Behavioural effects of a thoracic squeeze on healthy neonatal mammals of precocial species

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Abstract

Some neonatal farm mammals that are apparently healthy do not behave normally after birth and without intervention, often die. Recently, a thoracic squeeze has been applied to these neonates, which observed their entrance into a 'non-responsive state', from which they rapidly recover when the squeeze is released to express normal healthy behaviours. In addition, healthy newborn foals also become non-responsive during application of the thoracic squeeze.

To date, there have been no systematic studies into the effects of the thoracic squeeze on healthy neonates of other precocial mammalian species. This preliminary research aimed to describe the responses of healthy newborn piglets and calves to a standardised application of the thoracic squeeze, and evaluate the effectiveness of the method of squeeze application in eliciting a non-responsive state.

Neonatal piglets and calves were squeezed around the chest with either a soft fabric rope or a modified inflation cuff. Physiological parameters were monitored for abnormal changes, while induction behaviour and neural reflex activity were measured.

Behavioural data indicated that the thoracic squeeze was effective at eliciting a non-responsive state in all piglets in the study and maintaining this state in half of all the piglets induced, with reduced or absent reflexes in nearly all piglets. In contrast, 81% of the calves were induced into a lower-responsive state, with half of all calves squeezed maintaining this state for the full observation period and present reflexes observed for nearly all calves. Furthermore, the cuff appeared to be more effective at inducing both piglets and calves into a non-responsive or lower-responsive state than the rope, with a faster application and removal.

These findings suggest that the non-responsive state reported in foals can be generalised to neonates of other precocial species. The thoracic squeeze has been demonstrated to be inherently safe and effective at inducing and maintaining a non-responsive state in neonates. This pilot study provides a foundation for further research using the inflation cuff to explore the mechanisms underlying the thoracic squeeze and ways in which the squeeze can be effectively applied in an industry context.

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1. General Introduction

Some neonatal farm mammals that are apparently healthy do not behave normally after birth (Diesch and Mellor 2013; Aleman *et al.* 2017; Stilwell *et al.* 2019). This poses a problem because neonates must express a normal pattern of behaviours soon after birth in order to survive (Tuchscherer *et al.* 2000). The normal progression of these behaviours depends on important changes in neural activity that occur during the birth process. Specifically, a transition from unconsciousness before birth to consciousness after birth enables the newborn to 'wake up' and begin the pattern of behaviours that culminates in nursing from the dam (Mellor 2017). It is proposed that when this transition goes wrong, these behaviours become delayed or inhibited, leading to neonates developing morbidities and the working diagnosis of 'low vigour' (Tuchscherer *et al.* 2000; Baxter *et al.* 2008; Barrier *et al.* 2012). This contributes to significant animal losses within the production industries, and causes concern for the welfare of these animals (Mellor and Stafford 2004).

It is currently unknown why these abnormal behaviours occur in otherwise healthy neonates, but it has been proposed that the brains of these "dummy" neonates are not properly activated during the birth process (Mellor and Diesch 2006; Mellor 2017). Typical approaches to managing dummy neonates address only the clinical signs and require time, money, and intensive care from veterinary professionals (Aleman *et al.* 2017). In more recent years, a mechanistic approach to managing dummy neonates has been taken. Applying a squeeze around the chest (thoracic squeeze) of these dummy animals in the postnatal period has been observed to put them into a 'non-responsive state', from which they rapidly recover when the squeeze is released to express normal healthy behaviours (Aleman *et al.* 2017; Mellor 2017; Stilwell *et al.* 2019). In addition, healthy newborn foals also become non-responsive during application of the thoracic squeeze (Toth *et al.* 2012). Whether healthy neonates of other precocial mammalian species become non-responsiveness during application of a thoracic squeeze is currently unknown, but understanding the effects of the thoracic squeeze in other precocial neonatal mammals will provide a foundation to explore the mechanisms underlying the thoracic squeeze technique in neonatal mammals.

In the following chapter, I briefly review the literature relating to low vigour in neonatal mammals of precocial species, and its association with neonatal maladjustment syndrome (NMS) in foals. The various approaches to managing NMS are also discussed, with specific focus on the effects of a thoracic squeeze technique in NMS and healthy neonates and the questions raised by previous work that will be explored in this pilot study.

2. Literature review

2.1 Normal expression of survival-related behaviours in neonatal mammals of precocial species

Precocial mammals are characterised by their demonstrated capacity for consciousness shortly after birth. They are considered neurologically mature at birth, meaning they have the brain structures sufficiently developed to support consciousness in utero, but are actively kept unconscious until after birth (Mellor and Diesch 2006, 2007; Mellor and Lentle 2015). At birth, the survival of neonates such as piglets, lambs, calves, and foals, is challenged by a range of factors (Mellor and Stafford 2004). These factors include changes in ambient temperature, predation, injuries, mismothering, competition with littermates, and pathophysiological insult (Mellor and Stafford 2004; Baxter *et al.* 2008; Muns *et al.* 2016). These neonates must therefore express a specific pattern of behaviours soon after birth to ensure their survival (Baxter and Edwards 2018). These behaviours progress from uncoordinated to coordinated locomotion, searching for a functional teat, and sucking colostrum (Muns *et al.* 2016).

Vigorousness (occasionally referred to as vitality) is defined as the successful expression of these behaviours after birth (Tuchscherer *et al.* 2000). A neonate is considered to have high vigour when the latency from birth to the successful sucking of colostrum is shorter than the average for the species (Baxter *et al.* 2008). While the specific pattern of behaviours is relatively consistent among mammalian species, the latency to complete the sequence differs. In a normal population of horses with both healthy and unhealthy foals, it takes a range of 15-1200 minutes to complete the pattern, with an average time of 111 minutes (McCue and Ferris 2012), whereas in piglets the average range is 30-45 minutes (Herpin *et al.* 1996; Tuchscherer *et al.* 2000; Baxter *et al.* 2008). Healthy lambs have an average sucking latency of 68 minutes (Dwyer 2003), while a normal population of cattle containing both healthy and unhealthy calves take from 55 minutes to 12 hours, with a median of 4 hours (Ventorp and Michanek 1991).

This latency is influenced by the spatial relationships between dams and neonates typical for the species (Nowak *et al.* 2000). "Hiders" are neonates that are largely separated from their dam in the early postpartum period, due to concealment from potential predators. Calves are known hiders, resulting in the longer observed latency to the expression of survival behaviours (Edwards and Broom 1982; Nowak *et al.* 2000). "Followers", such as lambs, piglets, and foals, are neonates that spend little time separated from the dam during the early postpartum period. Their latencies to the expression of survival behaviours are shorter as a result of rapid and frequent nursing (Nowak *et al.* 2000; Broster *et al.* 2010).

When these behaviours are successfully completed, the chance of survival is significantly increased (Baxter and Edwards 2018). However, any impairment or delay in the sequence of survival-related behaviours can compromise survival.

2.2 The maintenance of unconsciousness before birth in healthy mammals

The normal progression of survival-related behaviours depends on important neural changes that occur during the birth process. Deviations from this behavioural pattern may be due to an impairment or delay in the transition from unconsciousness before birth to the onset of consciousness after birth.

In healthy precocial mammalian species, brain structures become sufficiently developed to support the capacity for consciousness in utero (Mellor and Diesch 2006; Mellor and Lentle 2015). However, the onset of consciousness in utero is actively inhibited before and during birth by a range of factors, in order to maintain motor and respiratory quiescence and reduce the energy costs of increased cognitive functions associated with consciousness (Mellor and Diesch 2006; Mellor 2017). These factors originate in the fetal brain and include the combined actions of a sleep-inducing agent (Adenosine), two neurosteroidal sedatives (Allopregnanolone and Pregnanolone), and a sleep-inducing hormone (Prostaglandin D_2) (Lee *et al.* 2002; Hunter *et al.* 2003; Nguyen *et al.* 2003). Unconsciousness is also facilitated by a placental peptide (Alvaro *et al.* 1993), as well as buoyancy, warmth, and cushioned tactile stimulation (Mellor and Gregory 2003). Adenosine is known to be the most powerful inhibitor of consciousness, with rapid changes occurring due to the short biological half-life (Hunter *et al.* 2003). Pregnane concentrations have also been observed to decline slower than other inhibitors after birth, suggesting at least a sedative, anaesthetic and analgesic effect occurs that persists for the first three days after birth (Nguyen *et al.* 2003).

These inhibitors of consciousness remain at elevated levels for the last third of gestation, and during labour (Mellor 2017). They act to induce a deeper non-responsive state during labour (Mellor and Diesch 2006). The fetus then passes through the cervix into the birth canal, where its head and chest are tightly compressed. It has been proposed that this compression activates a separate brain reflex that potently inhibits movement, and (with the input from the inhibitors) reinforces fetal unconsciousness (Mellor 2017). Furthermore, it is thought that this reflex pathway simultaneously prepares the fetal brain for the postnatal onset of consciousness once the compression is discontinued (Mellor 2017).

2.3 The onset of consciousness after birth in healthy newborn mammals

The onset of consciousness and thus survival-related behaviours after birth requires the coordination of multiple events. As the mammal is born, the inhibitors of consciousness rapidly decline, so that the fetal brain is no longer actively kept unconscious (Mellor 2017). This decline is thought to be facilitated by the cessation of thoracic compression once the fetus has passed through the birth canal (Mellor 2017). Simultaneously, this compression, combined with transient episodes of hypoxaemia during the second stage of labour, results in the rapid increase of factors that activate consciousness. This occurs by the activation of the locus coeruleus (Berridge and Waterhouse 2003). Locus coeruleus activity is responsible for the release of noradrenaline, which exerts excitatory effects on the fetal brain (Berridge and Waterhouse 2003). Another powerful neuroactive steroid, *17*β-*oestradiol,* has excitatory effects on the fetal and newborn brain (McEwen 2002).

Concentrations of both activating factors become progressively elevated during labour and thoracic compression, facilitating the transition to consciousness by preparing the brain to support an increase in behavioural arousal and the onset of continuous rhythmic breathing (Mellor and Gregory 2003).

The combined actions of elevating activators and withdrawal of inhibitors in the brain stimulates the onset of consciousness soon after birth (Berridge and Waterhouse 2003; Mellor and Diesch 2006). Elevated adenosine concentrations rapidly fall as fetal tissues become oxygenated with the establishment of continuous breathing (Mellor and Gregory 2003; Mellor and Diesch 2006). Separation from the placenta at birth, combined with the onset of continuous rhythmic breathing, ceases pregnanolone and allopregnanolone supply from the placenta to result in their slower decline (Nguyen *et al.* 2003; Mellor and Diesch 2006). This enables the elevated activatory concentrations to override the remaining low-level inhibition, resulting in the onset of consciousness after birth. The onset of survival-related behaviours occurs within minutes of birth, with young opening their eyes and attempting to stand in the first few minutes, followed by orientation to the mother and teat seeking behaviours (Nowak *et al.* 2000).

2.4 Effects of low vigour in neonates

Any underlying issue that impairs the expression of the specific pattern of neonatal behaviours results in a significant increase in the risk of morbidity and mortality. This impairment is recognized as low vigour, which is characterised by a delay in the onset of, or an inability to express, survival-related behaviours (Tuchscherer *et al.* 2000). Low vigour ultimately leads to hypothermia, starvation, and eventually death (Baxter *et al.* 2008). As a result, neonates likely experience welfare compromise from morbidity and eventual mortality (Mellor and Stafford 2004). Primary low vigour, where animals show characteristics of low vigour despite being apparently healthy, is becoming more relevant to production industries that are continually looking to improve their animal welfare standards. Both morbidity and mortality significantly contribute to production losses. Mean rates of neonatal mortality internationally range from 6-30% in lambs (Dalton *et al.* 1980; Mousa-

Balabel 2010; Hinch and Brien 2014), 6.7-15% in calves (Laster and Gregory 1973; Raboisson *et al.* 2013), up to 35% in foals (Linklater *et al.* 2004), and 4.5-28% in piglets around the world (KilBride *et al.* 2012; Muns *et al.* 2016).

2.5 Observations of low vigour in neonates

2.5.1 Low vigour in foals

Low vigour foals showing behaviours characteristic of a neurological disorder known as neonatal maladjustment syndrome (NMS) have been observed worldwide, particularly in countries where mares are closely monitored during foaling (Baird 1973). Foals with NMS express a delay or an inability to complete survival-related behaviours (Aleman *et al.* 2013; Aleman *et al.* 2017), and without intervention their health rapidly deteriorates (see section 2.7).

In warmer countries such as Australia, where mares are kept outdoors, the incidence of low vigour foals appears to be lower than in colder climates such as Europe (Baird 1973). However, this difference may be likely due to the level of monitoring, with indoor foaling mares receiving constant care and supervision compared to mares kept outdoors (Baird 1973). Furthermore, the prevalence of NMS in newborn foals has been reported only in thoroughbred horses, but has not yet been investigated in other breeds (Baird 1973). Foals born exhibiting characteristics of NMS were reported at a proportion of 1% out of all foals born (Mahaffey and Rossdale 1959), though this proportion may have increased in more recent years as the causes and clinical signs of NMS have become better recognised (Madigan *et al.* 2012; Aleman *et al.* 2013; Diesch and Mellor 2013; Stilwell *et al.* 2019).

2.5.2 Low vigour in piglets

Low vigour has been well recognised in piglets as a major contributor to mortality (Tuchscherer *et al.* 2000; Baxter *et al.* 2008; Muns *et al.* 2016; Baxter and Edwards 2018). However, in nearly all cases, low vigour occurred as a result of pathophysiology such as hypoxia, genetic disorders, physical malformations, and underlying disease, which resulted in either starvation or crushing (Muns *et al.* 2016). There are no reported cases of low vigour as a primary cause of morbidity or mortality, though Baxter et al. (2008) note a classification of piglet mortality as 'other' whereby piglets that showed no underlying disease or disorder demonstrated low vigour, and eventually died. Piglets that showed low vigour characteristics at birth had a higher risk of mortality within the first seven postnatal days (Baxter *et al.* 2008). These piglets are also often euthanised due to the severe welfare impact caused by starvation and hypothermia (Baxter *et al.* 2008).

2.5.3 Low vigour in calves

The incidence of low vigour is well reported in calves that have experienced pathophysiological insult (Murray and Leslie 2013). Dystocia rates have been steadily increasing in cows, leading to prolonged and assisted births that often result in the calf experiencing hypoxaemia-induced low vigour after birth (Murray and Leslie 2013). However, where low vigour in other neonates may be observed as minimal time spent standing and sucking milk, calves spend significantly less time with the dam, and as such, their behaviours cannot be compared to other neonates (Edwards and Broom 1982; Nowak *et al.* 2000). Nevertheless, low vigour has been observed more frequently in calves that had difficult births than calves born naturally without issue (Barrier *et al.* 2012). However, two calves were recently found to express NMS behaviours despite no presence of underlying abnormalities or pathophysiological insult after birth (Stilwell *et al.* 2019). These calves were born via caesarean section and displayed abnormal behaviours characteristic of NMS commonly recognised in newborn foals (Stilwell *et al.* 2019).

2.5.4 Low vigour in lambs

Low vigour in lambs is understood to be a main contributor to mortality rates (Wesselink *et al.* 1999; Matheson *et al.* 2011; Dwyer and Bünger 2012). In countries like New Zealand, sheep are predominantly kept outside for lambing. Outdoor births result in lambs being born in cold and wet environments that put them at a significantly higher risk for mortality than lambs born in sheltered, monitored areas (Nash *et al.* 1996). Furthermore, birth weight plays a significant role in the vigour of lambs at birth, with lighter lambs displaying lower vigour than heavier lambs (Nash *et al.* 1996). It is likely that lower birth weight lambs have experienced pathophysiological insult such as chronic hypoxaemia, particularly if there are multiple lambs (Mellor 1988).

There have been no reports of low vigour as a primary cause of morbidity or mortality in lambs. The only mention in literature is the notion that lambs born 'weak' have a higher mortality rate than lambs born 'strong' (Nash *et al.* 1996), which may suggest that lambs born exhibiting low vigour are more likely to die. Additionally, lambs experiencing pathophysiological insult may also show low vigour, leading to an increased risk of death rates (Wassmuth *et al.* 2001; Matheson *et al.* 2011; Dwyer and Bünger 2012).

2.6 Causes of low vigour in neonatal mammals

2.6.1 Pathophysiology

There are multiple causes of low vigour in neonates. One suite of causes, collectively termed 'pathophysiological impairments', reflect disruption of the normal physiological functions of the fetus and/or placenta resulting in intrauterine or intrapartum abnormalities which can cause postnatal morbidity or mortality (Mellor 1988). These pathophysiological impairments include chronic and acute hypoxaemia, and inadequate heat production (Mellor and Cockburn 1986; Mellor 1988). Chronic intrauterine hypoxaemia can occur from a small placenta that impedes oxygen and nutrient supply to the fetus, resulting in chronically low oxygen (Mellor 1983, 1988). The larger the litter size (as is the case in multiparous mammals such as pigs), the smaller the placenta is for later born fetuses (Blasco *et al.* 1995). In fetuses that survive the birth process under hypoxaemic conditions, the effects of chronic hypoxaemia continue postnatally (Mellor 1988). Hypoxaemia alone can induce low vigour in newborn mammals, resulting in a delay in the onset of survival-related behaviours at birth. Acute intrapartum hypoxaemia is another primary cause of low vigour in newborn mammals (Mellor 1988; Mellor and Gregory 2003; Barrier *et al.* 2012; Dwyer and Bünger 2012). This acute oxygen deficiency occurs during the birth process as a result from umbilical cord occlusion, or a protracted labour (Mellor 1988). Intrapartum hypoxaemia can result in low vigour newborns, due to the increased susceptibility to postnatal hypothermia through impaired heat production(Mellor and Stafford 2004).

The effects of pathophysiological impairments on vigour after birth have been evidenced in newborn piglets (Herpin *et al.* 1996; Baxter *et al.* 2008; Muns *et al.* 2016), calves (Vasseur *et al.* 2009; Barrier *et al.* 2012; Murray and Leslie 2013) and lambs (Mellor 1988; Wassmuth *et al.* 2001; Matheson *et al.* 2011; Dwyer and Bünger 2012).

