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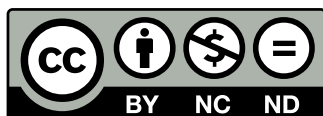
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### Summary

### Zusammenfassung



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## Optimal pain indicators for field trial assessment of analgesic efficacy in piglets undergoing surgical castration

### *Die geeignetsten Schmerz-Indikatoren zur Beurteilung der analgetischen Wirksamkeit bei der chirurgischen Kastration von Ferkeln im Feldversuch*

Meredith Sheil<sup>1</sup>, Adam Polkinghorne<sup>2,3</sup>

There is a growing demand for pain mitigation strategies that improve the welfare of piglets undergoing surgical castration in commercial pig production systems. While a range of potential anaesthetic and/or analgesic interventions have been trialled, efforts to confirm efficacy in field use, are stymied by the absence of 'gold-standard' methods to measure pain experienced by piglets during and after surgical castration. A review of the available literature in this field reveals that many methods commonly utilised to measure piglet pain lack sensitivity and/or specificity and may be unreliable. Measurement of biomarkers of physiological responses to pain, for example, appear to be readily confounded by similar responses to handling and restraint and/or to tissue trauma, which may occur in the absence of piglet pain. Similarly, it is challenging to accurately document pain-related behaviours in neonatal piglets following castration, since such behavioural disturbances are subtle, variably expressed and short-lived as compared with those undergoing handling only. Of the methods reviewed, nociceptive motor responses and/or vocal responses during the procedure, and targeted direct observation of specific pain-related behaviours, along with mechanical sensory testing for sensory hyperalgesia following the procedure, appear to be the most reliable methods for detection of pain in neonatal piglets, with significant differences most consistently observed between castrated and non-castrated animals, and/or those receiving analgesia/anaesthesia versus those left untreated. Understanding the strengths and weaknesses of current methods of measuring perioperative pain in piglets is critical to ongoing efforts by stakeholders to develop effective pain mitigation strategies.

**Keywords:** pain mitigation, anaesthesia, biomarker

Es besteht eine wachsende Nachfrage verschiedener Interessengruppen nach Strategien zur Schmerzlinderung nach einer chirurgischen Kastration in kommerziellen Schweineproduktionssystemen, die das Wohlbefinden von Ferkeln verbessern. Während eine Reihe potenzieller anästhetischer und/oder analgetischer Eingriffe erprobt wurden, fehlen „Goldstandard“-Methoden zur Messung der Schmerzen während und nach der chirurgischen Kastration zur Bestätigung der Wirksamkeit. Eine Übersicht der verfügbaren Literatur auf diesem Gebiet zeigt, dass viele Methoden, die üblicherweise zur Messung von Schmerzen verwendet werden, nicht genügend empfindlich und/oder spezifisch bzw. möglicherweise gar unzuverlässig sind. Die Messung von Biomarkern physiologischer Reaktionen auf Schmerzen kann beispielsweise leicht mit ähnlichen Reaktionen auf Gewebetraumata verwechselt werden. In ähnlicher Weise ist es schwierig, schmerzbedingte Verhaltensweisen bei neugeborenen Ferkeln zu dokumentieren, da solche Verhaltensstörungen subtil und kurzlebig sind. Von den untersuchten Methoden scheinen nozizeptive motorische Reaktionen, Stimmreaktionen während des

Verfahrens, die gezielte direkte Beobachtung spezifischer schmerzbedingter Verhaltensweisen sowie mechanische sensorische Tests auf sensorische Hyperalgesie die zuverlässigsten Methoden zum Nachweis von Schmerzen bei neugeborenen Ferkeln zu sein. Dabei konnten signifikante Unterschiede gefunden werden, am häufigsten zwischen kastrierten und nicht kastrierten Tieren und/oder solchen, die Analgesie/Anästhesie erhalten hatten. Das Verständnis der Stärken und Schwächen der derzeit verwendeten Methoden zur Messung perioperativer Schmerzen bei Ferkeln ist entscheidend, um wirksame Strategien zur Schmerzlinderung zu entwickeln.

