration was 62 nmol/L above ( $p<0.05$ ) the pretreatment value 30 minutes after administration of lignocaine and phenylbutazone (time 0), returning to pretreatment levels at 1.5 hours ( $p<0.05$ ). The mean cortisol concentration increased significantly to 141 nmol/L at 6.5 hours, returning to pretreatment values at 9 hours ( $p<0.05$ ). Thereafter, the mean cortisol concentration did not deviate significantly from the pretreatment levels throughout the subsequent period of study (Fig. 3.10).

The mean cortisol concentration of LA5:P DH calves was significantly elevated compared to LA5:P control calves at 6, 6.5 and 7.5 hours after treatment ( $p<0.05$ ), and was significantly elevated compared to DH calves at 6.5 and 7 hours ( $p<0.05$ ), but was significantly less than DH calves between 30 minutes and 4.5 hours. The mean cortisol concentration was not significantly different from LA5: DH during the study period (Fig. 3.10).

### **LA5:ACTH DH**

There were two transient peaks in the plasma cortisol concentration in LA5: ACTH DH calves; highest values were at 141 nmol/L at 1.5 hours and 61 nmol/L at 6.5 hours. The first peak occurred between 0 and 4 hours and the other peak between 5.5 and 10 hours. Otherwise the cortisol concentrations were not significantly different from pretreatment values (Fig. 3.11).

The mean cortisol concentrations in LA5: ACTH DH calves were significantly higher than those in LA5: ACTH calves between 6 and 9 hours after treatment ( $p<0.05$ ) (Fig. 3.11). The mean cortisol concentrations in LA5: ACTH DH calves were significantly higher compared to LA5: DH calves between 0.5 and 2.5 hours, and significantly lower between 5.5 and 6 hours after treatment ( $p<0.05$ ) (Figure 3.11).

### ***ACTH / ACTH***

Injection of ACTH caused a marked significant increase within the first 30 minutes after injection ( $p < 0.05$ ), which peaked at 134 nmol/L above the pretreatment level by 1 hour (Fig. 3.12). The mean cortisol concentration returned to the pretreatment value by 3.5 hours. A second significant elevation of 148 nmol/L above the mean pretreatment concentration ( $p < 0.05$ ) occurred between 6 and 9.5 hours in response to the second dose of ACTH at 6 hours. Thereafter, the mean cortisol concentration did not deviate significantly from the pretreatment level, except between 10.5 and 13, and at 18 hours ( $p < 0.05$ ) (Fig. 3.12).

### ***ACTH / DH***

Injection of ACTH caused a marked significant increase in the cortisol concentrations within the first 30 minutes after injection ( $p < 0.05$ ), reaching a peak at 151 nmol/L above the pretreatment level after 1 hour. The mean cortisol concentration returned to pretreatment values by 3.5 hours. After dehorning at 6 hours the cortisol concentrations began to rise significantly above pretreatment value, reaching a plateau, before returning to pretreatment concentration ( $p < 0.05$ ) at 10 hours. The concentration then returned to pretreatment values at 11.5 hours where it remained throughout the subsequent period of study (Fig. 3.13).

### ***LA5:DH + Cautery***

After the administration of local anaesthetic (lignocaine) the mean cortisol concentration increased significantly above pretreatment values. The cortisol concentrations remained elevated above pretreatment values between 0.0 and 0.5 hours and between 3 and 5 hours. Thereafter, the mean cortisol concentrations did not deviate significantly above pretreatment levels (Fig. 3.15).

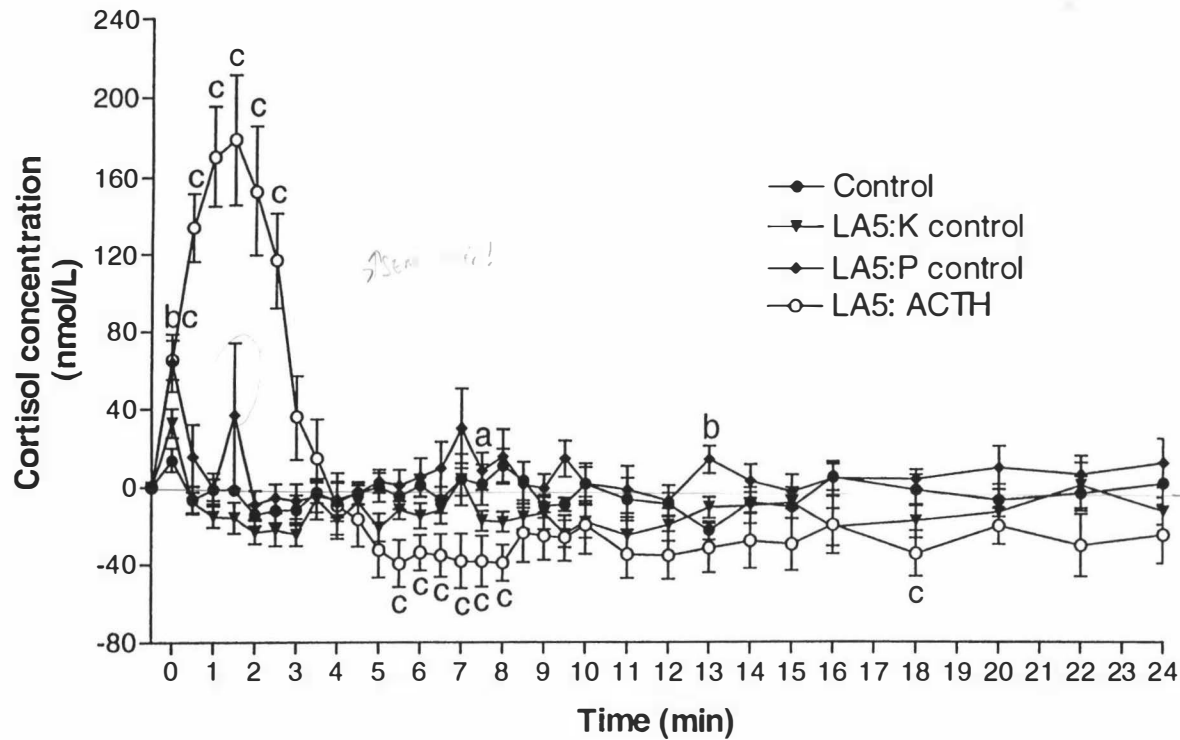
The mean cortisol concentrations of LA5:DH + cautery calves were only significantly higher than LA5: DH calves between 3.5 and 4.5 hours, thereafter

the cortisol concentrations of LA5: DH calves was significantly greater than LA5: DH + cautery calves from 6-8 to 9-9.5 hours (Fig. 3.15).

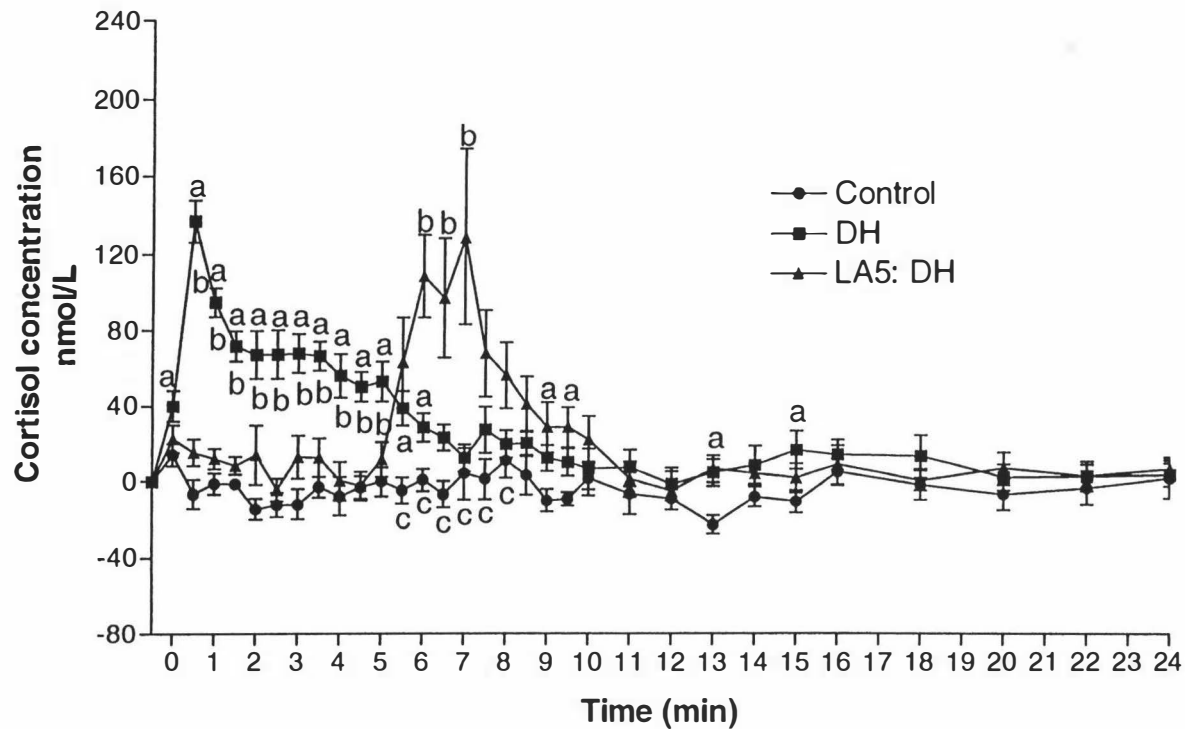
### **Integrated cortisol responses**

The integrated cortisol response was calculated for all treatments over the 24-hour study period. The integrated cortisol response was also calculated for specific periods for particular treatments (Table 3.3). The total integrated cortisol response of control handled calves was significantly different from LA5: ACTH, DH, LA5: DH, LA5:P DH, ACTH / DH and ACTH / ACTH calves over the 24 hour study period ( $p < 0.05$ ). The integrated cortisol response caused by dehorning (DH calves) was significantly greater than the response to LA5: DH + cautery ( $p < 0.05$ ). Furthermore, the integrated cortisol response to LA5: DH was similar to the integrated cortisol response to LA5:K DH and LA5:P DH treatments.