2.6.2 Abnormal onset of consciousness

More recently, low vigour has been attributed to the persistence after birth of factors that inhibit brain activity. This cause appears to act independently from pathophysiology or underlying disease to result in the expression of abnormal behaviours from birth (Madigan *et al.* 2012; Aleman *et al.* 2013; Mellor 2017). This type of low vigour, termed 'Neonatal maladjustment syndrome' (NMS) refers to behavioural/neurological and developmental impairments in newborn foals. It has been proposed that the abnormal behaviours characteristic of NMS are due to a failure in the birth process (section 2.2 and 2.3) that normally successfully "wakes up" the newborn after birth (Mellor 2017).

2.7 Association between low vigour and neonatal maladjustment syndrome

The similarity of behaviours expressed by NMS neonates and low vigour neonates (Tuchscherer et al., 2000; Bianco et al., 2017), suggests that the two disorders may be closely associated.

Similar to low vigour, NMS has been attributed to either various pathophysiological causes that are not considered recoverable, including acute and/or chronic hypoxia and ischemia (Palmer and Rossdale 1976; Diesch and Mellor 2013), or to other causes that suggest recovery is possible by therapeutic approaches (Toth *et al.* 2012; Diesch and Mellor 2013; Aleman *et al.* 2017).

The first group of NMS foals show abnormal behaviours immediately at birth due to difficulties during the birth process that lead to pathophysiological insult after birth(Diesch and Mellor 2013). Healthy newborn behaviours are completely inhibited in these foals, resulting in inadequate intake of colostrum and high mortality rates (Clément 1987).

The second group of otherwise healthy foals appear to have a normal birth, but soon after, display low vigour behaviours (Clément 1987). These foals exhibited a delayed onset of survival-related behaviours (Aleman *et al.* 2013; Diesch and Mellor 2013; Aleman *et al.* 2017), including reduced awareness of the environment, low-affinity for the mother, poor mother-foal bonding, and limited sucking. Abnormal behaviours characteristic of NMS in foals have also recently been observed in two calves born via caesarean section (Stilwell *et al.* 2019).

2.8 Current hypotheses on the cause of neonatal maladjustment syndrome in foals

In healthy newborn foals, brain inhibiting factors have been shown to peak at birth and rapidly decrease postnatally, with allopregnanolone and pregnanolone declining slower than other inhibitors (Nguyen *et al.* 2003; Aleman *et al.* 2013).

However, these inhibitor levels remain elevated long after birth in foals exhibiting characteristics of NMS (Madigan *et al.* 2012; Aleman *et al.* 2013). Preliminary research suggests that the persistence of brain inhibitors after birth may result in an inability to complete the sequence of newborn behaviours (Toth *et al.* 2012; Diesch and Mellor 2013; Aleman *et al.* 2017). A recent study looking into the role of brain inhibitors in the onset of survival-related behaviours in foals found evidence of neurosteroidal persistence as a potential cause of NMS (Madigan *et al.* 2012). Healthy, active foals injected with inhibitory steroids in the first few days after birth immediately showed abnormal behaviours characteristic of NMS, with healthy behaviours resuming once metabolism and withdrawal of the inhibitors occurred (Madigan *et al.* 2012). Furthermore, foals exhibiting abnormal behaviours from birth were also found to have elevated concentrations of brain inhibiting factors, suggesting a link between neurosteroidal persistence and low vigour in neonates (Aleman *et al.* 2013).

This neuroinhibitory persistence is proposed to be due to a lack of compression of the foal's body during passage through the birth canal (Mellor 2017). It was suggested that abnormal behaviours characteristic of NMS are associated with a rapid passage through the birth canal in some newborn foals (Clément 1987; Aleman *et al.* 2017). Although no relationship between NMS and a rapid birth is stated in literature, it is noted that NMS foals had successful, non-eventful births, but the duration of stage II labour appeared to be shorter than average (Clément 1987). This association between rapid birth and the low vigour state suggests that compression-induced brain activation that may occur during passage through the birth canal requires time to reach a sufficient level to be fully effective in its role of eliciting consciousness after birth (Lee *et al.* 2002; Hunter *et al.* 2003; Nguyen *et al.* 2003).

2.9 Traditional therapeutic approaches for managing neonatal maladjustment syndrome

Managing recoverable NMS foals has focused on addressing the clinical signs of the syndrome. Management practices involve intensive veterinary care practices for up to seven days after the birth of the foal, which is both costly and time consuming (Madigan *et al.* 2012; Aleman *et al.* 2017). When abnormal behaviours indicative of NMS are initially observed by horse owners, veterinary care is often quickly requested due to the requirement for trained professionals in accurately distinguishing the syndrome from other potential causes (Koterba *et al.* 1985; Aleman *et al.* 2017). NMS foals are then transferred to intensive care units at veterinary hospitals, where they are kept in temperature-controlled environments with minimal pathogen exposure (Aleman *et al.* 2017). Minimisation of exposure to pathogens is required because a lack of colostrum intake, and therefore an absence of colostrum-derived immunoglobulins, increases the foals' risk of infection and the associated complications (Koterba *et al.* 1985).

A common observation with NMS in newborn foals is the occurrence of convulsions within 24 hours of birth, with associated hypoglycaemia, hypothermia, metabolic disorders, and sepsis (Clément 1987). It is likely that these convulsions result from hypoglycaemia, as body reserves deplete rapidly after birth when metabolic heat production increases, thus requiring energy sources from colostrum to replenish these body stores (Mellor and Cockburn 1986; Clément 1987; Mellor 1988). If colostrum for NMS foals is not obtained, then foals will be unable to replenish their body energy reserves, resulting in hypoglycaemic-induced convulsions. As a result, temperature-controlled rooms are necessary to avoid hypothermia as a result of hypoglycaemia, as well as to reduce the need for increased metabolic heat production, which would require further energy stores gained from colostrum and milk (Mellor and Cockburn 1986; Mellor and Stafford 2004).

The lack of colostrum ingestion by foals occurs due to no presence of a sucking reflex (Aleman *et al.* 2013). As a result, during the foal's stay in an intensive care unit, accommodations must be available for the mare, where she may need to be milked to provide colostrum and milk for the foal (Aleman *et al.* 2017). This colostrum is then often fed to the foal through a nasogastric feeding tube, which, depending on the level of morbidity of the foal, may be removed and reinserted for each feed, thus requiring more care surrounding the type and size of the nasogastric tube used (Koterba and Drummond 1985). The foal's nutritional needs are specific depending on the severity of its clinical signs and if the mare is present to milk, and as such, other milk replacers may be used. The foal must be fed consistently throughout the day, often once every 1-2 hours for the first few days (Koterba and Drummond 1985). Nasal and pharyngeal irritation, colic, diarrhoea, gas bloating, and aspiration pneumonia are common side effects of nasogastric tube use in foals, which may lead to more intensive care to mitigate these effects (Koterba and Drummond 1985).

With the intensive care required for NMS foals, the number of trained professionals that must be available at all hours throughout each day increases (Aleman *et al.* 2017). Continuous care and observation often include intravenous administration of sedation, fluids, and other supportive medications (Aleman *et al.* 2017). Furthermore, foals that remain recumbent for multiple days until NMS behaviours appear to subside, often require physical therapy and supportive measures for body position (Aleman *et al.* 2017).

It is clear that management of foals exhibiting behaviours characteristic of NMS require intensive care that is costly in both time and money, and may result in euthanasia as owners cannot afford the care needed to support the foal until the NMS behaviours appear to resolve (Aleman *et al.* 2017). Alternative methods that can enable the foal to stand and suck colostrum from the mare rapidly after birth may significantly decrease the chance of secondary complications, and further reduce the costs and time associated with intensive care.

2.10 The thoracic squeeze as a therapeutic approach to managing neonatal maladjustment syndrome

Another therapeutic approach that is gaining popularity is known as a "thoracic squeeze" (Toth *et al.* 2012; Aleman *et al.* 2017). This approach focuses on the mechanisms underlying recoverable NMS rather than the clinical signs alone. It involves applying three loops of a soft rope around the thorax of the foal and firmly tightening it around the chest. The squeeze has been observed to cause these foals to rapidly go into a non-responsive state, with maintenance of this state occurring until the squeeze is removed. Removal of the squeeze then leads to the onset of behaviours equivalent to those expressed by healthy, active foals (Aleman *et al.* 2017). The same responses were observed in “dummy” calves that showed abnormal behaviours after caesarean section births (Stilwell *et al.* 2019), that immediately attempted to stand and suck milk from the dam upon removal of the squeeze.

The thoracic squeeze has also demonstrated to be effective at eliciting a non-responsive state in healthy newborn foals (Toth *et al.* 2012). Preliminary research found a distinctive change in behaviour, with foals collapsing into an apparently somnolent (sleep-like) state accompanied with a decrease in muscle tone. Slight decreases in heart rate and respiratory activity also occurred, with respiratory activity often switching to abdominal breathing soon after the application of the rope (Toth *et al.* 2012). This was further supported by reduced electroencephalographic (EEG) brain activity and no changes to venous blood pH for the duration of the squeeze (Toth *et al.* 2012).

It has been proposed that the thoracic squeeze mimics the compressive actions of the birth canal during the birth process (Mellor 2017). By redoing this compression artificially with a soft, fabric rope looped around the thorax, it is thought that the transition from unconsciousness to consciousness can be repeated, enabling the newborn to display normal, healthy behaviours upon removal of the squeeze (Mellor 2017).

2.11 Research aims and thesis structure

To date, there have been no systematic studies into the effects of the thoracic squeeze on healthy neonates of other precocial mammalian species such as piglets or calves. Furthermore, there have been no studies demonstrating the safety of the thoracic squeeze in neonatal mammals. In order to apply the thoracic squeeze as a therapy for addressing abnormal postnatal behaviour in low vigour neonates, there is a need to first demonstrate that the squeeze has few or no adverse effects on healthy neonates of these species. Such studies would provide insight into the mechanisms of the thoracic squeeze in both healthy and low vigour animals. Moreover, regardless of mechanisms, this technique may have significant practical benefits for animal production industries.

The following chapters relate to the project investigating the effects of a thoracic squeeze on healthy neonatal mammals of precocial species. The main aim of this study was to describe the responses of healthy newborn piglets and calves to a standardised application of the thoracic squeeze. A secondary aim was to evaluate the practical application of a rope and inflation cuff in applying a squeeze to newborn piglets and calves. The methods (Chapter 3) and results (Chapter 4) of the project are presented in the following sections, with a discussion (Chapter 5) using the results to address the study aims, followed by an explanation of what the results might mean in regard to potential mechanisms. The methodological limitations (Chapter 6) of the pilot study are evaluated, followed by future research (Chapter 7) which will use the results of this study to provide a basis for further investigation.

3. Methods

All procedures were approved by the Massey University Animal Ethics Committee (MUAEC Protocol 19/06).

3.1 Animals and care

Neonates of two precocial species, piglets (*Sus scrofa*) and calves (*Bos taurus*) were included in the study.

Seventeen piglets were sourced from Ratanui farm in Halcombe, Manawatu, over two months (May – June 2019). Piglets were selected between 12 and 36 hours old, to ensure they were established as healthy, having received sufficient colostrum (See inclusion criteria below). They were housed on site with their sows and litter in the farrowing stalls for the duration of the study. The sex of piglets was not recorded.

Sixteen calves were sourced from Dairy 1 Farm at Massey University over a period of three weeks in September 2019. They included a mix of bobby (unwanted) and replacement calves. Sex of the calves was not recorded. All calves were between 12 and 36 hours old at the commencement of the observation period.

Cows were routinely checked in the paddock twice daily by farm staff for newborn calves. Calves that were eligible for the study (See inclusion criteria below) were removed from the dam from between two and twenty four hours old (Jolanda Amoore, personal communication, September 2019) due to a once daily pick up of calves, and placed into an empty pen in the calf shed with dry woodchip bedding and shelter from the elements and kept there for the remainder of the study. The feeding status of all of the calves was unknown, in relation to whether or not they received colostrum from the dam before being moved to the calf shed, or whether they received colostrum or milk replacer in calf feeders by the farm staff.

No previous research has been done on the effects of the thoracic squeeze on piglets and calves. Previous research by Toth et al. (2012) used 8 healthy neonatal foals to generally describe the physiological and behavioural effects of a thoracic squeeze. In the absence of any guiding information, eight or nine animals per treatment was proposed to be sufficient to characterise responses of piglets and calves to the thoracic squeeze, and to explore variability in the response of the animals to the two application methods.

3.2 Inclusion criteria

To be included in the study, neonates had to be healthy and normally vigorous, having completed the necessary survival behaviours before application of the squeeze. For practical reasons, animals could not be observed from birth but were observed within the age range at which a healthy animal would have expressed these behaviours and the strict inclusion criteria meant that we could be assured of the health of animals included into the study. Criteria for each species are described below.

3.2.1 *Piglets*

Litters were first selected based on the desired age range, ensuring that the entire litter were older than 12 hours. Piglets were selected from litters of 12 or fewer to reduce the likelihood of applying the squeeze to piglets with pathophysiology, such as placental insufficiency or intrapartum hypoxaemia which are more common in larger litters (Mellor 1988; Svendsen 1992; Alonso-Spilsbury *et al.* 2007). Due to the limited availability of litters within the desired age range, up to 6 piglets from a single litter were selected. Within the litter, piglets which appeared to be the most vigorous (e.g. showing survival-related behaviours such as walking, teat seeking, and sucking milk) were chosen. Piglets that were smaller or appeared to show abnormal conformation (e.g. protruding forehead, shorter snout, leg deformities) were due to the likelihood of pathophysiological insult (Mellor 1988).

The inclusion criteria were: normal locomotion, socialisation, body posture, and rectal temperature. In terms of locomotion, piglets had to stand, walk, vocalise, or move their head or limbs when undisturbed or approached by researchers. Normal socialisation was demonstrated by seeking contact with dam or littermates, and normal body posture required a curled tail and relaxed, forward facing ears (K. Chidgey, personal communication, April 2019). Rectal temperature was measured once before inclusion into the study, and had to be within the normal range for piglets (38-39 degrees celcius) (Sipos *et al.* 2013). When all criteria were observed, the piglet was identified with tail paint applied at either the base of the neck or at the tail root.

3.2.2 *Calves*

The inclusion criteria used for calves differed to those used for piglets because their management and undisturbed behaviours differ (e.g. all calves were removed from their dam within 24 hours of birth and they are hiders). As calves are hiders, they do not readily stand and move when approached or touched by humans and are not curious to the same degree as piglets. As a result, the criteria were: normal locomotion, sucking reflex, body posture and rectal temperature. To assess locomotion, calves were encouraged to stand through touch or by manually assisting the calf to its feet and their movement around the pen was observed. Calves were only included if they could move freely without assistance. The presence of a sucking reflex was tested by feeding calves a small amount (approximately 200 ml) of warm colostrum or cold milk in a bottle supplied by the farm staff. As some calves may have not sucked from their dams, a weak sucking reflex was considered an acceptable standard for inclusion. Normal body posture was considered to be the head at the level of the body and not pulled back and up, while the calf was in sternal recumbency or standing on all four feet. Normal rectal temperature for calves ranges between 38 to 39 degrees Celsius (Mee 2008; Silva *et al.* 2016).

3.3 Experimental design and procedures

The order of testing on a given day was determined by the number of animals available that met the inclusion criteria. Animals were assigned to either the cuff or rope squeeze technique (see below), based on the order in which they were caught (piglets) or supplied (calves). All animals were squeezed only once to prevent an accumulative response. On each testing day, five to six piglets were squeezed, and two to three calves were squeezed.

3.3.1 *Piglets*

Piglets were allocated to one of two treatment groups related to the method of squeeze application (inflation cuff or rope). This allocation was determined by the number of litters available within the chosen age range each day, as well as the number of piglets within each litter that met the inclusion criteria. Due to the availability of litters within the desired age range, up to 6 piglets from one litter were used in the study in a single testing period. The availability of the equipment for the squeeze application also determined the treatment allocation. The inflation cuff required modification after initial testing, resulting in the use of the rope method on four of the piglets before the alternate allocation into each method of squeeze application could occur.

3.3.2 *Calves*

In a single testing day, 2-3 calves were ready for the study provided they met the inclusion criteria. Both methods of squeeze application were completed before testing on calves began. Therefore calves were alternately assigned to either the inflation cuff or rope method.

For both piglets and calves, a video camera was set up to record the undisturbed behaviour of all animals for up to 10 minutes, including their interactions with each other, the environment, and in the case of piglets, with the sow. Before applying the squeeze device, a pulse oximeter probe was attached to the ear (piglet) or teat (calf) and where possible, pulse rate and oxygen saturation were measured. Heart rate and respiratory rate for calves that met the inclusion criteria were obtained prior to treatment. This was not possible for piglets due to equipment malfunction or loud vocalisations from the piglets during measurement with the stethoscope. As vocalisations can change the normal pattern of breathing, and distort heart sounds during auscultation, these data were not included. These pre-squeeze values were used to provide pre-treatment values by which to monitor the physiological status of the animals throughout the squeeze.

3.3.3 *Applying the squeeze – Piglets*

The piglet was lifted from the floor of the farrowing crate and placed onto a wooden board lined with foam to reduce discomfort and conductive heat loss. The board was fixed securely to the bars of the farrowing crate. The squeeze device was firmly secured around the thorax (see below) and the squeeze applied. Upon application, a timer was started, and the time taken to achieve a non-responsive state was recorded. This state was characterised as lateral recumbency, closed eyes, and cessation of movement of limbs. A folded piece of vet wrap was placed over the eyes to reduce light stimulation. The squeeze was applied until the piglet met the discontinuation criteria (see below) or the maximum time of 10-minutes was reached. Additionally, if struggling and/or apparent wakefulness persisted for more than 3 minutes after application then the squeeze was discontinued.