**Schlüsselwörter:** Schmerzlinderung, Anästhesie, Biomarker

## Introduction

Surgical castration is a painful procedure employed in commercial swine facilities to remove the risk of 'boar taint', reduce undesirable behaviours and to prevent uncontrolled breeding. Concern exists over the welfare of animals that undergo these procedures given that it is reported to cause significant acute pain to the animal during the procedure, as well as post-operative pain in the hours and days after the procedure (Borell et al. 2009, Rault et al. 2011). Despite increasing efforts to develop and implement pain-mitigation strategies, a 2016 survey revealed that over half of the male pigs undergoing castration in surveyed countries in Europe, still received no analgesia or anaesthesia (De Briyne et al. 2016). Data prevalence for USA swine farms is lacking but is likely to have a similar prevalence of castrated pigs than in the European Union, with an even lower prevalence of analgesia/anaesthetic use (Rault et al. 2011, Wagner et al. 2020). The lack of effective, commercially viable, practical and simple to administer methods of anaesthesia is a key barrier to the adoption of on-farm pain mitigation (Wagner et al. 2020).

Pain-mitigation strategies must be targeted at mitigating both acute and post-operative pain. This generally involves the use of general or local anaesthesia for mitigation of acute procedural (neurally mediated) pain, and non-steroidal anti-inflammatory drugs (NSAIDs) or other long-acting analgesics for mitigation of post-operative (inflammatory mediated) pain. A number of such medication options have been explored to reduce perioperative pain experienced by piglets undergoing surgical castration (Borell et al. 2009, Dziamunhenga et al. 2014, O'Connor et al. 2014, Sheil and Polkinghorne 2020, Sutherland, 2015). General anaesthesia options trialled include the use of injectable agents or sedatives such as Ketamine, Azoperone and/or Buprenorphine (McGlone and Hellman 1988, Rintisch et al. 2012, Viscardi and Turner 2018a), as well as inhalable CO<sub>2</sub>, halothane and isoflurane (Kohler et al. 1998, Walker et al. 2004). Local anaesthetics such as procaine or lignocaine can be injected subcutaneously as well as into the testis or infundibulum, separately or in combination with NSAIDs (e.g. meloxicam) prior to the procedure (Courboulay et al. 2010, Hansson et al. 2011, Horn et al. 1999, Keita et al. 2010, Kluivers-Poodt et al. 2012, Leidig et al. 2009, Saller et al. 2020, Wavreille et al. 2012). More recently, topical local anaesthetics (applied pre-operatively or by direct wound instillation during or following the procedure) have also been trialled to mitigate peri-

operative pain (Gottardo et al. 2016, Lomax et al. 2017, Sheil et al. 2020, Sutherland et al. 2012).

While some of the options for pain mitigation in piglets undergoing surgical castration show potential, identifying options that are the most effective for pain mitigation while also being acceptable for use in a commercial swine facility (e.g. safe, practical, economically sustainable) is a major challenge for stakeholders worldwide. Many or most of the anaesthetic/analgesic options required skilled veterinary administration and prolonged or double handling which may not be practically or commercially viable. Furthermore, many or most of the medications are not registered for use for this indication and must therefore be used off-label under veterinary prescription (Castrum Consortium 2016, De Briyne et al. 2016, Wagner et al. 2020). Obtaining regulatory approval requires meeting high standards of proof of safety and efficacy, including in the field situation.

To gain desperately needed safety and efficacy data on the use of different pain medications, to date, researchers have employed a variety of methods to assess pain in piglets during and after castration. In general (Sheil and Polkinghorne 2020), these methods look to assess piglet pain using measurement of (i) potential physiological markers of piglet pain, such as plasma adrenaline, noradrenaline, cortisol, adrenocorticotrophic hormone and  $\beta$ -endorphin; (ii) motor and vocal responses during the surgical procedure; (iii) the sensitivity of piglet wounds after the procedure; and (iv) changes to the behaviour of piglets in the period of time immediately after castration and in the following hours and days. Unfortunately, there is both a wide variability in methods employed and in results reported, and a complete lack of information regarding the consistency, specificity or reliability of different methods used (Dziamunhenga et al. 2014, Sheil and Polkinghorne 2020). This poses a risk that the pain experienced by piglets and the efficacy of treatments may both be significantly misconstrued.