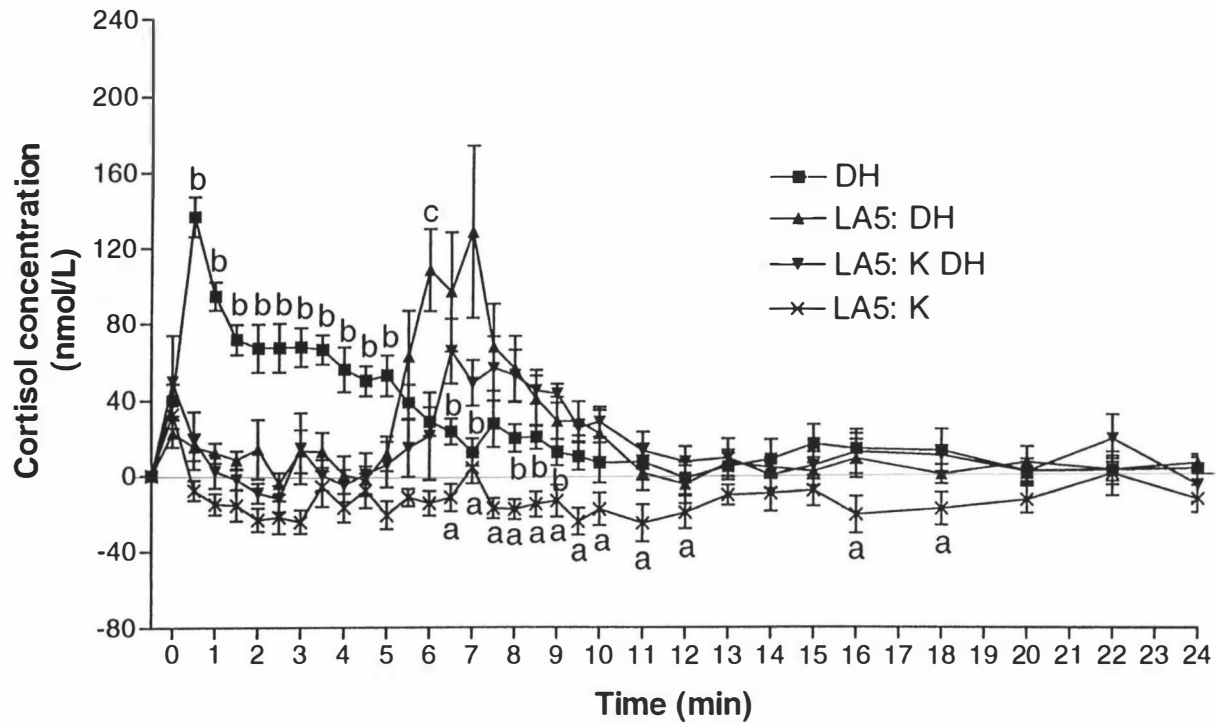
The integrated cortisol response caused by scoop dehorning was similar to the cortisol response of LA5: DH calves over the whole 24-hour study period. Furthermore, the integrated cortisol response caused by dehorning between 0 and 7 hours, was similar to the integrated cortisol response of the delayed cortisol response in LA5: DH calves, between 4.5 and 11 hours. The integrated cortisol response of LA5: ACTH DH calves was similar to LA5: DH calves over the 24-hour study period and the integrated cortisol response to LA5:ACTH DH between 5.5 and 11 hours only was similar to the integrated cortisol response of LA5: DH calves between 4.5 and 11 hours only. Furthermore, the integrated dehorning response, between 0 and 7 hours, was not significantly different from the integrated dehorning response of ACTH / DH calves, between 6 and 13 hours.



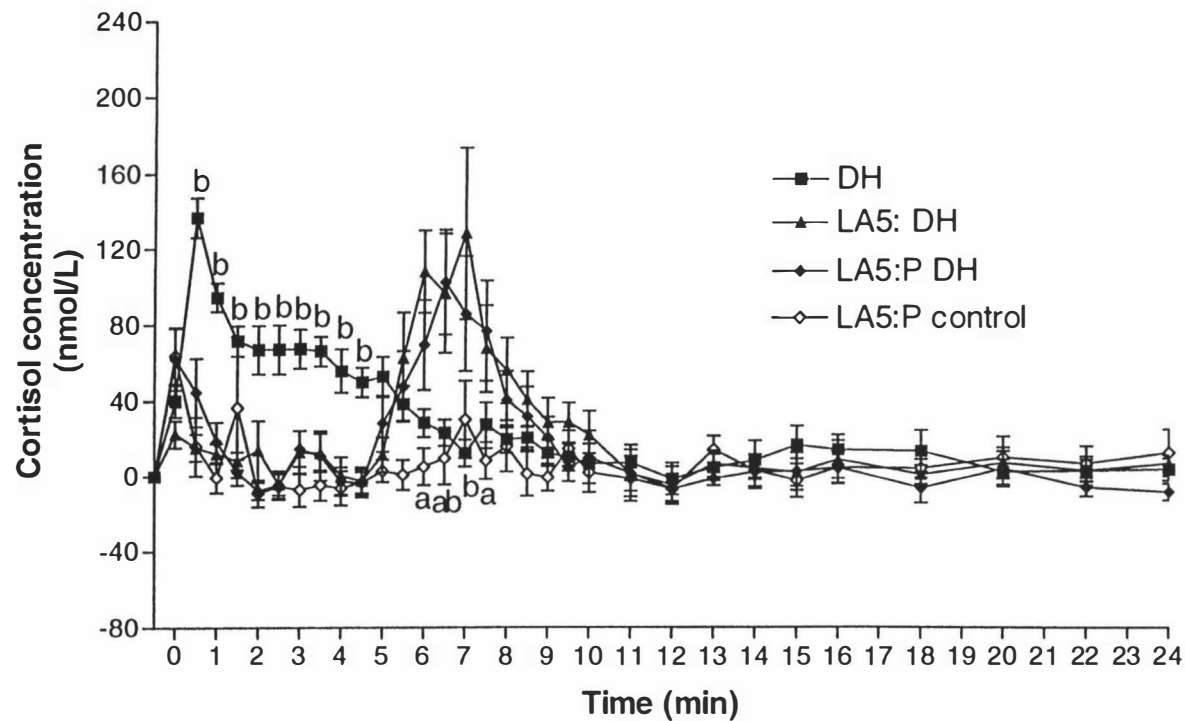
**Fig. 3.7:** Changes in plasma cortisol concentration in calves in response to control handling, local anaesthetic administration, NSAIDs administration, and ACTH administration. [a – LA5: K control significantly different from control; b – LA5: P control significantly different from control; c – LA5: ACTH significantly different from control]. *p < 0.05!*



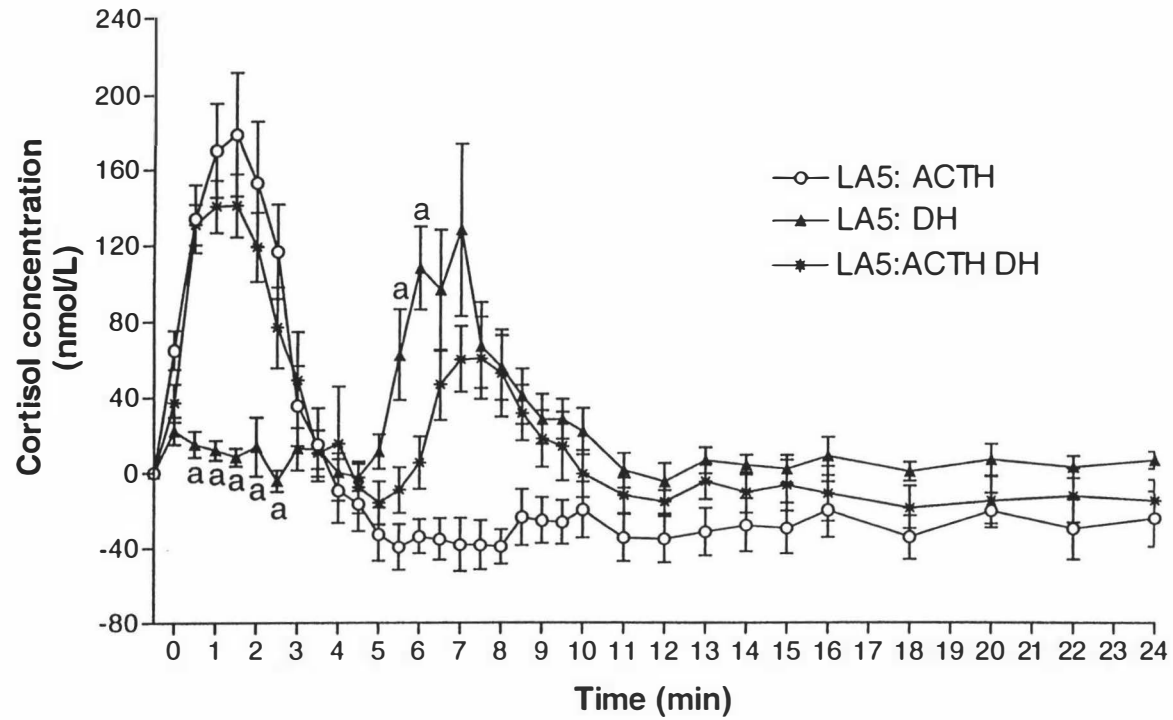
**Fig. 3.8:** Changes in plasma cortisol concentration in calves in response to control handling (control), dehorning (DH), and local anaesthetic (5 hours) plus dehorning (LA5: DH). [a - DH is significantly different from control; b - LA5: DH is significantly different from DH; c - LA5: DH is significantly from control].