3.3.4 *Applying the squeeze – Calves*

The calf was held in a standing position whilst the squeeze device was applied, before being allowed to lie in sternal recumbency either on a large memory foam dog bed, or on the floor, to reduce conductive heat loss and minimise discomfort. The squeeze device was firmly secured around the thorax while the calf was held and the squeeze applied. Upon application, a timer was started, and the time taken to achieve lateral recumbency and cessation of limb movements was recorded. Time to closed eyes was not measured for calves, due to this not being shown by any calf during their induction into a non-responsive state, and due to the fact that dozing or resting calves often do so with their eyes open (Jolanda Amoore, personal communication, September 2019). Nevertheless, a folded piece of vet wrap was placed over the eye to reduce any light stimulation. The squeeze was applied until the calf met the discontinuation criteria (see below) or the maximum time of 10-minutes was reached. Additionally, if struggling and/or apparent wakefulness persisted for more than 3 minutes after application then the squeeze was discontinued.

3.4 Squeeze treatments and application

3.4.1 *Rope*

For piglets, a soft polypropylene fabric rope of 1 cm diameter and approximately 1.5 metres in length was used. Vet wrap (a self-adhering elastic bandage that does not stick to the skin) was placed around the thorax to provide protection to the skin against any friction from the rope. A bowline knot was secured on one end, and the rope was placed between the front legs and looped through the knot at the wither (Figure 1). In the same direction, the rope was looped twice more around the thorax and secured with half hitches, ensuring each loop was evenly spaced across the thorax and would not slip down onto the abdomen. Each loop was then tightened to approximately 2.5kg weight measured with a luggage scale attached to the free end of the rope, or until a finger could no longer fit between the skin and the rope. The free end of the rope was pulled taut to prevent loosening of the squeeze. The timer was started when the first loop of the rope had been tightened. Removal of the squeeze was achieved by loosening and removing the rope from the thorax.

For the calves, a soft polyester fabric rope of 3 cm diameter and 5 metres in length was used. A small loop was already sewn into one end of the rope, which acted as the bowline knot. The rope squeeze was applied in same way as described for the piglets.

Figure 1: Rope applied to the thorax of a piglet, with pulse oximetry electrode attached to the ear.

3.4.2 *Inflation cuff*

Modified blood pressure inflation cuffs were created for each species (Figure 2). The external cuff was made from nylon, with the internal inflation balder made from elasticated rubber. To determine the appropriate size, measurements were taken from five newborns of each species within the 12–36-hour age range, which were not squeezed. Two measurements were taken: the heart girth in centimetres, referring to the circumference of the thorax directly behind the forelegs; and the length of the thorax in centimetres, measuring from directly behind the forelegs to the caudal end of the last rib. For piglets, the cuff measured 8cm in width (length of the thorax) and 36cm in length (thoracic circumference), and for the calves measured 17cm in width and 97cm in length.

For both species, the cuff was fitted closely around the thorax with velcro, and a nylon strap with an adjustable clip was placed around the shoulders and neck to prevent slipping of the cuff onto the abdomen (Figure 2). The device was inflated using different methods for the two species because of the pressure required to squeeze the thorax. For the piglets, a one-way valve pump with a sphygmomanometer attached was used to inflate the cuff to a minimum effective pressure of 180 mm Hg. For the calves, a diving tank with inflation and dumping valves was used to the inflate the cuff to a minimum effective pressure of 100 mm Hg (Figure 3). Timing was started when inflation of the cuff commenced. Removal of the squeeze was achieved by deflating the cuff and undoing the Velcro and shoulder strap.

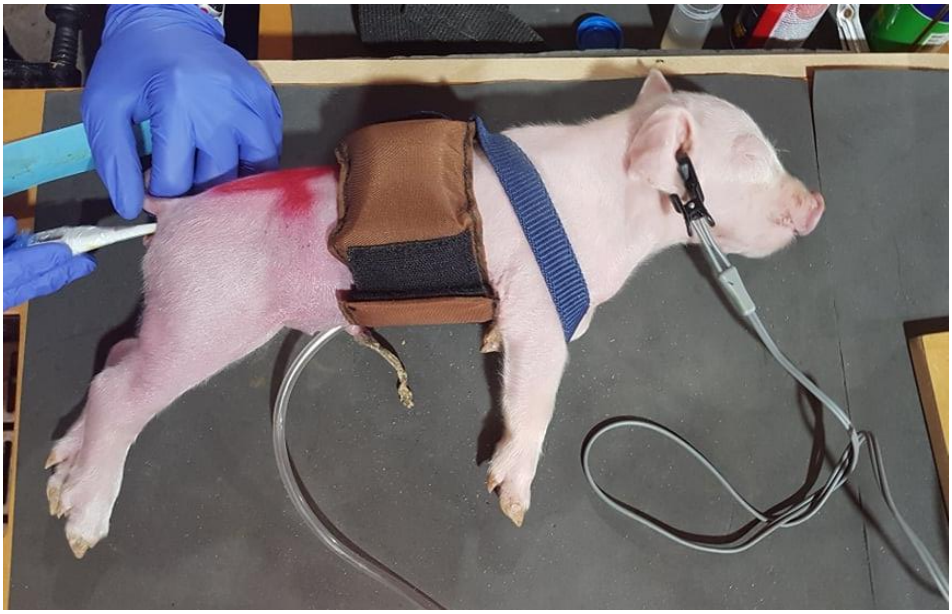

Figure 2: Inflation cuff applied to a piglet, secured around the thorax with velcro, and prevented from slipping onto the abdomen by a soft fabric strap. A rectal thermometer is being inserted into the rectum, and a pulse oximetry electrode is attached to the ear.

Figure 3: Inflation cuff placed on a calf ready to be inflated.

3.5 Criteria for discontinuing the squeeze

Physiological and behavioural variables were monitored at 2 time points during the squeeze (Table 1): 2 and 8 minutes after the start of squeeze application. Marked changes in any of these parameters triggered an immediate discontinuation of the squeeze. In particular, a sudden decrease or increase in heart rate or breathing rate suggested a detrimental change in physiological status and the squeeze was immediately discontinued. Often the sudden changes coincided with a complete absence of pedal and palpebral reflexes and absence of muscle tone.

Table 1: Physiological variables monitored before and twice during the squeeze at 2 and 8 minutes to decide whether to discontinue the squeeze due to physiological instability, and after the 10-minute squeeze duration to check recovery.

VARIABLE	DESCRIPTION
HEART RATE OR PULSE RATE	Heart rate (beats per minute) determined using a stethoscope placed on the chest between the forelimbs; recorded once before the squeeze application, and twice during the squeeze.
RECTAL TEMPERATURE	Temperature (°C) obtained using a lubricated thermometer inserted into the rectum; recorded once before the squeeze application, and twice during the squeeze.
OXYGEN SATURATION	Blood oxygen saturation (%) recorded where possible, using a pulse oximeter attached to either an ear (piglets) or teat (calves); recorded once before the squeeze application, and twice during the squeeze.
RESPIRATORY RATE	Respiration rate (breaths per minute) determined by visual assessment of chest movement, recorded once before the squeeze application, and twice during the squeeze.
MUSCLE TONE	Assessment of muscle tone (rigid or relaxed), determined by moving and bending the forelimbs and hindlimbs for resistance in movement, observed twice during the squeeze.
POSTURE	Assessment of body posture, determined by a standing or lying position, with lying including both sternal and lateral recumbency, evaluated twice during the squeeze.
HEAD POSITION	Position of the head, determined by a raised or dropped head, evaluated twice during the squeeze.
EYES	Eye status after application of the squeeze, determined as the eyes being open or closed, observed twice during the squeeze.
ORAL MUCOSA COLOUR	Determined by visual assessment of the gums and tongue colour (pink or blue), evaluated twice during the squeeze.

Discontinuation of the squeeze also occurred if the animal showed high intensity arousal before the end of the 10-minute period. This decision was based on the duration and intensity of arousal. If low-level transient arousal occurred, lasting only a few seconds with low intensity struggling, the squeeze was continued. If the arousal was of higher intensity and longer duration (lasting longer than 5 seconds with vocalising and righting onto all four feet was achieved), the squeeze was immediately discontinued.

3.6 Data collection

Behavioural responses and neural reflex activity following the application of the squeeze were assessed (Table 2), both in real time and by reference to video recordings. Latency to enter an apparently non-responsive state after the start of the squeeze was evaluated and reflexes assessed at 2 and 8 minutes. The timing and occurrence of arousals, urination and trembling were recorded continuously and later summarised over consecutive 2-minute intervals. The first interval included application of the squeeze and subsequent induction of a non-responsive state (for animals that were induced). Activity prior to the induction of a non-responsive state was excluded for consistency and the rate of each behaviour was calculated for each period. When discontinuation of the squeeze occurred, the subsequent 2-minute intervals were marked as 'missing' data.

Table 2: Behavioural responses recorded during the 10-minute thoracic squeeze for the purpose of analysis.

VARIABLE	DESCRIPTION
LATENCY TO LOSS OF POSTURE	Time to enter lateral recumbency from a standing or sternal recumbent position. The maximum time to latency was 180 seconds after the start of squeeze application, after which the squeeze was discontinued.
LATENCY TO EYE CLOSURE (PIGLETS ONLY)	Time for piglets to close their eyes, with eyes remaining closed for 5 seconds before latency recorded. The maximum time to latency was 180 seconds after the start of squeeze application, after which the squeeze was discontinued.

LATENCY TO CESSATION OF MOVEMENT	Time for the cessation of all limb and head movements. The maximum time to latency was 180 seconds after the start of squeeze application, after which the squeeze was discontinued.
PEDAL REFLEX	Status of reflex determined by pinching the interdigital cleft between the two claws of a front foot to elicit withdrawal of the limb; measured twice during the squeeze at 2 minutes and 8 minutes. *Present* was assigned for a full limb withdrawal response; *Reduced* was assigned for a slight limb withdrawal or a limb twitch response; and *Absent* was assigned for no muscle twitch or limb movement in response to the pedal test.
PALPEBRAL REFLEX	Status of reflex determined by lightly brushing or touching the eyelashes or skin caudal to the eye, to elicit a blinking or eye twitch response; assessed twice during the squeeze at 2 minutes and 8 minutes. *Present* was assigned for a full responsive twitch or blink response; *Reduced* was assigned for a small twitch response; and *Absent* was assigned for no muscle twitch in response to the palpebral test.
LOW INTENSITY AROUSAL	Low intensity, short duration movements. This was characterised by opening of the eyes (piglets) and vigorous movement of the limbs and head for 10 seconds or less, before a non-responsive state was resumed.
URINATION	Urination at any point during the 10-minute squeeze was noted. The timing and frequency were noted. Data collected was not analysed due to small samples.
TREMBLING	Trembling activity during the squeeze was noted when it occurred at any point during the 10-minute duration. Trembling was considered to include shivering thermogenesis, generalised muscle spasms, and whole-body tremors. As there was no way to determine the cause of the trembling activity, the label was used to cover all possible types of

	generalised muscle movements. Data collected was not analysed due to small samples.

3.7 Post-squeeze recovery

Upon deflation of the cuff or release of pressure on the rope, all animals were monitored for 5 minutes to ensure they had recovered from the squeeze. Piglets were allowed to stand on all four feet before being returned to the farrowing pen. When placed back into the farrowing stall, the quality of any exploratory behaviour, socialisation with the dam or littermates, and resting behaviour were observed, as well as ear position and tail orientation. Piglets were considered recovered when behaviours matched that which is normally observed in healthy piglets of this age.

Calves were encouraged into sternal recumbency and allowed to remain there for two minutes. Calves were then encouraged to stand by a researcher to assess their recovery. Warmed colostrum or milk was offered to each calf using a bottle with a rubber teat and sucking reflex and body posture were assessed. Normal posture was considered as a raised head and the calf was lying in sternal recumbency or standing and walking around the pen. The calf was considered recovered when all variables were observed to coincide with behaviours normally observed in healthy calves of this age.

3.8 Data analysis

Given that the aim of this preliminary study was simply to characterise the responses of piglets and calves to the thoracic squeeze, the data analysis is descriptive in nature and no statistical tests for significance were undertaken (Anderson 2019; McShane *et al.* 2019). We did not set out to test any specific hypotheses, and presenting the results of statistical tests of significance would be misleading given the small numbers of animals used in this study (Anderson 2019; McShane *et al.* 2019).

The success of the squeeze in inducing a non-responsive state and subsequent maintenance of non-responsiveness were categorised as follows:

A: No induction of a non-responsive state (using the criteria by 180 seconds after the start of application and the squeeze was discontinued).

B: Induction of a non-responsive state occurred within 180 seconds of application of the squeeze, but was not maintained for the full 10-minute observation period due to high intensity arousals (As described in section 3.5).

C: Induction a non-responsive state occurred within 180 seconds of application of the squeeze and was maintained for the 10-minute squeeze duration.

D: Induction of a non-responsive state occurred within 180 seconds of application of the squeeze, but the squeeze was discontinued due to concerns based on physiological monitoring criteria outlined in Table 1 and section 3.5.

The numbers and proportions (and 95% confidence intervals) of animals in each category were compared. The relationships between the induction categories or the method of squeeze application and variables describing the latency to induction (loss of posture, movement and eye closure) were explored using Kaplan-Meier graphs, median values and ranges. The relationships between the induction categories or the method of squeeze application and the presence/absence of pedal and palpebral reflexes and the frequency of low intensity arousals during the squeeze were explored graphically. All summary statistics and graphical representations were produced using R Studio version 1.2.1335 (RStudio Team 2018).

4. Results

4.1 Piglets

4.1.1 Success and maintenance of induction (categories)

A total of 17 piglets were included in the study, with nine piglets being squeezed using the inflation cuff method and eight piglets with the rope method (Table 3). All piglets were successfully induced, thus there were no Category A piglets.

Overall, 8 of 17 (Categories B and D: 47.1%) piglets did not complete the 10-minute squeeze period (Table 3). Of the piglets squeezed with the cuff, the squeeze was discontinued for three piglets. The squeeze was discontinued for one piglet due to a rapid decline in physiological parameters at 265 seconds and the other two piglets due to high intensity arousals in which they righted to their feet, at 115 and 160 seconds. In the rope group, 5 out of 8 squeezes were discontinued due to high intensity arousals.

Table 3: Number and proportion of piglets and calves in each induction category for each method of squeeze application, with 95% confidence intervals presented for the proportions in each induction category. Category A refers to animals that were not induced into a non-responsive state; Category B refers to animals that were induced but had the squeeze discontinued due to high intensity arousals; Category C refers to animals that were induced and maintained in a non-responsive state for the full 10 minutes; Category D refers to animals that were induced but discontinued due to physiological instability.

Category	A	B	C	D	TOTAL
Piglets					
Cuff	0	2	6	1	9
Rope	0	5	3	0	8
Total	**0**	**7**	**9**	**1**	**17**
Percentage *(95% CI)*	**0%** *(0-20%)*	**41.2%** *(18-67%)*	**52.9%** *(28-77%)*	**5.9%** *(15-29%)*	
Calves	A	B	C	D	TOTAL
Cuff	1	2	3	2	8
Rope	2	2	4	0	8
Total	**3**	**4**	**7**	**2**	**16**
Percentage *(95% CI)*	**18.8%** *(4-46%)*	**25%** *(7-52%)*	**43.7%** *(20-70%)*	**12.5%** *(2-38%)*	

Discontinuation of the squeeze always occurred before 300 seconds. Piglets that stayed non-responsive up to approximately the 240 second point stayed that way for the full 10-minute duration (Figure 4).

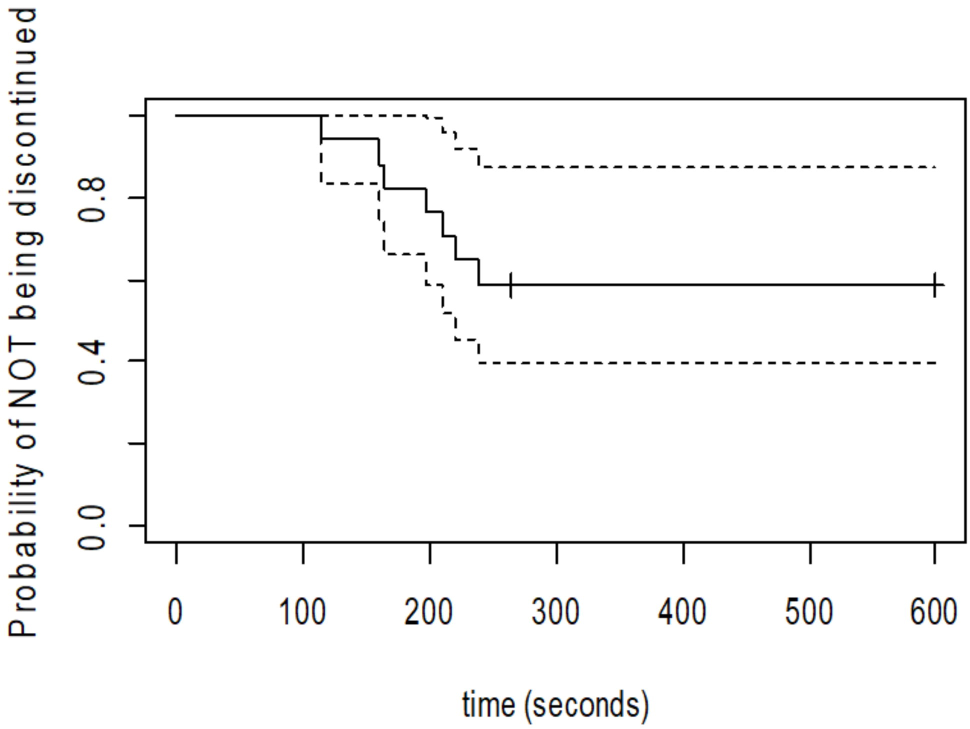

Figure 4: Kaplan-Meier graph of the time to discontinuation of the squeeze in all 17 piglets. Dashed lines are 95% confidence intervals, vertical lines indicate right-censored piglets (left vertical line is one D category piglet removed from the study due to changes in physiological variables). The other vertical line represents all 9 C category piglets that may have continued past the 10-minute observation period.