To be valuable as indicators of pain mitigation, measures must be capable of consistently detecting a significant difference in pain-associated responses during and/or following castration as compared with pre-operative values, and/or as compared between castrated and non-castrated piglets. Secondly, variables must optimally be physiologically and/or clinically relevant to the evaluation of the type of pain being measured e.g., intraoperative pain or post-operative pain. Ideally, these measures (i) must be practically measured within the study without being confounded by the assessment of other variables; and; (ii) have the ability to be measured using

**TABLE 1:** Summary of, and recommendations regarding, indicator methods used for field assessment of castration-associated pain in neonatal piglets

Method	Method Details	Sensitivity (to detect change in piglets undergoing castration)	Specificity (to pain)	Reproducibility	Recommendation	Comment
Physiological response	Markers of HPA axis/SNS activation (adrenalcorticotrophic hormone/cortisol/adrenalin)	High	Low	Moderate	Not recommended for assessment of pain mitigation via general or local anaesthesia (blockade of neural pain transmission) May provide indication of efficacy for NSAIDs (blockade of inflammatory-induced pain)	Confounded by extraneous factors such as duration of restraint/surgical stress response/degree of bleeding or tissue trauma.
	Markers of neuropeptide/inflammatory response (TNF- $\alpha$ , IL-1 $\beta$ , C-reactive protein)	Moderate	Low	Moderate		
Nociceptor motor response	Scored via NRS/VAS or ordinal scale	High	High	High	Recommended	Optimally, scoring restricted to time of acute pain generation.
Nociceptive vocal responses	Measured via peak dB, total vocal response (such as area under the dB/time waveform), the frequency (Hz) of call with the highest intensity (dB (A)), rate of high frequency calls (>1000 Hz) or stress vocalisations using the STREMOD system	High	Moderate (depending on assessment method)	Moderate (depending on assessment method)	Recommended with qualification	Sensitivity/specificity may be reduced in non-acoustically separated environment
Mechanical wound sensory testing	Measured using von-Frey, needlestick or pressure algometry	High	High (to evoked pain/hyperalgesia)	High (von-Frey)	Recommended (von-Frey)	Optimally should be used in combination with a method to assess spontaneous pain
			Low (to spontaneous pain)	Low (pressure algometry)	Not recommended (pressure algometry)	
Post-operative pain behaviour	General postures and behaviours (time spent lying, standing sitting, nursing etc.)	Moderate	Low	Low	Not recommended	Confounded by neonatal piglet response to restraint, handling and separation from sow
	Specific pain associated behaviours (Huddling up, prostration, tremors/trembling, stiffness, scratching abnormal gait)	Low (Evident in first minutes and hours following castration, when recorded by direct quiet observation).	High	Moderate (depending on assessment method)	Recommended	Continuous video recording techniques appear insensitive to acute pain related behaviours, however, may be sensitive to subacute behavioural abnormalities (scratching/tail-wagging)
Facial grimace score	Assessed via – orbital tightening, ear position, cheek tightening/nose bulge	Moderate	Low	Low	Further development/evidence required	May be impacted by body weight or activity state
Infra-red thermography	Reduction in skin surface temperature secondary to pain-related activation of SNS	High	Low	Low	Not recommended	Confounded by piglet response to stress and inflammatory response to tissue trauma

an analytical method or measurement device/subjective assessment tool that has sufficient validation (Ison et al. 2016).

To support the development of effective pain mitigation strategies in neonatal piglets, we recently completed and reported a comprehensive review of the strengths and weaknesses of these methods for in-field use to assess pain in piglets during and after castration (Sheil and Polkinghorne 2020). Here, we summarise the outcomes of this review and provide a series of recommendations on the optimal methods currently available

for assessing efficacy of anaesthesia/analgesia for peri-operative castration pain mitigation in neonatal piglets.

### Identification of the optimal methods for assessment of pain during castration

A summary of the relative sensitivity, specificity and reproducibility of different methods for assessing pain in piglets based on a comprehensive review of the available literature (Sheil and Polkinghorne 2020) is presented in Table 1.

