Scientific Advisory Committee on Animal Health and Welfare (SACAHW)

Public Consultation on SACAHW Opinion on animal husbandry practices

Teagasc submissions
5 February 2016

teagasc
Agriculture and Food Development Authority

Teagasc responses to the recommendations are highlighted in blue font

Scientific Advisory Committee on Animal Health and Welfare (SACAHW)

Public Consultation on SACAHW Opinion on animal husbandry practices

The following is an opinion of the SACAHW on an issue raised by the Department

> **Opinion taking account of the scientific appropriateness of the time limits set out in the Animal Health and Welfare (Operations and Procedures) (No. 2) Regulations 2014 on (i) castration of cattle and sheep, (ii) dehorning/disbudding of calves, and (iii) tail docking of sheep, S.I. 127 of 2014.**

Background

When the Animal Health and Welfare Act 2013 was being prepared, it was evident that the legislative framework underpinning certain routine animal husbandry practices on farms needed to be brought into line with best scientific knowledge and practice. Rather than delaying progress on the AHW Act, it was decided to take forward the existing standards and have them evaluated by referring them for the advice of the SACAHW. The opinion and recommendations of the SACAHW were considered by the Farm Animal Welfare Advisory Council (FAWAC) at its meeting on 11th December 2015. In additional to consideration by FAWAC, a wider consultation is being undertaken to enable views of all stakeholder interest groups and individuals to be harnessed.

This consultation process presents stakeholders with a unique opportunity to contribute to the decision-making process. The Department is anxious that as wide a view as possible is canvassed and that the legislation is drafted in an informed manner, having regard to the submissions received. All views received will be examined with a view to setting out a proposed course of action in mid 2016.

The SACAHW opinion and recommendations are attached and interested parties are invited to submit comments for consideration.

Submissions can be submitted by email to: niamh.cunningham@agriculture.gov.ie

Alternatively written submissions can be submitted to:

Ms Niamh Cunningham
Animal Health & Welfare Division, 4th Floor
Agriculture House
Kildare Street
Dublin 2
Telephone 01 607 2680

Annex I

Scientific Advisory Committee on Animal Health and Welfare Opinion of the Committee, taking account of current practice in other jurisdictions and recognising the need to provide for necessary husbandry practices, on the scientific appropriateness of the time limits set out in the Animal Health and Welfare (Operations and Procedures) (No. 2) Regulations 2014 on i) castration of cattle and sheep, ii) dehorning/disbudding of cattle, and iii) tail docking of sheep.

This task involves multiple variables, the Opinion is presented according to current knowledge on each of the specified husbandry procedures, according to species.

Castration of Cattle

Castration of cattle is usually performed in order to prevent sexual behaviour, reduce aggression, and increase handling safety. In Ireland cattle can be castrated, other than by a veterinary practitioner, before it attains 6 months of age using a Burdizzo or before it attains 8 days of age using a rubber ring (S.I. 127 of 2014), in both cases without the use of anaesthesia and analgesia (S.I. 107 of 2014).

Numerous scientific studies have investigated acute and chronic pain, distress and performance relating to the age and method of castration. All methods of castration cause significant acute pain and distress, while castration with a rubber ring is also associated with chronic pain. The younger the calf is castrated the less stressful the procedure (Bretschneider, 2005). Castration in 6 day old calves is less painful than in 6 week old calves (Robertson et al., 1994), while castration by Burdizzo is less stressful and less painful in calves 6-8 weeks old than in calves 5.5-6 months old (Ting et al., 2005; Dockweiler et al., 2013). Ting el al. (2005) also found that there was no effect of castration on the overall 42-day growth trends of calves in the study groups (1.5-5.5 months).

Calves castrated at or close to birth reach the same live weight at weaning as calves castrated at weaning (Bretschneider, 2005). Counter to current beliefs, castration-associated weight loss increases with age of calf. Therefore castration after puberty does not confer a benefit on performance related to the anabolic properties of testosterone (Bretschneider, 2005).

Pain relief is recommended, although opinions vary on the efficacy of local anaesthetic (LA) alone and in combination with analgesia (ketoprofen). One study found that ketoprofen alone was more effective in minimising acute stress and behavioural responses to pain (Ting et al., 2003).

Legal provisions from other jurisdictions vary in the requirements relating to castration; however most of those surveyed required LA in all ages. Where there was a requirement for LA for all ages, some jurisdictions did not apply an upper age limit for carrying out the procedure but restricted it to a veterinary procedure (Sweden, Norway), while other competent authorities stipulated age restrictions (2-4 weeks), training requirements, and veterinary involvement (Denmark, Switzerland). In the United Kingdom, castration is permitted by rubber ring up to 7 days and Burdizzo up to 2 months without a requirement for LA, while in New Zealand, castration without pain relief is permitted up to 6 months of age (except where high tension bands are used).

Key Issues

- Regulations from other European jurisdictions with high welfare standards require the administration of pain relief for castration, irrespective of the age of the calf.
- Castration is a painful procedure causing acute pain and distress, but LA and analgesia is not currently required in Ireland for castration of calves by rubber ring at less than 8 days of age and by Burdizzo before it attains 6 months.
- Some methods such as the rubber ring are also associated with chronic pain.
- Given the research pertaining to weights achieved at weaning for calves castrated at different ages, there is no benefit in delaying castration from birth or close to birth until weaning.
- Castration is more painful and stressful in older calves than in younger calves.
- While there are recognised pharmacological methods for the elimination of acute pain following castration, the cost and availability of these products may be problematic.
- One local anaesthetic product, Adrenacaine, and 28 NSAIDs, authorised for use in cattle in Ireland, are suitable for the management of pain associated with the disruption of sensitive tissues. All products are designated Prescription Only Medicines (POM) and as such may be administered by a farmer but only in accordance with a veterinary prescription. Adrenacaine and 24 of the NSAIDs are injectable products and thus require training in terms of administration. Additionally it may be that not all authorised products are marketed in Ireland at any point in time.
- Given experiences surrounding the use of LA for disbudding of calves, farmers may be reluctant and lack the requisite knowledge to use LA for castrating calves.

Recommendations

- Taking into account that younger calves experience less acute responses than older calves, calves castrated by Burdizzo without the use of anaesthetic should be castrated as young as possible.

1. **Teagasc Response (Please see Appendix I with supplementary information)**

We believe that no change in the current status should be made until such time as more objective scientific data are available to better inform policy. We do not support the recommendation.

We wish to highlight concerns with regard to the age of castration of younger versus older calves and highlight areas where we believe further research studies are warranted. The scientific basis for our decisions are as follows:

The impact of castration procedures on cattle welfare (i.e., the stress response) can be assessed using evidence based scientific methods that quantify changes in behavioural, physiological and immunological functions. To fully understand this process, it is imperative to firstly examine the basic biology of the animals, in terms of how individual biological processes can be altered in response to the stress, pain and inflammation elicited following tissue injury; and understand how these processes interact and integrate with each other during the process of coping to minimise or alleviate the negative effects.

Robertson et al. (1994) examined the effects of three methods of castration without analgesia using rubber rings, burdizzo or surgery in Ayrshire bull calves at the three different ages (6, 21, and 42 days) and observed their behaviours over a three-hour period post-castration. The authors reported significantly higher frequencies of restlessness, tail wagging and foot stamping following rubber ring castration than with either surgical or burdizzo castration across all ages. There was a delayed onset of abnormal postures across all ages of calves castrated by rubber rings. The abnormal standing postures in these calves increased from 24 min after castration, followed by increased abnormal lying postures from 90 min to 180 min after castration. An interesting observation was that the burdizzo castrated calves adopted an immobilised "statue" standing, whereas the rubber ring castrated calves displayed greater active behaviours. Robertson et al. (1994) concluded that 1) all methods of castration caused acute pain irrespective of age, and 2) burdizzo castration produced the least pain, but the effect was more pronounced in younger calves.

We examined the effect of age at castration on physiological, immunological stress indices, behavioural responses using 60 Holstein-Friesian bull calves that were sourced so that they were in one of five age groups for Burdizzo castration on day 0 (N= 10 per treatment) : 1.5, 2.5, 3.5, 4.5, and 5.5 mo of age, or were sham castrated at 5.5 months of age (Ting et al., 2005; 2010). Castration was shown to be stressful across all ages between 1.5 and 5.5 mo-old, as indicated by the increased (~180 to >300% mean increase) integrated plasma cortisol response for the first 3 h after treatment relative to the intact controls. However by reducing the age at castration, the integrated cortisol response was markedly reduced (by nearly half) in the 1.5 mo-old and by one-third in 4.5 mo-old castrates, but the ~20% reduction observed in the 2.5 and 3.5 mo-old castrates were not significant. The peak cortisol responses to castration were reduced by castrating calves at younger ages. However, there was no evidence to suggest that the welfare (including performance and immune responses) of calves was adversely affected by burdizzo castration from 1.5 to 5.5 months of age (without use of LA) (Ting et al., 2005). This is an important consideration; the cortisol response was short-lived, there was no adverse effect on immune variables and performance at the age range studied.

In a second study (Ting et al., 2010) the effects of Burdizzo castration on the thermal nociception induced by a CO_2 laser device (heat beam) was investigated by comparing castrated against intact calves at 5.5 mo of age; and the effects of castration at several ages between 1.5 to 5.5 mo on thermal nociception. The hindlimb skin temperatures in the 1.5 and 2.5 mo-old calves were markedly increased compared with baseline following castration. The majority of the calves responded to the laser heat beam by either moving their leg off the ground or lifting it, with little to no kicking response. Within 5.5 mo-old calves, more leg moving, and less leg lifting were observed at 24 and 48 h in castrated than in intact animals. There were no differences among the 3.5 and 4.5 mo-old calves compared with 5.5 mo-old calves in terms of the type of leg responses to the laser at any stage of the study. The 2.5 mo-old calves had less leg moving responses at 48 h than 5.5 mo-old calves. The 1.5 mo-old calves had less leg-lift responses than the calves in all other castration groups 72 h before treatment; and these calves had greater proportions of non-responding leg reactions to the laser (i.e., where the maximum latency of 25 seconds was recorded) at 24 and 48 h after castration than the 3.5 and 4.5 mo-old calves.

By contrast, while the skin temperatures in the intact 5.5 mo-old calves remained unchanged throughout the study period, the 5.5 mo-old castrated calves had marginally (< 1 to 2 ^{o}C) reduced skin temperatures following castration. The precise reasons for these observations are unclear. Variations in skin temperature are likely due to changes in tissue perfusion, metabolism, and blood flow in the superficial veins, and sympathetic nerve activity. However, the question remains to be addressed concerning the physiological developmental stage of calves at 1.5 mo of age versus older calves (2-6 mo-old) and their physiological responses to castration procedures, since the laser-based thermal nociceptive assay used in the study by Ting et al. (2010) was influenced by the initial skin temperature and the age of calves, particularly in calves less than two months of age, which had lower skin temperatures and longer reaction latency to the laser heat beam. This finding would suggest physiologically immaturity of younger compared with older calves.

A misconception that castration and dehorning is less stressful for young animals likely arose because in older animals have a greater peak of plasma cortisol after castration, and calves lose less weight after castration than older animals (Bretschneider, 2005; Stafford and Mellor, 2011). However, as mentioned previously, a high peak of cortisol after a known stressor may be a sign of a well-developed HPA axis

(Moberg and Mench, 2000; Mitra et al., 2009). In fact, Murray and Leslie (2013) suggest that pain may be even greater among neonatal animals compared with mature animals because their nervous system and HPA axis are not developed. Kampen et al. (2006) reported that immune parameters in young calves differ from what is found in older calves and adult animals, and we believe that these age-related factors should be taken into consideration when assessing immunological responses in young calves in response to castration.