4.1.2 Relationship between induction category and time to induction and behaviour during the squeeze

4.1.2.1 Time to eyes closed

Category B piglets (induced but discontinued) appeared to take longer to close their eyes after the application of the squeeze (Med= 32s, Range= 15-90) than did Category C piglets (Med= 21s, Range= 11-90) (Figure 5).

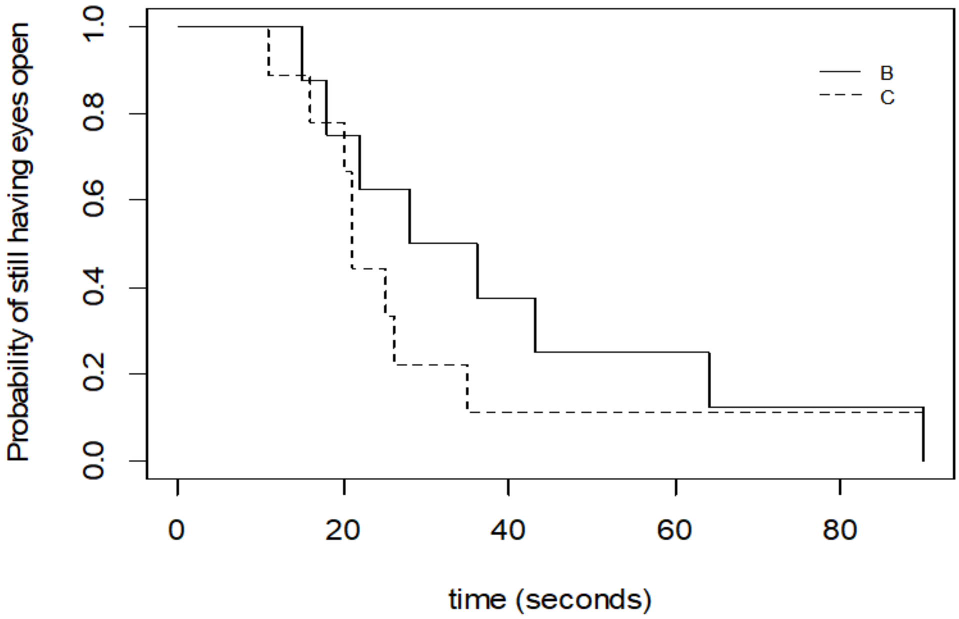

Figure 5: Kaplan-Meier graph of the latency to eyes closed in 16 piglets that were successfully induced into a non-responsive state with either the cuff or the rope, and that did not have the squeeze discontinued for health reasons. C group piglets maintained a non-responsive state for the full 10 minute squeeze period, whereas the squeeze was discontinued in B group piglets.

4.1.2.2 Time to loss of posture

Category B piglets for which the squeeze was discontinued before the end of the 10-minute period lost posture as quickly (Med= 37s, Range= 10-108) as Category C piglets that stayed non-responsive for the full 10 minutes (Med= 30s, Range= 11-69) but time to loss of posture was less variable in Category C piglets (Figure 6).

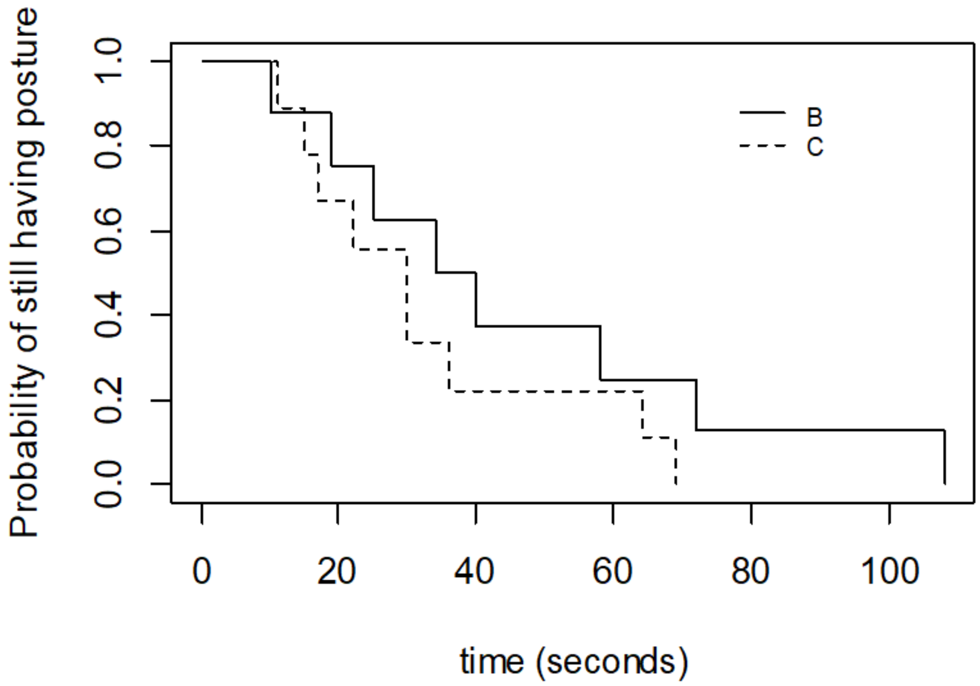

Figure 6: Kaplan-Meier graph of the latency to lose posture in 16 piglets that were successfully induced into a non-responsive state with either the cuff or the rope, and that did not have the squeeze discontinued for health reasons. C group piglets maintained a non-responsive state for the full 10 minute squeeze period, whereas the squeeze was discontinued in B group piglets.

4.1.2.3 Time to cessation of movement

Category B piglets (induced but discontinued) appeared to take longer to cease moving after the application of the squeeze (Med= 58.5s, Range= 22-108) than did Category C piglets (Med= 32s, Range= 12-129) (Figure 7).

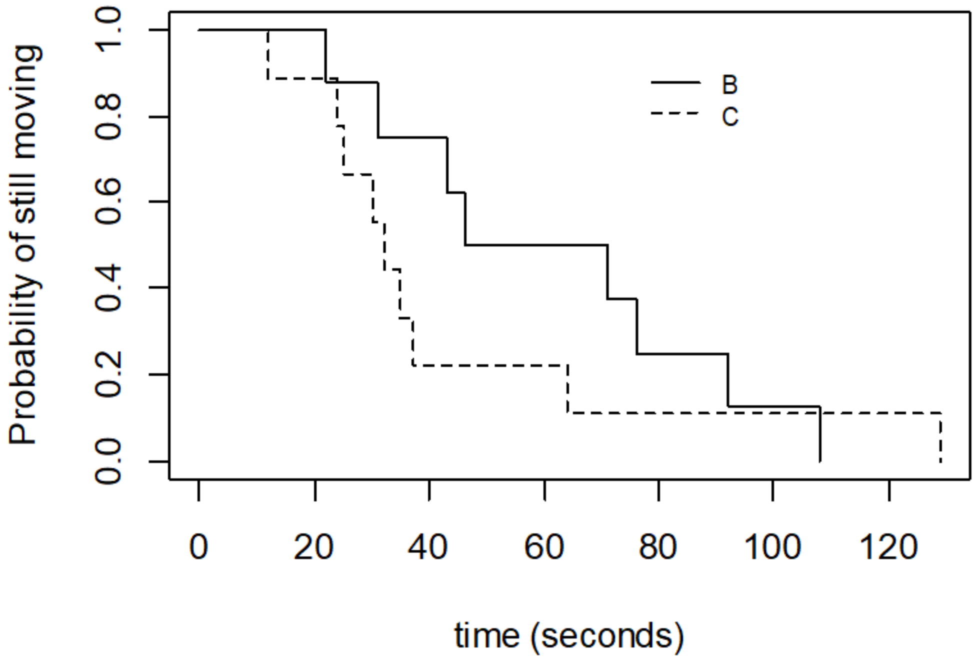

Figure 7: Kaplan-Meier graph of the latency to cessation of movement in 16 piglets that were successfully induced into a non-responsive state with either the cuff or the rope, and that did not have the squeeze discontinued for health reasons. C group piglets maintained a non-responsive state for the full 10 minute squeeze period, whereas the squeeze was discontinued in B group piglets.

4.1.2.4 Low intensity arousals

A comparison of the rate of low intensity arousals of Category B and C piglets could only be conducted for the time blocks during which piglets in both groups were still being observed (up to 4 minutes after application of the squeeze). Piglets that were subsequently discontinued (Category B) showed more low intensity arousals, particularly in the 2-4 minute block than did piglets that were maintained in a ‘non-responsive state’ for the full 10 minutes (Category C) (Figure 8). Piglets that were going to have the squeeze discontinued also showed greater variability in their rate of arousals for block two, compared to the little variability shown by piglets that continued for the full 10-minutes.

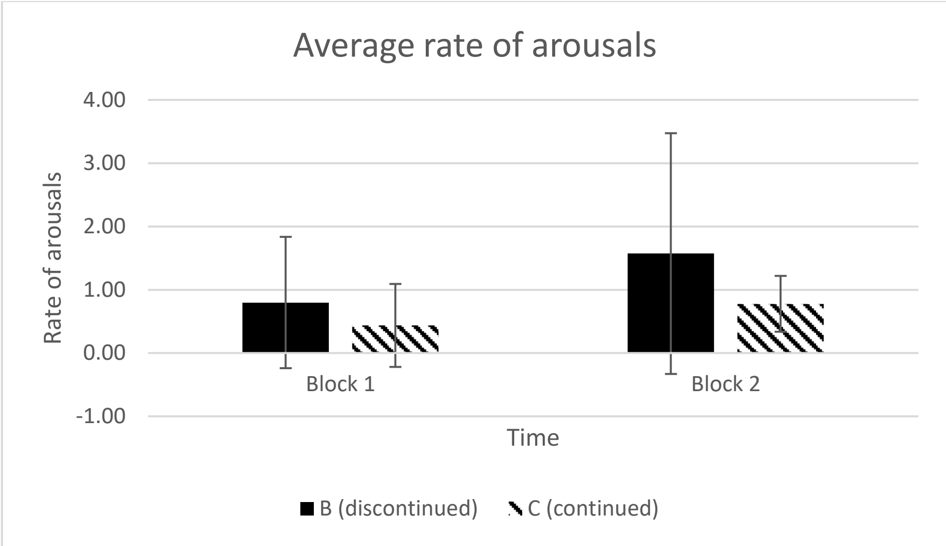

Figure 8: Average (± SD) rate of arousals per 2 minutes calculated for the first two time blocks in piglets for which the squeezed was maintained for the full observation period (9 Category C piglets) and piglets for which the squeeze was discontinued (7 Category B piglets). The duration of the first time block varied depending on how long it took for the individual piglet to be induced while block 2 was two minutes long. Only two time blocks (up to 4 minutes after start of squeeze application) are shown because all discontinuations had occurred by this point so there were no further data for Category B piglets.

4.1.3 Effect of method of application on induction, discontinuation, and behaviour during squeeze

4.1.3.1 Time to eyes closed

7 out of 9 piglets squeezed with the cuff appeared to close their eyes sooner (Med= 21s, Range= 11-90) than piglets squeezed with the rope (Med= 31.5s, Range= 20-64 seconds) (Figure 9), with the 2 remaining cuff piglets taking 90 seconds to close their eyes.

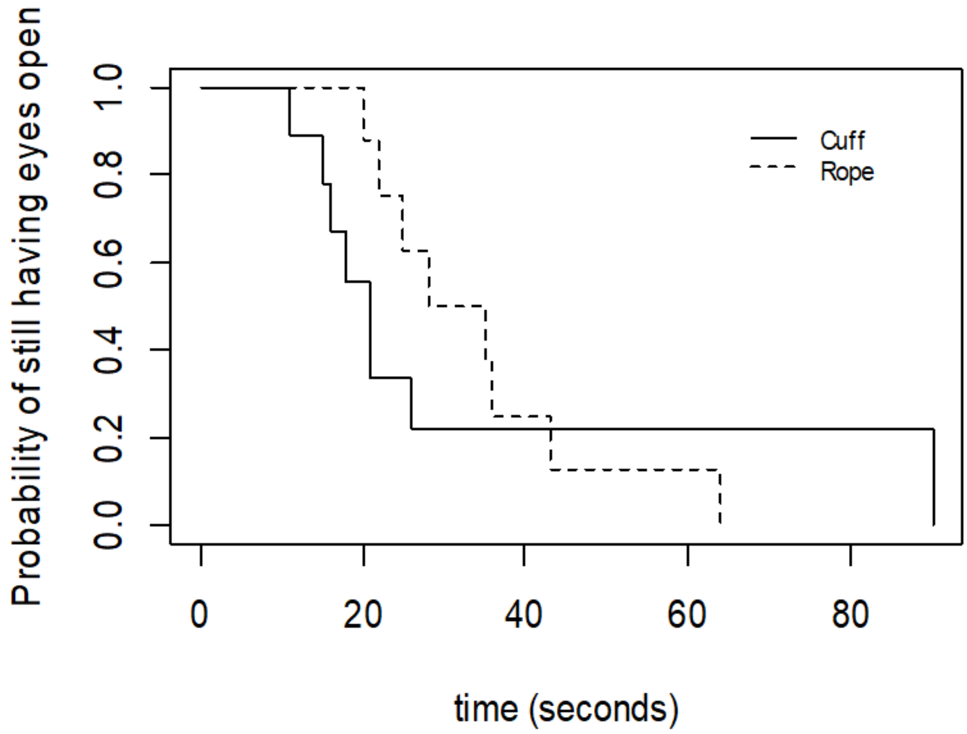

Figure 9: Kaplan-Meier graph of the latency to close their eyes in 17 piglets that were successfully induced into a non-responsive state with either the cuff or the rope.

4.1.3.2 Time to loss of posture

The time taken for piglets to lose posture appeared to be shorter when the squeeze was applied with the cuff (Med= 25s, Range= 10-69) than when applied with the rope (Med= 49s, Range= 19-108) and the time to loss of posture was less variable with the cuff (Figure 10).

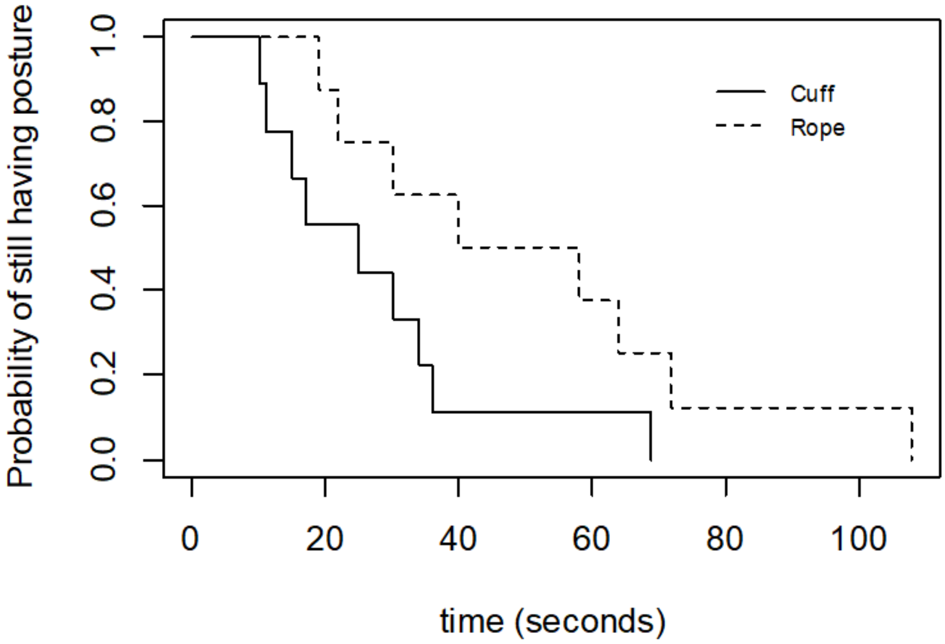

Figure 10: Kaplan-Meier graph of the latency to lose posture in 17 piglets that were successfully induced into a non-responsive state with either the cuff or the rope.

4.1.3.3 Time to cessation of movement

Piglets squeezed with the cuff appeared to stop moving sooner (Med= 31s, Range= 12-129) than piglets squeezed with the rope (Med= 55s, Range= 30-108 seconds) (Figure 11).

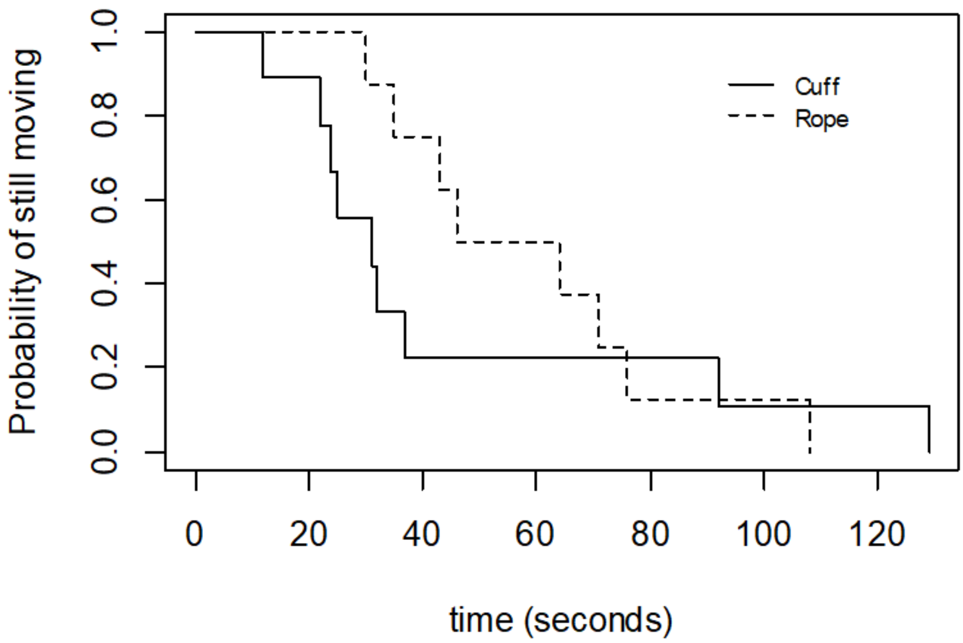

Figure 11: Kaplan-Meier graph of the latency to cessation of movement in 17 piglets that were successfully induced into a non-responsive state with either the cuff or the rope.