### Physiological responses to castration

Physiological responses to castration in piglets have been widely reported (Table 1, and as reviewed by Dziamunhenga et al. 2014, O'Connor et al. 2014, Rault et al. 2011, Sheil and Polkinghorne 2020, Sutherland 2015). Activation of the hypothalamus-pituitary-adrenal axis (HPA-axis), sympathetic nervous system (SNS) and release of opiate neuropeptides occurs in response to stress, pain and tissue trauma, which also initiates an inflammatory response. Although a relatively short-lived (0–3 hr) physiological response can be detected following castration in piglets, unfortunately however, due to the aforementioned confounders, these markers show low specificity and cannot be relied upon to indicate pain. Surgical incision under general anaesthetic (i.e. in the absence of pain) increases stress hormone expression, similar to the pain response (Lykkegaard et al. 2005). Further, when duration of handling and restraint is similar, sham-handled control piglets show similar expression levels of cortisol and  $\beta$ -endorphin, as well as markers of the inflammatory response to castrated piglets following the procedure (Hay et al. 2003, Marchant-Forde et al. 2009, Moya et al. 2008). For this reason, physiological responses may be unreliable indicators of efficacy of pain-mitigation. This is particularly the case for assessment of the efficacy of anaesthetics (local or general), which prevent pain via blockade of neural transmission without necessarily having impact on the physiological (humoral) response to surgical incision and tissue trauma induced by cytokine- or other mediator release from damaged cells at the incision site (Desborough 2000). Haemorrhage alone, for example, without pain, is known to result in an increase in ACTH, cortisol,  $\beta$ -endorphin concentration, as well as tissue content of pro-inflammatory cytokines; (including tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\alpha$  (IL-1 $\alpha$ ), IL-6 and IL10), and opiates have a proposed role in regulating the hemodynamic response to blood loss (Molina 2001).

It should be noted that NSAIDs and local anaesthetics block pain by different mechanisms. This has important implications for the use of biomarkers of HPA axis, neuroendocrine and/or inflammatory cascade activation as indicators of pain and pain mitigation. NSAIDs block the conversion of arachidonic acid to prostaglandins by cyclooxygenase enzymes (COX), preventing activation of the inflammatory cascade and release of pain-inducing inflammatory mediators which contribute to post-operative (inflammatory) pain (Vane and Botting 1998). Prostaglandins also directly stimulate ACTH and cortisol release, and thus directly mitigate the humoral aspect of the surgical stress response to tissue trauma (Aloisi et al. 2011, Zacharieva et al. 1992), separate from mitigating pain. Nevertheless, a reduction in cortisol following NSAID administration may be anticipated to indicate a collateral reduction in production of prostaglandins and other associated pain-inducing inflammatory mediators, and hence also an associated decrease in inflammatory-induced pain in piglets post castration. In this setting, therefore, cortisol or ACTH levels may provide an indirect biomarker of pain mitigation in piglets following NSAID administration.

Local anaesthetics, on the other hand, block nerve fibre conduction of pain signals. In doing so, local anaesthetics do not primarily affect the cytokine/inflammatory response to tissue trauma or associated HPA-axis

activation which means that biomarkers associated with the surgical stress response may be elevated even when pain induced by them is blocked. A further confounder to the use of local anaesthetics comes from the common co-administration of adrenaline or nor-adrenaline to enhance the effects of local anaesthetics and minimise the risk of systemic absorption. This may clearly confound their use as indicators of pain secondary to activation of the SNS. Adrenaline and nor-adrenaline, may have centrally and/or peripheral effects to stimulate corticotrophin releasing hormone and increase the breakdown of proopiomelanocortins into ACTH and  $\beta$ -endorphins (Labrie et al. 1984, Liu et al. 1991, Slominski et al. 2013). Adrenaline administered exogenously may thus further confound the use of markers of endogenous HPA-axis and SNS activation and opiate-peptide production in castrated piglets.

Based on our review (Sheil and Polkinghorne 2020), it is apparent that biomarkers of activation of the HPA axis, SNS, opiate neuropeptides and immune response, lack specificity as indicators of pain associated with neonatal piglet castration. Pain biomarkers may have some role in assessment of post-operative inflammatory pain mitigation following NSAID administration, however, are poor markers of efficacy of pain mitigation for local or general anaesthetics.