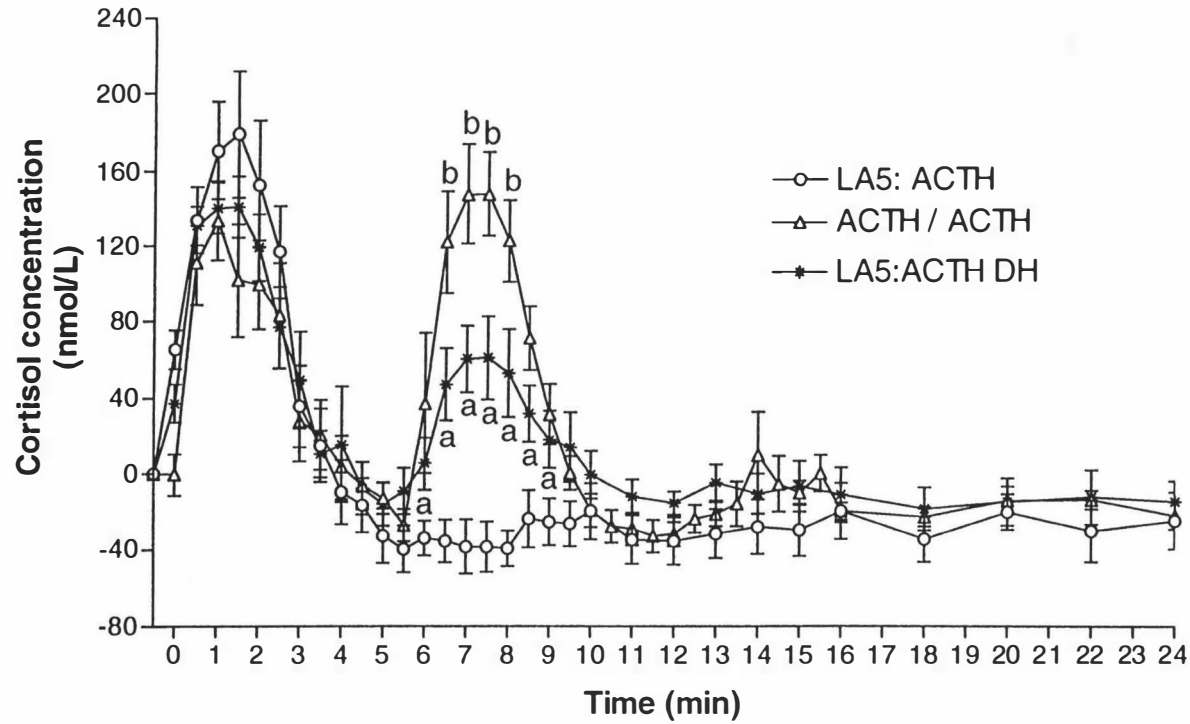
**Fig. 3.9:** Changes in plasma cortisol concentration in calves in response to dehorning (DH), local anaesthetic (5 hours) plus dehorning (LA5: DH), local anaesthetic (5 hours), ketoprofen plus dehorning (LA6:K DH), and local anaesthetic (5 hours) plus ketoprofen (LA5: K control). [a – LA5: K control is significantly different from LA5:K DH; b – DH is significantly different from LA5: K DH; c – LA5: DH is significantly different from LA5: K DH].



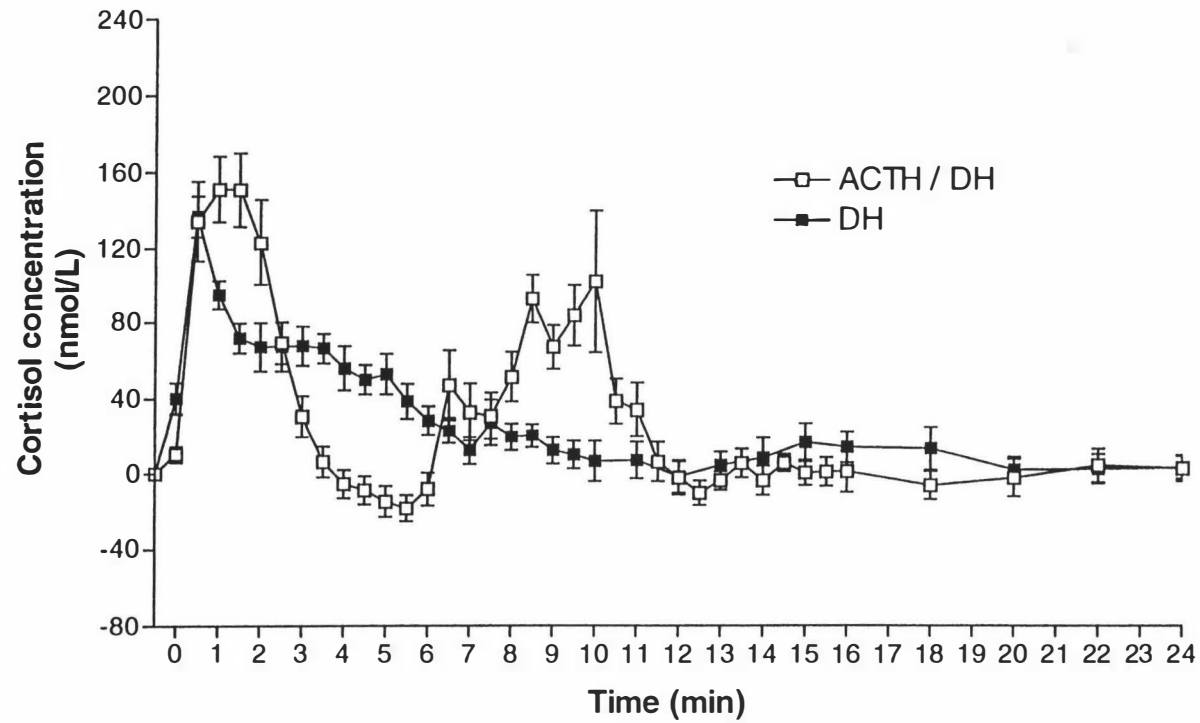
**Fig. 3.10:** Changes in plasma cortisol concentration in calves in response to dehorning (DH), local anaesthetic (5 hours) plus dehorning (LA5: DH), local anaesthetic (5 hours) plus phenylbutazone (LA5:P control), and local anaesthetic (5 hours), phenylbutazone plus dehorning (LA5:P DH). [a – LA5:P control is significantly different from LA5:P DH; b - DH is significantly different from LA5: P DH].



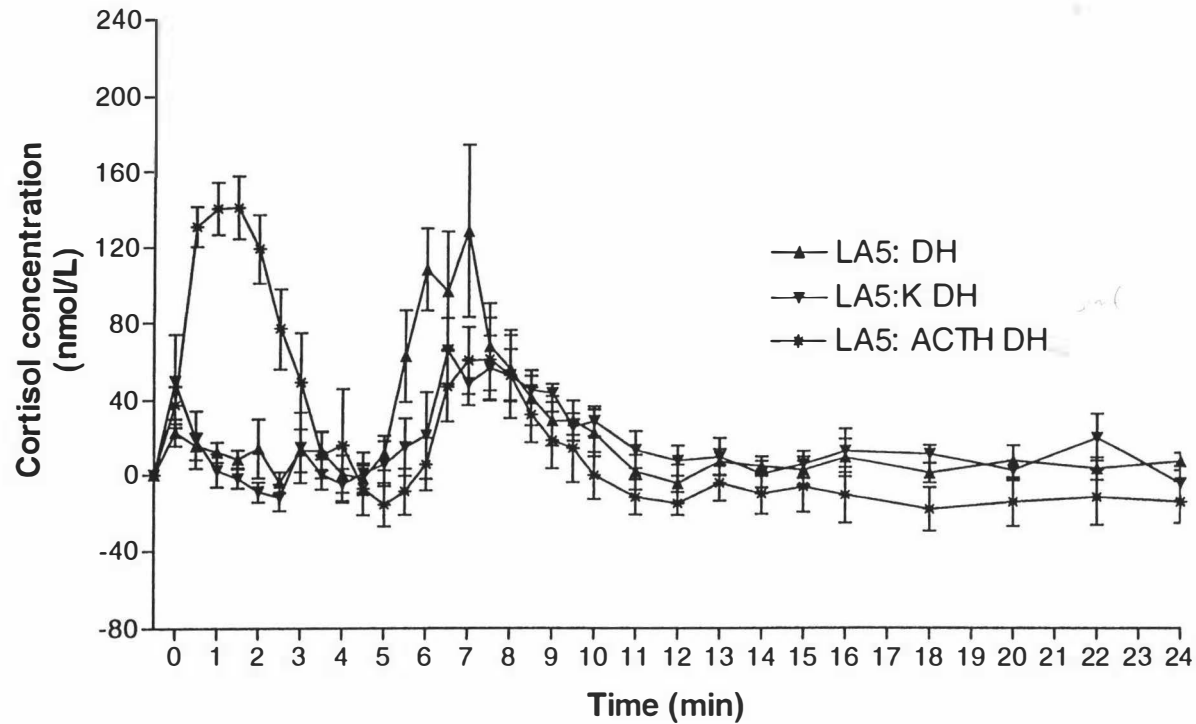
**Fig. 3.11:** Changes in plasma cortisol concentration in calves in response to local anaesthetic (5 hours) plus ACTH (LA5: ACTH), local anaesthetic (5 hours) plus dehorning (LA5: DH), and local anaesthetic (5 hours), ACTH plus dehorning (LA5:ACTH DH). [a – LA5: DH is significantly different from LA5:ACTH DH].