Furthermore, in cattle, most of the immune system maturity is seen by 5 to 8 months of age. For example, T cells (CD4+, CD8+ and TCRγδ+ cells) do not reach peak levels until the animal is 8 months of age (Cortese, 2009). Research has demonstrated that from birth, there is a decrease in immune responses until day 3 in calves, when they are at their lowest levels (Cortese, 2009). By day 5, these responses are back to the level of immune responses seen on the day of birth. Procedures like disbudding, castration and movement need to be considered as stressors that have the potential to decrease immune system function temporarily in younger calves.

The source of calves for Irish beef production come from the national dairy and beef herd. There are a wide range of beef production systems in use with the two predominant systems for steer beef production being the grass based suckler calf-to-beef systems (20 month steer beef (suckler bred); 24 month steer beef (suckler); 28 month steer beef (suckler) and dairy calf-to-beef systems (23 month early maturing steer beef (dairy calf to beef); 24 month Friesian steer beef (dairy calf to beef) and 26 month early maturing steer beef (dairy calf to beef).

Most castration studies have been conducted using Holstein-Friesian or dairy breed bull calves younger than 6 mo-old; there are limited data in the literature on the responses of young suckler beef breeds, and in particular continental beef breeds, to castration procedures (see reviews by Bretschneider, 2005 (studies conducted from 1963 to 2000) and Coetzee, 2013 (studies conducted from 1992 to 2011). Considering the inherent differences in husbandy and nutritional management of suckled beef calves versus dairy calf production systems we believe that research investigating the responses of suckled beef calves to castration procedures is warranted.

More recently, and to our knowledge, we are the first group to report (Johnston et al., 2015, 2016) transcriptional down-regulation of genes involved in cell signalling and immune responses in Jersey compared with Holstein-Friesian calves. Due to the differential expression of these immunological genes, we have reported functional differences in immune activity between these two breeds have been reported, including decreased monocyte and T lymphocyte chemotaxis and phagocytosis. Furthermore, the putative risk factors of disease between non-weaned beef and non-weaned dairy calves cannot be estimated because of the inherent differences in their management. Dairy-bred calves are artificially reared, whereas beef calves are generally reared with their dams. Relative to dairy calves there is much less research carried out on beef calves. As part of the DAFM funded RSF project (11/S/131 €asyCalf, led by Dr. Earley, Teagasc, AGRIC, Grange, Co. Meath) the development of immunocompetence from birth to weaning is currently under investigation in suckled beef and artificially reared dairy calves from birth to weaning. We believe that the outcomes of this study will yield important information on the development of immunocompetence in suckled beef and artificially reared dairy calves. This new knowledge of breed-specific immune responses could enable improved health management practices which could be better tailored towards specific husbandry management practices, including disbudding and castration, and disease sensitivities of particular breeds of interest.

Neuroendocrine interactions predominantly occur at the level of the hypothalamic–pituitary axis (HPA), thus pituitary-derived hormones can clearly mediate the effects of the central nervous system (CNS) on immune responses (Gupta et al., 2007a, 2007b; Lynch et al., 2011). The CNS regulation of the immune system acts principally via the hypothalamic–pituitary axis (Carroll and Burdick Sanchez, 2014). This cross regulation (neuro-endocrine-immune) is critical for homeostasis and has profound effects on the health and behaviour of animals. Activation of the HPA axis is the main defining feature of the stress response (Carroll and Burdick Sanchez, 2014).

To monitor the stress response of animals measuring glucocorticoids and HPA axis activity is the standard approach. Through dynamic testing methodologies, the HPA axis can be pharmacologically stimulated with the use of exogenous bovine corticotropin-releasing hormone (bCRH) or adrenocorticotropic hormone (ACTH), and animal responses at both the pituitary and adrenal levels can be successfully assessed, which has been shown by our group previously (Fisher et al., 2002, Gupta et al., 2004, Gupta et al., 2007b) and others (Curley et al., 2008) to be appropriate for investigation into the bovine stress response. Additionally, the HPA axis is also an excellent example of a negative feedback system in which the end product (i.e. cortisol) inhibits the initiating substance (Fisher et al., 1997; Gupta et al., 2004; 2007b).

A combined dexamethasone-suppression/CRH-stimulation test has been widely used in the past to assess dysregulation of the hypothalamic-pituitary-adrenal system (Gaignage et al., 1991) and has been shown by our group to provide an independent method to test the sensitivity of the pituitary and adrenal gland during chronic housing stress (Gupta et al., 2007b). To our knowledge this approach has not been investigated in the calf castration model, or indeed in young calves. We believe research efforts should test HPA responsiveness in calves ranging in age from 1 mo-old to 6 mo-old, in a calf castration model, using appropriate age matched controls. In addition, a focus on characterizing HPA function together with cortisol:dehydroepiandrosterone ratio (DHEA, a precursor of cortisol), previously suggested as an effective biomarker of transport stress (Buckham Sporer et al., 2008) and weaning stress (Lynch et al., 2011) in cattle, should be investigated.

Additionally, on a practical implications level, this research should be designed and conducted to incorporate typical 'on farm' practices, in order to provide practical information and solutions which could be readily disseminated to, and used by farmers to improve pre- and post-castration cattle management practices.

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- Recognising the need to provide for necessary husbandry practices, and taking into account practices in other jurisdictions, it is recommended that the upper age limit for castration of a bull using a Burdizzo without the use of LA be reduced from the current position of 6 months to as close as possible to 2 months. Such a proposed reduction in the upper age limit should be preceded by a communication and education campaign informing farmers of the benefits and justification for earlier castration of calves.

2. Teagasc Response

There are limited studies on the use of LA on the physiological and behavioural responses of calves to burdizzo castration in the age range 2 to 6 mo-old. The majority of studies have been conducted in dairy calves older than 6-mo using LA (ref to Review by Coetzee, 2013). If the upper age limit (2-mo old) for castration of a bull using a burdizzo without the use of LA is being considered, we believe that research is required to support this recommendation for both dairy and suckler beef calves.

We would recommend that no change in the current status with regard to age at castration should be made until such time as more objective scientific data are available to better inform policy. We do not support the recommendation.

The impact of castration procedures on cattle welfare (i.e., the stress response) can be assessed using evidence based scientific methods that quantify the changes in behavioural, physiological and immunological functions in the body, with the objectives to determine: 1) the degree of difficulties (i.e., the stress, pain and inflammation) the animal is experiencing following castration, and 2) the adequacy of the coping mechanisms in response to the injury. To fully understand this process, it is imperative to firstly examine the basic biology of the animals, in terms of how individual biological processes can be altered in response to the stress, pain and inflammation elicited following tissue injury; and understand how these processes interact and integrate with each other during the process of coping to minimise or alleviate the negative effects. The subjective emotional experience of castration pain is probably one of the most significant determinants of acute distress in cattle. However, suitable methods for measurement of the emotional distress (e.g., see Désiré et al., 2002) associated with the perception of castration pain in cattle have yet to be fully developed. Furthermore, the behaviour-based approach is likely to be the most practical, non-invasive method for the assessment of castration stress and pain in cattle. The usefulness of a subjective pain scoring system based on objective ethological data should be further examined (e.g., see Kent et al. (2004) and Grant et al. (2004) for sheep). More recently, Gigliuto et al. (2014) reported that a universal method for identifying and recording pain objectively in large animal models has not yet been developed and suggested that further studies are necessary for the assessment of pain.

We believe that there is a need to have more objective assessments of the pain-induced behavioural responses of younger calves to castration procedures; Automatic recording enables high-resolution, continuous, (sometimes real-time) analysis of physiological and behavioural data from individual animals. It also significantly improves the objectivity of behavioural data. Appropriate methodologies for sensor-based and automated data collection that could be exploited include accelerometry (IceTag pedometers; Lynch et al., 2012) and imaging techniques (Van Hertem et al., 2013a, 2013b).

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- The age limit for non-surgical castration of a bull using a rubber ring should remain unchanged

3. Teagasc Response

We agree with the recommendation on the use of a rubber ring. However we would like to highlight that plasma cortisol concentrations are in a state of flux in young calves during the first week of life.

Plasma cortisol concentrations decrease during the first week of life in neonatal calves and transiently decrease after intake of colostrum, milk or milk replacer (Ronge and Blum, 1988, Baumrucker and Blum, 1994, Lee et al., 1995 and Hadorn et al., 1997). Plasma cortisol concentrations during the first week of life were reported to be higher in calves fed only milk replacer than in those fed colostrum (Hammon and Blum, 1998, Kühne et al., 2000 and Rauprich et al., 2000).

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Delaying castration

There is a general perception among producers that delaying castration could extend the production advantages of keeping animals as bulls until weaning or beyond puberty (Keane and Drennan, 1998). However, a number of studies have shown that there is no advantage in delaying castration of bulls from birth up to 17 months of age in terms of liveweight, growth rate, or carcass weight at slaughter (Worrell et al., 1987; Bagley et al., 1989; Parrassin et al., 1999; Keane, 1999; Knight et al., 1999a,b).
Keane (1999) reported that Burdizzo castration of spring-born calves in their first autumn (complete castration) at five to six months of age did not significantly affect the overall 347-d liveweight gain compared with: 1) delayed unilateral castration - the right testicle removed in autumn and left testicle the following spring with ~178 d apart, or 2) split castration in spring with about one month interval between removal of each testicle. Furthermore, the author reported no interaction between castration treatment and breed type (Friesian versus Charolais × Friesian) was found. Knight et al. (1999a,b) reported that the age at surgical castration (range: 7 to 15 months versus 17 months) of post-pubertal bulls had no effect on either liveweight or carcass weight when the animals were slaughtered at the same age of 22 months.

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Banding castration

We believe that the banding of older cattle also needs to be considered. We have listed a series of studies that addressed the banding of older cattle (Pang et al. 2005, 2006, 2009a, 2009b and 2011).
Teagasc conducted an on-farm study (Pang et al., 2008) which compared burdizzo with banding as castration methods using 12 month old bulls. Animals weighing 399 kg from four different farms were assigned, to one of three treatment groups: banding castration (Banding; 80 bulls), burdizzo castration (Burdizzo; 83 bulls), or controls (control intact; 80 bulls). Local anaesthesia was given with injection of 2% lignocaine (local anaesthetic). Animals were injected 15 minutes prior to banding or burdizzo castration. The band was applied using the Callicrate Smart Bander. Covexin-8 containing tetanus toxoid was administrated 4 weeks before (primary dose) and on the day of castration (booster). All animals were turned out to pasture on the day of castration. Overall intact bulls grew faster than castrates and performed better than either castration treatment. There was no difference between the performance of Band and Burdizzo castrates during the period 15 to 84 days post-castration.

The study by Pang et al. (2008) was selected as one of the top ten in medicine and surgery of food producing animals in 2008. The selection criteria included excellence in study design, statistical analysis and a high likelihood that the results would impact food animal practice.
The selection of the papers was undertaken by Professor Constable, a leading US veterinary scientist and Head of the Department of Veterinary Clinical Science in Perdue University. Professor Constable screened the top 50 veterinary journals and 40 animal agricultural journals as part of the selection

process. The evidenced-based articles reviewed dealt with important topics likely to become "difference makers" and that serve as models for research that scientists should aspire to undertake. Professor Constable highlighted the paper by Pang et al. (2008) at the American College of Veterinary Internal Medicine Forum in Montreal (2008) in a talk titled "Top ten evidenced based papers in food animal medicine and surgery for 2008". The selection of this paper demonstrates the research to be internationally competitive, and it reflects well on the quality of research conducted in Teagasc.