### Piglet motor response to castration

Castration without anaesthesia induces a piglet's nociceptive withdrawal response to acute pain, involving protracted violent struggling and escape behaviour and a loud vocal response (Hansson et al. 2011, Leidig et al. 2009, Nyborg et al. 2000, Saller et al. 2020, Walker et al. 2004). The nociceptive motor response can be graded using a range of validated methods (Dobromylskyj et al. 2001) such as (i) ordinal scales (Leidig et al. 2009) (ii) focal assessments (Keita et al. 2010, Nyborg et al. 2000), (iii) visual analogue scales (VAS) (Hansson et al. 2011), or; (iv) the use of numerical rating scales (NRS) (Lomax et al. 2017, Walker et al. 2004). Despite a range of different methodologies used for assessment, studies have consistently reported a significant increase in nociceptive motor response in castrated versus sham-castrated piglets and/or a reduction in these responses in castrated animals following the administration of general (Walker et al. 2004), injected local anaesthesia (Hansson et al. 2011, Horn et al. 1999, Leidig et al. 2009, Nyborg et al. 2000, Saller et al. 2020) or topical anaesthesia applied via wound instillation (Sheil et al. 2020). Good correlation has been reported in piglets castrated under Ketamine-Azoperone general anaesthesia, between thresholds for nociceptive flexor reflex amplitudes (initiated by electrical stimulation distal from the wound site and measured using electromyography), traditional intra-operative controls of analgesia (interdigital reflex) and defence reactions initiated by surgical stimulation including; incisions in the scrotal skin, in the tunica vaginalis and in the testis, pulling off the spermatic cord, clamping and cutting off the spermatic cord and final wound disinfection (Rintisch et al. 2012). The nociceptive withdrawal response to clamping the interdigital space of the hind claw (interdigital reflex) is also recommended as a method of testing adequacy of general anaesthesia, prior to castration of piglets (Saller et al. 2020). In review (Sheil and Polkinghorne 2020), assessment of the nociceptive motor response is concluded to provide

a relatively consistent and sensitive method of assessing acute procedural pain and pain-mitigating effects of anaesthetic/analgesic treatments in neonatal piglets undergoing castration.

#### **Piglet vocal response to castration**

A number of studies have reported that piglets undergoing castration squeal more often, more loudly and/or at higher frequency than those undergoing sham-handling (Marchant-Forde et al. 2009, Taylor et al. 2001, Weary et al. 1998, Wemelsfelder and van Putten 1985). Furthermore, the vocalisation sound parameters of the castration responses can be comprehensively distinguished from that emitted by handling alone (Marx et al. 2003). Analysis of these parameters have revealed that a single event in a call, such as peak level or peak frequency are considered to provide more consistent results than parameters that describe an average, such as weighted frequency and main frequency (Marx et al. 2003).

Most studies have shown local and general anaesthetics mitigate the piglet vocal response to castration (Hansson et al. 2011, Leidig et al. 2009, Marx et al. 2003, Sheil et al. 2020; Sutherland et al. 2012, White et al. 1995) while NSAID treatment has little impact (Hansson et al. 2011, Kluivers-Poodt et al. 2012, O'Connor et al. 2014, Reiner et al. 2012, Sutherland et al. 2012). This is not unexpected as NSAIDs do not block the nerve conduction of incisional pain signals occurring acutely at the time of tissue trauma (O'Connor et al. 2014). NSAIDs are more likely to affect post-operative inflammatory pain stimuli that are transmitted as a consequence of the later production of cytokines and prostaglandins that occurs secondary to disruption of cell membranes (Coetzee 2013). It should nevertheless be noted that while there is consistency in the reported outcomes, it is difficult to combine these data or quantify the effect of pain mitigation interventions, since the actual metrics reported are diverse (Dzikamunhenga et al. 2014, O'Connor et al. 2016). Another challenge with measurement of pig vocalisation is that regulatory safety and efficacy trials require demonstration of the efficacy of drugs in field situations. In most cases, studies of pig vocalisation response to castration have been recorded in rooms acoustically isolated from farrowing pens where piglet castration usually takes place (Sheil and Polkinghorne 2020). Measurement of vocal responses in commercial farm settings must account for normal background noise levels and the confounding effects of the sow and littermates on piglet vocal responses. In this respect, vocal response measurements may be less sensitive in regulatory field trial settings compared to acoustically separated research environments. In review however, it was concluded that with careful application to ensure targeting of the measurement period to coincide with the time points of pain generation, and avoidance of confounding factors such as the duration of restraint or recordings, several measures of piglet vocalisation in response to castration including the peak decibel (dB), total vocal response (such as area under the dB/time waveform), the frequency (Hz) of call with the highest intensity (dB [A]), rate of high frequency calls (>1000 Hz) or stress vocalisations using automated software (e.g. STREMOD system) appear to provide a relatively consistent and sensitive method of assessing procedural pain associated with castration, and pain

mitigation in neonatal piglets (Sheil and Polkinghorne 2020).

