RESEARCH REPORT
ANIMAL WELFARE

Title: **Reviewing the State of Scientific Evidence and Developing Recommendations about Pain Mitigation Strategies in Piglets Undergoing Routine Processing Procedures NPB #12-186**

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Non-Technical Summary

The following non-technical summary describes the recommendations from a review and decision-making process conducted between August 2012 and August 2013 by the panel[1] at the request of the National Pork Board. The objectives of the project were to assess research describing the efficacy of pain mitigation strategies in piglets and the development of recommendations for use where appropriate, and provide guidance for future funding priorities. Details of the process used to reach the conclusions are available in the full text.

CURRENT RECOMMENDATIONS FOR PAIN MITIGATION STRATEGIES

The transparent development of guidelines for industry stakeholders is an important process. For this project, we used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) process, which is an internationally recognized approach to developing guidelines in healthcare. Examples of this approach are available from the World Health Organization[2], the American College of Physicians[3], The Agency for Healthcare Research and Quality[4], and the US Centers for Disease Control and Prevention[5].

The rationale for choosing the GRADE approach is the transparency and inclusivity of diverse and informed perspectives that the process brings to guideline development. The process acknowledges the importance of scientific evidence and the potential for bias in scientific information. Importantly, the GRADE process explicitly includes transparent articulation of the ethical and non-ethical values and preferences that created the recommendations. A key concept in recommendations is that, while scientific evidence is global, decisions are local. The concept that scientific evidence is global means that if the same approach to searching, extracting, and analyzing the data from relevant studies were employed by others, then they would reach the same conclusions about the effects of the interventions. However, decision making is local because it is informed by local challenges, values, and preferences as well as by other limitations of a particular review process.

As a means of illustrating the idea of global evidence and local decisions, a good example relates to the use of wildlife culling as a method of control for countries that have wildlife reservoirs of tuberculosis in livestock. In the United Kingdom and New Zealand, wildlife are important reservoirs for tuberculosis (e.g., possums in New Zealand and badgers in the United Kingdom). Evidence that culling is an effective approach may be similar; however, the decision to employ culling is influenced by local values and preferences. In New Zealand, the possum is a feral species, and widespread opposition to culling the species is not observed. Indeed, culling is viewed positively by livestock producers and conservationists, as it would protect both native and domestic species. However, in the United Kingdom, culling of badgers is viewed negatively and is not employed due to local opposition to the approach. Therefore, although the evidence for culling is similar, the decisions differ based on local preferences.

Not only are recommendations local, they can be time specific and have an expiration date. Because recommendations involve scientific evidence, values, and preferences, the balance of benefits, harms, and resources change over time, and thus, recommendations should not be viewed as standing for all time. As such, the recommendations made by the panel are relevant to the current state of knowledge and resource availability. Timelines for revision and factors that might influence changes in the recommendations are

[1]Iowa State University (ISU) Steering Committee: Coetzee, J.F [1] Johnson, A. K. [1], Karriker, L. A. [1], McKean, J. [1], Millman, S. T. [1] and external participants (in alphabetical order): Anthony, R. [2], Bergamasco, L. [3], Lemke, K. [4], Marchant-Forde, J.N. [5], Martineau, G.S. [6], Noem, G. [7], Pajor, E.A. [8], Rutherford, K. [9], Scholl, B. [10], Sprague, M. [11], Sutherland, M. [12], von Borell, E. [13]

[2]http://www.who.int/influenza/human_animal_interface/guidelines/pharmamanagement/en/

[3]http://www.gradeworkinggroup.org/publications/Qaseem_ACP-COPD_Annals2007.pdf

[4]http://www.ahrq.gov/downloads/pub/evidence/pdf/phe/phe.pdf

[5]http://www.cdc.gov/vaccines/recs/acip/GRADE/about.htm

included. The GRADE tables presented in the report should be used to understand the recommendations. The summary recommendations are the following:

The panel's current position is a strong recommendation against the use of a CO_2/O_2 general anesthesia mixture for pain mitigation during castration in piglets between 1 and 28 days old.

The panel's current position is a weak recommendation for the use of non-steroidal anti-inflammatory drugs (NSAIDs) for pain mitigation during castration in piglets between 1 and 28 days old.

The panel's current position is a weak recommendation against the use of lidocaine as a pain mitigation strategy for piglets undergoing castration.