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- The use of analgesia (i.e. NSAIDs) at castration is recommended for all ages.

4. Teagasc Response

We believe that future studies should examine the effect of NSAID administration at the time of the castration procedure, and by other routes, so that the results are relevant to farm production settings. We would recommend that no change in the current status should be made until such time as more objective scientific data are available to better inform policy. We do not support the recommendation.

Studies examining the effect of non-steroidal anti-inflammatory drugs (NSAIDs) on physiological and behavioural responses of calves to castration have involved administration of the analgesic 20 minutes by the i.v. route before the start of the procedure. This protocol, used in research settings, and based on the pharmacokinetics of the NSAID, is used to ensure adequate analgesic drug concentrations in the tissues at the time of castration. However, this significantly diminishes the external validity of these studies because such a delay is impractical in field situations.

Although administration of NSAID's are associated with a decrease in plasma cortisol concentration after castration, most studies have not addressed the practical or production implications of these interventions and routes of administration (s.c., i.m., topical as opposed to i.v.) in a commercial farming situation, and in young and older animals. We believe that studies examining the health and performance effects of newer drugs with extended durations of activity are also needed.

Our studies have reported that local anaesthetic administration is more effective at reducing the cortisol response following surgical compared with burdizzo castration (Earley and Crowe, 2002), and causes increased scrotal swelling when used in conjunction with burdizzo castration (Fisher et al., 1997). Increased scrotal inflammation will prolong the pain response (Fisher et al., 1997). The prolonged increase in scrotal circumference of burdizzo + LA castrates is probably related to the technique of local anaesthetic administration. Local anaesthetic or agents introduced with it, remaining in the testicle after the disruption of venous and lymphatic drainage, may have contributed to the inflammatory reaction.

Prolonged scrotal swelling as a result of the use of this method of induction of local anaesthesia in conjunction with burdizzo castration is undesirable, and could be considered to be welfare unfriendly.

The study by Earley and Crowe (2002) reported that surgical castration induced a significant elevation in cortisol secretion in 6 mo-old calves; the rise in cortisol was reduced to control levels by the administration of ketoprofen but not local anaesthetic (Table 1). Thus, it was concluded that systemic analgesia using ketoprofen was more effective than local anaesthesia during castration at alleviating the stress response.

Table 1. Mean ± SE area under the 12-h cortisol curve (AUC), peak cortisol, and interval to peak cortisol concentrations of 5.5 month-old Friesian calves left untreated (C), surgically castrated (S), surgically castrated following ketoprofen (S + K), surgically castrated following local anaesthetic administration (S + LA), or surgically castrated following local anaesthetic and ketoprofen (S + LA + K; n = 8/treatment group).

Plasma cortisol	Treatment				
	C	S	S + K	S + LA	S + LA + K
AUC, $ng/mL^{-1}\cdot h$	56.8 ± 5.37^{a}	**176.1** ± 27.68^{d}	**78.1** ± 13.87^{ab}	130.8 ± 15.18^{cd}	117.6 ± 19.76^{bc}
Peak, ng/mL	19.0 ± 4.63^{a}	45.8 ± 6.16^{b}	24.7 ± 5.12^{a}	22.1± 2.69^{a}	28.8 ± 4.23^{a}
Interval to peak, h	–	0.31 ± 0.04^{a}	0.29 ± 0.04^{a}	2.63± 0.77^{b}	4.61 ± 1.75^{b}

^{a,b,c,d} Means within a row without common superscripts are different ($P < 0.05$)
Source: Earley and Crowe (2002)

Fisher et al. (1997) reported that the provision of local anaesthesia by intratesticular and s.c. administration of lignocaine reduced the cortisol response in the first 1.5 h after castration with either method, but had little effect thereafter. Burdizzo castration caused scrotal swelling which was prolonged in animals administered local anaesthetic.

A study by Stafford et al. (2002) using different calf castration procedures reported that when local anaesthetic (Lidocaine, 3 mL/testicle, 20 min before castration) was given before castration (surgery castration by traction of spermatic cords and burdizzo clamp castration) the cortisol response was not significantly different from that of the corresponding control calves. Thus, local anaesthetic did not reduce the plasma cortisol response to surgical castration with traction or burdizzo with clamp, but when it was combined with ketoprofen it reduced the cortisol response.

The duration of anaesthesia following the regional infiltration of lignocaine without an added vasoconstrictive agent is approximately 1 h. For castration of calves/cattle, a local anaesthetic such as bupivicaine, which has a duration of action twice that of lignocaine should be investigated.

A study by Pang et al. (2011) showed that banding or burdizzo castration does not induce a general systemic inflammation and does not significantly affect peripheral leukocyte inflammatory cytokine gene expression in 5.5 month old calves. The study showed that systemic inflammatory markers are not altered by burdizzo or banding castration in 5.5 month old calves compared with intact controls.

We believe that further research is required on the use of LA, and in particular the response of younger calves (2-6 mo-old) to burdizzo castration. In addition, the Teagasc responses outlined previously are also pertinent to this recommendation.

New and advanced molecular approaches for assessing stress-immune status and function are being used widely to re-evaluate some of the old beliefs about the young animal's immune system and hypothamic-piuitary-adrenal (HPA) responsiveness. We believe it is essential to elucidate the molecular mechanisms that underlie stress responses to castration, and intervention strategies in young and old animals. To gain a greater understanding of the complex interactions genome-scale high-throughput functional genomics methods such as RNA-seq and complementary bioinformatics are essential and have the potential for identifying diagnostic markers or signatures that are indicative of the stress response and of its severity. We have already applied this state of the art technology for the investigation of stress responses to animal transport of young bulls (Buckham-sporer et al., 2007), weaning stress in beef (O'Loughlin et al.,2011, 2012) and dairy calves (Johnston et al., 2016).

Functional genomics approaches such as RNA-seq, generate genome-scale expression data for both mRNA and non-coding RNA, and applying this method to castration research can expedite the development of stress biomarkers and procedural outcomes. RNA-seq is currently the best approach to perform transcriptome profiling that uses deep-sequencing technologies revolutionizing our view of the extent and complexity of bovine transcriptome. Studies have been conducted in Teagasc, AGRIC, Grange, Co. Meath (McCabe et al., 2012; O'Loughlin et al., 2012; Keogh et al., 2016; McGettigan et al., 2016; Johnston et al., 2016) using this methodology in cattle in a range of tissue types and generated sequences on an unprecedented scale at a fraction of the cost of previous approaches. Arriving at a list of expressed or differentially expressed genes (DEG) identified by high-throughput approaches requires a systems biology bioinformatic examination, in the context of identifying molecular pathways and gene networks, to decipher the underlying regulatory mechanisms that are responsible for a given phenotype or biological response. We believe that it is essential to examine the molecular mechanisms regulating the stress response to castration in beef and dairy calves using RNA-seq and bioinformatic analyses. To our knowledge the application of RNA-seq technology has not been investigated to-date in a calf castration model.

We also believe that a collective examination of blood immune cell markers, neutrophil and lymphocyte number, functional activity (neutrophil phagocytosis), and proportion of CD4+ and WC1+ lymphocytes (Burton et al., 2005) would be useful support stress biomarkers to examine in the calf castration model. Failure of these blood stress-immune biomarkers to return to baseline levels as time progresses post-castration would signal disturbed homeostasis. We have applied this approach when investigating stress responses to abrupt weaning of suckled beef calves (Lynch et al., 2010, 2011). To our knowledge, these functional stress-immune biomarkers have not been investigated in response to castration stress. We believe that examination of the biomarker panel consisting of neutrophil and lymphocyte number, percentage neutrophils performing phagocytosis, and percentage CD4+ and WC1+ lymphocytes, neutrophil trafficking (L-selectin) would be indicative of calves sensitive to stress and consequently more susceptible to infection post-castration.

We also believe that research is needed to guide the timing of vaccination protocols against calf pneumonia, and more especially the management of young calves prior to and post castration. There is clear evidence that endemic infection, namely the bovine respiratory disease complex (BRDC) represents a major threat to animal health (Lorenz et al., 2011a; 2011b; O'Neill et al., 2014; Murray et al., 2015). Peak incidence of bovine respiratory disease (BRD) occurs in young calves, less than 6 months of age, often in the face of pre-existing maternally derived antibody (MDA) (Patel and Didlick, 2004). Many vaccine protocols have been developed to vaccinate young calves at frequencies as often as weekly during the first and second months of age. Finding any experimental studies that support this timing is difficult. Vaccination of calves is complicated by the presence of significant levels of maternally derived antibodies that persist in calves, colostral and neonatal hormonal factors, the lack of full immune competence, and husbandry stressors. Considering the differences in passive immune status both within and between artificially reared dairy and suckled beef calves (Todd et al., 2015), the response to castration may vary substantially, and thus, susceptibility to infectious disease.

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- The Farm Animal Welfare Advisory Council should be requested to develop detailed guidelines on the castration of calves, taking into account these recommendations (e.g. effects of castration on stress and pain responses and growth rates, the justification for earlier castration, recommendations for pain relief, appropriate handling and castration techniques).

5. Teagasc Response

We support this recommendation, however we believe that further research is necessary to inform the preparation of the guidelines and the associated procedures.

Disbudding of Calves

Disbudding is performed for economic and practical reasons: to prevent bullying and injury to other animals (with implications for productivity and carcass damage respectively) and human safety during handling. There are three techniques used: cautery, surgical and caustic paste. Cautery is recommended by the European Food Safety Authority (EFSA) and other authority organisations, and is the only method of disbudding allowed in Ireland under S.I. 127 of 2014, which permits disbudding of calves up to 28 days old by thermal cauterisation. Either local anaesthetic (LA) or analgesia is required for disbudding on calves that have attained the age of 15 days.

There is variation in the suggested upper age limits for disbudding of calves ranging from 6 to 8 weeks of age. These age limits are not based on empirical evidence, but rather on opinion related to the physical development of horns in calves and that the horn buds become attached to the underlying periosteum at approximately 2 months of age. It is stated that the development of horns in some beef breeds occurs much later than in dairy herds but there are no empirical data to support this. The degree of tissue damage associated with disbudding is determined by the stage of development of the horn bud e.g. in younger calves the burning of the vessels surrounding the horn bud is sufficient, whereas the whole bud needs to be removed (by levering it out from the side) when the horn is further developed.
Disbudding is painful and stressful, but is less painful than dehorning. Pain relief is recommended in all ages.

Research by Dwane et al. (2013) using focus groups reported that some Irish beef farmers were reluctant to use LA and opted to disbud calves less than 14 days old in order to avoid using it, even when the horn bud had not erupted. This may indicate a lack of knowledge concerning aspects of the disbudding procedure and the application of LA.

Legal provisions in other jurisdictions show a range of age limits set for disbudding and the administration of LA (Table 2; ALCASDE, 2009). These age limits, where specified, range from 3 weeks to 6 months. In accordance with EFSA opinion, many countries surveyed now require the use of LA for disbudding, irrespective of age. However, almost as many other countries allow disbudding without LA for some of or the entire allowable age limit. Switzerland provides specific training on pain management and licences farmers to administer LA.