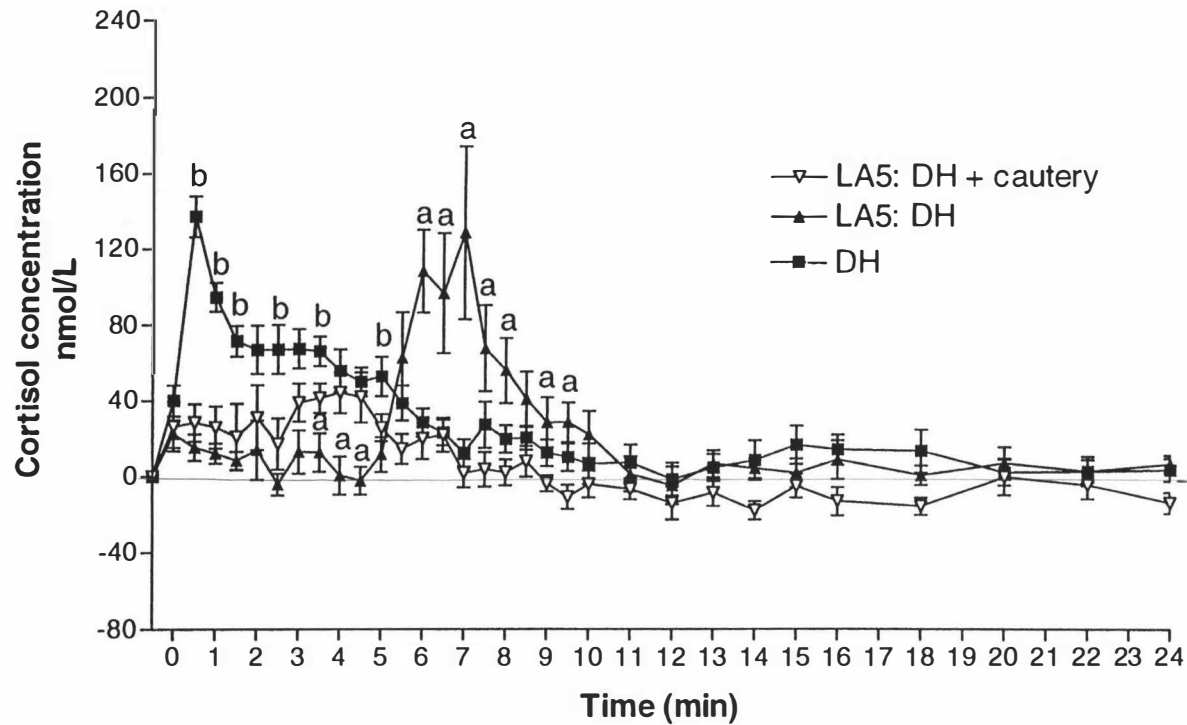
**Fig. 3.12:** Changes in plasma cortisol concentration in calves in response to local anaesthetic (5 hours) and ACTH (LA5: ACTH), local anaesthetic (5 hours), ACTH plus dehorning (LA5:ACTH DH), and ACTH (t=0) plus ACTH at 6 hours (ACTH / ACTH). [a – LA5: ACTH is significantly different from LA5:ACTH DH; b - ACTH / ACTH is significantly different from LA5:ACTH DH].



**Fig. 3.13:** Changes in plasma cortisol concentration in calves in response to dehorning (DH) and ACTH plus dehorning at 6 hours (ACTH / DH).



**Fig. 3.14:** Changes in plasma cortisol concentrations in calves in response to local anaesthetic (5 hours), ketoprofen plus dehorning (LA5:K DH), local anaesthetic (5 hours) plus dehorning (LA5: DH), and local anaesthetic (5 hours), ACTH plus dehorning (LA5: ACTH DH).



**Fig. 3.15:** Changes in plasma cortisol concentration in calves in response to dehorning (DH), local anaesthetic (5 hours) plus dehorning (LA5: DH), and injection of local anaesthetic (5 hours), dehorning plus cautery (LA5:DH + cautery). [a – LA5: DH is significantly from LA5:DH + cautery; b – DH is significantly different from LA5:DH + cautery].

Treatment	N	Integrated cortisol Duration (hours)	Response
Control	8	24	24,160 ± 2,629
LA5:K control	9	24	30,748 ± 5,261
LA5:P control	8	24	29,824 ± 6,383
LA5: ACTH	8	24	70,844 ± 7,357 <sup>a</sup>
DH	8	24	44,907 ± 4,171 <sup>a</sup>
		first 7 hours	26,066 ± 2,225
LA5: DH	8	24	39,006 ± 6,130 <sup>a</sup>
		4.5 – 11hrs	21,880 ± 4,452
LA5:ACTH DH	9	24	61,150 ± 6,901
		5.5 - 11hrs	13,591 ± 2,859
LA5:K DH	8	24	35,293 ± 6,057
LA5:P DH	10	24	40,916 ± 5,268 <sup>a</sup>
ACTH / DH	9	24	54,638 ± 2,438 <sup>a</sup>
		6 – 13hrs	20,201 ± 2,413
ACTH / ACTH	8	24	63,738 ± 6,784 <sup>a</sup>
		5.5 - 11hrs	23,769 ± 4,016
LA5:DH+cautery	10	24	31,616 ± 2,507 <sup>b</sup>

**Table 3.3:** The mean ( ± SEM) integrated cortisol response of each group over the trial period of 24 hours or specific time. [a - significantly different from control; b – DH is significantly from LA5: DH + cautery (p < 0.05)].

### 3.5 Discussion

The following conclusions were drawn from the present study which used plasma cortisol concentrations as an indicator of pain-induced distress to compare different methods for alleviating the distress caused by dehorning in calves. First, ketoprofen and local anaesthetic administered prior to dehorning only marginally reduced the delayed cortisol response which occurred once the local anaesthetic wore off, compared to that seen in calves given local anaesthetic only prior to dehorning. Furthermore, phenylbutazone and local anaesthetic given prior to dehorning in calves virtually abolished the cortisol response to dehorning for the period of action of the local anaesthetic, but did not further reduce the delayed cortisol response compared to that in calves injected with local anaesthetic only. Second, administering ACTH and local anaesthetic prior to dehorning to approximately simulate the cortisol surge caused by dehorning only marginally reduced the delayed cortisol response compared to that seen in calves given local anaesthetic only. Third, cauterising the scoop wounds plus giving local anaesthetic significantly reduced the pain-induced distress caused by dehorning, both by the action of the local anaesthetic and the absence of a delayed cortisol rise once the local anaesthetic wore off. Fourth, once the elevated cortisol concentrations caused by the different dehorning treatments had returned to pretreatment values few further significant changes in concentrations were observed in any of the groups for the rest of the 24-hour study period.

The format of this discussion is as follows: The cortisol responses to dehorning with and without local anaesthetic and possible explanations for the observed differences will be considered. The cortisol responses to dehorning using the NSAIDs ketoprofen and phenylbutazone in combination with local anaesthetic will be compared and the implications of the differences evaluated. The cortisol response to dehorning with local anaesthetic using ACTH to induce a cortisol surge will be compared with the cortisol responses of those groups just named and the significance of the findings assessed. Finally, the cortisol response to dehorning using local anaesthetic and cautery will be discussed.

### ***Cortisol response to dehorning with and without local anaesthetic***

Scoop dehorning produces a cortisol response where the plasma cortisol concentrations peak at 30 minutes, plateau and then return to pretreatment values approximately 7 hours after treatment (Fig. 3.8). The pattern and duration of the cortisol response caused by scoop dehorning in the present study (Fig. 3.8) are consistent with those found in previous studies involving calves of varying ages (1-2, 3-4, and 5-6 months) and sex (Petrie *et al.*, 1996a; McMeekan *et al.*, 1997, 1998a, b; Sylvester *et al.*, 1998b). The initial rise in the cortisol concentration in calves dehorned is likely to be due to maximal secretion from the HPA-axis as the slope and magnitude of this rise are similar to those caused by injecting ACTH which elicits a maximal cortisol response (Fig. 3.13) (Petrie *et al.*, 1996a; Sylvester *et al.*, 1998b).

In the present study, lignocaine (duration of action 2 hours) was injected 30 minutes prior to treatment followed by an injection of bupivacaine (duration of action 3-4 hours) 2 hours after treatment, so that animals experienced nerve block for up to 5 hours after treatment. This local anaesthetic regimen virtually abolished the cortisol response to dehorning for the first 5 hours after treatment. However, once the local anaesthetic began to wear off the cortisol concentration began to rise and remained elevated during the period between 5.5 and 8 hours after treatment, thereafter remaining at pretreatment values throughout the 24-hour study period. A similar cortisol response after scoop dehorning with local anaesthetic has been shown in previous studies, using local anaesthetic of varying durations of action (2, 4, or 8 hours) (Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a, b; Sylvester *et al.*, 1998a). Furthermore, the cortisol response to dehorning with local anaesthetic is consistent within calves of varying ages (1-2, 3-4, and 5-6 months) and sex (Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a, b; Sylvester *et al.*, 1998a). The duration of action of the local anaesthetic was demonstrated in each case by a transient rise in the cortisol concentrations, a change in behaviour, and by an increase in the sensitivity to a needle prick test of the skin surrounding the amputation wounds once the local anaesthetic wore off.

The integrated cortisol response to dehorning in calves is similar to the integrated cortisol response of calves dehorned with local anaesthetic in three out of four studies, but the patterns of the cortisol responses differed (Table 3.3, Fig. 3.8) (Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a, b; Sylvester *et al.*, 1996a). Furthermore, the duration of the cortisol response to dehorning alone is similar to the duration of the rise in plasma cortisol concentrations once the local anaesthetic wears off in calves dehorned after a prior injection of local anaesthetic. Therefore, it seems that giving local anaesthetic has the effect of delaying the cortisol response to dehorning for the duration of action of the local anaesthetic used, and not affecting its overall magnitude or duration.