4.1.2.4 Time to discontinuation

Piglets for which the squeeze was discontinued before the end of the 10 minute period, for reasons of physiological instability or because they aroused, apparently did so earlier if they were squeezed with the cuff. The median time to discontinuation of the squeeze for the cuff was 160 seconds (n=3; Range= 115-265) and 210 seconds (n=5; Range= 165-239) for the rope.

4.1.2.5 Reflex responses during squeeze

At the 2-minute testing point for both pedal and palpebral reflexes, the squeeze had already been discontinued for one piglet in the cuff group and another couldn't be tested. All seven piglets tested in the cuff group showed reduced (n=3) or absent (n=4) pedal responses. In the rope group, all four 'excluded' piglets shown in Figure 12 were because testing was not logistically possible. In the rope group 2 of the 4 tested had reduced pedal responses and one had no response (Figure 12).

At the 8-minute testing point, all 'excluded' piglets in both groups were because the squeeze had already been discontinued. Of those piglets still remaining at 8 minutes, 2 of 6 piglets tested in the cuff group showed present pedal responses and 4 out of 6 were reduced or absent. In the rope group, 1 of 3 tested had a present response while the remaining two piglets showed no response (Figure 12).

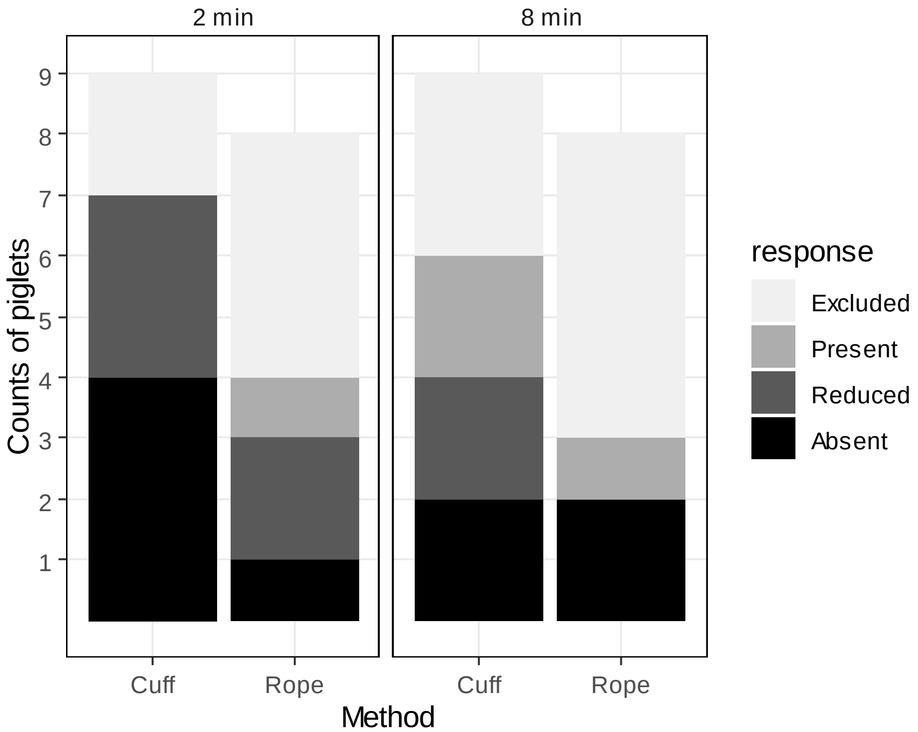

Figure 12: Number of piglets exhibiting each level of pedal responses at 2 minutes and 8 minutes into the thoracic squeeze between methods. 'Excluded' refers to piglets that did not have their reflexes tested for logistical reasons or because the squeeze had already been discontinued. Present refers to a full limb withdrawal; Reduced refers to a slight limb withdrawal; Absent refers to no limb responses to reflex testing.

At 2 minutes, more piglets squeezed with the cuff had reduced or absent palpebral responses (7 out of 7 tested) compared to piglets squeezed with the rope (3 out of 4 tested). At 8 minutes, 2 out of 6 piglets tested in the cuff group showed present responses while 4 out of 6 were reduced or absent. In the rope group, 1 of 3 tested had a present response while the remaining two piglets showed no response (Figure 13).

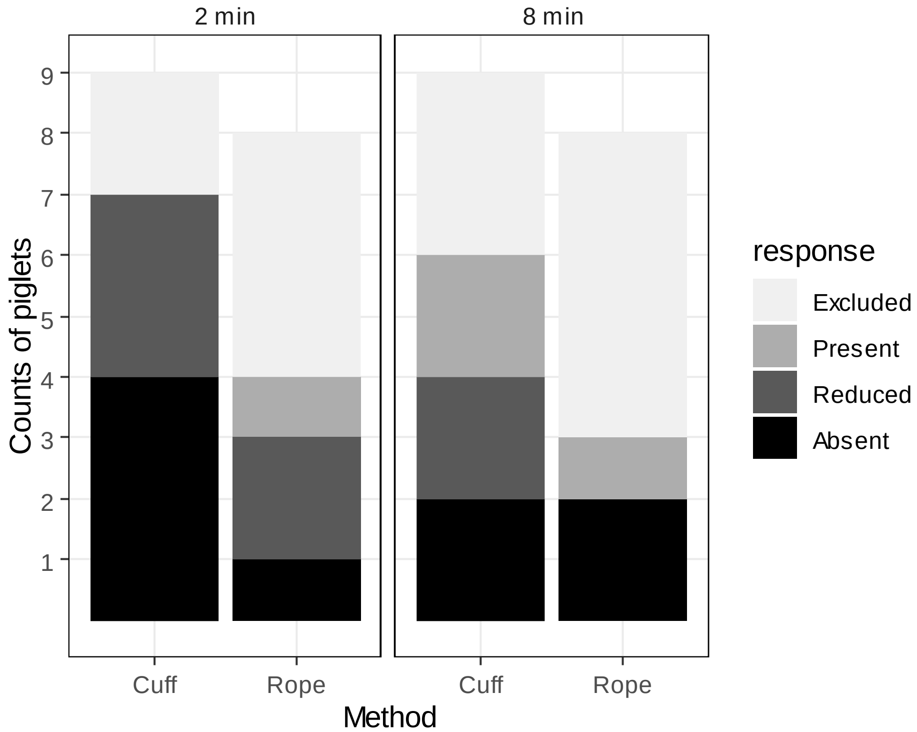

Figure 13: Number of piglets exhibiting each level of palpebral responses at 2 minutes and 8 minutes into the thoracic squeeze. 'Excluded' refers to piglets that did not have their reflexes tested for logistical reasons or because the squeeze had already been discontinued. Present refers to a full limb withdrawal; Reduced refers to a slight limb withdrawal; Absent refers to no limb responses to reflex testing.

4.1.3.6 Low intensity arousals

There appeared to be little difference in the rate (per 2 minutes) of low intensity arousals between application methods although there was an upward trend in the rate of arousals in the rope group in those piglets that were still non-responsive towards the end of the 10-minute observation period (n=3).

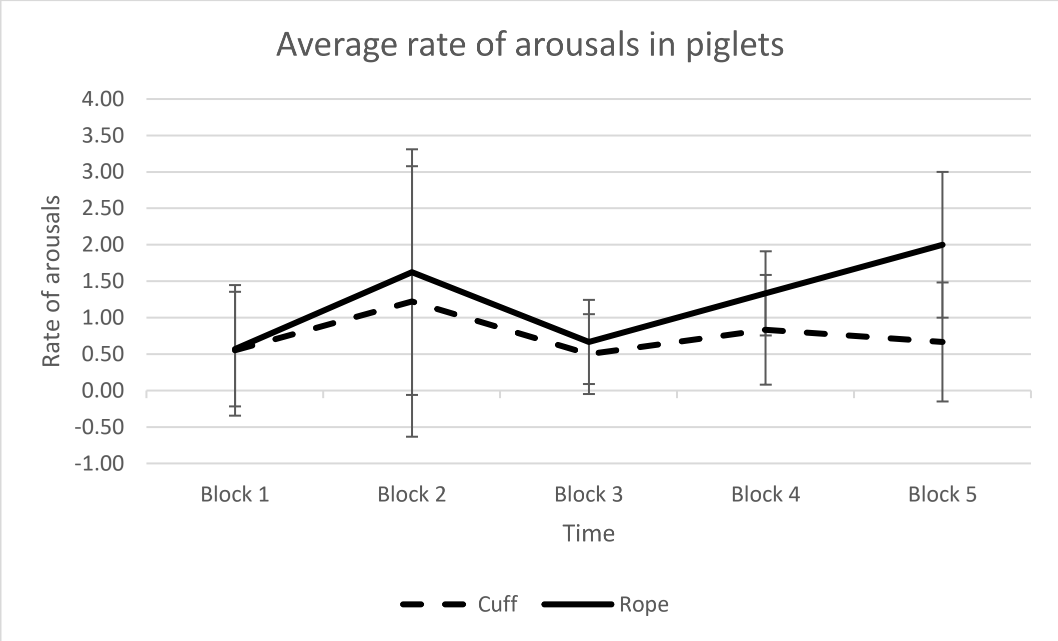

Figure 14: Average (±SD) rate of low intensity arousals per 2 minutes calculated for five time blocks over the 10-minute observation period. The duration of block 1 varied depending on how long it took for the individual piglet to be induced while all other blocks were two minutes long. The number of piglets in each time block dropped from 17 piglets in block 1 to 9 piglets in block 3 as the squeeze was discontinued throughout the 10-minutes.

4.1.4 Other behaviour

Urination occurred rarely in the piglets. Only 2 out of 9 piglets squeezed with the cuff and 2 out of 8 piglets squeezed with the rope urinated and only once each during the 10-minute observation period.

Trembling activity was also rare in the piglets and was not observed in piglets squeezed with the cuff. Two piglets in the rope group exhibited trembling during the 10-minute observation period, with trembling occurring repeatedly (in three out of five two-minute periods) in one piglet and once in the other piglet.

4.2 Calves

4.2.1 Success and maintenance of induction (categories)

A total of 16 calves were included in the study, with eight calves being squeezed using the inflation cuff method and eight calves with the rope method (Table 3). There were three category A calves (18.8%) that were not successfully induced.

Overall, 6 out of 16 calves (Category B and D: 37.5%) did not complete the 10-minute squeeze period (Table 3). These calves were induced (46%) but had the squeeze discontinued before the end of the 10 minute period. Overall, 7 out of 16 calves (43.7%) were successfully induced and stayed in for the full 10 minutes.

Of the eight calves squeezed with the cuff, the squeeze was discontinued for four calves. Two were for a rapid decline in physiological parameters at 322 and 332 seconds and the other two calves due to high intensity arousals in which they righted to their feet, at 351 and 464 seconds. In the rope group, the squeeze was discontinued for 2 out of 8 calves due to high intensity arousals at 411 and 510 seconds.

Discontinuation of the squeeze in calves due to high intensity arousals occurred consistently between 360 and 510 seconds, whereas discontinuation of the squeeze due to physiological instability occurred before 360 seconds (Figure 15).

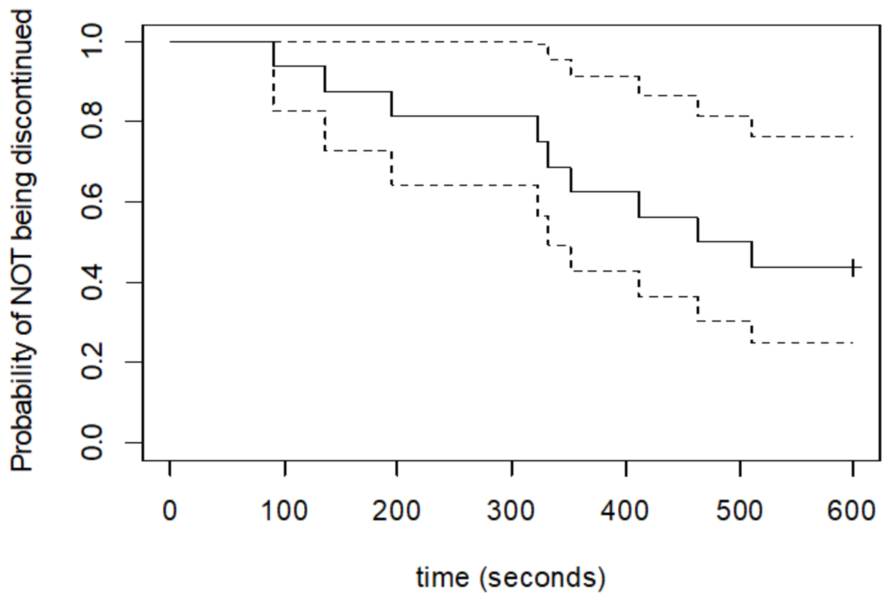

Figure 15: Kaplan-Meier graph of the time taken for the squeeze to be discontinued in all 16 calves. Dashed lines are 95% confidence intervals, the vertical line indicates right-censored calves, which represents all 7 C category calves that may have continued past the 10-minute observation period.

4.2.2 Relationship between induction category and time to induction and behaviour during the squeeze

4.2.2.1 Time to eyes closed

No calves that were induced into a non-responsive state after application of the squeeze closed their eyes. As a result, latency to eye closure was not considered an indicator of non-responsiveness in calves.

4.2.2.2 Time to loss of posture

Category B calves (n=4) that had the squeeze discontinued before the end of the 10-minute period lost posture sooner (Med= 15.5s, Range= 5-97) than Category C calves (n=7) that stayed non-responsive for the full 10 minutes (Med= 27s, Range= 11-96) (Figure 16). Most category B calves that lost posture did so within 20 seconds of application of the squeeze. However two calves did not lose posture until 96 and 97 seconds after the squeeze application. In contrast, category C calves showed an even spread of times to loss of posture.

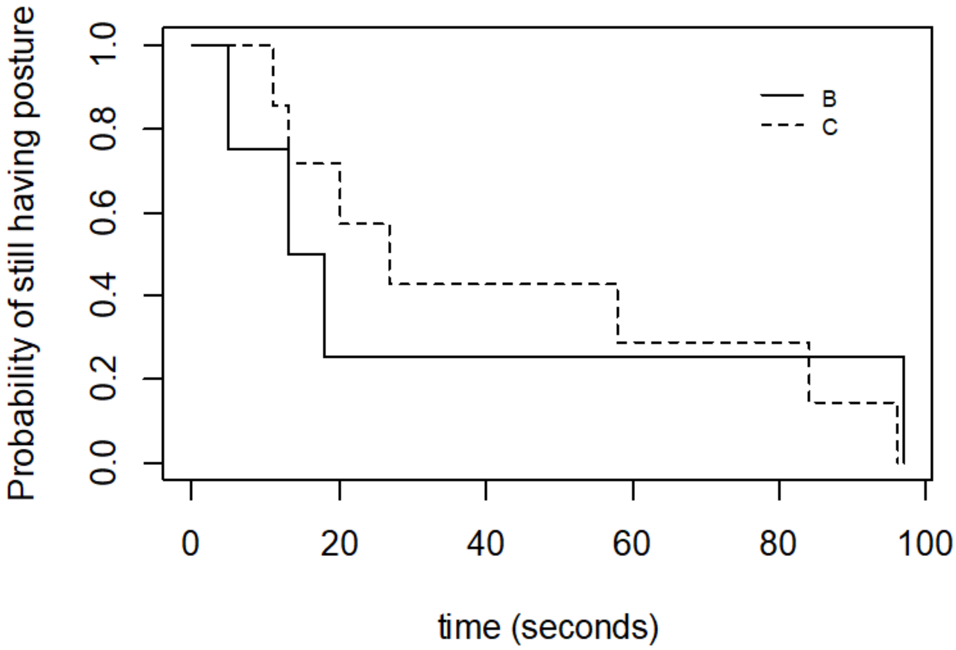

Figure 16: Kaplan-Meier graph of the latency to lose posture in 11 calves that were successfully induced into a non-responsive state with either the cuff or the rope, and that did not have the squeeze discontinued for health reasons. C group calves maintained a non-responsive state for the full 10 minute squeeze period, whereas the squeeze was discontinued for B group calves.

4.2.2.3 Time to cessation of movement

Category B calves (induced but discontinued) appeared to take shorter to cease moving after the application of the squeeze (Med= 26.5s, Range= 13-108) than did category C calves (induced for the full 10-minute observation period) (Med= 40s, Range= 20-115) (Figure 17). Most category B calves that ceased moving did so within 20 seconds of application of the squeeze. However two calves did not cease moving until 96 and 97 seconds after the squeeze application. In contrast, category C calves showed an even spread of times to cessation of movement.

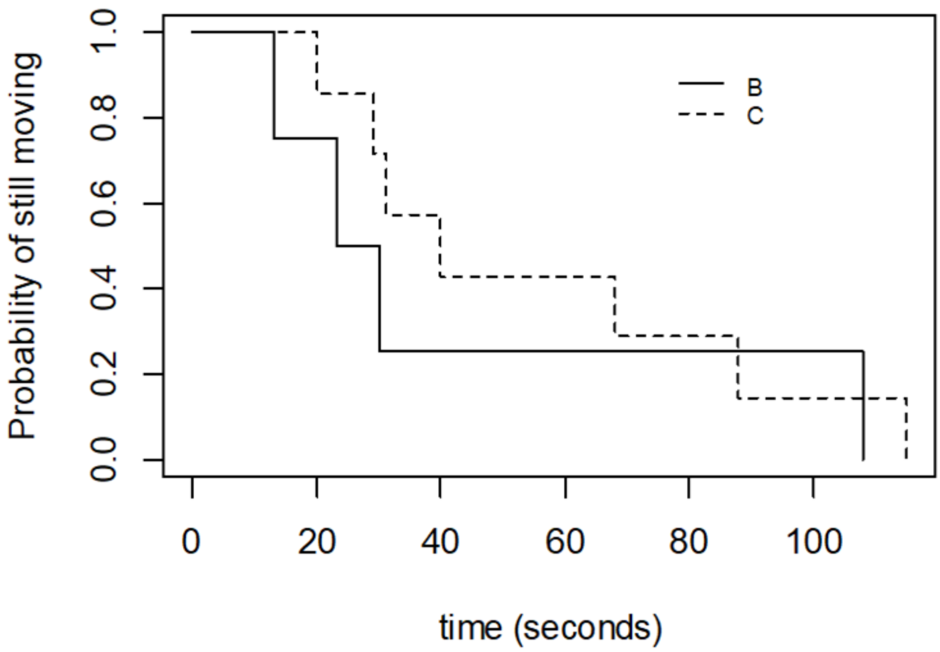

Figure 17: Kaplan-Meier graph of the latency to cessation of movement in 11 calves that were successfully induced into a non-responsive state with either the cuff or the rope, and that did not have the squeeze discontinued for health reasons. C group calves maintained a non-responsive state for the full 10 minute squeeze period, whereas the squeeze was discontinued for B group calves.