Key Issues

- Regulations from other European jurisdictions with high welfare standards require the administration of anaesthesia for disbudding, irrespective of the age of the calf.
- Lack of consensus over the appropriate upper age limit for disbudding of calves.
- Lack of empirical data regarding the possible later development of horn buds in certain breeds of cattle.
- Both disbudding and dehorning are painful procedures, irrespective of the age of the animal. However there is a wide variation in the legislative requirements in other countries.
- Adequacy of current training of farmers in administering LA for the purpose of disbudding.
- Disbudding prior to development of the horn buds to avoid use of LA.
- Disbudding late, once the horns have attached to the underlying periosteum.
- One local anaesthetic product, Adrenacaine, and 28 NSAIDs, authorised for use in cattle in Ireland, are suitable for the management of pain associated with the disruption of sensitive

tissues. All products are designated Prescription Only Medicines (POM) and as such may be administered by a farmer but only in accordance with a veterinary prescription. Adrenacaine and 24 of the NSAIDs are injectable products and thus require training in terms of administration. Additionally it may be that not all authorised products are marketed in Ireland at any point in time.

Recommendations

- The upper age limit of disbudding of a bovine should remain unchanged at 28 days subject to research on the development of the horn bud in different breeds and genetic lines.

1. Teagasc response (Please see Appendix II with supplementary information)

We support the recommendation. We agree that setting definitive ages for disbudding or dehorning is difficult since no published data are available on the development of horns in suckler beef breeds with anecdotal evidence to suggest that bud development occurs much later than in the dairy breeds. Most studies in the literature have been conducted using dairy calves (Appendix II).

- Pain relief is recommended for all ages.

2. Teagasc response

3. We would recommend that no change in the current status should be made until such time as more objective scientific data are available to better inform policy.

4. We believe that future studies should examine the effect of NSAID administration at the time of the procedure, and by other routes (e.g. topical anaesthetic formulation (Tri-Solfen) available in Australia), so that the results are relevant to farm production settings, experience of the farmer, skills of the farmer with the administration of NSAIDs and LAs and to breed and age of calf. Products need to be made available to support the recommendation (see Response 6 below).

- The Farm Animal Welfare Advisory Council should be requested to develop detailed guidelines on the disbudding of calves (e.g. justification, anatomy, physiology, behaviour, pain, handling, techniques for disbudding and LA use, equipment).

5. Teagasc response

We agree with this recommendation.

- Training in the use of local anaesthetic for disbudding should be included in the curriculum of agriculture and animal science education programmes.

6. Teagasc response

We agree with this recommendation. Two products are currently recommended, Willcain and Adrenacaine, for the provision of local anaesthesia for disbudding of calves.
http://www.veterinaryireland.ie/images/stories/VI_links/pdf/Use%20of%20Local%20Anaesthetics.pdf

In Australia, topical anaesthetic formulation (Tri-Solfen) is available. Tri-Solfen is a local anaesthetic and antiseptic gel spray for use on lambs to provide pain relief following mulesing, in Australia. It has also been developed to reduce blood loss and infection in order to improve wound healing. The product contains two proven topical local anaesthetics; fast-acting Lignocaine for immediate pain relief and long-acting Bupivacaine for prolonged post-operative pain relief. Adrenaline is included for its ability to reduce the shock and stress of blood loss, whilst prolonging the anaesthetic action. Tri-Solfen also contains Cetrimide, an antiseptic widely used to cleanse skin and wounds and provides protection from bacterial contamination. The product's gel base adheres well to the wound and acts as a barrier to environmental stimuli to improve wound healing.

A Finnish study by Hokkanen et al. (2014) reported that oromucosal detomidine gel is an effective sedative for calves prior to infiltration of local anaesthetics and to disbudding. The authors suggested that this non-invasive and user-friendly oromucosal sedation method for calves could ease the use of local anaesthetics. Detomidine is a potent α2-adrenoceptor agonist that is used commonly for sedation or premedication in horses and cattle, including calves (Peshin et al. 1991).

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We recommend that similar type products be made available in Ireland, if possible.

- SACAHAW recommends that breeding for polledness should be included as part of holistic breeding programs taking into account other economically important traits.

7. Teagasc response

We believe that selection and breeding of polled stock, when available, should be considered as an alternative because it eliminates both animal pain and production expenses associated with disbudding/dehorning.

- SACAHAW recommends that research be carried out –
    - to determine the age range in the development of horn buds for different breeds and genetic lines of cattle.
    - into alternative methods of cautery disbudding.

8. Teagasc response

Teagasc supports this recommendation that further research is necessary.

We would recommend that no change in the current status should be made until such time as more objective scientific data are available to better inform policy.

Tail docking of Sheep

Tail docking is performed to prevent the accumulation of faeces on the tail and anogenital area and thus to reduce the risk of fly strike and cutaneous myiasis. It is also performed for management of ewes at mating and lambing. The risk of fly strike depends on climatic conditions, sheep breed and the production system. For example, early lambs are not tail docked because they are born in winter and slaughtered in the spring, before fly strike emerges. The relationship between tail docking and faecal build-up is unclear, as is the relationship between cleanliness and fly strike. In Ireland tail docking of sheep, without the use of anaesthesia/analgesia, is permitted in sheep that have not attained the age of eight days using a rubber ring to constrict the flow of blood to the tail.

Tail docking is normally performed within the first 48h of birth, using a rubber ring, thermocautery or excision, retaining enough of the tail to cover the anogenital region. Ram lambs are normally castrated at the same time.

Empirical data suggest than younger lambs suffer from the same pain responses as older lambs (Dwyer, 2008) and are not less sensitive to acute pain than older lambs (Guesgen et al., 2011). These findings support the view that the current legislation which limits the use of the rubber ring to the first week of life appears to be based on the erroneous impression that lambs of this age feel less pain than older lambs (FAWC, 1994).

Local anaesthetic (LA) reduces the effects of acute pain, and may reduce the time course and behavioural responses associated with chronic pain. The combined method of Burdizzo application across the full width of the tail just proximal to and following rubber ring application also reduces the level of acute pain by crushing the afferent nerves from the tail.
In countries surveyed where tail docking of lambs is permitted, the maximum age at which a rubber ring can be applied is 7 days, with none of these countries requiring the use of LA for this procedure.

The EFSA Scientific Opinion on sheep welfare (2014) reports that experts consider painful management procedures such as tail docking to have significant welfare consequences for lambs.

Key Issues

- Regulations from other European jurisdictions with high welfare standards tend to allow tail docking of lambs without the use of local anaesthesia up to 7 days of age. Tail docking is banned by both Sweden and Norway, except for therapeutic reasons.

- Tail docking is a painful procedure and ram lambs that are castrated at the same time are likely to suffer considerable acute and chronic pain.

- Research has demonstrated that young neonatal lambs show similar pain responses to older lambs following tail docking.

- Excessive docking of tails may increase the risk of rectal prolapse, while little is known about other effects of short docking.

- Limited evidence evaluating the reduction in fly strike associated with tail docking with only one of three studies demonstrating reduced strike in docked sheep.

- Lack of research about the importance of the tail in other contexts such as social communication or fly control.

- Currently there are no local anaesthetics or NSAIDs specifically authorised for use in sheep in Ireland. In practice the situation is the same as already described in cattle as the products authorised in cattle may be prescribed by veterinary practitioners under the ‘cascade’ arrangements, when no authorised product is available.

Recommendations

- The upper age limit of tail docking by rubber ring of a lamb before it attains the age of 8 days should remain unchanged.

1. Teagasc response

We agree with this recommendation

- Application of a Burdizzo across the width of the tail proximal to and following rubber ring application is recommended in order to reduce acute pain and accelerate healing.

2. Teagasc response

We do not support the recommendation and propose that further research is required.

Tail docking is widely considered to reduce the level of faecal soiling and subsequently decrease fly-strike, which is a serious welfare problem. However, we believe that there is a lack of empirical evidence linking docking with reduced fly-strike at flock level and other management strategies aimed at reducing susceptibility to strike or decreasing fly numbers may be equally, if not more effective.

We believe that management strategies aimed at controlling "fly-strike" should be considered.

While it is recognised that the process of castration and tail docking itself causes stress, and evidence that immune stressors are relatively prevalent in lambs, the potential for stressors to modulate acute pain following castration and/or tail docking in lambs has not been fully investigated.

It is important to understand the interactions between stressors, such as isolation or infection, and a lamb's response to painful stimuli in order to reveal how stress and pain systems interact in this species, and to inform husbandry recommendations concerning the likely influence of a lamb's underlying health and separation from the dam on it's response to castration and tail docking.

The handling and short-term removal of the lamb from the dam to carry out castration and/or tail docking is another source of stress to the lamb.

Lambs may be immunologically stressed around the time of castration or tail docking as a result of sub-clinical disease. Infections such as 'watery mouth', pneumonia, 'navel ill' and 'joint ill' are common (Barlow et al. 1987; Watkins and Sharp 1998; Mellor and Stafford 2000, 2004; Roger 2008).

The present DAFM FAWAC guidelines (Animal Welfare Guidelines For SHEEP FARMERS) states:
Sheep's tails should not be docked routinely, only if there is a real threat of fly strike. Sheep farmers should consider carefully whether tail docking within a particular flock is necessary. Tail docking may be carried out only if failure to do so would lead to subsequent welfare problems because of dirty tails and potential fly strike. If it is considered that both tail docking and castration are necessary, thought should be given to performing both operations simultaneously so as to minimise disruption through repeated handling and the potential for mis-mothering and distress. In any case the use of tail docking should be carried out before seven days of age.

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The Farm Animal Welfare Advisory Council should be requested to develop detailed guidelines on tail docking in lambs (e.g. justification, methods, pain relief, alternatives to tail docking i.e. fly control, etc.).

3. Teagasc response

We feel that objective scientific data are necessary to inform the preparation of detailed guidelines on tail docking in lambs and with particular relevance to sheep breeds and climatic conditions.

- SACAHAW recommends that research should be carried out on the prevalence of flystrike in Ireland and the relationship between tail-docking and the risk of flystrike.

4. Teagasc response

We agree with this recommendation.

Castration of sheep

Ram lambs are castrated to prevent indiscriminate breeding, to avoid aggressiveness between males, and to improve performance. Castration is typically conducted in neonatal ram lambs at, or shortly after birth using rubber rings. It is normally conducted at the same time as tail docking. In Ireland the non-surgical castration of a ram is permitted, without the use of anaesthesia/analgesia, by use of a Burdizzo where the ram has not attained the age of 3 months, or, where a rubber ring to constrict the flow of blood to the scrotum is used, before the ram attains the age of 8 days.

Older lambs have more severe scrotal lesions and more chronic pain following castration than younger lambs.

Local anaesthetic reduces the effects of acute pain, and may reduce the time course and behavioural responses associated with chronic pain. The combined method of Burdizzo application across the scrotum after rubber ring also reduces the level of acute pain.

The EFSA Scientific Opinion on sheep welfare (2014) reports that experts consider painful management procedures such as castration to have significant welfare consequences for lambs.

Key Issues

- Regulations from other European jurisdictions with high welfare standards require the administration of pain relief for castration, irrespective of the age of the lamb.
- Lack of consensus on the need to castrate.
- Some methods such as the rubber ring are associated with acute and chronic pain.
- Castration in ram lambs is typically conducted at the same time as tail docking and is likely to cause considerable acute and chronic pain.
- Currently there are no local anaesthetics or NSAIDs specifically authorised for use in sheep in Ireland. In practice the situation is the same as already described in cattle as the products authorised in cattle may be prescribed by veterinary practitioners under the 'cascade' arrangements, when no authorised product is available.