The initial nociceptor barrage caused by dehorning in calves may stimulate stress-induced analgesia through the release of endogenous endorphins (Petrie *et al.*, 1996a). Subsequently, these animals may experience less pain following the release of these endorphins. Local anaesthetic given prior to dehorning in calves may prevent the stimulation of stress-induced analgesia or delay its activation, hence these animals may perceive more pain once the local anaesthetic begins to wear off, consequently resulting in a greater cortisol response at this time. However, plasma  $\beta$ -endorphin concentrations in 6-month-old steers were not elevated by scoop dehorning which may suggest that dehorning does not activate endogenous antinociceptive mechanisms (Cooper *et al.*, 1995), although  $\beta$ -endorphin concentrations within the brain may be more indicative of the activation of stress-induced analgesia. Naloxone, an opioid antagonist, did not affect the cortisol response caused by ring castration plus tailing in lambs (Wood *et al.*, 1991), suggesting minimal endogenous analgesic effects of this type. Therefore, if ring castration plus tailing, a procedure that causes acute pain-induced distress in lambs, does not activate endogenous antinociceptive mechanisms then, similarly, dehorning in calves may not activate these mechanisms.

The rise in the cortisol concentration once the local anaesthetic wears off in dehorned calves first given local anaesthetic may be a result of calves reacting to pain which they are experiencing for the first time (McMeekan *et al.*, 1998a).

Moreover, this delayed rise in cortisol concentrations may be in response to the novel experience of noxious input at a cognitive level as the HPA-axis responds sensitively to physiological and psychological stimuli (Levine, 1985; Rushen, 1986). Studies have shown that glucocorticoid levels in animals increase in response to novel and unpredictable stressors (Bassett and Cairncross, 1973; Wiepkema and Koolhaas, 1993).

It has been suggested that the cortisol response to dehorning consists of two phases: an initial peak due primarily to the nociceptor barrage caused by horn amputation, followed by a plateau phase due mainly to inflammation-related pain (McMeekan *et al.*, 1998b). A biphasic response, consisting of an initial phase caused by direct nociception followed by an inflammatory phase is also supported by results of a formalin test used in mice (Hunnskaar and Hole, 1987). Local anaesthetic given prior to dehorning blocks nerve impulses and hence stimulation of the HPA-axis; consequently cortisol secretion is not elevated for the duration of action of the local anaesthetic. Therefore, if the second phase of the dehorning response is due mainly to inflammation-related pain, the minimising of the secretion of cortisol (a potent and natural anti-inflammatory agent) by the nerve block action of the local anaesthetic in dehorned calves with local anaesthetic, may delay the resolution of inflammation during the period of action of the local anaesthetic (McMeekan *et al.*, 1998a).

### ***Cortisol response to dehorning using NSAIDs and local anaesthetic***

The effects of ketoprofen, an NSAID with both anti-inflammatory and antinociceptive properties, and phenylbutazone, a predominantly anti-inflammatory NSAID, were examined to assess whether the delayed cortisol response observed in calves dehorned with local anaesthetic is due mainly to unresolved inflammation. NSAIDs were originally thought to provide analgesia solely through peripheral mechanisms related to the inhibition of prostaglandin synthesis (Cashman, 1996; Cherng *et al.*, 1996). However, both peripheral and central analgesic actions of NSAIDs have now been described (McCormack and Brune, 1991; Urquhart, 1993; McCormack, 1994; Vasko, 1995; Cashman, 1996; Cherng *et al.*, 1996; Johnston and Budenberg, 1997). Evidence suggests that NSAIDs can act centrally, at the supraspinal or spinal level, by inhibiting

central prostaglandin synthesis (Urquhart, 1993; Vasko, 1995; Cashman, 1996; Johnston and Budsberg, 1997). McCormack and Brune (1991) define the actions of NSAIDs as analgesic when related to the peripheral inhibition of prostaglandins and antinociceptive when describing the central action of NSAIDs.

#### *Ketoprofen and local anaesthetic*

Ketoprofen plus local anaesthetic (duration of action 5 hours) given prior to dehorning delayed the rise in cortisol concentrations, but once the local anaesthetic wore off the response was similar to that in calves given local anaesthetic only and the overall cortisol response was only marginally reduced (Fig. 3.9, Table 3.3). In a previous study local anaesthetic with a duration of action of 2 or 4 hours, plus ketoprofen, given prior to dehorning virtually abolished the cortisol response to scoop dehorning in calves (McMeekan *et al.*, 1998b).

It was postulated that the delayed cortisol response observed in calves given local anaesthetic prior to dehorning is stimulated by inflammation-related pain, which remains unresolved during the period of action of the local anaesthetic due to low cortisol concentrations (McMeekan *et al.*, 1998a). Therefore, the anti-inflammatory actions of ketoprofen in calves given local anaesthetic plus ketoprofen prior to dehorning should resolve the inflammation caused by dehorning, thereby preventing a delayed cortisol response. Furthermore, the duration of action of the local anaesthetic should have no effect on the efficiency of ketoprofen, as ketoprofen should resolve the inflammation caused by dehorning during the period of action of the local anaesthetic. Therefore, the delayed cortisol response observed in calves dehorned with local anaesthetic may also be stimulated by the conscious perception of pain from the dehorning wounds, which was experienced in these calves for the first time when the local anaesthetic begins to wear off. Consequently, it may be the antinociceptive action of ketoprofen that is important in alleviating the pain-induced distress caused by dehorning in these calves, as ketoprofen has been shown to have a central component to its analgesic action in addition to its peripheral action (Mehlich *et al.*, 1984; Willer *et al.*, 1989; Urquhart, 1993; McCormack, 1994).

The greater effectiveness of ketoprofen plus local anaesthetic (duration of action 2 hours) (McMeekan *et al.*, 1998b) compared to ketoprofen plus local anaesthetic (duration of action 5 hours) (Fig. 3.9) in alleviating the pain-induced distress caused by dehorning may be influenced by a central antinociceptive effect of the ketoprofen and the different durations of action of the local anaesthetic. Calves given local anaesthetic (duration of action 2 hours) plus ketoprofen prior to dehorning may still have a high pain-threshold once the local anaesthetic wears off due to high levels of circulating ketoprofen in the blood. Thus, once the local anaesthetic wears off after 2 hours, the calves would still experience only minimal if any pain-induced distress. However, in the present study the circulating levels of ketoprofen in the blood would have been much lower after 5 hours when the local anaesthetic wore off and consequently the pain-threshold of these animals may be reduced to a level where they experience pain in response to the dehorning wounds resulting in a transient rise in cortisol concentrations. The short elimination half-life of ketoprofen, of approximately 42 minutes when intravenously injected into 20 week old calves (Landoni *et al.*, 1995), further supports this explanation.

#### *Phenylbutazone plus local anaesthetic*

In the present study, local anaesthetic and phenylbutazone given prior to dehorning resulted in a cortisol response similar to that of control calves for the duration of action of the local anaesthetic. This response was followed by a rise in the cortisol concentrations during the period between 5.5 and 8.5 hours and a subsequent return to pretreatment values at 9 hours, where they remained throughout the rest of the study period. Thus, local anaesthetic plus phenylbutazone given prior to dehorning results in a cortisol response similar to that in calves dehorned in combination with local anaesthetic only (Fig. 3.10).

Phenylbutazone injected intravenously has an elimination half-life of more than 30 hours in cows (De Backer *et al.*, 1980). Therefore, in the present study the anti-inflammatory action of phenylbutazone would have been expected to last throughout the 24-hour study period. Studies have shown phenylbutazone to be 80 times less potent than ketoprofen in reducing inflammation caused by carrageen-induced abscesses in rats and 800-1500 times less potent than

ketoprofen in inhibiting prostaglandin synthesis (Kantor, 1986). The lower anti-inflammatory potency of phenylbutazone compared to that of ketoprofen may explain why phenylbutazone had virtually no effect on the delayed cortisol response. Alternatively, it may be because, unlike ketoprofen, phenylbutazone has apparently little, if any, central antinociceptive effects.

### ***Cortisol response to dehorning giving local anaesthetic and ACTH***

In the present study calves dehorned with local anaesthetic were given an injection of synthetic ACTH to elicit a cortisol response, which would approximately simulate the cortisol response observed in calves dehorned without local anaesthetic. This was done in order to assess whether the usual cortisol response to dehorning alone provides any anti-inflammatory benefits.

The integrated cortisol response elicited by the dose of ACTH used in the present study is equivalent to the integrated cortisol response elicited by scoop dehorning (Table 3.3), but the pattern and the duration of the responses differ (Fig. 3.13) (Petrie *et al.*, 1996a, b; Sylvester *et al.*, 1998b). ACTH and local anaesthetic administered prior to dehorning stimulated an initial marked cortisol response which was similar to the initial cortisol response observed in LA5: ACTH calves (Fig. 3.11). Six hours after treatment in LA5: ACTH DH calves the cortisol concentrations increased for a second time, returning to pretreatment values 10 hours after treatment. This second cortisol surge began about 1 hour later and was significantly smaller in magnitude when compared to the delayed cortisol response observed in calves given local anaesthetic only prior to dehorning (Fig. 3.11).

The difference in the magnitude of the delayed cortisol response observed in LA5: ACTH DH calves compared to calves given local anaesthetic only prior to dehorning may be due to the actions of cortisol, a potent anti-inflammatory agent (Johnston and Budsberg, 1997). Alternatively, the reduction in the delayed cortisol response may have been due to the local anaesthetic acting longer in LA5: ACTH DH calves. This seems unlikely however, as the calves injected with local anaesthetic (duration of action 5 hours) and ketoprofen prior

to dehorning showed a similar delayed cortisol response to that in LA5: ACTH DH calves which supports an anti-inflammatory effect (Fig. 3.14).