### **Identification of the optimal methods for assessment of post-operative pain**

A variety of methods have been utilised to assess post-operative pain in piglets. Our review of these methods (Sheil and Polkinghorne 2020) has revealed that post-operative pain control is optimally evidenced by a reduction in peripheral afferent nerve sensitisation combined with an associated reduction in specific pain-related behaviours. The key findings of this analysis are summarised in Table 1.

#### **Mechanical nociceptive sensory testing**

Afferent nerve sensitisation, resulting in hyperalgesic responses to sensory stimuli, is considered to be a primary underlying mechanism responsible for the development and persistence of post-operative pain (Amaya et al. 2013, Brennan et al. 1996, 2005). Post-operative hyperalgesic responses can be most specifically and sensitively identified using quantitative sensory testing (QST) (Brennan et al. 1996, Curatolo et al. 2000, Ison et al. 2016), providing evidence of a lower threshold for nociceptive responses to a mechanical, thermal or chemical stimulus. Assessment of nociceptive responses to von Frey mechanical stimulation at the wound site (primary mechanical hyperalgesia) is a well-established, widely used method of assessment of post-incisional pain, and efficacy of anaesthetics/analgesics (Whiteside et al. 2004), including in neonatal rats and humans (De Lima et al. 1999, Fitzgerald et al. 1989), with similar methods recently also developed in pigs (Castel et al. 2014, 2017, Janczak et al. 2012). Von Frey filaments or 'hairs' are a set of calibrated filaments that bend when a certain pressure is reached, allowing a reproducible mechanical stimulus to be delivered, graduating from that inducing a light-touch sensation through to a pain-weighted stimulation of skin or tissues. In non-verbal humans and animals, the reflex nociceptive response is assessed using similar validated grading schemes (NRS, VAS, ordinal scale) as are used for measurement of the piglet nociceptive motor response during castration. Studies in neonatal piglets post-castration (Lomax et al. 2017, Sheil et al. 2020) using Von-Frey stimulation and grading of the nociceptive motor response, have identified a significant reduction in threshold for nociceptive motor response (equating to an increase incidence and/or grade of motor response to 300g von-Frey filament and needlestick stimulation of the wound site) in castrated piglets as compared to sham handled controls (Lomax et al. 2017), and those treated with injected local (Lomax et al. 2017) or topical anaesthesia (Lomax et al. 2017, Sheil et al. 2020), and is concluded to provide a relatively sensitive and specific method of assessment of incisional pain, and efficacy of pain mitigation post castration in neonatal piglets.

Pressure algometry is an alternative to Von Frey filaments for mechanical nociceptive testing. This method is designed to indicate hyperalgesia through detecting a lowering of the threshold for a blunt stimulus applied with increasing pressure over time, rather than as brief punctate touch stimulus at a predetermined pressure (as occurs with Von Frey assessment) (Curatolo et al. 2000).

Pressure algometry is generally applied using a hand-held device to hard surfaces such as the sternum, or pincer device to softer/movable tissues such as the ear lobe. Although this method has been trialled for QST in piglets following castration (Gottardo et al. 2016), issues with the algometry tip inducing significant skin indentation were noted. Furthermore, confounding factors such as a piglet's age and weight affect responses to pressure algometry, particularly in the first week of life where responses were not repeatable (Janczak et al. 2012). On this basis, QST using von Frey filaments and needlestick should be considered the most robust method for measuring incisional pain and pain-mitigation in piglets following castration. It should be noted, however, that QST methods examine evoked pain responses as compared with "spontaneous" or "at-rest" pain responses. This provides an indication of mechanisms underlying post-incisional pain, however such elicited responses may be present in the absence of spontaneous (at-rest) pain (Ison et al. 2016). For this reason, it is advised to combine such assessments with assessment of post-operative pain-related behaviour.