Recommendations

- When castration is deemed appropriate, it should be performed in lambs as young as possible.

1. Teagasc response

We believe that research is necessary to support this recommendation. Comprehensive studies involving cortisol, haptoglobin, haematology, active behavioural responses and growth parameters would provide a clearer picture of the overall animal response.
Consideration needs to be given to the problems of practicality in achieving effective local anaesthetic infiltration of the scrotum and tail of young lambs, particularly given the numbers of animals that are typically treated on farms.
Furthermore, regulatory issues also limit the use of analgesic treatments in sheep.

- The upper age limits of castration by rubber ring of a lamb before it attains the age of 8 days and of castration by Burdizzo of a lamb before it attains the age of 3 months should remain unchanged.

2. Teagasc response

We believe that this recommendation should stand until research to the contrary is available.

- Application of a Burdizzo across the width of the scrotum proximal to and following rubber ring application is recommended in order to reduce acute pain and accelerate healing.

3. Teagasc response

We believe that research is necessary to support this recommendation.

- The Farm Animal Welfare Advisory Council should be requested to develop detailed guidelines on the castration of lambs (e.g. justification, methods, pain relief, alternatives to castration i.e. management).

4. Teagasc response

We believe that research is necessary to support this recommendation.

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Appendix I

Teagasc published (castration) studies

Earley B, Crowe MA. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. . J Anim Sci. 2002 Apr;80(4):1044-52.

Fisher AD, Crowe MA, O'Nualláin EM, Monaghan ML, Prendiville DJ, O'Kiely P, Enright WJ. 1997. Effects of suppressing cortisol following castration of bull calves on adrenocorticotropic hormone, in vitro interferon-gamma production, leukocytes, acute-phase proteins, growth, and feed intake. J Anim Sci. 1997 Jul;75(7):1899-908.

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Ting ST, Earley B, Crowe MA.2003. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. J Anim Sci. 2003 May;81(5):1253-64.

Ting ST, Earley B, Crowe MA. 2004. Effect of cortisol infusion patterns and castration on metabolic and immunological indices of stress response in cattle. Domest Anim Endocrinol. 2004 May;26(4):329-49.

Ting, S.T.L., Earley, B., Veissier, I., Gupta, S., Crowea, M.A. 2005. Effects of age of Holstein-Friesian calves on plasma cortisol, acute-phase proteins, immunological function, scrotal measurements and growth in response to Burdizzo castration. Animal Science, 80, 377-386

Ting, S. T. L.; Earley, B.; Veissier, I.; Gupta, S.; Crowe, M. A. 2010. Title Effects of Burdizzo castration on CO_2 laser induced thermal nociception of Holstein-Friesian calves of different ages. Applied Animal Behaviour Science 2010 Vol. 126 No. 1/2 pp. 12-18

Abstracts

Fisher AD, Crowe MA, O'Nualláin EM, Monaghan ML, Prendiville DJ, O'Kiely P, Enright WJ. 1997. **Effects of suppressing cortisol following castration of bull calves on adrenocorticotropic hormone, in vitro interferon-gamma production, leukocytes, acute-phase proteins, growth, and feed intake.** J Anim Sci. 1997 Jul;75(7):1899-908.

The objective was to determine the effects of reducing the plasma cortisol rise in calves following castration on plasma ACTH concentrations, keyhole limpet hemocyanin (KLH)- and concanavalin A (Con A)-induced in vitro interferon (IFN)-gamma production, white blood cell (WBC) numbers, neutrophil:lymphocyte (N:L) ratio, plasma haptoglobin and fibrinogen concentrations, ADG, and ADFI. Forty 5-mo-old Friesian bull calves (169 +/- 1.7 kg) were assigned to four treatments: 1) control (CON); 2) oral metyrapone administration (MET); 3) surgical castration at 0 h on d 0 (SURG); and 4) oral metyrapone administration and surgical castration (MET+SURG). Cortisol, ACTH, IFN-gamma production, haptoglobin, fibrinogen, ADFI, and ADG were not different between CON and MET animals. The MET+SURG calves had lower (P < .001) peak and mean cortisol during .25 to 1.5 h than SURG animals, but area under the cortisol vs time curve from 0 to 12 h did not differ (P > .39) between SURG and MET+SURG calves. Peak ACTH concentrations and area under the ACTH vs time curve from 0 to 6 h were greater (P < .05) for MET+SURG than for SURG calves. There were no differences between MET+SURG and SURG animals in IFN-gamma production, WBC numbers, and ADFI. On d 1, MET+SURG and SURG animals had lower (P < .01) KLH- and Con A-induced IFN-gamma production and higher (P < .05) neutrophil numbers and N:L ratio compared with CON animals. Plasma haptoglobin on d 1 and 3 and fibrinogen concentrations on d 3 and 7 were elevated (P < .05) for MET+SURG and SURG compared with CON animals, whereas SURG animals had greater (P < .05) haptoglobin and fibrinogen concentrations than MET+SURG animals on d 7. The ADG of SURG calves was lower (P < .05) than that of MET+SURG calves during d 0 to 7. Metyrapone treatment partially suppressed cortisol and increased ACTH in castrated calves but did not alter the castration-induced suppression of IFN-gamma and increases in neutrophil numbers and the N:L ratio.

In conclusion, a reduction in the initial surge in plasma cortisol concentrations following surgical castration in calves resulted in increases in the magnitude and duration of elevated ACTH and some alleviation of the castration-induced reductions in ADG and ADFI, but did not alter the subsequent castration-induced suppression of in vitro interferon-γ production and increases in the N:L ratio, haptoglobin and fibrinogen. The administration of 3 g of metyrapone every 4 h for 48 h to bull calves of 170 kg BW is not sufficient to suppress fully the rise in plasma cortisol concentrations following surgical castration. Although this level of metyrapone administration does not affect the post-castration suppression of in vitro interferon-γ production, this may be linked to the increased secretion of hormones of the hypothalamic and pituitary elements of the hypothalamic-pituitary-adrenal axis, due to a decrease in negative feedback from cortisol.

Fisher AD, Crowe MA, O'Nualláin EM, Monaghan ML, Larkin JA, O'Kiely P, Enright WJ. 1997. **Effects of cortisol on in vitro interferon-gamma production, acute-phase proteins, growth, and feed intake in a calf castration model.** J Anim Sci. 1997 Apr;75(4):1041-7.

The objective of this study was to determine the effects of castration, with its presumed pain and inflammatory effects, including increased cortisol, and elevated cortisol per se on in vitro interferon-gamma (IFN-gamma) production, ADG, ADFI, and plasma haptoglobin and fibrinogen. Thirty Friesian bull calves (174 +/- 3.8 kg) were assigned to three treatments (given on d 0): 1) control (CON); 2) i.v. cortisol administration to mimic castration-induced increases in cortisol (CORT); and 3) surgical castration (SURG). Blood samples were collected for 12 h on d 0 and at 24 and 72 h after treatment for cortisol determination. Keyhole limpet hemocyanin (KLH)- and concanavalin A (Con A)-induced in vitro IFN-gamma production in blood, and plasma haptoglobin and fibrinogen were measured in blood samples taken before treatment on d 0 and on d 1 and 3. On d 0, CORT and SURG animals had higher peak cortisol (P < .001) and area under the cortisol curve (P < .001) than CON animals. There were no differences (P > .05) between CON, CORT, and SURG animals in cortisol at 24 and 72 h. There were no differences (P > .05) between CON and CORT animals in IFN-gamma production, haptoglobin, fibrinogen, ADG, and ADFI. Compared with CON animals, SURG animals had lower (P < .05) KLH-induced IFN-gamma on d 1 and CON A-induced IFN-gamma on d 1 and 3. Haptoglobin concentrations were greater (P < .05) for SURG than for CON animals on d 1 and 3. Fibrinogen concentrations were greater (P < .001) for SURG than for CON animals on d 3. The SURG animals had lower (P < .01) ADG and ADFI during d 0 to 7 than CON animals. In conclusion, castration decreased IFN-gamma production, ADG, and ADFI and increased haptoglobin and fibrinogen, and these effects seemed to be independent of plasma cortisol concentrations.

In conclusion, surgical castration induced increases in plasma haptoglobin and fibrinogen, and decreases in in vitro interferon-γ production, ADG and ADFI, which were not mediated by the castration-induced rise in plasma cortisol alone. Factors other than increased plasma cortisol alone are responsible for the suppression in performance and immune function (as measured by reduction in interferon-γ production) following surgical castration of calves. The reduction in interferon-γ production occurred 24 and 72 hours after castration concurrent with increased plasma APP concentrations, and may be associated with the inflammatory process following castration.

Earley B, Crowe MA. 2002**Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses.** . J Anim Sci. 2002 Apr;80(4):1044-52.

To determine the effects of the anti-inflammatory ketoprofen, alone or with local anesthesia (LA) during castration on cortisol, immune, and acute phase responses, 40 Friesian calves (215 +/- 3.5 kg) were assigned as follows: 1) control, 2) surgical castration (SURG), 3) SURG following ketoprofen (3 mg/kg BW i.v.; SURG + K), 4) SURG following LA (9 mL of 2% lidocaine hydrochloride to each testis; SURG + LA), or 5) SURG following LA and K (SURG + LA + K). Total cortisol response was greater (P < 0.05) in SURG, SURG + LA, and SURG + K + LA calves than in control calves and was not different between control and SURG + K calves. The interval to peak cortisol was longer (P < 0.05) for SURG + K + LA calves than for either SURG or SURG + K calves. On d 3, KLH-induced interferon-gamma production was lower (P < 0.05) in SURG calves than in control calves, whereas concanavalin A-induced interferon-gamma production was lower (P < 0.05) in all castration groups than in control. On d 1 after surgery, fibrinogen was higher (P < 0.05) in SURG and SURG + LA calves than in control calves, whereas SURG + LA + K calves had lower (P < 0.05) fibrinogen than did SURG calves. Haptoglobin was higher (P < 0.05) in SURG calves on d 1, 3, and 7 than in control calves. On d 1 after surgery, SURG + K and SURG + LA + K calves had lower (P < 0.05) haptoglobin concentrations than SURG calves, whereas SURG + K calves had lower (P < 0.05) levels than SURG calves on d 3. In conclusion, surgical castration induced a significant elevation in cortisol secretion; the rise in cortisol was reduced to control levels by the administration of ketoprofen but not local anaesthetic. Thus, systemic analgesia using ketoprofen is more effective than local anesthesia during castration to alleviate the associated stress response.

Ting ST, Earley B, Hughes JM, Crowe MA. 2003. **Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior.** J Anim Sci. 2003 May;81(5):1281-93.