4.2.2.4 Low intensity arousals

A comparison of the rate of low intensity arousals of Category B and C calves could only be conducted for the time blocks during which calves in both groups were still being observed (up to 6 minutes). Calves for which the squeeze was subsequently discontinued (Category B) showed more low intensity arousals with greater individual variability in block 1 and 3, but showed less arousals in block 2 compared to calves that were maintained in a 'non-responsive state' for the full 10 minutes (Category C) (Figure 18).

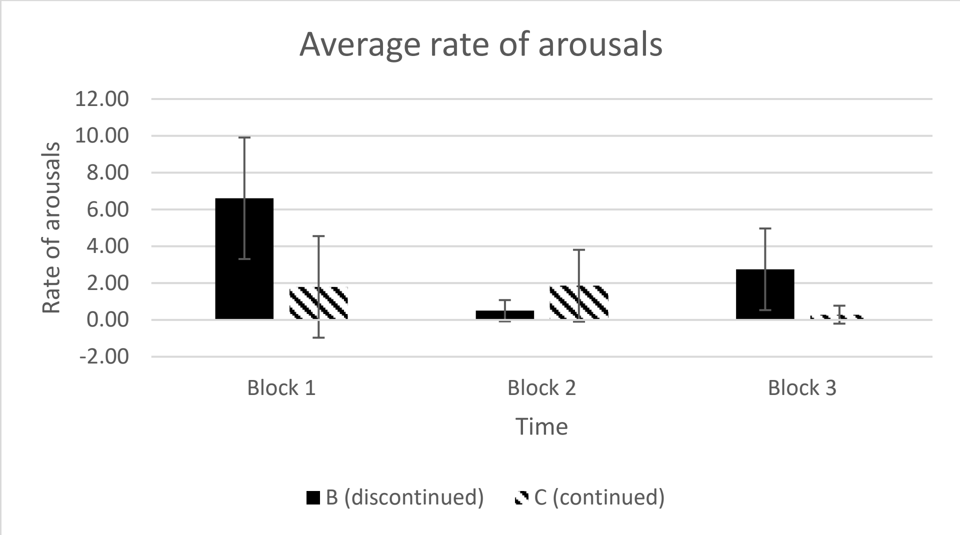

Figure 18: Average (± SD) rate of arousals per 2 minutes calculated for the first three time blocks in calves for which the squeezed was maintained for the full observation period (7 Category C calves) and calves for which the squeeze was discontinued (4 Category B calves). The duration of the first time block varied depending on how long it took for the individual calf to be induced while block 2 was two minutes long. Only three time blocks (up to 6 minutes after start of squeeze application) are shown because almost all discontinuations had occurred by this point so there were not enough further data for Category B calves.

4.2.3 Effect of method of application on induction, discontinuation, and behaviour during the squeeze

4.2.3.1 Time to loss of posture

The time taken for calves to lose posture appeared to be shorter when the squeeze was applied with the cuff (Med= 14s, Range= 5-58) than when applied with the rope (Med=27s, Range= 11-97) and the time to loss of posture was less variable with the cuff (Figure 19).

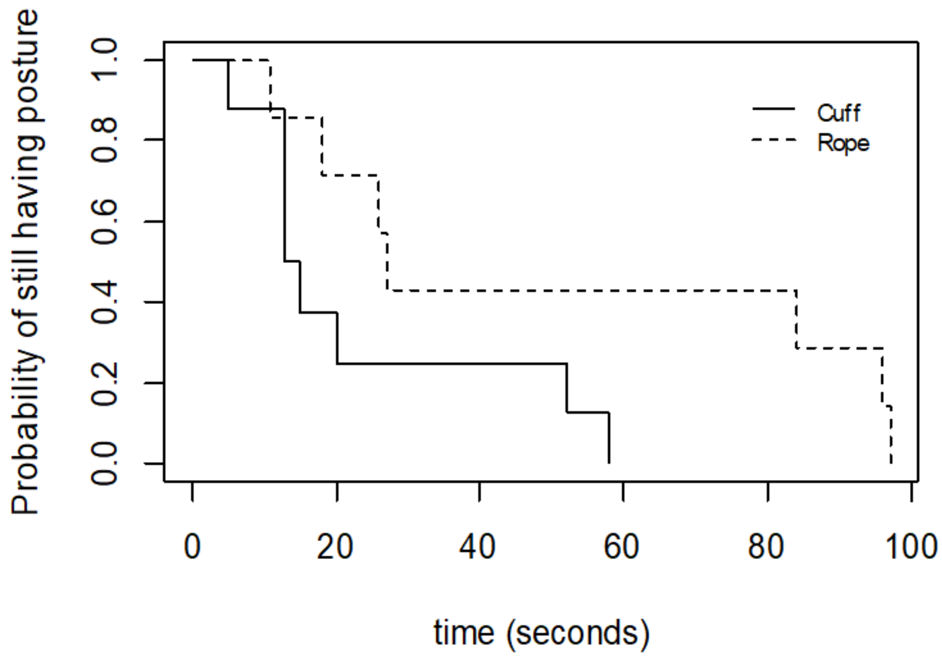

Figure 19: Kaplan-Meier graph of the latency to lose posture in 13 calves that were successfully induced into a non-responsive state with either the cuff or the rope.

4.2.3.2 Time to cessation of movement

Calves squeezed with the cuff appeared to stop moving sooner (Med= 31s, Range= 13-68) than those squeezed with the rope (Med= 54s, Range= 23-115 seconds) (Figure 20).

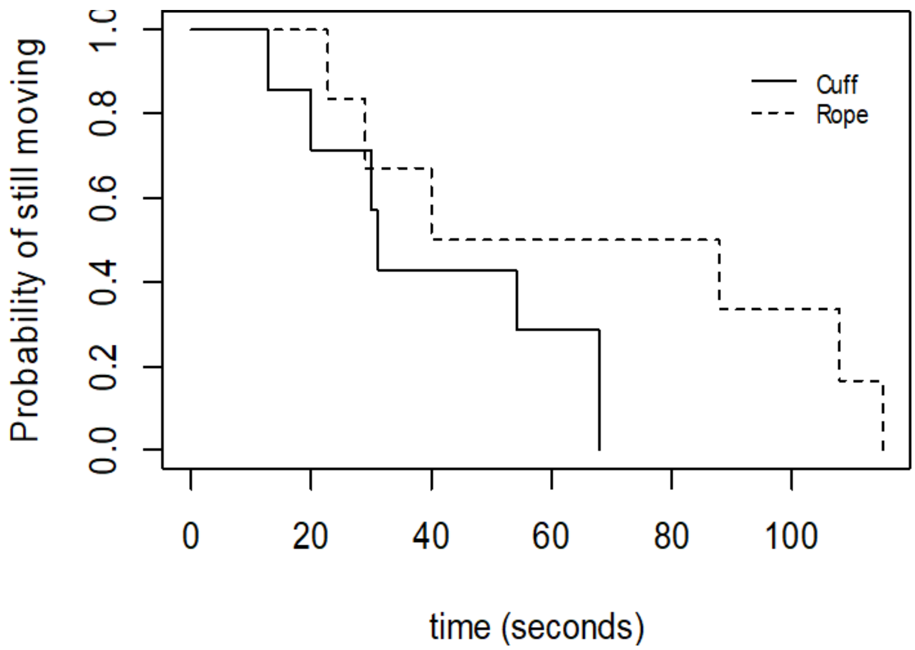

Figure 20: Kaplan-Meier graph of the latency to cessation of movement in 13 calves that were successfully induced into a non-responsive state with either the cuff or the rope.

4.2.3.3 Time to discontinuation of the squeeze

Calves for which the squeeze was discontinued before the end of the 10-minute period, for reasons of physiological instability (Category D), or because they aroused after induction (Category B), apparently did so sooner if they were squeezed with the cuff than with the rope. The median time to discontinuation of the squeeze with the cuff was 341.5 seconds (n= 4; Range= 332-464 s), and 460.5 seconds (n= 2; Range= 411-510 s) for the rope.

4.2.3.4 Reflex responses during the squeeze

At the 2-minute testing point, the squeeze had already been terminated for one cuff calf due to no induction into a non-responsive state (Category A). Likewise, the squeeze had already been terminated for two of the rope calves due to no induction into a non-responsive state (Category A), and thus reflexes could not be tested.

At 2 minutes, all but 1 out of 7 tested in the cuff group showed a present pedal response. In the rope group, all six calves tested showed a present pedal response (Figure 21).

At the 8-minute testing point, all 'excluded' calves were because the squeeze had already been discontinued. In the cuff group, 2 of the 3 calves tested showed present responses and 1 out of 3 was reduced. In the rope group, 4 of 5 tested had a present response while the remaining one calf showed a reduced response (Figure 21).

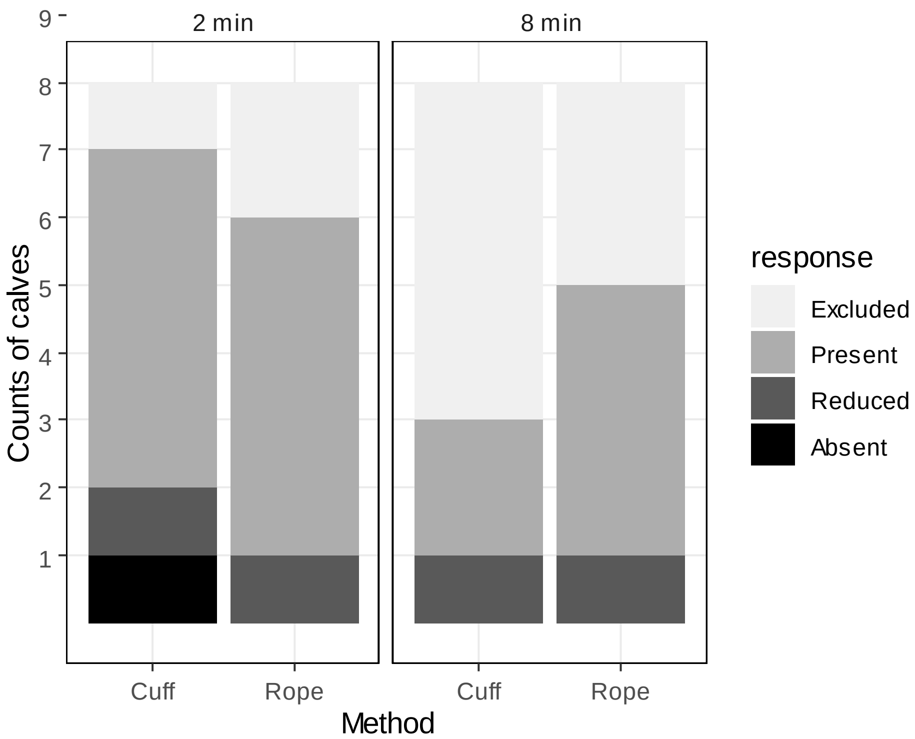

Figure 21: Number of calves exhibiting each level of pedal responses at 2 minutes and 8 minutes into the thoracic squeeze between methods. 'Excluded' refers to calves that did not have their reflexes tested for logistical reasons or because the squeeze had already been discontinued. Present refers to a full limb withdrawal; Reduced refers to a slight limb withdrawal; Absent refers to no limb responses to reflex testing.

At 2 minutes, 2 out of 7 calves in the cuff group showed reduced palpebral responses and 1 showed no response. In the rope group, all six calves tested showed presented palpebral responses (Figure 22).

At 8 minutes, 2 of 3 calves tested in the cuff group showed reduced palpebral responses and 1 out of 3 was present. In the rope group, 5 of 5 tested had a present response (Figure 22).

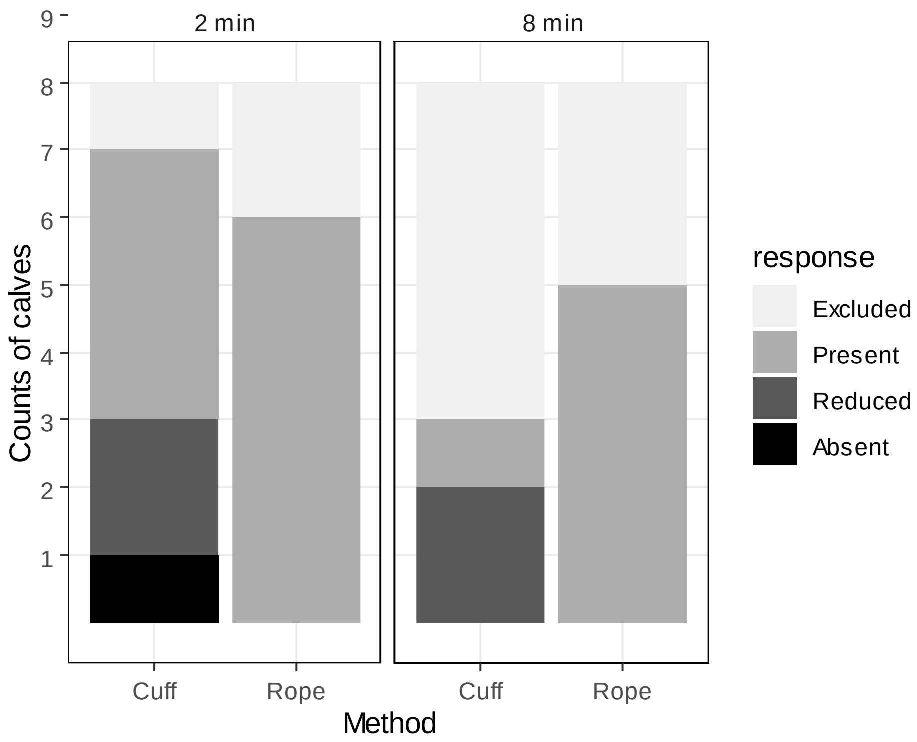

Figure 22: Number of calves exhibiting each level of palpebral responses at 2 minutes and 8 minutes into the thoracic squeeze between methods. 'Excluded' refers to calves that did not have their reflexes tested for logistical reasons or because the squeeze had already been discontinued. Present refers to a full limb withdrawal; Reduced refers to a slight limb withdrawal; Absent refers to no limb responses to reflex testing.

4.2.3.5 Low intensity arousals

There appeared to be little difference in the rate (per 2 minutes) of low intensity arousals between application methods for the full 10-minute observation period. The number of arousals was somewhat higher and more variable among individuals in the first block of time that included their induction into a non-responsive state than in subsequent time blocks (Figure 23).

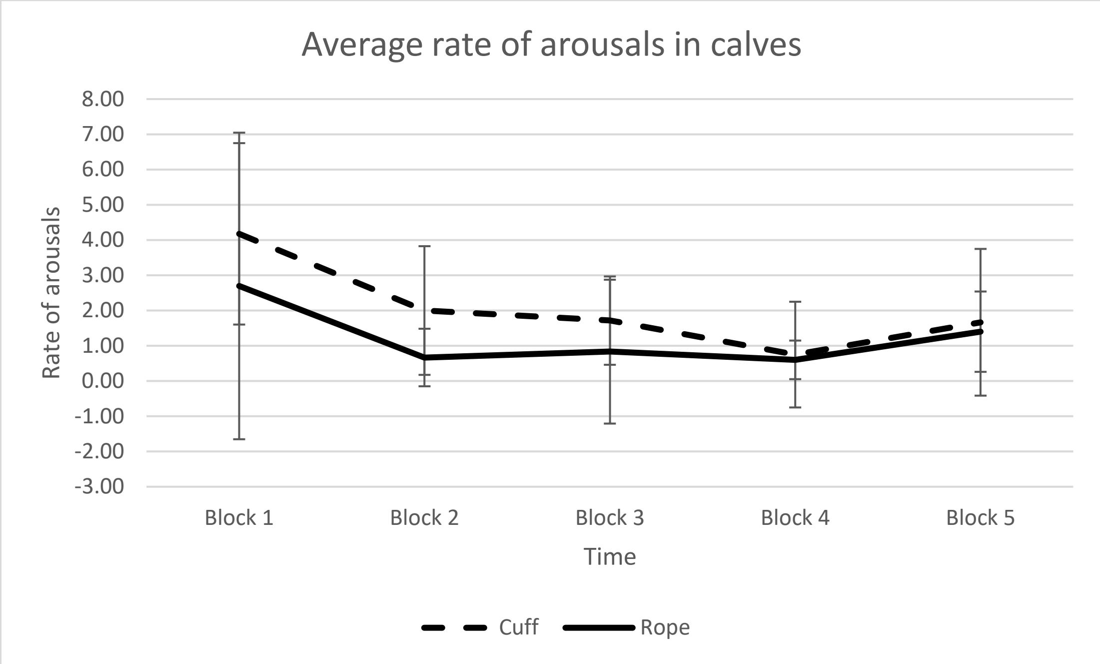

Figure 23: Average (±SD) rate of low intensity arousals per 2 minutes calculated for five time blocks over the 10 minute observation period. The duration of block 1 varied depending on how long it took for the individual calf to be induced while all other blocks were two minutes long. The number of calves in each time block dropped from 13 calves in block 1 to 8 calves in block 5 (7 category C calves and 1 category B calf) as the squeeze was discontinued in calves throughout the 10-minutes.