#### Post-operative pain-related behaviour

In general, measures of behaviour have proven to be more reliable indicators of pain than physiological measures in animals following castration (Rault et al. 2011, Sheil and Polkinghorne 2020). In other animal species, behaviours such as decreased or abnormal locomotion, turning the head towards the rump, abnormal postures including prostration (standing or sitting with head below the shoulders), hunching (standing with kyphosis), stiffness (lying with legs tense and extended or walking with a stiff gait), increased or reduced movements of the tail are considered indicators of pain resulting from castration (Almond et al. 2015, Crowe 2011, Lomax and Windsor 2013, Mellor and Stafford 2000, Sheil et al. 2020). More diffuse and variable responses may occur in neonatal animals due to immaturity of neuronal pathways involved with pain processing (Hatfield 2014).

Consistent with this, a similar range of individual 'pain-specific' behaviours have been described in neonatal piglets following castration, however they appear to be transient, subtle and variably expressed. First detailed in an ethogram by Hay et al. (Hay et al. 2003), and subsequently examined by a number of investigators (using an ethogram with only minor variations) (Hansson et al. 2011, Keita et al. 2010, Kluivers-Poodt et al. 2013, Moya et al. 2008), acute pain-specific behaviours have been evident in the first minutes and up to 1–2 hours post-procedure including: tremors/trembling, spasms, "huddled up" posture, "prostration" and "stiffness", as well as later identified "kyphosis" and gait abnormalities (Gottardo et al. 2016, Lonardi et al. 2015). Additionally, increased tail wagging and/or scratching are reported in the later hours (from 2 hours and peaking at 24 hours) following the procedure (Hay et al. 2003, Viscardi and Turner 2018a).

Earlier studies identified a number of behaviours thought to be indicative of pain in piglets, including changes in posture, position and nursing behaviour, with reduced standing and increased lying (away from heat), and reduced nursing in the early hours

(3–6 hrs) following the procedure as compared with uncastrated controls, effects that were ameliorated by use of lignocaine local anaesthesia prior to castration (McGlone and Hellman 1988, McGlone et al. 1993). A subsequent study (Taylor et al. 2001), however, reported differently, documenting decreased lying, increased sitting and increased nursing in piglets post castration as compared with uncastrated controls. In all cases, however, the authors reported that effects, although statistically significant, were marginal and/or of low magnitude (Table 1). Separate to the pain-specific behaviours already mentioned, Hay et al. recorded, but did not find significant differences in a range of indices of piglet posture, position, and 'non-specific' behaviours (such as walking, running, sleeping, playing, exploring and aggression) in neonatal piglets post-castration as compared with sham handled controls, and concluded that these were not reliable indicators of pain in piglets post-castration (Hay et al. 2003). These data indicate that general postures and behaviour, including nursing, may be affected by a multitude of factors in addition to pain, in this setting, including stress of separation from the sow, and restraint and handling. Furthermore, responses may vary considerably between piglets. With regard to suckling, for example, inappetence or immobility may predispose to decrease suckling, however neonatal animals may also increase suckling such as to "self-medicate", as suckling may activate natural pain-modulating mechanisms and have quite profound analgesic action (Blass and Watt 1999, Shann 2007).

On review of available data (Sheil and Polkinghorne 2020), we concluded that acute pain-related behaviour post-castration in neonatal piglets appears to be transient, subtle and variably expressed. The most consistent and reliable evidence for pain was a statistically significant increase in total "pain-specific" behaviours including tremors/trembling, huddled-up, stiffness, prostration, kyphosis, scratching, and stiff/abnormal gait, as compared with sham handled animals in the early minutes and up to 1–2 hours following castration. Increased tail-wagging and scratching may be seen at later time points. Other general indices of piglet posture, position, and 'non-specific' behaviours are confounded by the piglet response to handling and restraint and are not reliable indicators of pain in piglets post-castration.

The majority of investigators that have identified a significant increase in acute "pain-specific" behaviours in piglets in the first minutes and 1–2 hours following castration have employed direct observation methods, with trained observers using scan sampling and/or focal assessments (Burkemper et al. 2020, Gottardo et al. 2016, Hansson et al. 2011, Keita et al. 2010, Moya et al. 2008). Although there are no validated methods of behavioural assessment for use in piglets, continuous recording, scan sampling and focal assessment are all well described methodologies of behavioural assessment (Lehner 1992). Whilst continuous sampling may be considered the gold standard, this method suffers from the need to use video-tape recording with off-line analysis, as opposed to live, or direct observation. Unfortunately, possibly due to the subtlety and nature of expression of acute pain in piglets, to date, methods using video recording and off-line analysis have not proven sensitive enough to detect behavioural differ-