CURRENT RECOMMENDATIONS FOR FUNDING PRIORITIES

The goal of periodic review of research and guideline development is to determine "Have we answered the question?" and, if the answer is "no," to identify what is needed to move the area forward. This goal should not be interpreted as meaning that more money and targeted research will necessarily make decision making less difficult. Clearly, decision making is not only informed science but is also influenced by values and preferences, reflecting a balance of benefits, harms, and availability of resources. When large variations in values and preferences occur, it is possible that even high-quality scientific evidence will make little difference in the decision-making outcome. Despite this caveat, the panel concluded that it is valid to provide guidance for future research. The panel concluded that pain mitigation will become a higher-priority area for consumers and retailers, as animal well-being considerations move beyond gestation sow housing.

Much of the work reviewed was categorized in the first stages of research (i.e., hypothesis generating). In other words, the work reviewed frequently provided clues as to ways pain associated with management procedures in piglets might be measured. The panel's opinion was that the next step in the research process should be validating measures of pain in controlled piglet-specific models. The research community could then use these validated pain measures when conducting pain mitigation hypothesis-testing studies. The panel concluded that the single greatest barrier to reaching strong recommendations was uncertainty about the validity and expected variations in pain assessments. As with other animal health research, uncertainty about the measurements of the outcomes of interest, in this case pain, made interpretation of the reviewed research extremely difficult; unless this deficiency is addressed, the problem will continue.

Infectious disease research has a long history of developing diagnostic tests to identify infection as the first critical stage of research. Once tests are available that can identify infected animals or herds, researchers are able to conduct hypothesis-driven studies that assess interventions with knowledge that what was measured is known. This research model, validate measurements then test mitigation strategies, has lead to the eventual control of numerous diseases. The committee concluded that this concept similarly applies to pain research. The panel therefore recommended that a funding priority should address the development of a validated porcine pain model that enables the consistent measurement of pain through behavioral, biochemical, and physiological means. The model should establish a correlation of these measures. Once such a model is available, and the variations in behavioral, biochemical, and physiological measures of pain are established, then appropriately powered hypothesis-driven studies that assess interventions can be conducted.

With respect to hypothesis-driven research, the factors that establish a credible evidence base in all areas of research apply to animal welfare. Well-executed and comprehensively reported studies that *a priori* specify a null hypothesis and the magnitude of an alternative hypothesis are needed. Such studies enable end users to understand potential biases and the role that chance plays in the results. As publication bias is

a major issue in assessing outcomes, efforts must be made to have all results from well-executed, adequately powered studies indexed in searchable databases. If studies are prospectively designed to detect a meaningful difference in a validated outcome and no differences are observed, publication should not be hampered. When studies are conducted without knowledge of the variations in an outcome or conducted without defining the magnitude of differences in outcomes that are considered to be clinically important, then rejection by journals is more difficult to criticize.

This priority may require a shift in focus for some funding agencies that shy away from funding basic research, such as diagnostic test development. However, basic research must precede applied research. It is also possible that some agencies are expecting others to fund basic research. However, the current funding situation for animal welfare in the US means that funding for basic work is, by and large, unavailable.

For hypothesis-testing studies, researchers should consider assessing piglets that receive multiple procedures, as occurs on the farm. Researchers should also give serious consideration to assessing the effects of interventions on acute and chronic pain. If studies only report mitigation of either acute or chronic pain, the industry will remain unaware as to how to assess these types of pain and which intervention is preferred.

Technical Summary

SUMMARY OF REQUEST

The National Pork Board (NPB) requested a project that would compile, review, and summarize the existing scientific literature about pain mitigation strategies in piglets and identify future research needs.

BACKGROUND TO PAINFUL PROCEDURES IN SWINE PRODUCTION

In swine production, multiple painful procedures, including castration, tail docking, teeth clipping, and ear notching, are conducted on piglets in the first few days of life. The rationale for these procedures differs. Castration reduces boar taint, as males mature to market weights used in the US. In the US, all male swine except breeding stock are castrated. Alternatives to castration, such as immunocastration, are becoming commercially available. Tail docking is conducted to prevent pigs from biting the tails of their pen mates, resulting in tail infections that can cause abscesses, paralysis, or death. Management alternatives to tail docking to eliminate this multi-causal event and provide consistent results are not available. Piglet teeth clipping is conducted to reduce the impact of bites on mammary glands during nursing and on littermates during teat competition. Ear notch identification allows for individual pig identification and is used in the US by breed registries and the US government (USDA, 2012). Individual producers may use ear notches to identify date of birth or other management information on individual pigs or groups.

