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Effect of castration method on neurohormonal and electroencephalographic stress indicators in Holstein calves of different ages

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ABSTRACT

As public concern for food animal welfare increases, a need to validate objective pain assessment tools exists in order to formulate animal welfare policies and facilitate regulatory approval of compounds to alleviate pain in livestock in the United States. The aims of this study were (1) to compare the physiological response to pain induced by surgical and nonsurgical (band) castration in calves and (2) to elucidate age-related differences in pain response of calves subjected to different castration methods. Seventy-six Holstein bull calves were blocked by age (≤8-wk and ≥6-mo-old) and randomly assigned to 1 of 4 treatment groups: control (n = 20), castration by banding (n = 18), cut-and-clamp surgical castration (n = 20), and cut-and-pull surgical castration (n = 18). Measurements included electroencephalogram, heart rate variability, infrared thermography, electrodermal activity, and concentrations of serum cortisol and plasma substance P before, during, and within 20 min following castration. Electroencephalogram recordings showed desynchronization for all treatments, consistent with increased arousal; yet the magnitude of desynchronization was greatest for 6-mo-old calves castrated by cut-and-clamp. Additionally, older calves in the cut-and-pull group showed greater desynchronization than younger calves in the same group. Based on the heart rate variability analysis, 6-mo-old calves in the control or cut-and-pull castration groups showed greater sympathetic tone than younger calves in the same treatment groups. Overall, younger calves showed lower electrodermal activity than older calves. Regardless of treatment, concentrations of cortisol and plasma substance P were greater in 6-mo-old calves relative to their younger counterparts, indicating a more robust response to all treatments in older calves. In summary, neurohormonal and electroencephalographic stress responses of calves to castration were age-specific. Castration by cut-and-clamp showed the most pronounced stress response in 6-mo-old calves. These findings provide evidence that support welfare policies recommending castration at an early age and the use of analgesic compounds at the time of surgical castration especially in older calves. However, the potential long-term negative consequences of early untreated pain must be considered and warrant further investigation.

Key words: calf, castration, electroencephalogram, pain

INTRODUCTION

Castration is a common husbandry procedure used to decrease aggressive behavior, prevent unwanted matings, and modify carcass characteristics in cattle (Stafford, 2007). Currently, in the United States, less than 20% of producers provide pain relief at the time of castration (Coetzee et al., 2010). Consequently, castration without analgesia is considered standard industry practice in the United States at present. As the public's concern for food animal welfare increases, objective and noninvasive approaches of pain measurement are critical to assist in the development of science-based animal welfare policies. Unlike in Europe, currently no analgesic compounds have been approved for the alleviation of pain in food animals in the United States (AVMA, 2012). In accordance with United States Food and Drug Administration Guidance Document 123, validated methods of pain assessment must be used to prove that a pharmaceutical is efficacious before this can be labeled as an analgesic (FDA, 2006). This

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study was designed to assess the physiological response of calves of different ages to surgical and nonsurgical castration-induced pain by measuring their electroencephalogram, heart rate variability, infrared thermography, electrodermal activity, and plasma concentrations of cortisol and substance P.

The electroencephalogram (**EEG**) records the electrical activity of the cerebral cortical neurons which are believed to play a role in pain perception (Johnson, 2007). Activity is measured by 4 bands, namely low-frequency δ and θ and high-frequency α and β. In general terms, a typical EEG response to pain involves a decrease in total power, an increase in 95% spectral edge (the 95th percentile of the power spectrum), and an increase in median frequency (the statistical median of the power spectrum; Murrell and Johnson, 2006). This response is attributed to a decrease in low-frequency activity and an increase in high-frequency activity. This phenomenon is termed desynchronization and has been associated with increased arousal (Murrell and Johnson, 2006). A study performed in cattle during castration showed an association between EEG responses and cortisol concentrations, thereby suggesting that EEG may be useful in assessing increases in brain electrical activity associated with pain (Bergamasco et al., 2011).

Heart rate variability has also been used to measure stress in animals (von Borell et al., 2007). Heart rate variability assesses the function of the autonomic nervous system, especially the balance between sympathetic and vagal tone (Saul, 1990; von Borell et al., 2007). Some common variables assessed in heart rate variability analysis include mean heart rate, low-frequency power, high-frequency power, low frequency to high frequency ratio, and number of successive R-R interval differences greater than 50 ms (**NN50**). A study on the effects of castration showed an increase in heart rate for calves castrated with and without local anesthetic, but no change for calves in the control groups (Stewart et al., 2010a). This study also found a decreased low frequency to high frequency ratio, indicative of increased vagal tone (Stewart et al., 2010a). This increase in parasympathetic activity may be associated with the deep visceral pain likely experienced when the spermatic cords are torn (Stewart et al., 2010a).

Infrared thermography is a technique used to measure the temperature of body areas. Ocular temperature has been shown to respond to castration-induced pain in a stimulus-dependent manner, suggesting it may be a useful tool for pain measurement (Stewart et al., 2010a). Electrodermal activity is the measurable change in skin electrical conductance in response to sympathetic stimulation (Critchley, 2005). Electrodermal activity is considered to represent brain stem reticular activation, and therefore may indicate the complex modalities of behavior and emotions in animals (Sequeira et al., 2009).

Pain causes activation of the hypothalamo-pituitary-adrenal axis, which is the neuroendocrine stress pathway (Cardo et al., 2011). The activity of this pathway can be measured by its end product, cortisol (Kent et al., 1993). Stressful situations augment the physiological secretion of cortisol and cause episodic peaks in concentrations, making it a useful stress indicator (Minton, 1994; Grandin, 1997; Bosch et al., 2009). Substance P has been described as a modulator of nociception, and has also been involved with transmission of painful stimuli to the central nervous system (DeVane, 2001). Previous studies have reported elevated substance P concentrations in calves following castration and dehorning (Coetzee et al., 2008, 2012).

In the present study, we examined physiological indicators of stress and pain induced by 1 of 2 surgical castration methods or a nonsurgical castration method applied to either young (≤8-wk-old) or older (≥6-mo-old) calves. To our knowledge, no reports using EEG to measure pain response in calves of different age groups subjected to surgical and nonsurgical castration have been published. The aims of the present study were (1) to compare the physiological response to pain induced by surgical and nonsurgical (band) castration in calves and (2) to elucidate age-related differences in pain response of calves subjected to different castration methods.

MATERIALS AND METHODS

Animals and Housing

Forty Holstein bull calves aged approximately 8 wk (30–60 kg on arrival) and 40 Holstein bull calves aged approximately 6 mo (110–200 kg on arrival) were enrolled in the study. Eight-week-old calves arrived from Gill, CO on May 24, 2011. Six-month-old calves arrived from Conway Springs, KS on June 1, 2011. Processing procedures occurred 1 d after arrival. All calves received a single subcutaneous dose of the antibiotic oxytetracycline at 300 mg/mL (Noromycin 300 LA, Norbrook Inc., Lenexa, KS), a single subcutaneous dose of an 8-way clostridial vaccine (Covexin 8, Intervet/Schering-Plough Animal Health, Summit, NJ), and a single subcutaneous dose of a bovine rhinopneumonitis vaccine (Bovi-Shield GOLD 5, Pfizer Animal Health, New York, NY) to prevent these common bovine diseases. A topical pour-on comprised of 5% permethrin and 5% piperonyl butoxide (Ultra Boss Pour-On Insecticide, Intervet/Schering-Plough Animal Health) was applied to all calves upon arrival and repeated as needed for fly control. Six-week-old calves were given