To determine the effects of burdizzo castration alone or in combination with ketoprofen (K), local anesthesia (LA), or caudal epidural anesthesia (EPI) on plasma cortisol, acute-phase proteins, interferon-gamma production, growth, and behavior of beef cattle, 50 Holstein x Friesian bulls (13 mo old, 307 +/- 5.3 kg) were assigned to (n = 10/treatment): 1) control (handled; C); 2) burdizzo castration (B); 3) B following K (3 mg/ kg of BW i.v.; BK); 4) B following LA (8 mL into each testis and 3 mL s.c. along the line where the jaws of the burdizzo were applied with 2% lidocaine HCl; BLA); and 5) B following EPI (0.05 mg/kg of BW of xylazine HCl and 0.4 mg/kg of BW of lidocaine HCl as caudal epidural; BEPI). The area under the cortisol curve against time was lower (P < 0.05) in BK than in B, BLA, or BEPI animals. On d 1 after treatment, plasma haptoglobin concentrations were higher (P < 0.05) in B, BLA, and BEPI than in BK animals. On d 3, haptoglobin and plasma fibrinogen concentrations were higher (P < 0.05) in all castration groups than in C. On d 7, haptoglobin and fibrinogen concentrations remained higher (P < 0.05) in BLA than in B and C animals. On d 1, concanavalin A-induced interferon-gamma production was lower (P < 0.05) in B, BLA, and BEPI than in C, but there was no difference between BK and C animals. From d -1 to 35, ADG was lower (P < 0.05) in B, BLA, and BEPI animals, but not in BK compared with C animals. Overall, there was a higher (P < 0.05) incidence of combined abnormal postures in B than in C, BK and BEPI animals. Although the use of K and EPI decreased (P < 0.05) these postures compared with B alone or B with LA, there was no difference between the K and EPI treatment. In conclusion, burdizzo castration increased plasma cortisol and acute-phase proteins, and suppressed immune function and growth rates. Local anesthesia prolonged the increase in acute-phase proteins. Ketoprofen was more effective than LA or EPI in decreasing cortisol and partially reversed the reduction in ADG following castration. The use of K or EPI was more effective than LA in decreasing pain-associated behavioral responses observed during the first 6 h after treatment. Systemic analgesia with ketoprofen, a non-steroidal antiinflammatory drug, was more effective in reducing inflammatory responses associated with castration than LA or EPI.

Ting ST, Earley B, Crowe MA.2003. **Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior**. J Anim Sci. 2003 May;81(5):1253-64.

To determine the effect of repeated ketoprofen (K) administration to surgically castrated bulls on cortisol, acute-phase proteins, immune function, feed intake, growth and behavior, 50 Holstein x Friesian bulls (11 mo old; 300 +/- 3.3 kg) were assigned to one of five treatments: 1) untreated control (C); 2) surgical castration at 0 min (S); 3) S following an i.v. injection of 3 mg/kg of BW of K at -20 min (SK1); 4) S following 1.5 mg/kg of BW of K at -20 and 0 min (SK2); or 5) S following 1.5 mg/kg of BW of K at -20 and 0 min and 3 mg/kg of BW of K at 24 h (SK3). Castration acutely increased plasma cortisol concentrations in S- and K-treated animals compared with C, with no differences in peak and interval to peak cortisol responses among the castration groups. Overall, the integrated cortisol response was greater (P < 0.05) in the castrates than in C, whereas K treatments decreased (P < 0.05) this response compared with S alone, with no differences between K treatments. Plasma haptoglobin and fibrinogen concentrations were increased (P < 0.05) on d 3 in the castration groups compared with C as the result of tissue trauma induced by castration, whereas SK1 and SK2 had lower (P < 0.05) haptoglobin concentrations than S animals. On d 1, concanavalin A-induced interferon-gamma production was suppressed (P < 0.05) in S and SK3 compared with C, SK1, and SK2 animals. Overall from d 1 to 33, DMI were lower (P < 0.05) in S, SK1, and SK3 than in C animals. From d -1 to 35, ADG were lower (P < 0.05) in S, SK2, and SK3 compared with C animals. A higher (P < 0.05) incidence of standing postures and lower incidence of lying postures was observed in S compared with C during the first 6 h after treatment. However, the higher (P = 0.02) incidence of abnormal standing activities observed for S was reversed (P < 0.05) by the K treatments. In conclusion, surgical castration increased plasma cortisol and acute-phase proteins and decreased immune function, feed intake, and growth rate. Ketoprofen effectively reduced the cortisol response to castration, but there was no advantage in treating with two split doses of K (1.5 mg/kg of BW per dose). A repeated K dose 24 h after treatment (3 mg/kg of BW) had no influence on changes in acute-phase proteins and immune response. Systemic analgesia with K is an effective method for alleviating acute inflammatory stress associated with castration.

Ting ST, Earley B, Crowe MA. 2004. **Effect of cortisol infusion patterns and castration on metabolic and immunological indices of stress response in cattle.** Domest Anim Endocrinol. 2004 May;26(4):329-49.

This study tested the hypotheses that: (1) either acute stress induced by Burdizzo castration, or cortisol infusion would modulate plasma glucose, insulin and growth hormone (GH) concentrations; and (2) immune modulation induced by cortisol would be dependent on the pattern, intensity and duration of circulating cortisol concentrations. Fifty 9.2-month-old Holstein x Friesian bulls (232 +/- 2.0 kg) were blocked by weight and randomly assigned to one of five treatments (n = 10 per treatment): (1) sham handled control; (2) Burdizzo castration; (3) hydrocortisone infusion to mimic the castration-induced secretion pattern of cortisol; (4) hourly pulse infusion of hydrocortisone; and (5) sustained infusion of hydrocortisone for 8h. Blood samples were collected intensively on day 0, and weekly from days 1 to 35. Castration acutely increased plasma cortisol, GH and haptoglobin concentrations, suppressed lymphocyte in vitro interferon-gamma (IFN-gamma) production, but had no effect on plasma glucose and insulin concentrations. Cortisol infusion to simulate the castration-induced secretion pattern of cortisol, and pulse infusion of cortisol did not suppress the IFN-gamma production. A sustained infusion of cortisol resulted in the transient suppression of IFN-gamma production. Moreover, the sustained cortisol infusion resulted in increased plasma glucose, insulin and GH concentrations. The overall 14-day feed intakes and 35-day growth rates were not affected by treatments. In conclusion, cortisol infusion to induce immune suppression in vivo occurred only at pharmacological doses. Within physiological ranges, cortisol was not associated with the suppression of immune function, indicating that during castration cortisol per se is not responsible for the suppression of in vitro IFN-gamma production.

Ting, S.T.L., Earley, B., Veissier, I., Gupta, S., Crowea, M.A. 2005. **Effects of age of Holstein-Friesian calves on plasma cortisol, acute-phase proteins, immunological function, scrotal measurements and growth in response to Burdizzo castration.** Animal Science, 80, 377-386

To determine the effect of age at castration on physiological and immunological stress indices, 60 Holstein-Friesian bull calves were sourced so that they were in one of five age groups for Burdizzo castration on day 0 (16 July 2002; no. = 10 per treatment) : 1·5, 2·5, 3·5, 4·5, and 5·5 months of age (mean body weight ± s.e. = 63 ± 2·5, 89 ± 3·7, 104 ± 3·7, 142 ± 3·6, 169 ± 8·1 kg, respectively), or were sham castrated at 5·5 months of age (171 ± 2·9 kg body weight) to serve as intact controls specific to this age group. Blood samples were collected at 15- to 30-min intervals from 2 h before until 8 h after treatment, with further samples collected at 10 and 12 h on day 0, and on days 1, 2 and 3, and weekly from days 7 to 35 after treatment. Following castration, peak plasma cortisol responses were significantly greater in 5·5-month-old castrates than intact calves, and all calves castrated at a younger age had reduced peak responses, with the greatest reduction in 1·5-month-old castrates. Overall, the integrated cortisol responses for the first 3 h after castration were three-fold greater in 5·5-month-old castrates than intact calves. While the integrated cortisol responses were reduced by proportionately 0·46 and 0·35 in 1·5- and 4·5-month-old castrates, the lower responses observed in 2·5- and 3·5-month-old castrates were not significantly different from the 5·5-month-old castrates. The integrated cortisol responses for the next 9 h after castration were not different among treatment groups. On day 3 after castration, peak plasma haptoglobin and fibrinogen concentrations were significantly greater in 5·5-month-old castrates than intact calves, but the concentrations were markedly reduced in calves castrated at 1·5 and 2·5 months than when castrated at 5·5 months of age. On day 1, phytohaemagglutinin-induced in vitro interferon-γ production was suppressed in 5·5-month-old castrates compared with intact calves. Scrotal circumferences increased in all castrates on day 1 and 7 and were greater in 5·5- month-old castrates than intact calves, but the swelling was reduced in the 1·5- compared with 5·5-month-old castrates. The temperature differences between the core body and scrotal skin were greater on day 2 and 3 in the 1·5-month-old than all other castrates. There was no effect of castration on the overall 42-day growth rates of calves. In conclusion, the physiological stress and inflammation caused by Burdizzo castration, indicated by increased plasma cortisol, acute-phase proteins, scrotal swelling, and depressed temperature differences between the core body and scrotal skin were reduced by castrating calves at 1·5 months rather than at 5·5 months of age.

Ting, S. T. L.; Earley, B.; Veissier, I.; Gupta, S.; Crowe, M. A. 2010. **Title Effects of Burdizzo castration on CO2 laser induced thermal nociception of Holstein-Friesian calves of different ages.** Applied Animal Behaviour Science 2010 Vol. 126 No. 1/2 pp. 12-18

The objective was to investigate the effects of Burdizzo castration on the thermal nociception (stress-induced hypoalgesia) of Holstein-Friesian bull calves of different ages. Calves castrated at 5.5 mo of age were compared with either intact calves of the same age, or calves castrated at 1.5, 2.5, 3.5, and 4.5 mo of age (n=10 calves/treatment). Treatments were conducted on the same day for all calves. The baseline surface skin temperatures on the caudal part of metatarsi, and the latency of the calves to perform hind leg withdrawal (i.e., thermal nociception threshold) in response to a CO2 laser beam applied on the same area were measured 72 h before, and 12, 24 and 48 h after treatment. The thermal nociception threshold varied inversely with the baseline skin temperature (pooled correlations, r=-0.45, -0.31 and -0.48 at 12, 24 and 48 h, respectively; P<0.01). There were no differences (P>0.05) in the skin temperatures between castrated and intact calves at 5.5 mo of age. Calves castrated at 1.5 mo-old had consistently lower skin temperatures than all other castrated calves throughout the study. These calves had markedly increased skin temperatures following castration, while the opposite trend was observed in older castrated calves, and no change was observed in intact calves. At -72 h, the 1.5 mo-old calves had higher thermal nociception thresholds than older calves. In all calves, the thermal nociception threshold increased after treatment. Calves castrated at 5.5 mo of age tended to display higher thermal nociception threshold than intact calves. However, variations in the initial skin temperature accounted for these differences between treatments or interactions between time and treatment. In conclusion, the laser-based thermal nociception assay can be influenced by the surface skin temperature of the hind legs and the age of animals, particularly in calves less than 2 mo of age which have lower skin temperatures and longer latency to respond to the laser. Burdizzo castration increased the skin temperature of 1.5 mo-old calves, but had the opposite effects on older calves. Within the temporal limits of this study, no conclusive evidence was found to support the presence of acute stress-induced hypoalgesia following castration.