Corticosteroids have been shown to suppress the transmission of impulses in thin unmyelinated C-fibres, which is reversed completely within 60 minutes of the corticosteroids being removed (Johansson *et al.*, 1990). Therefore, a suppressive action of corticosteroid on C-fibres and consequently a reduced noxious input from the injured site may explain the reduced cortisol response in LA5:ACTH DH calves once the local anaesthetic wears off. However, Johansson *et al.* (1990) applied the corticosteroid directly onto the dissected nerve fibres, whereas in the present study cortisol is not likely to have accumulated in the injured area at equivalent concentrations, hence such an effect probably did not occur here.

It could be argued that the large initial cortisol surge stimulated by the injection of ACTH in LA5: ACTH DH calves may result in exhaustion of the adrenal cortex thus resulting in a reduced cortisol response once the local anaesthetic wears off. However, calves injected with ACTH at 0 hours and at 6 hours showed no indication of exhaustion of the adrenal cortex, as the first and second cortisol surges had similar magnitudes and durations (Fig. 3.12). Furthermore, previous studies have shown that repeated injections of ACTH do not reduce the secretory ability of the adrenal gland in cows or lambs (Fox *et al.*, 1980; Mellor and Murray, 1989b; Verkerk, 1995).

The prior injection of ACTH and the consequently elevated cortisol secretion in LA5: ACTH DH calves may inhibit endogenous ACTH production, thus reducing the responsiveness of the adrenal glands to further stimulation once the exogenous ACTH has been eliminated, as secretion of ACTH is under the control of corticosteroid negative feedback inhibition (Jones *et al.*, 1977). ACTH given prior to dehorning with and without local anaesthetic elicited a marked cortisol response which in some cases declined significantly after returning to pretreatment values (Fig. 3.7) (Petrie *et al.*, 1996b; Sylvester *et al.*, 1998b). In the present study, and in that of Petrie *et al.* (1996b), the pretreatment cortisol concentrations of the calves given ACTH were double that

of control handled animals. Therefore, it is likely that the cortisol concentrations of these calves undershot pretreatment values once the exogenous ACTH had been eliminated due to higher mean pretreatment cortisol concentrations rather than as a consequence of negative feedback inhibiting cortisol secretion. However, this model is not consistent for all ACTH-induced cortisol responses in calves (Petrie *et al.*, 1996a; Sylvester *et al.*, 1998b).

In the present study, calves were injected with ACTH at time 0 and dehorned 6 hours later, to determine whether the prior ACTH-induced cortisol surge would affect the pattern, magnitude or duration of the cortisol response to subsequent dehorning. The cortisol response stimulated by the ACTH injection at time 0 was similar to the cortisol response elicited in LA5: ACTH calves. The pattern of the cortisol response stimulated by dehorning 6 hours after injecting ACTH was distinctly different from that of calves dehorned at time 0 (Fig. 3.13). The initial peak cortisol concentration was absent, as the cortisol concentrations gradually rose to a plateau before returning to baseline values. Lingering anti-inflammatory effects from the initial cortisol surge may have reduced the initial pain-induced distress experienced by calves dehorned at 6 hours. However, in calves given ketoprofen prior to dehorning the initial peak cortisol concentrations were not significantly different from calves dehorned only (McMeekan *et al.*, 1998b). Furthermore, the integrated cortisol response and the duration of the response to dehorning in ACTH/DH calves were similar to those of DH calves.

Ketoprofen plus local anaesthetic (duration of action 5 hours) and ACTH plus local anaesthetic (duration of action 5 hours) given prior to dehorning reduce and delay the cortisol response elicited once the local anaesthetic wears off compared to those features of the response in calves given local anaesthetic only prior to dehorning (Fig. 3.14). The similarity between these two responses may be a coincidence, or ketoprofen and cortisol (elicited by injecting ACTH) may have a common mechanism of action that is absent when local anaesthetic and phenylbutazone are given together, such as antinociceptive properties. The antinociceptive actions of ketoprofen are well established in the literature (Mehlich *et al.*, 1984; Willer *et al.*, 1989; Urquhart, 1993;

McCormack, 1994), but the analgesic properties of glucocorticoids, like cortisol, have not apparently being examined separately (Dart, 1992). Alternative explanation for the differing response is phenylbutazone may not be as potent an anti-inflammatory agent as ketoprofen and cortisol.

### ***Cortisol response to dehorning using local anaesthetic and cautery***

Administering local anaesthetic before and cauterising the amputation wounds after dehorning was conducted to further evaluate whether the reduction in the pain-induced distress achieved by this alleviation strategy was as effective as reported by Sylvester *et al.* (1998a). In the present study, the cortisol concentrations of LA5: DH + cautery calves were significantly elevated above LA5: DH levels for one hour and above control values for approximately 5 hours (Fig. 3.15), but the magnitude and the integrated cortisol response was significantly reduced compared to dehorning alone (Table 3.3). Previously, the cortisol response to dehorning has been virtually abolished by cauterising the scoop wounds in combination with giving local anaesthetic of 2 hour duration of action (Sylvester *et al.*, 1998a). However, in the present study the cortisol concentration of LA5: DH + cautery calves at time 0 was high compared to the pretreatment cortisol concentration (Fig. 3.15), probably due to handling and administration of local anaesthetic. Therefore, if the time 0 cortisol concentration was used as the pretreatment value there would be no significant change in the cortisol response to LA5: DH + cautery, hence the results would broadly agree with Sylvester *et al.* (1998a).

Cauterising the amputation wounds caused by dehorning in combination with using local anaesthetic prevents the delayed cortisol response seen in LA5: DH calves for at least 24 hours after (Fig. 3.15) (Sylvester *et al.*, 1998a). Cautery is likely to cause third degree burns, which destroy the epidermis and the dermis of the skin, thereby destroying the nociceptors in the burned areas and preventing nociception from the injured tissues (Bonica, 1990). Therefore, if nociception from the injured area is prevented due to cautery, animals are unlikely to perceive noxious input from the site of injury once the local anaesthetic wears off, thereby preventing stimulation of the HPA-axis and subsequent cortisol release. However, cautery is unlikely to cause evenly

distributed damage to the whole amputation wound. The cauterised dehorning wounds are likely to exhibit first, second and third degree burns. First degree burns involve the most superficial layers of the skin and second-degree burns can be either superficial or deep, but neither first degree nor second degree burns destroy the nociceptors in the injured area (Geiser and Walker, 1984). Furthermore, third degree burns do not destroy the bone (Geiser and Walker, 1984) and the nociceptors within the bone. Therefore, LA5: DH + cauterity calves may experience noxious sensory input from the dehorning wounds once the local anaesthetic wears off but not enough to stimulate a cortisol response (Fig. 3.15).

In conclusion, phenylbutazone was not effective and ketoprofen and an ACTH-induced cortisol surge only produced a small reduction in the delayed cortisol response to dehorning which occurred when the local anaesthetic wore off 5 hours after amputation dehorning. This suggests that the delayed cortisol response seen in calves when the local anaesthetic wears off is not primarily due to inflammation-related pain. Giving local anaesthetic prior to dehorning and cauterising the amputation wounds substantially reduced the delayed cortisol response to dehorning, which occurred when the local anaesthetic wore off 5 hours after amputation dehorning.

## CHAPTER FOUR: General Discussion

### 4.1 Major Conclusions

#### *Ring castration and tailing of lambs*

In the present study the effects of using local anaesthetic or a castration clamp to alleviate the pain-induced distress caused by ring castration plus tailing in lambs was assessed by measuring the plasma cortisol concentrations following castration plus tailing. The major conclusions were as follows:

1. Ring castration plus tailing alone caused significant pain-induced distress for a period of approximately three hours, as reported by others (Mellor and Murray, 1989; Lester *et al.*, 1991a; Wood *et al.*, 1991; Kent *et al.*, 1993, 1995; Dinniss *et al.*, 1997b).
2. Local anaesthetic injected into the scrotal neck just *before* ring castration plus tailing significantly reduced the magnitude and the overall cortisol response to ring castration plus tailing.
3. Injecting local anaesthetic into the testes just *after* ring castration plus tailing only marginally (non-significant) reduced the magnitude and the integrated cortisol response to ring castration plus tailing.
4. Applying the castration clamp for 6 seconds across the full width of the scrotal neck in combination with ring castration plus tailing in lambs did not reduce the cortisol response to this procedure.
5. Applying the castration clamp for 10 seconds across the full width of the scrotal neck after ring castration plus tail docking in lambs effected a small reduction in the cortisol response to this procedure.
6. A comparison over a six week period between the healing of the castration wounds of lambs ring castrated plus tailed or ring and clamp

castrated plus ring tailed showed no greater adverse effects using the ring plus clamp method.

In the present study, local anaesthetic injected into the scrotal neck just before ring castration plus tailing significantly alleviated the overall pain-induced distress caused by ring castration plus tailing. Local anaesthetic injected into both the scrotal neck may block noxious sensory input from the scrotum and testes, thereby significantly reducing the pain experienced by these lambs (Dinniss *et al.*, 1997a). However, local anaesthetic injected into each testis just before castration only marginally reduced the pain-induced distress caused by ring castration plus tailing. Other nerves are also involved in the afferent activity from the scrotal area, such as nerves that originate from the scrotal plexus and other afferent visceral nerves, which may not be effectively blocked by injecting local anaesthetic into the testes (Cottrell and Molony, 1995). Hence, injecting local anaesthetic into the testes only may block a limited amount of the overall noxious sensory input caused by ring castration plus tailing.

Local anaesthetic injected into the testes or the scrotal neck 15 minutes prior to treatment has been shown to virtually abolish the cortisol response to ring castration (Dinniss *et al.*, 1997a). However, in the present study, where local anaesthetic was injected into the same locations immediately before or after ring application, the pain-induced distress caused by ring castration *plus* tailing was only partially alleviated. Local anaesthetic injected 15 minutes prior to treatment compared to a few seconds (as was the case in the present study) would allow more time for the local anaesthetic to diffuse through the tissues, consequently anaesthetising the tissues of and within the scrotum more effectively. Kent *et al.*, (1998) achieved a 77% reduction in the cortisol response by administering local anaesthetic immediately before or after ring application for castration only. Therefore, in the present study local anaesthetic injected into the scrotum or the testes of lambs may have been more effective than was first apparent due to unmodified noxious sensory input from the tail contributing to the cortisol response.