APPROACH TO THE REQUEST

A steering committee coordinated the project. Under the guidance of the steering committee, a systematic review of the scientific literature was conducted to identify gaps in procedures, interventions, and outcomes. This approach produced empirical evidence about research gaps. At a meeting that involved the steering committee and invited external experts and stakeholders, the GRADE[6] decision-making process was used to reach recommendations about the use of pain mitigation strategies in piglets. The GRADE process enabled the development of recommendations for or against the adoption of identified pain mitigation strategies, a rationale for the strength of these recommendations, identification of research areas that would strengthen science-based decisions, and identification of areas where recommendations could not be made due to the absence of scientific evidence.

THE RESEARCH LANDSCAPE FOR WELFARE TOPICS IN FOOD-PRODUCING LIVESTOCK PRODUCTION

In the last decade, the swine industry has recognized the importance of high-quality research to inform science-based decisions about swine welfare and pork production. The list of swine welfare issues continues to grow due to an increasing interest within animal agriculture as well as from stakeholders. Welfare topics identified to date include gestation sow housing, transportation, sow lameness, on-farm euthanasia, and routine piglet management procedures. The impact of providing animal movement capabilities is of interest to consumers, as illustrated by ballot initiatives that focus on hens kept in conventional, caged housing systems and gestating sows housed in stalls (Mench et al., 2009).

Juxtaposed to the growing number of research questions is funding for such studies. For swine, major sources of funding are limited to the NPB checkoff-funded program, which has had an active animal welfare research program since 2000. In 2012, the priority areas were euthanasia, sow housing, transportation of weaned and feeder pigs, and pain mitigation (not in order of priority). The US Department of Agriculture (USDA) external funding group, the National Institute of Food and Agriculture (NIFA), has never explicitly excluded questions of animal welfare from funding nor is animal welfare a program area. However, the USDA did have an internal funding program relevant to animal welfare: USDA-Agriculture Research Service (ARS) National Program NP105 Animal Well-Being and Stress Control Systems, under which

[6]The Grading of Recommendations Assessment, Development and Evaluation

ARS research units at Purdue, Texas Tech, Missouri, and Georgia Universities were funded. This program merged with National Program 101 in approximately 2006. At the US federal level, animal welfare continues to fall within the boundaries of larger areas of animal, plant, forestry, and environmental research.

A search of the USDA Current Research Information System (CRIS) database with the terms "piglet" and "pain" identified 12 studies, and a search with terms "piglet" and "processing" identified 36 studies. Within these searches, one grant appeared to directly address a review of pain at processing, but not pain mitigation. No publications were reported for that grant after 2004. In 2013, projects with animal welfare emphasis could be submitted to the *Engineering, Products* and *Processes* program of USDA. This program aims to address engineering projects to inform changes in agriculturally relevant plant, animal, forestry, and natural resource systems. Within this program, approved projects on animal welfare must contribute to improved animal welfare within production systems as they relate to handling, containment, feeding, housing, or harvesting practices and technologies. Public scrutiny of US farm animal production is high, and many standard industry practices are increasingly being questioned on ethical grounds. Concerns not only include food safety, environmental impact, and the sustainability of many of the industry's practices, but also animal welfare. Concern for the welfare of livestock animals raised in intensive animal agriculture in the US has become a voter's issue. For example, in 2008, Proposition 2 in California banned veal crates, gestation stalls for sows, and battery cages for hens. Oregon, Colorado, New Jersey, Arizona, and Florida have also seen a rise in awareness among consumers towards the welfare of animals and new laws to protect their interests (Mench, 2003; Croney and Millman, 2007; Swanson, 2008). Thus, as long as we farm animals, we shoulder the responsibility to ensure that they are spared unnecessary suffering and that their physical and mental states are good. The modern animal welfare legislation movement, for example, suggests that animals "should be protected from suffering and harm not for the benefit of us humans, as in earlier anthropocentric conceptions, but in their own interest" (Würbel, 2009).

The juxtaposition of the need for increased information to answer societal questions and the level of funding availability warrants frequent review of funding outcomes and priorities. For all funding areas, periodic reviews should be performed to determine whether the desired goal has been achieved (i.e., whether the research has answered the current questions) or whether refinement of the research question is required to ensure that funding is directed to the highest-priority areas.