ences between castrated and non-castrated piglets in the early minutes and hours following the procedure, when such pain is most acute. For example, using a video-tape and off-line analysis method, Viscardi and Turner (2018a) demonstrated a significant difference in the proportion of time engaged in pain related behaviour when comparing piglets castrated without anaesthesia/analgesia versus sham controls across all study timepoints, however, at individual time points this difference was only significant at 24 hours following the procedure, but not at earlier time points. Similar findings were described in their separate study (Viscardi and Turner 2018b) with a significant difference in proportion of time engaged in pain related behaviour when analysing all time points together, but only seen at 2, 7 and 24 hours on individual time points (Fig. 2 and 4 of publication). Furthermore, these differences pertained primarily to increased tail wagging and scratching at the latter time points. These behaviours may indicate itch or irritation rather than pain, and thus may be less amenable to analgesic medications. Video assessment therefore appears to be of greater sensitivity for detecting subacute behavioural abnormalities including tail-wagging and scratching, which are generally only increased from several hours following castration, peaking at 24 hours.

## Other pain indicators

A number of alternative methods have been examined as pain-indicators in piglets with variable results. Infra-red thermography measurement of skin temperature has been used to assess loss of heat from the body's periphery. This is hypothesized to occur due to peripheral vasoconstriction, secondary to activation of the SNS in response to pain (McCafferty 2007). Similar results have been observed for piglets undergoing castration, with lidocaine and meloxicam treatment prior to castration mitigating this effect somewhat (Bates et al. 2014, Bonastre et al. 2016), however conflicting results have been reported (Coetzee 2019, Lonardi et al. 2015). There are a number of known confounders to temperature measurements which may affect studies of the pain relief efficacy of different methods, however, with (i) body temperature in piglets potentially affected by the stress response to restraint and handling, and/or the post-surgical inflammatory response (Lonardi et al. 2015); and (ii) NSAIDs having an antipyretic effect, and anaesthetics and adrenaline having vaso-active effects, which could confound assessments of the efficacy of such treatments to reduce piglet pain. Piglet facial grimace scores (PGS) have also been used to assess castration related pain in piglets. Piglet facial expressions, including the assessment of orbital tightening, ear position, cheek tightening/nose bulging, can be captured and assessed at various stages of surgical castration (Viscardi et al. 2017) with the initial study showing a strong correlation between piglet grimaces and piglet pain-related behaviours. A subsequent study, however, has raised questions about its specificity with evidence that facial grimacing may be confounded by piglet body weight and/or activity state (Viscardi and Turner 2018b). Inter-operator reproducibility of the PGS amongst investigators is also of concern (Gottardo et al. 2016).

## Recommendations and future directions

Our review (Sheil and Polkinghorne 2020) has highlighted that there is considerable variability in the sensitivity and specificity of different pain-indicators used for castration pain assessment in neonatal piglets (Table 1). This poses significant challenges identifying methods sufficiently reliable to assess the efficacy of pain-mitigation interventions to meet field-trial regulatory requirements. In the absence of a gold-standard method, from the body of available literature, our review found that the most reliable indicators of pain for in-field analgesic efficacy assessment include; nociceptive motor and vocal responses during the procedure; and, for assessment of post-operative pain, a combination of mechanical (von-Frey) sensory testing and direct observational assessment and scoring of acute "pain-specific" behaviours. Understanding the need for flexibility to accommodate different pain-mitigation methods and on-farm analysis scenarios, there is nevertheless the need to better standardise methods of assessing these indicators. The use of such methods for in-field assessments is anticipated to assist stakeholders in the development of pain mitigation strategies that will improve the welfare of piglets undergoing surgical castration in commercial pig facilities.

## Ethical approval

Not applicable.

## Conflict of Interest

This work was supported by Animal Ethics Pty Ltd, for the development of methodologies for use in analgesic efficacy trials to international VICH regulatory requirements for the topical anaesthetic product, "Tri-Solfen"®. Dr Sheil is Founding Director, Chief Medical Officer and indirect shareholder of Animal Ethics Pty Ltd.

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## Author Contributions

Conceptualization, methodology, supervision, project administration, funding acquisition: MS.  
Data collection, writing review and editing: MS, AP.  
Writing original draft preparation: AP.  
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