Pang, W.Y., Earley, B., Gath, V., Crowe, M.A. 2005. **Effect of banding or burdizzo castration on plasma testosterone, acute-phase proteins, scrotal circumference, growth, and health of bulls.** Livestock Science, 117, 79–87 . DOI: http://dx.doi.org/10.1016/j.livsci.2007.11.012

The objective was to assess the effect of banding or burdizzo castration performed on farms on plasma testosterone, acute-phase proteins, scrotal circumferences, growth, and well-being of bulls. 243 Continental bulls (12 months; 399.2±5.72 kg) from three different farms were allocated at random, after stratification on weight within breed type, to one of three treatment groups: banding castration (BAND; n=80), burdizzo castration (BURD; n=83), or controls (CON; n=80). The castration methods were conducted under local anaesthesia, and tetanus toxoid vaccine and antibiotic were also injected at castration. BAND and BURD castrates had lower (P<0.001) plasma testosterone concentration than control bulls, with no difference between BAND and BURD castrates on 28 d post-castration. From days 0 to 14 post-castration, BAND (P=0.0002) and BURD (P<0.0001) castrates had lower average daily gain (ADG) than CON bulls, no difference (P=0.46) was found between BAND and BURD castrates. From days 15 to 28, BAND castrates had lower ADG compared with BURD castrates (P=0.03) and CON bulls (P=0.01), while no difference (P=0.76) was found between BURD and CON. From days 29 to 56, BAND (P=0.01) and BURD (P=0.002) castrates had lower ADG than CON bulls, no difference (P=0.55) was found between BAND and BURD. From days 57 to 84, the ADG of BAND castrates was not different compared with BURD castrates (P=0.12) and CON bulls (P=0.38), while BURD had lower (P=0.02) ADG compared with CON. The integrated ADG from day 0 to 112 of BAND (P=0.0001) and BURD (P=0.02) groups were lower compared with CON, while there was no difference (P=0.09) between BAND and BURD castrates. On d 14 post-castration, BAND castrates had lower scrotal temperature than BURD (P<0.0001) and CON (P<0.0001), and BURD castrates had greater (P<0.006) scrotal temperature than CON; BAND castrates had lower scrotal latitudinal and longitudinal circumferences than BURD castrates (P<0.001) and CON bulls (P<0.001), and BURD castrates had greater (P<0.001) scrotal latitudinal and longitudinal circumferences than CON bulls. BAND (P<0.0001) and BURD (P=0.01) castrates had greater glucose concentration than CON bulls, and BAND castrates had greater (P=0.04) glucose concentration than BURD. In conclusion, BAND or BURD castration significantly reduced plasma testosterone concentration; reduced average daily weight gain mainly during the first 2 weeks, which was not compensated during the subsequent 16 weeks; increased withdrawal of stored energy and increased plasma protein concentration. BURD showed an advantage over BAND in growth during days 15 to 28 following castration

Pang WY, Earley B, Sweeney T, Crowe MA. 2006. **Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-gamma production, acute-phase proteins, feed intake, and growth.** J Anim Sci. 2006 Feb;84(2):351-9.

The objective of this study was to determine the effect of carprofen (C) administration before banding or burdizzo castration of bulls on cortisol, in vitro interferon-gamma (IFN-gamma) production, acute-phase proteins, feed intake, and growth. Fifty Holstein Friesian bulls (5.5 mo old; 191 +/- 3.7 kg) were blocked by weight and assigned randomly to 1 of 5 treatments (n = 10/treatment): 1) untreated control (2) banding castration at 0 min (Band); 3) Band following an i.v. injection of 1.4 mg/kg of BW of C at -20 min (Band+C); 4) Burdizzo castration at 0 min (Burd); or 5) Burd following 1.4 mg/kg of BW of C at -20 min (Burd+C). Castration acutely increased plasma cortisol concentrations compared with control; no significant differences occurred in peak and interval to peak cortisol responses between Band and Band+C or Burd and Burd+C groups. The administration of C in Band+C reduced (P < 0.05) the cortisol concentration between 6 and 12 h postcastration compared with Band animals. Overall, the integrated cortisol response was greater (P < 0.05) in the castrates than in control, whereas C treatments tended to reduce this response compared with Band (P = 0.08) and Burd (P = 0.07), respectively. Plasma fibrinogen was elevated in Band animals on d 14 and in Burd animals on d 3 and 14. Carprofen administration reduced Band- and Burd-induced fibrinogen production on d 14 and 3, respectively. Plasma haptoglobin was elevated in Band animals on d 3 and 35 compared with control, and C administration was effective in reducing the haptoglobin elevation on d 35 in Band+C compared with Band. There were no differences among treatments in in vitro IFN-gamma production induced by concanavalin A and phytohemagglutinin on d 1 and 2. Overall from d -1 to 16, there were no DMI differences among treatments. From d -1 to 35, there were no ADG differences among treatments. In conclusion, banding and burdizzo castration increased plasma cortisol with no change in in vitro IFN-gamma production. Carprofen (1.4 mg/kg of BW) tended to reduce the integrated cortisol response, and it reduced cortisol secretion in banded animals between 6 and 12 h postcastration. There was an increased acute-phase protein production following castration; this response was effectively moderated by the administration of C before castration.

Pang WY, Earley B, Sweeney T, Pirani S, Gath V, Crowe MA. 2009. **Effects of banding or burdizzo castration of bulls on neutrophil phagocytosis and respiratory burst, CD62-L expression, and serum interleukin-8 concentration**. J Anim Sci. 2009 Oct;87(10):3187-95. doi: 10.2527/jas.2009-1905. Epub 2009 Jul 17.

The objective was to investigate measures of neutrophil function in response to banding or burdizzo castration of bulls. Thirty-two Holstein-Friesian bulls (14 mo old, 505 +/- 7.8 kg of BW) were assigned to 1 of 4 treatment groups: 1) sham-handled control (CON); 2) banding castration alone (BAND); 3) burdizzo castration alone (BURD); or 4) cortisol infusion (CORT) as a further control group. For each group on d -14, 8 animals (2 animals/treatment) were tied up in tie stalls (day of treatment = d 0). At -2, 2, 6, 12, 24, 48, 72, and 144 h relative to treatment time, blood samples were collected for analyses of neutrophil phagocytosis and respiratory burst, neutrophil CD62-L expression, and serum IL-8 concentration. Leukocyte counts, phagocytosis activity, and CD62-L expression were similar (P > 0.05) among the 4 treatment groups. The BURD castrates had greater burst activity compared with BAND castrates (P = 0.048) and CON (P = 0.01) at 72 h posttreatment. The BURD castrates had a greater percentage of granulocyte positive leukocytes (Gr%; P < 0.01) at 2 h posttreatment compared with CON and CORT bulls. The BURD castrates had greater (P < 0.05) Gr% compared with BAND, CON, and CORT animals at 24, 48, and 72 h posttreatment. The BURD and BAND castrates had greater Gr% (P < 0.05) compared with CORT bulls at 144 h posttreatment. In general, BAND, BURD, and CORT did not affect serum IL-8 concentration. Banding castration, BURD, and CORT did not induce leukocytosis, whereas BURD induced a modest neutrophilia. Neutrophil functioning in terms of phagocytosis and respiratory burst and serum IL-8 concentration were not compromised by BAND, BURD, and CORT. These findings indicate nonsurgical castration is unlikely to induce a severe acute systemic inflammatory response in terms of neutrophil function.

Pang W, Earley B, Sweeney T, Gath V, Crowe MA. 2009. **Temporal patterns of inflammatory gene expression in local tissues after banding or burdizzo castration in cattle.** BMC Vet Res. 2009 Sep 23;5:36. doi: 10.1186/1746-6148-5-36.

Castration of male cattle has been shown to elicit inflammatory reactions and acute inflammation is initiated and sustained by the participation of cytokines. METHODS: Sixty continental x beef bulls (Mean age 12 +/- (s.e.) 0.2 months; Mean weight 341 +/- (s.e.) 3.0 kg) were blocked by weight and randomly assigned to one of three treatments (n = 20 animals per treatment): 1) untreated control (Con); 2) banding castration at 0 min (Band); 3) Burdizzo castration at 0 min (Burd). Samples of the testis, epididymis and scrotal skin were collected surgically from 5 animals from each group at 12 h, 24 h, 7 d, and 14 d post-treatment, and analysed using real-time PCR. A repeated measurement analysis (Proc GLM) was performed using SAS. If there was no treatment and time interaction, main effects of treatment by time were tested by ANOVA. RESULTS: Electrophoresis data showed that by 7 d post-castration RNA isolated from all the testicle samples of the Burd castrated animals, the epididymis and middle scrotum samples from Band castrates were degraded. Transitory effects were observed in the gene expression of IFN-gamma, IL-6, IL-8 and TNF-alpha at 12 h and 24 h post treatment. Burd castrates had greater ($P < 0.05$) testicular IFN-gamma mRNA levels compared with Band and Con animals, but lower ($P < 0.05$) testicular TNF-alpha mRNA levels compared with Con animals. Band castrates had greater ($P < 0.05$) testicular IL-6 mRNA levels than Burd castrates at 12 h post-castration. Burd castrates had greater ($P < 0.05$) testicular IL-8 mRNA levels than Band and Con animals at 24 h post-castration. In the epididymis, Burd castrates had greater ($P < 0.05$) IL-6 mRNA (both at 12 h and 24 h post treatment) and IL-8 mRNA (12 h post treatment) levels compared with Band and Con animals; Burd castrates had greater ($P = 0.049$) IL-10 mRNA levels than Band castrates at 12 h post-castration. CONCLUSION: Banding castration caused more inflammatory associated gene expression changes to the epididymis and scrotum than burdizzo. Burdizzo caused more severe acute inflammatory responses, in terms of pro-inflammatory cytokine gene expression, in the testis and epididymis than banding.

Pang WY, Earley B, Murray M, Sweeney T, Gath V, Crowe MA. 2011. **Banding or Burdizzo castration and carprofen administration on peripheral leukocyte inflammatory cytokine transcripts**. Res Vet Sci. 2011 Feb;90(1):127-32. doi: 10.1016/j.rvsc.2010.04.023. Epub 2010 May 20.

The objective was to investigate if Banding or Burdizzo castration of bulls would alter the gene expression profile of a range of peripheral leukocyte inflammatory cytokines (IL-1, IL-6, IL-8, IL-10, interferon-γ and tumor necrosis factor-α) and to determine if the administration of carprofen (C) before castration would affect the expression of these genes. Thirty Holstein-Friesian bulls (5.5 months; Mean 191±(SEM) 3.7 kg) were blocked by weight and randomly assigned to one of five treatments: (1) untreated control (CON); (2) Banding castration at 0 min (BAND); (3) BAND following an i.v. injection of 1.4 mg/kg BW of carprofen (C) at -20 min (BAND+C); (4) Burdizzo castration at 0 min (BURD); or (5) BURD following 1.4 mg/kg BW of carprofen at -20 min (BURD+C). Blood samples were collected at 1 h before castration and 6, 24 and 48 h post-castration for routine hematology and quantitative real-time PCR analysis of cytokine gene expression analysis. Generally, there were no differences ($P>0.05$) among treatment groups in hematological variables following castration. Cortisol concentrations were unchanged throughout the experimental period in CON bulls. BURD animals had greater cortisol concentrations than BAND and CON animals at 6 h post treatment. Transitory effects were observed only in the expression of IL-6 and TNF-α. The relative expression of IL-6 was greater in the BURD than in the BAND treatment ($P<0.05$) at 24 h post-castration and was greater in the BURD+C group than in the BURD group ($P<0.05$) at 48 h. The relative expression of TNF-α was greater in BAND than in the BURD group ($P<0.05$) at 48 h. In conclusion, these findings indicate that Banding or Burdizzo castration did not have any major effect on peripheral leukocyte inflammatory cytokine gene expression; Banding castration caused a greater pro-inflammatory cytokine gene expression reaction than Burdizzo castration and carprofen administration can affect IL-6 gene expression levels in BURD castrated animals.