Applying the castration clamp for 6 seconds across the full width of the scrotal neck distal to the ring did not alleviate the pain-induced distress caused by ring castration plus tailing in lambs. Furthermore, applying the castration clamp for 10 seconds in the same manner effected only a small reduction in the pain-induced distress associated with ring castration plus tailing. Applying the castration clamp to the neck of the scrotum and the tail for 6 or 10 seconds after ring castration plus tailing in five to six day old lambs has been found to significantly reduce the cortisol response to this procedure (Kent *et al.*, 1993, 1995), but in the present study continued noxious sensory input from the tail would have contributed to the cortisol response and may have reduced any benefits achieved from disabling the innervation from the scrotal area using the clamp. Dinniss *et al.* (1997b) found no significant difference was found when comparing the cortisol responses elicited by ring plus clamp castration and ring tailing with ring plus clamp castration and tailing, but in that study the clamp was applied in the traditional way which leaves a gap of uncrushed tissue (Table 2.1). Alternatively, the castration clamp may not be as effective at disabling the innervation from the scrotal area in older and larger lambs, such as three to six week old lambs which were used in the present study, as the pressure of the jaws of the clamp would be distributed over a larger area (Hosie *et al.*, 1996).

Local anaesthetic injected into the neck of the scrotum was the most effective method, in the present study, for alleviating the pain-induced distress associated with ring castration plus tailing. However, many impracticalities associated with using local anaesthetic on the farm will need to be overcome before it becomes a viable method of alleviating the pain-induced distress associated with ring castration plus tailing. So far, a high-pressure needless injection has been developed for administering local anaesthetic for castration plus tailing of lambs, which provides comparable analgesia to the conventional needle and syringe but with greatly reduced risk of infection (Kent *et al.*, 1998).

### ***Dehorning of calves***

In the present study the effects of using local anaesthetic and NSAIDs or an ACTH stimulated cortisol surge to alleviate the pain-induced distress caused by

dehorning was assessed by measuring the plasma cortisol concentrations following dehorning. The major conclusions are as follows:

1. Scoop dehorning of calves without analgesia resulted in a cortisol response indicative of acute pain-induced distress for a period of 7 hours, as reported previously (Petrie *et al.*, 1996a; McMeekan *et al.*, 1997, 1998a, b; Sylvester *et al.*, 1998a, b).
2. Injecting local anaesthetic prior to dehorning virtually abolished the cortisol response to dehorning throughout the 5-hour period of its action, but once the local anaesthetic wore off the plasma cortisol concentrations exhibited a transient rise (delayed cortisol response). The resultant integrated cortisol response to dehorning with local anaesthetic was similar to that of calves dehorned without local anaesthetic.
3. Ketoprofen and local anaesthetic injected prior to dehorning marginally reduced the delayed cortisol response compared to that seen in calves dehorned with a prior injection of local anaesthetic only.
4. Phenylbutazone and local anaesthetic given prior to dehorning was ineffective in reducing the delayed cortisol response compared to that in calves given an injection of local anaesthetic only prior to dehorning.
5. ACTH and local anaesthetic injected prior to dehorning, to approximately simulate the cortisol response to dehorning, marginally reduced the delayed cortisol response compared to that seen in dehorned calves given local anaesthetic only.
6. Injecting local anaesthetic and cauterising the amputation wounds caused by scoop dehorning significantly reduced the pain-induced distress caused by dehorning with and without a prior injection of local anaesthetic.

In the present study local anaesthetic (lignocaine) of 2 hour duration of action was given 20 minutes prior to treatment and bupivacaine (duration of action 3-4

hours) was given two hours after treatment, thereby providing calves with 5 hours of regional analgesia after treatment. Local anaesthetic and ketoprofen given to calves prior to dehorning reduced the delayed cortisol response by 1 hour. The magnitude of this response compared to that in calves dehorned with a prior injection of local anaesthetic only, probably due to the anti-inflammatory action of ketoprofen or to its central antinociceptive action, or both. Phenylbutazone and local anaesthetic given prior to dehorning did not further alleviate the pain-induced distress caused by dehorning compared to that in calves given local anaesthetic only. Phenylbutazone may be less effective than ketoprofen due to phenylbutazone having a lower anti-inflammatory potency compared to ketoprofen (Kantor, 1986), and no antinociceptive action. An ACTH-induced cortisol surge, used to approximately simulate the cortisol response to dehorning, reduced the delayed cortisol response after dehorning compared to that in calves given local anaesthetic only prior to dehorning. The anti-inflammatory potency of ketoprofen and cortisol may therefore both be superior to the potency of phenylbutazone under these circumstances.

In the present study, ketoprofen plus local anaesthetic (duration of action 5 hours) given prior to dehorning was less effective at reducing the cortisol response to dehorning when compared to ketoprofen plus local anaesthetic of 2 hour duration of action reported by McMeekan *et al.* (1998b). Therefore, the duration of action of the local anaesthetic may influence the effectiveness of ketoprofen. However, if the transient rise in the cortisol concentrations seen in calves given local anaesthetic prior to dehorning when the local anaesthetic wears off is stimulated by inflammation-related pain, then ketoprofen should help in resolving this inflammation during the period of action of the local anaesthetic. So the transient rise in cortisol concentrations elicited after the local anaesthetic wears off may be in response to the novelty of pain experienced for the first time by these calves as local anaesthetic blocks the initial pain response. Therefore, it may be the antinociceptive action of ketoprofen that is responsible for the reduction in the delayed cortisol response once the local anaesthetic wears off in the present study and the virtual

abolition of the cortisol response to dehorning reported in previous work (McMeekan *et al.*, 1998b).

Ketoprofen and an ACTH-induced cortisol surge only marginally reduced and phenylbutazone was ineffective at reducing the delayed cortisol response after dehorning compared to that in dehorned calves given local anaesthetic only. This suggests that this delayed cortisol response is not solely stimulated by inflammation-related pain, but has a central component. Therefore the effects of local anaesthetic in combination with NSAIDs on the dehorning cortisol response need to be assessed further before any new recommendations can be made. Cauterising the amputation wounds and giving local anaesthetic was the most effective method of alleviating the pain-induced distress caused by dehorning in the present study, as a transient rise in cortisol concentrations was not observed when the local anaesthetic wore off in these calves, confirming the results reported by Sylvester *et al.* (1998a).

## **4.2 Experimental Design, Limitations and Future Directions**

### ***Lamb studies***

The number of groups and the number of animals per group used in the present study was sufficient to answer the proposed questions. However, if money was not a limitation, more treatments could have been added to the experimental design for further comparisons.

In the first lamb study local anaesthetic was injected into the scrotal neck or the testes just before or just after ring castration plus tailing, but none was injected into the tail. Future studies could assess the extent of the noxious sensory input from the tail by comparing the cortisol response to ring castration plus tailing after or before giving local anaesthetic into the scrotal neck or the testes with the cortisol response resulting from injecting local anaesthetic into the tail and the scrotal neck or testes. Furthermore, the cortisol response to ring castration plus tailing with local anaesthetic injected into the tail only may help

to further distinguish the component of noxious sensory input from the tail compared to that of the scrotum.

The second set of lamb trials carried out in the present study, compared the cortisol response to ring castration plus tailing with ring and clamp castration plus ring tailing. An injection of ACTH and ring castration plus tailing were used as positive controls in this study, as the cortisol responses to these treatments are well established in the literature (Mellor and Murray, 1989b; Lester *et al.*, 1991a; Dinniss *et al.*, 1997a). Control handling, a negative control group, was omitted from this study because of financial limitations, but the experiment already contained two positive controls so a third control group was not considered to be necessary. The ring and clamp method was used to alleviate the pain-induced distress to ring castration plus tailing, but the tail was not clamped because this was thought to be excessively noxious as it often involves crushing bone. However, further studies could assess the extent to which the continued noxious sensory input from the tail contributes to the cortisol response by comparing the cortisol response to ring and clamp castration plus ring tailing only with ring and clamp castration plus tailing. Dinniss *et al.* (1997b) compared the cortisol response of lambs ring castrated plus tailed after applying the clamp to the scrotal neck or the scrotal neck and tail and found no significant differences between these procedures. However, in the present study the clamp was applied across the full width of the scrotal neck, whereas Dinniss *et al.* (1997b) applied the clamp in the traditional way which leaves a gap of uncrushed tissue and intact nerves and thereby the potential for continued noxious input from the scrotum.

Observations of the healing of the scrotal wounds of lambs castrated using the ring method or the ring and clamp method were recorded for up to 6 weeks. However, not all lambs could be observed for up to 6 weeks. It would have been preferable to observe the scrotal wounds of all lambs until all the scrotums had sloughed off, for consistency. Observations ceased when it appeared as though no further complications would arise from the castration wounds. In the present study only superficial wound healing was reported. The chronic response to castration could be further investigated by using

histological techniques to examine whether neuroma formation occurs and if so whether the method used increases the risk of this.

### ***Calf study***

In the present study a local anaesthetic control group was not included, due to limited calf availability and financial limitations. However, a local anaesthetic control group may have been beneficial for comparison with some of the other treatments. The cortisol responses to dehorning, dehorning with local anaesthetic and local anaesthetic controls are well established in the literature (Petrie *et al.*, 1996a; McMeekan *et al.*, 1997, 1998a, b; Sylvester *et al.*, 1998a, b). Furthermore, it is well established that local anaesthetic and its injection have minimal effect on the cortisol response compared to control handling.