4.2.4 Other behaviour

Urination did not occur in any calves during the observation period in either the cuff or rope method.

Trembling activity was also rare in the calves. Five calves exhibited trembling activity during the 10-minute observation period, one in a calf squeezed with the cuff, and four observed in calves squeezed with the rope.

5. Discussion

The main aim of this study was to describe the responses of healthy newborn piglets and calves to a standardised application of the thoracic squeeze. A secondary aim was to evaluate the effect of method of application of the squeeze on those responses: a soft fabric rope or a modified inflation cuff.

This is the first study to provide a detailed characterisation of behavioural responses to the thoracic squeeze in healthy neonatal mammals of any precocial species. Previous research has reported physiological stability during application of the squeeze in healthy and low vigour foals and calves (Toth *et al.* 2012; Aleman *et al.* 2017; Stilwell *et al.* 2019). However, these studies have not given details of the squeeze application nor the behavioural responses to application of the squeeze, including the proportion of squeezes resulting in successful induction, the duration for which a lower- or non-responsive state was maintained (i.e. any information about discontinuation) and the level of responsiveness during the squeeze (e.g. reflexes indicative of neural activity). Thus, few direct comparisons to previous work can be made. The following sections will discuss the results in the context of addressing the study aims, followed by what the results suggest about the mechanisms of the squeeze, and lastly, what the results mean for applying the thoracic squeeze in an industry setting.

5.1 A generalised behavioural response to the thoracic squeeze

As in foals, neonates of other precocial species that are otherwise healthy sometimes show abnormal behaviours after birth (Stilwell *et al.* 2019). The thoracic squeeze appears to be a useful therapeutic approach for recovering such foals, characterized as having Neonatal Maladjustment Syndrome (NMS), as well as several calves showing similar abnormal behaviours soon after birth (Aleman *et al.* 2017; Stilwell *et al.* 2019). Interestingly, applying the squeeze to healthy foals in the early post-natal period caused comparable behavioural responses to those shown by NMS foals. Healthy foals reportedly responded by entering into a non-responsive state characterised by sternal recumbency, reduced muscle tone and sleep-like brain electrical (EEG) activity after application of a thoracic squeeze (Toth *et al.* 2012).

Similarly, in the current study, all piglets and more than 80% of calves were induced into a less- or non-responsive state within 3 minutes after the start of application of the thoracic squeeze. This suggests that this response may represent a generalised phenomenon in neonates of precocial mammalian species. However, no information has been presented in previous studies on the proportion of foals (healthy or otherwise) squeezed that were successfully induced compared to the total number of foals squeezed. Systematic studies reporting those data are needed for foals to fill this knowledge gap. Discussion of the potential mechanisms underlying the response are discussed in section 5.5.3 below and possible applications of the squeeze explored in section 5.6.

5.2 Behavioural responses to the thoracic squeeze

5.2.1 Piglets

All piglets were induced into a non-responsive state within 2.5 minutes of the squeeze application, regardless of the method of squeeze. While in this state, most piglets had reduced or absent reflexes at 2 minutes, while approximately two-thirds of those that continued to the full 10-minutes still showed reduced or absent reflexes towards the end of the squeeze period.

This change in reflex responses suggest that all piglets were in a fully non-responsive state similar to what was reported in healthy foals (Toth *et al.* 2012). These reflexes are commonly used to assess the depth of unconsciousness in animals under general anaesthesia, and has more recently been explored in animals after stunning for slaughter (Verhoeven *et al.* 2015). A pedal reflex assesses the depth of unconsciousness at the spinal cord level, whereas a palpebral reflex assesses the depth of unconsciousness at the brainstem level (Verhoeven *et al.* 2015). Indicators of unconsciousness in animals under general anaesthesia have been reported as an absence of pedal and palpebral reflexes (Verhoeven *et al.* 2015;

Bradbury and Clutton 2016). Therefore, a reduction or complete absence of responses to pedal and palpebral reflex tests after application of the thoracic squeeze suggests that the piglets are in a state of unconsciousness. However more research is needed to assess the depth of unconsciousness (see section 7: Future research).

Approximately half of all piglets that were induced into a non-responsive state spontaneously aroused before the end of the 10-minute squeeze application period and the squeeze was discontinued. Full arousal in piglets was characterized by opening of the eyes, righting onto all four feet and loud vocalisations and no attempt was made to re-apply the squeeze once these behavioural signs were shown. Whether the piglets could have been re-induced was not explored in this study due to the potential for cumulative effects of the squeeze to affect the results, as well as the lack of information regarding the safety of multiple squeezes on a neonate.

In previous studies of healthy foals and abnormally behaving foals and calves, no mention was made of arousals during squeeze application (Toth *et al.* 2012; Aleman *et al.* 2017; Stilwell *et al.* 2019). All squeezed animals were reported to stay in a non-responsive state for 20 minutes. Thus, it is not possible to comment on whether the failure of all piglets to maintain the non-responsive state in this study relates to species differences, differences in the way the squeeze was applied or the animals were managed during the squeeze, or the way the data were collected and reported.

In the piglets, arousal and discontinuation always occurred before 5 minutes after the start of application of the squeeze. Piglets that stayed in a non-responsive state for more than four minutes stayed that way for the full 10-minutes. It is unknown how long the non-responsive state would have lasted if the piglets were left past the 10-minute observation period. The number of low intensity arousals stayed relatively constant over the 10-minutes in those that stayed in the whole time.

The 10-minute squeeze duration was decided upon before beginning the study with the aim of reducing the risk that physiological disturbances that might occur with a protracted squeeze. This duration was based on reports of the non-responsive state being maintained for 20 minutes in foals (Toth *et al.* 2012; Aleman *et al.* 2017) and was kept shorter than the reported length of stage II labour (the total time a fetus is squeezed during passage through the birth canal) in piglets and calves. The duration of stage II labour is between 38-114 minutes in cows (Doornbos *et al.* 1984; Schuenemann *et al.* 2011; Campler *et al.* 2015) and between 12-20 minutes between successive births of piglets in sows (Van Dijk *et al.* 2005; Alonso-Spilsbury *et al.* 2007; Vanderhaeghe *et al.* 2013). Limiting the duration of the thoracic squeeze to 10 minutes ensured that the squeeze would be shorter than the duration of stage II labour of both species, and thus would protect against any physiological disturbances that may occur from a protracted squeeze.

Piglets that spontaneously aroused and for which the squeeze was discontinued took longer to be induced into a non-responsive state than piglets that remained in a non-responsive state for the full 10-minutes, as indicated by the latency to close their eyes and cease moving. The median time to loss of posture was similar across the two groups but there was more variability among the piglets that subsequently aroused. Furthermore, piglets for which the squeeze was later discontinued showed more low intensity arousals in the first four minutes after application of the squeeze (prior to full arousal) than piglets that continued for the full 10-minutes.

These indicators of subsequent arousal suggest that the duration of a thoracic squeeze in piglets may be predicted by differences in their behaviours immediately after application of the squeeze. The basis of this variability among individuals is unknown but it raises a valuable question for future research looking into why some neonates appear to be more susceptible to the effects of the squeeze than others. A possible reason for this may be the inconsistency in the application of the rope in the same way for each neonate. Ensuring the loops of the ropes covered the area of the thorax without slipping down onto the abdomen, or collecting too closely together on the thorax was difficult in the piglets. However, this does not explain why some piglets squeezed with the cuff were also less susceptible to the effects of the thoracic squeeze.

5.2.2 Calves

Approximately 20% of the calves were not induced into an apparently different state of responsiveness within 3 minutes of application of the squeeze, indicated by no collapse into lateral recumbency or remaining in sternal recumbency, had persistent righting attempts and eventually made it onto all four feet. The 13 calves that were induced all did so within 2 minutes of the squeeze application, regardless of the method of squeeze. While in this state of apparently altered responsiveness, nearly all calves had normal pedal and palpebral reflexes at 2-minutes, while approximately three-quarters of those that continued to the full 10-minutes still showed normal reflexes towards the end of the observation period. None of the 13 induced calves closed their eyes during their induction. The lack of change in reflexes after application of the squeeze suggests that the calves were not induced into a fully non-responsive state. The reduced or absent reflexes observed in animals under general anaesthesia are considered indicators of unconsciousness (Verhoeven *et al.* 2015; Bradbury and Clutton 2016), with the presence of reflexes suggesting that the animal is not at a depth sufficient to be considered fully unconscious (Verhoeven *et al.* 2015; Bradbury and Clutton 2016). Instead, based on the available data, the calves should be described as responding to the squeeze by entering a state of lower-responsiveness.

It is unclear why some of the calves weren't induced by the squeeze. It may be due to a difference in the application of the squeeze which resulted in an inefficient pressure applied to the thorax. It is possible that loops of the rope slipped on the thorax which reduced the pressure applied to the whole area of the thorax. More information is needed to understand why the squeeze appeared to have no reducing effect on the responsiveness of these calves.

Approximately one third (4 out of 13) of all calves that were induced later spontaneously aroused and the squeeze was discontinued. Half of induced calves remained under the squeeze for the full 10-minute period. Arousal in these calves was characterized by visible movements of the limbs, righting attempts and standing on all four feet. Calves that fully aroused did so in the second half of squeeze application, 6 - 8.5 minutes after the start, and the time to arousal was more variable in the calves than in the piglets. As these are the first detailed data on this phenomenon, whether the observed success of induction and duration for which a less responsive state was maintained is typical of calves, healthy or otherwise, is unknown.

Calves which spontaneously aroused during the squeeze had a shorter latency to induction than calves that remained under the squeeze for the full 10-minutes, as indicated by the calf remaining in lateral recumbency with no movement of the limbs, no righting attempts or standing on all four feet. Furthermore, calves which later aroused showed more low intensity arousals in the first 2 minutes, and at 4-6 minutes after application of the squeeze than calves that did not arouse during the full 10-minute squeeze.

Although the between-group difference in induction speed was in the opposite direction to that observed in piglets, these findings indicate that the latency to induction was faster with the cuff, and suggest that the rate of low intensity arousals early in the squeeze predict the likelihood of subsequent arousal in both piglets and calves. This information could be useful for therapeutic applications of the squeeze (see section 5.6).

5.3 Evaluating the safety of the thoracic squeeze

In order to apply the thoracic squeeze as a therapy for addressing abnormal postnatal behaviour in low vigour neonates, there is a need to first demonstrate that it is safe. The first step is to demonstrate that the squeeze has few or no adverse effects on healthy neonates of these species.

Out of 17 piglets squeezed, one piglet in the cuff group had the squeeze discontinued due to physiological instability at approximately 4.5 minutes. There were no marked changes in heart rate or breathing rate, but the piglet started gasping, which suggests it may have become hypoxic (low blood or tissue oxygen levels) during the squeeze. Gasping, defined as increased respiratory effort in order to reoxygenate, has been reported to sometimes occur during periods of hypoxia (Elnazir *et al.* 1996; Fewell 2005). Due to equipment malfunction, blood oxygen saturation could not be recorded in the piglets, but there was no change in the pink colour of the oral mucosa which suggests that the hypoxia was minor (Cotton and Grunstein 1980; Bureau *et al.* 1984; Powell *et al.* 1998). Upon immediate discontinuation of the squeeze, the piglet was observed to recover quickly to pass the recovery criteria within the 5-minute the post-squeeze recovery period, supporting the idea that the squeeze had only minor, transient effects on physiological status (see section 3.7).

Likewise, of 16 calves squeezed, few were discontinued due to physiological instability. One calf squeezed with the cuff showed a marked increase in breathing rate at approximately 5.5 minutes, which suggests a hyperventilation response to the squeeze application. This may be indicative of developing hypoxia (Cotton and Grunstein 1980; Bureau *et al.* 1984; Powell *et al.* 1998), potentially from the thoracic restriction imposed by the cuff if breathing is not switched from the thorax to the abdomen. This may have resulted in restricted blood flow to the brain or heart due to inefficient oxygen supply. The other calf, also squeezed with the cuff, showed a noticeable decrease in heartrate at approximately 5.5 minutes point. This sudden change in cardiac activity may be due to the secondary phase of hypoxia reported in newborn mammals, in which the release of hormones cause a rapid fall in heart rate in order to induce unconsciousness and protect the brain from hypoxic damage (Dawes *et al.* 1959; Elnazir *et al.* 1996). This hypoxia may have been due to inefficient breathing by the calf during the thoracic squeeze. The recovery of these calves after the squeeze was removed was more difficult to evaluate than in piglets due to the naturally less vigorous nature of calves (Edwards and Broom 1982; Nowak *et al.* 2000), but both calves passed all of the recovery criteria (see section 3.7) within the 5-minute post-squeeze recovery period.

These observations suggest that the squeeze rarely had marked effects on the physiological status of piglets or calves when applied up to a maximum of 10 minutes. In addition, there were no long lasting ill-effects of the squeeze on the few animals that showed a negative physiological response to the squeeze. However, the observations emphasise the importance of regular monitoring throughout the squeeze to ensure that any changes in physiological status can be detected quickly and the squeeze discontinued. Due to equipment failure, the physiological monitoring during this study did not include continuous oxygen saturation levels, which would have provided immediate and continuous information about the oxygen status of the neonate during the squeeze.

Overall, the low number of squeeze discontinuations that occurred in this study due to physiological instability suggests that the thoracic squeeze is generally safe for healthy neonatal piglets and calves of this age, provided caveats are in place to regularly monitor physiological variables and ensure the squeeze is immediately discontinued for any change in physiological status of the neonate. More detailed pre-squeeze health assessments for the inclusion criteria need to be developed to ensure all animals squeezed are healthy. Included in these criteria should be ensuring that all neonatal calves are fed prior to the squeeze (see section 7: Future research).

5.3.1 Methods of squeeze application

The second aim of the study was to evaluate the responses of piglets and calves to two methods of squeeze application: a soft fabric rope and an inflation cuff. All previous studies have used a rope to apply the squeeze. Previously, this rope method was applied to newborn foals (Toth *et al.* 2012) and recently in two newborn calves (Stilwell *et al.* 2019). Though the rope was reported to be successful at inducing a non-responsive state in the foals and calves, in the researcher's experience, it was difficult to maintain an even pressure on all three loops of the rope. Thus, the inflation cuff was developed in order to provide a more uniform and even squeeze and enable the safety of the thoracic squeeze to be assessed.

5.3.1.1 Piglets

Induction into a non-responsive state occurred sooner in piglets squeezed with the cuff than in piglets squeezed with the rope and fewer piglets spontaneously aroused when squeezed with the cuff. However, piglets which spontaneously aroused did so sooner if they were squeezed with the cuff than if they were squeezed with the rope. The rate of arousals over the 10-minute period did not differ markedly between the two methods of squeeze application for those piglets that stayed non-responsive throughout, though the rate of arousals appeared to increase slightly for piglets squeezed with the rope towards the end of the period.

These findings suggest that the cuff provided a more effective squeeze than the rope. The cuff was faster to apply than the rope, which may explain some of the difference in the latency to induction into a non-responsive state between the two methods. The cuff also appeared to apply a more uniform squeeze and even tactile pressure around the thorax compared the rope. It is unclear as to why piglets squeezed with the cuff were also discontinued sooner than piglets squeezed with the rope. The earlier discontinuation observed in piglets squeezed with the cuff may be because they were also induced sooner than piglets squeezed with the rope. It is possible that the time to discontinuation of the squeeze may be more even between methods of squeeze application when time to induction is taken into consideration.

Another possible explanation for the cuff functioning more effectively than the rope may the smaller thoracic size of piglets compared to foals and calves. Foals and calves have a larger thoracic circumference and greater body weight. The rope method can therefore be applied using sufficient force around the thorax without the risk of injury. On the contrary, the force applied to the thorax using the rope in piglets may not have been sufficient to induce a non-responsive state that persisted for the full 10-minutes when compared to the uniform squeeze provided by the cuff. Piglets typically weigh approximately 1.3-1.4 kilograms at birth with a mean weight gain of 100 grams by 24 hours old (Baxter *et al.* 2008; Decaluwé *et al.* 2014). As a result, the 2.5kg tension applied to the free end of the rope in the piglets was required to apply sufficient force on the thorax to elicit a behavioural response from the piglet to the squeeze and the risk of injury may have been greater than when piglets were squeezed with the cuff.

In this study, trembling, characterised as generalised shivering and muscle movements, was observed in two piglets squeezed with the rope. It is possible that trembling in these piglets may be explained by shivering thermogenesis, but this would have been reflected in a noticeable reduction in rectal temperature which did not occur. Trembling has been also reported as a pain-related behaviour in newborn piglets following painful procedures (Llamas Moya *et al.* 2008). It is possible that the pressure of the rope, which was concentrated to single loops of the rope around the thorax was substantial enough to cause a pain response in the piglets. However, this did not appear to arouse the piglets out of the non-responsive state.

5.3.1.2 Calves

As in piglets, induction into a lower responsive state occurred sooner in calves squeezed with the cuff than in calves squeezed with the rope, with less inter-individual variability in the latency to induction in the cuff group. There was no difference in the number of animals that spontaneously aroused with each method, but calves squeezed with the cuff that aroused did so sooner than calves squeezed with the rope. There was no difference in the rate of low intensity arousals throughout the 10-minute period between calves squeezed with the cuff and calves squeezed with the rope.

These findings suggest that a more consistent response to the thoracic squeeze occurs in calves, and that one method of squeeze application is not better at inducing and maintaining a non-responsive state than the other. However, the cuff was easier and quicker to apply to the thorax than the rope, which may explain why calves squeezed with the cuff were induced sooner into a lower responsive state than calves squeezed with the rope. The earlier induction may also explain the earlier discontinuation in calves squeezed with the rope. Furthermore, the cuff was quicker to remove from the thorax for calves that were discontinued due to physiological instability or due to spontaneous arousal, suggesting that the cuff may be a more practical way to apply a thoracic squeeze to neonatal calves than the rope.

5.4 Comparisons between piglets and calves

Comparisons of the thoracic squeeze between piglets and calves is limited by the differences in their early postnatal behaviours. This may explain why the responses to the squeeze in all piglets were more consistent than the responses observed in the calves.