Summary of Teagasc castration studies:

1. Depending on the techniques used (Burdizzo versus surgical), and the age of animals, castration in male cattle causes varying degrees of acute distress by inference from increased plasma cortisol concentrations, acute-phase protein production, leukocytosis with lymphopenia, temporal suppression of immune function (indicated by in vitro interferon-gamma production from stimulated lymphocytes in whole blood culture), increased scrotal swelling, depressed temperature gradient between the core body and scrotal skin, and increased expression of pain-associated behaviours.

2. Cortisol infusion (as hydrocortisone) to induce immunosuppression in vivo occurred only at pharmacological doses. Within physiological ranges, cortisol was not associated with the suppression of immune function, indicating that during castration, cortisol elevation per se is not responsible for the suppression of immunity.

3. Systemic analgesia with ketoprofen, a non-steroidal anti-inflammatory drug, is an effective method for alleviating the cortisol response, acute-phase protein production, and the suppression of immune function associated with surgical castration.

4. Repeated pre-emptive intravenous administration of ketoprofen (1.5 mg/kg of BW per dose at 20 and 0 min and 3 mg/kg of BW at 24 h) had no beneficial effects compared with a single treatment (3 mg/kg of BW at 20 min) on the changes in acute-phase proteins and immunity following surgical castration.

5. Ketoprofen was more effective in alleviating the acute cortisol response and suppression of immune function associated with Burdizzo castration than either use of local anaesthesia or caudal epidural anaesthesia.

6. Ketoprofen and epidural anaesthesia were more effective than local anaesthesia in minimising pain-associated behavioural displays during the first 6 hours after castration.

7. Collectively, the results of physiological, immunological and behavioural indices of welfare indicate that whilst local anaesthesia suppressed the initial cortisol response, overall, it failed to provide effective analgesia for alleviating castration-induced distress in cattle.

8. There was a trend for increased thermal nociceptive threshold following Burdizzo castration in 5.5 month-old calves. However, this effect was partly accounted for by the variations in skin temperature. The 1.5 month-old calves had consistently lower baseline surface skin temperatures than the calves in all other castration groups throughout the study. Consequently, these calves had greater thermal nociceptive threshold than calves in all other castration groups before treatment at –72 h. There was no effect of age at castration on thermal nociceptive threshold following castration.

9. The pattern and duration of castration-induced plasma cortisol elevation measured in Burd animals were in agreement with previous findings (Fisher et al., 1997; Ting et al., 2003). Carprofen tended to reduce the integrated cortisol response (AUC, 0-12 h), but had no effect on peak plasma cortisol and the interval to peak cortisol in castrated animals.

10. Banding or burdizzo castration in general does not induce a systemic inflammation and had no effect on peripheral leukocyte inflammatory cytokines gene expression.

11. Cytokine gene expression of peripheral leukocytes during castration was investigated and a significant change of IL-6 transcripts was found at both 24 h and 48 h post-castration. At 24 h post-castration, the relative quantity of IL-6 mRNA was greater in Burd than in Band which is in agreement with the findings that Band induced a greater cortisol secretion than Burd and that Burd caused less chronic inflammation than Band.

12. Banding and burdizzo castrations (of 5.5 or 14 months old bulls) acutely increased the secretion of cortisol. For 5.5 months old animals, there were no differences between banding and burdizzo castrated animals, except banding castrates had a greater cortisol response (i.e., area under cortisol curve) than burdizzo castrates from 0 to 4 hr post-castration. For 14 months old animals, there were no differences between banding and burdizzo castrated animals, except for 24 h post-castration, burdizzo castrates had greater cortisol concentration compared with banding castrates.

13. For 12 month old bulls, banding or burdizzo castration retarded ADG mainly during the first two weeks, which was not compensated for over a 16 week finishing period, post-castration. Burd showed an advantage over Band in growth during 3 to 4 weeks following castration. Band caused a relatively short reduction in growth rate than Burd (Band: 8 weeks versus Burd: 12 weeks) and there were no differences between Band and Burd castrates during 5 to 16 weeks post-castration in terms of ADG.

14. Carprofen administration (i.v. 1.4 mg/kg BW at -20 min) tended to reduce the overall cortisol response, had no effect on the initial peak response to castration, and failed to suppress the initial cortisol rise (from 0 to 6 h).

Appendix II

Disbudding/dehorning research

Several options are used to prevent horn growth (disbudding) including heat cauterization and chemical cauterization (caustic paste). Heat and chemical cauterization are often the methods of choice for younger calves (2 to 8 weeks of age; when horn buds are 5 to 10 mm long). When horns become longer and a disbudding iron is not effective, they are removed by amputation (dehorning; Weaver, 1986). Hot-iron disbudding is done by applying a device, electric or butane-gas heated to over 600^{o}C, over the horn bud destroying the growing tissue at its base. This method is performed when horn-buds are evident by palpation which usually occurs at an age of 2–8 weeks.

Hot-iron disbudding causes severe pain-related distress that is demonstrated by significant plasma cortisol rise (Boandl *et al*., 1989; Morisse *et al*., 1995; Graf and Senn, 1999; Stilwell *et al*., 2010) and behavioural changes (Faulkner and Weary, 2000; Stafford *et al*., 2000; 2003; Stafford and Mellor, 2005; Vickers *et al*., 2005; Doherty *et al*., 2007). Local anaesthesia (cornual nerve blocking) has been shown to delay the pain responses for at least 2 h (Graf and Senn, 1999; Vickers *et al*., 2005; Doherty *et al*., 2007). All these studies show that applying the hot-iron with no anaesthesia causes pain so that significant physical restraint is necessary to carry out the procedure. In a large survey in the USA, anaesthetics were used by only 12.4% of dairy owners and analgesics by 1.8% (Fulwider *et al*., 2008).

Currently the legislation concerning dehorning of cattle requires that once calves are over 2 weeks of age dehorning may only be performed in association with local anaesthesia. Dehorning or disbudding of horned cattle is a mandatory requirement in many countries to reduce the risk of injuries to humans or other animals (Marshall, 1977; Vowles, 1976). There is considerable variation both between breeds and within breeds between individuals in the age at which disbudding becomes impossible and amputation (dehorning) becomes the necessary method. Dehorning of older cattle is carried out by various methods and includes: 1), dehorning scoop that consists of two interlocking semicircular blades attached to handles that amputate the horn close to the underlying bone. 2), Guillotine shears / crange device. 3), saw -where the horn is cut close to the skull bone using a tenon saw. 4), foetotomy wire – where the horn is cut close to the skull bones by repeated sawing with a foetotomy wire. 5), cryosurgery (Bengtsson *et al*., 1996) - however this method was unreliable (60% effective at best) and too difficult to implement in practice and has not been published on since.

The cortisol responses of male Friesian calves (5 to 6 mo of age) to amputation dehorning by each of the first 4 methods listed were similar, suggesting that the degree of distress and pain caused by the different methods of dehorning are similar (Sylvester *et al*., 1998a). With scoop dehorning which may cause either shallow or deep impact on the underlying bone and surrounding skin, the depth of the wound did not affect the cortisol response (McMeekan *et al*., 1997).

When dehorning cattle, lignocaine local anaesthetic (LA) was somewhat effective at reducing cortisol rises and adverse behaviours during cautery or caustic paste disbudding for a period of 1.5 to 2 h (Morisse *et al*., 1995; Petrie *et al*., 1996, Graf and Senn, 1999; Grondahl-Neilsen *et al*., 1999) in young calves (4-8 weeks). Although it produced no benefit when used in conjunction with xylazine sedative (Vickers *et al*., 2005) in 1 to 5 week old calves. When ketoprofen (3 mg/kg BW orally in milk 2h pre, 2 h post and 7 h post dehorning) was used in conjunction with xylazine sedative and lignocaine LA an effective 24h period of pain alleviation was achieved (Faulkner and Weary, 2000).

In the case of the more severe amputation dehorning (scoop) in older calves, local anaesthesia (Lignocaine or Bupivicaine) was only partially effective at alleviating pain for a period of 2 to 4 hours, respectively (Petrie et a., 1996, Sylvester *et al*., 1998b, McMeekan *et al*., 1998a, b). When these two anaesthetic agents were administered sequentially (Lignocaine at –15 min relative to horn amputation, and bupivicaine 2h post amputation) a 5 h period of pain alleviation was achieved (Sutherland *et al*., 2002 a, b). However prolonging the local anaesthesia effectively only deferred the rise in cortisol without completely preventing it (McMeekan *et al*, 1998b, Sutherland *et al*., 2002a,b). Use of the non-steroidal anti-inflammatory (NSAID), ketoprofen, administered 15 to 20 min before de-horning had little effect on peak cortisol, but ensured a rapid reduction in cortisol to pre-treatment baseline concentrations (McMeekan *et al*., 1998b). However, when phenylbutazone (a NSAID) was used, it failed to prevent the inflammation-related cortisol response, suggesting that as in other species it is mainly anti-inflammatory rather than analgesic (Chambers *et al*., 2002). In the calf dehorning model the combined use of pre-emptive ketoprofen and lignocaine together virtually eliminated the cortisol response to dehorning (McMeekan *et al*., 1998b; Sutherland *et al*., 2002b). Indeed the behaviour of calves receiving this combined ketoprofen and local anaesthesia was similar to that of controls over the acute and chronic period following dehorning (McMeekan *et al*., 1999). However where ketoprofen was used in conjunction with longer acting local anaesthetics (bupivacaine; 4h; or lignocaine followed by bupivacaine; 5 h) there was a significant cortisol response once the local anaesthesia wore off (Sutherland *et al*., 2002b). Similarly the use of ketoprofen in combination with local anaesthesia during scoop amputation dehorning was effective at maintaining low cortisol up to 8 hours post dehorning (Stafford *et al*., 2003). In the same study Stafford *et al*. (2003), clearly demonstrated that xylazine sedation, or xlyazine sedation with local anaesthesia (lignocaine) were effective at alleviating pain for only 2 or 3 h, respectively. Heat cauterization of the amputation wound in association with local anaesthesia was also very effective (Sutherland 1998a). Unfortunately there is a complete absence of literature available on other methods of amputation dehorning (foetotomy wire, saw, guillotine crange) and alleviation of the associated pain. Stilwell *et al.* (2009) reported that caustic paste (Sodium hydroxide) disbudding causes distress for at least 3 h and that local anaesthesia is effective in controlling pain for the first hour but discomfort returns after the nerve blocking subsides. Sodium hydroxide, which is commonly used for calf disbudding, is a very strong (pH 14) and corrosive alkali. Nerve blocking may not be completely effective after chemical tissue damage due to caustic paste. Regional anaesthesia, together with a non-steroidal-anti-inflammatory drug (NSAID), was shown to reduce plasma cortisol in calves disbudded using caustic paste.

In conclusion, for disbudding (cautery or caustic) of horns in young calves the use of ketoprofen along with local anaesthesia is beneficial. For horn amputation (scoop) the most effective option of achieving pain relief appears to be lignocaine LA in conjunction with Ketoprofen NSAID (McMeekan *et al*., 1998b; Stafford *et al*., 2003). Unfortunately Ketoprofen is one of the more expensive NSAIDs available, and further work is required to determine if any cheaper alternatives are effective. The recommended methods for dehorning of calves are by scoop dehorners, gouging knife or heat cautery, as soon as the horn buds are detectable. The method of choice must be able to remove all horn-growing tissue in one action with minimal damage to adjacent tissues.

Dehorning/disbudding references

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