In the present study ketoprofen plus local anaesthetic (duration of action 5 hours) given prior to dehorning was less effective in reducing the cortisol response caused by dehorning compared to a previous study in which ketoprofen plus local anaesthetic (duration of action 2 hours) virtually abolished the cortisol response to dehorning (McMeekan *et al.*, 1998b). Therefore, future studies could assess what effect the duration of action of local anaesthetic has on the effectiveness of ketoprofen on the post dehorning distress response. In other words, was the reduced effectiveness of ketoprofen and local anaesthetic (duration of action 5 hours) in alleviating the post dehorning distress response due to reduced levels of ketoprofen in the blood once the local anaesthetic wore off?

Group 1: LA (Lignocaine - duration of action 2 hours) plus ketoprofen and then scoop dehorned.

Group 2: LA (Lignocaine + bupivacaine - duration of action 5 hours) plus ketoprofen and then scoop dehorned.

Group 3: LA (Bupivacaine + bupivacaine - duration of action 8 hours) plus ketoprofen and then scoop dehorned.

Group 4: LA (Lignocaine + bupivacaine - duration of action 5 hours), scoop dehorned plus ketoprofen at 4.5 hours after dehorning.

In the present study the anti-inflammatory agents ketoprofen, phenylbutazone and cortisol (stimulated by injecting ACTH) did not significantly reduce the *overall* delayed cortisol response compared to that of calves given local anaesthetic only prior to dehorning (Table 3.3). This suggests that this delayed cortisol response is not stimulated by inflammation-related pain, but may be in response to the sensation of pain experienced by these calves for the first time when the local anaesthetic wears off. Therefore, it may be the antinociceptive action of ketoprofen not its anti-inflammatory actions that is responsible for reducing the cortisol response to dehorning. Future studies could assess whether it is the antinociceptive action of ketoprofen that is responsible for virtually abolishing the cortisol response to dehorning when given in combination with a short acting local anaesthetic (McMeekan *et al.*, 1998b), by comparing ketoprofen with a opioid and phenylbutazone, which has no antinociceptive action.

Group 5: LA (Lignocaine - duration of action 2 hours) plus ketoprofen and then scoop dehorned.

Group 6: LA (Lignocaine - duration of action 2 hours) plus phenylbutazone and then scoop dehorned.

Group 7: LA (Lignocaine - duration of action 2 hours) plus an opioid analgesic and then scoop dehorned.

### **General**

A consideration and a potential limitation is whether the pretreatment cortisol concentrations taken at the start of the trial were a true representation of basal cortisol levels. The pretreatment cortisol concentration is subtracted from the subsequent cortisol concentrations. Therefore, if the pretreatment cortisol concentrations are high the following cortisol concentrations and subsequently the response to a treatment may be under-estimated. To avoid this, individual animals that had particularly high pretreatment cortisol concentrations (lambs - 13 out of 130; calves - 17 out of 120) were excluded from this study. Furthermore, the pretreatment cortisol concentrations were mostly similar to the final cortisol concentrations, even 24 hours after treatment, suggesting that the pretreatment cortisol concentrations were usually close to basal concentrations.

Blood sampling order may be expected to influence the cortisol response. However, no relationship between plasma cortisol concentration and the order in which the pretreatment blood samples was taken was found in either the lamb or the calf studies (Fig. 2.6; Fig. 3.6). In the present lamb studies there were two or three lambs in each bleeding pen, hence blood sampling order may have been expected to influence the cortisol response. However, each lamb only took approximately 15 seconds to bleed and hence a maximum of 1 minute would have been spent in each pen by the bleeders. One minute is not a sufficient amount of time to observe a cortisol response to a stressor (Dinniss, 1995). However, as in the present calf study there were fifteen calves per pen, a sufficient amount of time would have elapsed between the first and last calf being bled to observe a change in the cortisol concentrations in response blood sampling order (McMeekan, 1997). Nevertheless, no effect of blood sampling order was observed in the present calf study.

To further assess the distress caused by the different husbandry practices examined here additional indices of distress could be used. The measurement of catecholamine levels may give a better indication of an animal's immediate response to a stressor compared to cortisol which usually takes minutes, not seconds, to be released in response to a stimulus. In the present study, the effectiveness of local anaesthetic when injected into the scrotal neck or the testes just before or after ring castration plus tailing was assessed, compared to the standard practice where local anaesthetic is injected approximately 15 minutes prior to treatment, using cortisol as an index of distress. The measurement of catecholamines may allow determination of whether or not the local anaesthetic is effective immediately after castration plus tailing compared to 20 minutes later. The measurement of heart rate is a physically non-invasive measure of distress as well as instantaneous, hence measuring heart rate may give another perspective to the pain-induced distress caused by different husbandry procedures.

### 4.3 Practical Considerations

In the present study local anaesthetic injected into the scrotal neck just before ring castration plus tailing reduced the pain-induced distress caused by this procedure. Injecting local anaesthetic and subsequently applying the rings is more practical than injecting local anaesthetic 15-20 minutes prior to castration plus tailing and consequently having to handle animals twice. However, the continued impracticalities of using local anaesthetic include cost, knowledge of the neural anatomy so that local anaesthetic is injected effectively, and hygiene problems. The use of a high-pressure needleless injection to administer local anaesthetic has been found to effectively reduce the pain associated with ring castration (Kent *et al.*, 1998). However, the other impracticalities associated with local anaesthetic or NSAIDs used to alleviate the pain-induced distress caused by castration plus tailing in lambs or dehorning in calves need to be overcome before using injectable analgesics on the farm become a viable option.

The local anaesthetic and NSAIDs combinations used in the present study to alleviate the pain-induced distress caused by dehorning did not significantly reduce the overall distress response, hence are not recommended. Local anaesthetic injected prior to dehorning alleviates the initial nociceptor barrage to dehorning, but overall does not reduce the pain associated with dehorning (Table 3.3) (Petrie *et al.*, 1996a; McMeekan *et al.*, 1998a, b). However, in previous studies local anaesthetic (duration of action 2 hours) and ketoprofen injected prior to dehorning abolished the distress response associated with dehorning (McMeekan *et al.*, 1998b). Therefore, the duration of the local anaesthetic action in combination with NSAIDs needs to be further investigated (as above).

The combined ring plus clamp method used to alleviate the pain-induced distress caused by ring castration plus tailing cannot be recommended for 3-6 week old lambs. Applying the clamp for 10 seconds across the full width of the scrotal neck after ring castration plus tailing reduced the cortisol response to this procedure, but only marginally. Furthermore, impracticalities associated

with applying the clamp include the variation in size of the castration clamp in relation to the varying size of the lamb scrotum's and the technique used to apply the castration clamp can differ markedly between users making the alleviation benefits of the combined method inconsistent. Evaluation of castration clamps showed that the crushing pressure applied by castration clamps even those of the same size could vary by over 100% (Hosie *et al.*, 1996). Furthermore, a certain amount of skill and awareness of the underlying anatomy are required for effective application of the castration clamp (Wood and Molony, 1992).

#### **4.4 Personal Comments**

Often I have been asked whether the short term pain caused by castration and tailing in lambs or dehorning in calves is significant enough to warrant concern and the subsequent expense of alleviating this pain. The husbandry procedures such as dehorning, castration plus tailing are generally performed for the welfare of the animal as well for hygiene, safety for the stock handler and production reasons, hence are considered essential practices. Therefore, if alternative methods that do not cause distress or cause less distress can be found it is our responsibility to alleviate the distress caused by these husbandry practices to the best of our ability.

**Appendix 1: Summary of the different strategies reported in the literature to alleviate the pain-induced distress caused by ring castration plus tailing in lambs**

Reference	Treatment	Location of injection	Time injected
Wood <i>et al.</i> , 1991	Ring	Each spermatic cord, scrotal neck, and each testis. Tail.	15-20 mins (prior)
Dinniss <i>et al.</i> , 1997a	Ring	Scrotal neck. Spermatic cords. Scrotal neck and spermatic cords. Testes.	15 mins (prior)
	Clamp (10 sec)	Scrotal neck. Spermatic cords.	
	Clamp plus ring (10 sec)	Scrotal neck. Spermatic cords.	
Graham <i>et al.</i> , 1997	Ring / ring plus clamp (10 sec) / cautery	Subcutaneous. Epidural	1-2 mins (prior)
Molony <i>et al.</i> , 1997	Ring plus clamp (10 sec)	Testes	1-2 mins (prior)
	Clamp (10 sec)	Testes	
Kent <i>et al.</i> , 1998	Ring	Spermatic cords. Testes*. Scrotal neck*. Tail. Tail*.	Just before or just after
	Ring plus clamp (10 sec)	Scrotal neck*. Tail*	

\* A high pressure needleless injection was used instead of a conventional needle and syringe.

**Table 1:** Comparison of the different local anaesthetic strategies used to alleviate the pain-induced distress caused by ring castration plus tailing

References	Castration	Tailing
Kent <i>et al.</i> , 1993	Ring plus clamp <sup>#</sup> (6 sec)	Ring plus clamp (6 seconds)
Kent <i>et al.</i> , 1995	Ring plus clamp <sup>#</sup> (10 sec)	Ring plus clamp (10 seconds)
Dinniss <i>et al.</i> , 1997a	Clamp* (1, 5, 10 sec) plus ring	
Dinniss <i>et al.</i> , 1997b	Ring plus clamp* (6 sec) Ring plus clamp* (6 seconds)	Ring plus clamp (6 seconds) Ring
Graham <i>et al.</i> , 1997		Ring plus clamp (10 seconds)
Molony <i>et al.</i> , 1997	(same as Kent <i>et al.</i> , 1995)	
Kent <i>et al.</i> , 1998	Ring plus clamp <sup>+</sup> (10 sec)	Ring plus clamp <sup>+</sup> (10 sec)

\* Clamp applied using the conventional technique.

# Clamp applied across the full width of the scrotal neck.

+ Clamp applied across the full width of the scrotal neck, but proximal to the ring.

**Table 2:** Comparison of the different ring plus clamp approaches

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