Piglets are followers (Edwards and Broom 1982; Broster *et al.* 2010) and show distinctive high vigour behaviours consistently throughout the postnatal period, indicated by standing, coordinated locomotion, locating a functioning teat, sucking colostrum, and competition with littermates. As a result, their behavioural change from a vigorous, conscious state to a non-responsive state after application of the squeeze was apparent.

In contrast, calves are hiders (Edwards and Broom 1982; Nowak *et al.* 2000), and do not show distinctive high vigour behaviours consistently, instead spending more time in lying down than piglets (Edwards and Broom 1982; Nowak *et al.* 2000). The calves in this study were also observed to doze with their eyes open (J. Amoore, Personal communication, September 2019) which further limited the characterisation of their induction. Furthermore, the feeding status of the calves before their inclusion into the study was unknown, which may have affected their vigour. Calves that had never sucked from the dam or received colostrum in the calf shed may have been weaker and therefore shown less vigour than calves that had received colostrum from the dam or from calf feeders.

Based on these differences between piglets and calves, and how distinctive the change from a conscious and vigorous state to a non-responsive state was in the piglets, it cannot be concluded that the calves were induced into a non-responsive state in this study. The present reflexes observed in nearly all calves at both 2 minutes and 8 minutes after application of the squeeze suggests that whatever state they were in was light and easily arousable. Instead, it is recommended that the calves are considered to be in a state of lower-responsiveness.

On the other hand, it can be concluded that the piglets were induced into a non-responsive state after application of the squeeze. The distinctive change from a vigorous and conscious state to a generally quiescent and non-responsive state, combined with reduced and absent reflexes observed in nearly all piglets at both 2 and 8 minutes after application of the squeeze, supports the idea that all piglets were induced into a non-responsive state.

5.5 What these observations suggest about mechanisms of the thoracic squeeze

The thoracic squeeze has demonstrated a generalised effect of non-responsiveness in piglets and foals and lower-responsiveness in calves. However, the mechanisms underlying the thoracic squeeze and the cause for the induction into a non-responsive state are unknown. To date, no research has explored the potential mechanisms. What is currently understood from this study and previous research is that the non-responsive state elicited by the thoracic squeeze is unlikely to be due to fainting or tonic immobility.

5.5.1 Fainting

Fainting was an unlikely cause for the non-responsive state observed in all animals in the study. The physiological variables (heart rate, respiratory rate, mucous membrane colour, muscle tone and, where possible, oxygen saturation levels) monitored throughout the squeeze provided indirect evidence that the induction into a non-responsive state was unlikely to occur as a result of fainting. Category D animals (one piglet and two calves) for which the squeeze was discontinued due to a marked change in physiological variables (e.g. decrease in heart rate, increase in breathing rate, and gasping) did not show a rapid change in oxygen saturation or colouring of the oral mucosa, suggesting that fainting was an unlikely cause for the non-responsive state observed in all animals. Upon application of the squeeze, all animals switched from thoracic to abdominal breathing, but breathing depth and rate and heart rate did not change significantly. This is consistent with the research done in foals and calves that reported a switch from thoracic to abdominal breathing (Toth *et al.* 2012; Stilwell *et al.* 2019). Though venous blood pH and electroencephalogram (EEG) recordings weren't measured in the piglets and calves of this study, they were measured previously in healthy neonatal foals (Toth *et al.* 2012). Venous pH was reported to show no marked changes, and EEG recordings showed a decrease in brain electrical activity to resemble sleep-like brain activity

(Toth *et al.* 2012), suggesting fainting was not a result of the thoracic squeeze application. The physiological parameters monitored throughout the 10-minute squeeze for the piglets and calves of this study provided enough information about the physiological status of the animal to ensure that any marked changes in variables resulted in an immediate discontinuation.

5.5.2 Tonic immobility

Likewise, the observed response in piglets and calves is unlikely to reflect tonic immobility. Animals that are induced into a state of tonic immobility have a characteristic increase in muscle tone (Hoagland 1928; Moore and Amstey 1962), often reported as a fear-like paralysis. All animals in the study showed a decrease in muscle tone that suggests tonic immobility was not a result of the squeeze. An interesting characteristic of tonic immobility is the lack of reactivity to environmental stimuli (Fraser 1960). When animals are induced into a non-responsive state, a decrease in stimulatory reactivity would be expected. However, the incidence of arousals in all animals in this study suggests a complete abolition of environmental reactivity did not occur, supporting the idea that tonic immobility was not a cause for the non-responsive state observed in the neonates. Furthermore, tonic immobility induced in farm animals (Fraser 1960) has been observed to inhibit movement during an initial change into a tonic immobility state, even when strongly encouraged by researchers. Removal of the thoracic squeeze resulted in a rapid attempt to stand and walk, suggesting that the animals were not in a state of paralysis with inhibited muscle movement.

In this study, application of the thoracic squeeze was done on standing animals that were gently placed into lateral recumbency upon an apparent loss of posture, paired with eye closure and a reduction in muscle tone. This non-responsive state continued for up to 10-minutes for over half of all piglets squeezed. In contrast, tonic immobility appears to be transient in all animals that are manually restrained into the fear state (Erhard and Mendl 1999). Pigs that were placed on their backs into a V-shaped restraint with a weighted bag placed under the chin were observed to stay in a state of tonic immobility for only a short duration (approximately 120 s) before the study was discontinued due to persistent struggling (Erhard and Mendl 1999). Though characteristics of the tonic immobility test in pigs is similar to the responses observed in some of the piglets and calves in this study, it appears likely that the responses observed after application of the thoracic squeeze were not as a result of tonic immobility.

5.5.3 Mechanisms underlying the thoracic squeeze

The generalised effect of the thoracic squeeze in neonates of precocial mammals supports the current hypothesis regarding the therapeutic effect of the squeeze on neonates expressing abnormal behaviours. It was speculated that the postnatal thoracic squeeze mimics the natural thoracic compression that occurs during passage of a fetus through the birth canal (Aleman *et al.* 2017; Mellor 2017). Interest in this was further stimulated by the responses of NMS foals to the squeeze, which apparently reset brain function to result in the onset of healthy, vigorous postnatal behaviours (Aleman *et al.* 2017). It is thought that these foals are born in an altered state of consciousness that inhibits the expression of survival-related behaviours because of a lack of a sufficient squeeze during passage through the birth canal (Mellor 2017). By simulating the natural thoracic compression postnatally with an artificial squeeze, these foals can repeat the transitional process from unconsciousness to full consciousness, thus removing the inhibition of the expression of survival-related behaviours. This hypothesis has yet to be investigated, but the results of the pilot study in piglets and calves provide more information in support of this hypothesis. Furthermore, the recent report of two NMS calves expressing healthy behaviours after a thoracic squeeze (Stilwell *et al.* 2019) suggests that this technique could be used as a therapeutic approach in neonates of other precocial mammals that show NMS-like behaviours. However, further research is required to determine if NMS-like behaviours are present in apparently healthy piglets and a greater number of calves (see section 7: Future research).

5.6 Possible applications for the thoracic squeeze

The findings of this pilot study suggest that there are applications for the thoracic squeeze in an industry setting. The lower- and non-responsive states demonstrated in the neonates in this study suggest that the thoracic squeeze may be useful as a therapeutic approach for neonatal mammals of precocial species that show NMS-like behaviours as has been reported in foals and calves (Aleman *et al.* 2017; Stilwell *et al.* 2019). Furthermore, the cuff was observed to be more practical in its application and effects, providing a faster induction and a more uniform application than the rope. Thus, it is recommended that the inflation cuff be used as a therapeutic approach in the future.

Another possible use for the thoracic squeeze technique involves a non-chemical, benign form of restraint. All piglets and most of the calves were induced into a non-responsive or lower-responsive state, of which they showed general motor quiescence with distinctive indicators of high intensity arousals that warranted a discontinuation of the squeeze. This suggests that the squeeze may provide an effective form of restraint, particularly for the purpose of conducting husbandry procedures in neonates.

Typical piglet husbandry procedures involve tail docking, castration (outside of NZ), teeth clipping, and iron infusion within the first postnatal days (Dzikamunhenga *et al.* 2014; Sutherland 2015), all of which take less than 5 minutes to complete (K. Chidgey, personal communication, May 2019). In the piglets of this study, those that had the squeeze discontinued all did so before 5 minutes after application of the squeeze. This suggests that the squeeze application could be shortened to approximately 4 minutes to enable husbandry procedures to be completed with greater ease, with certainty that the piglet will remain in a non-responsive state for the full 4 minutes. However, more information is required to determine if piglets in a non-responsive state can experience pain. During the squeeze, reduced and absent reflexes were observed in nearly all piglets, suggesting that pain thresholds may be altered after application of the thoracic squeeze. More research is needed before this method can be applied in an industry setting for the purpose of husbandry procedures (see section 7: Future research).

6. Methodological limitations

The study was conducted under normal farm management conditions, which resulted in a number of limitations/uncontrolled variables that may have influenced the results. In particular, a number of environmental factors appeared to influence the duration of the thoracic squeeze.

6.1 Stimulation by environmental factors

There was no way to control variables such as temperature, light, sound, and mechanical stimulation, which are all known stimulators of conscious behaviours in neonates (Fraser and Broom 1998; Mellor and Gregory 2003). Previous research was conducted in a clinical setting where many variables were controlled for, which may explain the lack of information regarding arousals and incidence of discontinuation in foals and calves (Toth *et al.* 2012; Aleman *et al.* 2017; Stilwell *et al.* 2019).

To try and minimise conduction heat loss, the piglets were placed onto a board lined with insulated foam, and the calves were placed onto the shed bedding or a dog bed, with a blanket placed over them during the squeeze. To mitigate light stimulation, a folded cloth was placed over the eye once the non-responsive state was achieved. There was no way to reduce sound stimulation. Mechanical stimulation (reflex testing) was planned at the beginning of the study to determine how responsive the neonates were to external stimulation, and therefore was controlled in this study.

Though these factors may have influenced the results in this study, approximately half of the neonates in each species maintained a non-responsive state for the full 10-minute observation period, suggesting that this technique may be applied in an industry setting.

6.2 Access to animals for the study

The number of animals for this pilot study were limited. Piglets on production farms are born year-round. However the rate of litters born in one period at Ratanui farm followed a seasonal pattern for 2019, with birth rates declining rapidly from July to September and increasing again from mid-September onwards (D. Navarro, Personal communication, July 2019). As a result, the number of piglets that could be used in the study was restricted by the number of litters available within the desired age range, which was usually limited to one litter per visit. This added a further limitation on the selection of individual piglets for the study. To mitigate this, only the most vigorous piglets (piglets that demonstrated coordinated locomotion, sow interactions and competition for the best teats) were selected. Furthermore, the research team was restricted to conducting the study on certain days so as to avoid interfering with normal farm management practices.

Calves are born seasonally, with the large majority of calves born in Winter/Spring transition months (August to October). The calves used in the study could not be accessed until the later calving due to manufacturing of the inflation cuff and sourcing of a suitable farm. On average, 2 calves for use in the study were born in a 24-hour period, with some days having no available calves. This limited the number of calves available to be squeezed in the time available.

The small number of animals used for each method of squeeze application in the study aligns with the 8 healthy foals used in a previous study (Toth et al., 2012). As the main aim of the project was to determine if the thoracic squeeze would elicit a non-responsive state in the chosen species, these sample sizes were sufficient to address the study aims.

6.3 Allocation of piglets into one of two methods of squeeze application

Piglets were allocated to one of two treatment groups related to the method of squeeze application (inflation cuff or rope). This allocation was not randomised, due to the availability of the equipment for the squeeze. The inflation cuff required modification after initial testing, resulting in the use of the rope method on four of the piglets before the remaining piglets were alternately allocated into each method of squeeze application. However, the lack of randomisation in the piglets was unlikely to have an effect on the results, due to the strict inclusion criteria that had to be passed before application of the squeeze which resulted in less variability among individual animals.

6.4 Feeding status of calves

The feeding status of all of the calves in the study was unknown, in relation to whether or not they received colostrum from the dam before being moved to the calf shed, or if they received colostrum or milk replacer in calf feeders by the farm staff. It is unclear how this may have affected calf vigour before and during the squeeze. This therefore limited the ability to distinguish behavioural differences after application of the thoracic squeeze, which made the responses of the calves to the thoracic squeeze appear less distinctive than the responses of the piglets. However, feeding approximately 200ml of warmed colostrum or milk to the calves before the squeeze reduced this limitation.

7. Future Research

This project has demonstrated the thoracic squeeze technique in newborn piglets and calves, and suggests that the non-responsive state achieved by application of a squeeze may be a general phenomenon in other mammals of precocial species. As a pilot study, the results have provided a basis for a multiple avenues of research in the future.

7.1 Investigating the mechanisms underlying the thoracic squeeze

It would be beneficial to explore the potential mechanisms underlying the thoracic squeeze technique to provide a more detailed picture of how the squeeze acts to elicit such a response as has been demonstrated previously (Toth *et al.* 2012; Aleman *et al.* 2017; Stilwell *et al.* 2019) and now in this study. Methods to investigate this include measuring the concentrations of brain inhibiting factors in a healthy neonate immediately after birth, followed by another assessment of concentrations during and after removal of the squeeze, to investigate the effect of brain inhibitory levels on the non-responsive state elicited during the thoracic squeeze. Continuous electroencephalogram (EEG) recordings alongside inhibitory concentration analysis would provide detailed insight about the type of state that neonates enter into after application of the thoracic squeeze.

7.2 Exploring pain thresholds in piglets after application of the thoracic squeeze

The reduced and absent reflexes observed in the piglets in the non-responsive state suggests that sensitivity to noxious stimuli may be reduced during the squeeze as has been reported in animals under general anaesthesia (Verhoeven *et al.* 2015; Bradbury and Clutton 2016). A method to assess this includes the recording of evoked potentials after application of a low-level noxious stimulus to a limb during the squeeze. These recordings could be done before, during the squeeze, and after its removal, and would provide more detail into the effects of the squeeze on brain electrical activity, as well as the responsiveness of the neonate to external stimuli during the squeeze. Exploring this would enhance the applicability of the thoracic squeeze in an industry setting, with the potential for the thoracic squeeze to be used as a non-chemical form of restraint for husbandry procedures.

7.3 Developing detailed indicators of pathophysiological low vigour before applying the thoracic squeeze

It would be valuable to explore ways of developing a more extensive inclusion criteria that can be easy applied in an industry setting. The inclusion criteria developed for this study was rigorous and all of piglets and calves included in the study were expressing vigorous and healthy behaviours before application of the squeeze. However, three of the neonates (one piglets and two calves) developed physiological instability and the squeeze was immediately discontinued. It is possible that these neonates had experienced pathophysiological insult or had other morbidities that were undetectable using the inclusion criteria. Current methods for detecting pathophysiology soon after birth include testing lactate levels and packed cell volume for chronic oxygen deficiency, and lactate levels alone for acute oxygen deficiency (Mellor 1988). However these processes require expertise and specialist equipment which is not feasible in an industry setting. An extensive inclusion criteria that readily distinguishes neonates with pathophysiological morbidities and is easily applicable in an industry setting would enable a more widespread and accepted use of the thoracic squeeze as a form of restraint in healthy animals, or as a therapeutic approach to managing NMS in neonates. Methods of achieving this could include behavioural observations of low vigour neonates that are clinically assessed to have pathophysiology in order to find definitive behavioural characteristics or visual features that distinguish neonates with pathophysiology from others that have NMS. Ensuring that the feeding status of all neonates is known and neonates are fed prior to the squeeze will control for low vigour behaviours caused by starvation.

7.4 Prevalence of NMS behaviours in neonates of precocial species

Although observations of NMS have been reported in a range of precocial neonatal mammals (Aleman *et al.* 2017; Stilwell *et al.* 2019), there have been no systematic studies looking into the prevalence of NMS in neonates of precocial species other than what has been reported in thoroughbred horses (Baird 1973). The observation of low vigour in other apparently healthy precocial species suggests that the magnitude of the problems associated with NMS are not specific to horses, and may affect a greater proportion of neonates than is currently known. Understanding the proportion of newborns that may express NMS behaviours would enable the thoracic squeeze to be used as a therapeutic approach in more neonatal mammals of precocial species and in an industry setting. This could influence the mortality rates in the production industries, reduce the welfare impact of low vigour and increase production rates, whilst keeping treatment costs to a minimum.

8. Conclusions

The thoracic squeeze elicited a non-responsive state in all piglets in this study. In contrast, not all calves were induced, and those that were entered what was apparently a 'less-responsive state' than before the squeeze. Unlike the piglets, most induced calves maintained reflex responses to tactile stimulation throughout the squeeze, suggesting that a non-responsive state was not achieved during the squeeze. However, because 'hider' calves are normally less behaviourally active and responsive than 'follower' piglets in the early post-natal period, it was more difficult to determine the effects of the squeeze on their awareness and responsiveness.

In both species, only about half of those neonates induced stayed in the less/non-responsive state for the full 10-minute squeeze period. Those that aroused early showed different behavioural responses at induction and exhibited more low intensity arousals in the lead-up to full arousal and discontinuation of the squeeze. Thus, it may be possible to identify individuals likely to arouse, which is useful to know for practical applications of the squeeze such as restraint for husbandry procedures

The inflation cuff appeared to more practical in its application and therefore at inducing neonates into a non-responsive state than the rope and was faster and easier to apply consistently and to remove. Thus, it is recommended that the inflation cuff be used in future research.

These findings suggest that the non-responsive state previously reported in foals undergoing a thoracic squeeze in the early post-natal period may represent a generalised phenomenon in neonates of precocial mammalian species. The thoracic squeeze has been demonstrated to be generally safe at inducing and maintaining a lower- or non-responsive state in apparently healthy piglets and calves, although close physiological monitoring is recommended before, during and after the squeeze. This pilot study therefore provides a foundation for further research using the inflation cuff to explore the mechanisms underlying the thoracic squeeze and ways in which the squeeze can be effectively applied in an industry context.

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