EXTENDED SUMMARY OF OBJECTIVE 1- IDENTIFICATION OF RESEARCH GAPS

Pain mitigation will likely become a higher priority area for consumers and retailers as attention moves to areas beyond gestation sow housing. The European Union requires anesthesia during castration or cessation of surgical castration, and this has focused discussion in the US on this topic. Castration in the US is physiologically required due to the current and increasing slaughter weights for market swine. Studies on pain mitigation for castration will be a priority unless the US uniformly adopts immunocastration or chooses to not castrate and totally eliminates the procedure by marketing younger, immature pigs. A body of processing experiments has been identified that directly applies to the young piglet. Forty-two castration experiments were identified that assessed the impact of acute pain mitigation. These 42 experiments assessed numerous pharmacological interventions: general anesthesia, local anesthetic applications, and administration of non-steroidal anti-inflammatory drugs (NSAIDs). Only 10 experiments were identified that assessed pain mitigation strategies for tail docking. Two experiments assessed pain mitigation associated with teeth clipping and ear notching. Of the three broad types of interventions (i.e., general anesthesia, NSAIDs, and local anesthetics), the largest research base available is for general anesthesia during castration. Within general anesthesia, few specific intervention protocols (dose, route, duration, etc.) have been assessed in a large number of trials. The specific protocol with the greatest number of experiments regards the use of the NSAID meloxicam administered at a dose of 0.4 mg/kg intramuscularly. We speculate that the difference among the availability of studies for the different intervention types is attributable to funding opportunities or perhaps convenience of administering the intervention. The

manufacturer of meloxicam has supported several studies of its use in piglets. However, manufacturers of local anesthetic and general anesthesia products do not appear to support research into their applications as pain mitigation tools. This could be because most of these compounds are no longer under patent protection, and a market for using these compounds routinely in swine operations has not been established. Thus, the costs associated with obtaining a drug approval for analgesia may not be offset by drug sales, resulting in an uncertain return on investment.

Another gap identified by the review was the absence of interventions that combine multimodal pain protocols that would address pain caused by the actual procedure as well as subsequent pain. Most routine piglet surgeries result in pain at the time of the procedure as well as pain after the procedure. Ideally, pain mitigation strategies would alleviate all pain caused by the procedure.

A procedural question arises as to whether research should be directed toward establishing that these procedures are painful as an initial step, or to make the assumption that the procedures require pain mitigation. The current literature review did not seek to quantify the number of studies that addressed the question "Is castration/tail docking/ear notching/teeth clipping painful?" as there was an *a priori* assumption that this has already been established. Given that the validity of this assumption was not a topic of debate at the panel meeting, available resources should be directed toward pain mitigation strategies rather than pain validation studies.

However, without validated outcomes to objectively recognize and quantify pain perception in animals, addressing questions about animal welfare becomes difficult. As a result, it is necessary to ensure that we have achieved basic knowledge of validated measures of pain. We will then be ready for "field setting" studies. In this step, the pain mitigation strategies can be assessed to test the most effective/feasible/cost-effective pharmacological intervention(s) for pain mitigation in routine piglet processing procedures.

EXTENDED SUMMARY OF OBJECTIVE 2- RECOMMENDATIONS FOR INTERVENTIONS

Making transparent recommendations about the adoption of interventions requires the assessment of four aspects of decision making: 1) quality of evidence about critical outcomes, 2) assessment of benefits and harms, 3) consideration of values and preferences of those affected by the decision, and, where appropriate, 4) resource implications. It is possible that, using the same evidence base, different groups will reach different recommendations because of their understanding about the balance of benefits and harms, or different values and preferences. Similarly, with the same evidence at different times, recommendations may differ because values and preferences change or resources, such as new drugs, may become available that were not previously considered.

Scientific information should be transparently included in the recommendation-making process in conjunction with other societal values. Too often, groups hide behind "no evidence" as an excuse for not making a decision when the motivation was the expected impact on resources or values. The GRADE process aims to force panels to explicitly address these issues separately and articulate the rationale for the recommendation. Although we expect that good evidence is needed to make strong recommendations, this is not always the case. For example, strong recommendations are made for the adoption of internal biocontainment, although the evidence base is fairly weak. This weak evidence base is a function of the difficulty of defining and studying biocontainment. Similarly, empirical evidence for values and preferences is preferable but is not always necessary. For example, most analysts would agree that a systematic review of multiple, large, randomly selected surveys is not needed to document the preferences of parents to avoid a treatment during pregnancy that could result in genetic abnormalities; this is considered a standard value and preference.

The panel concluded there was likely a large variation in consumer values and preferences with respect to the use of pain mitigation strategies in piglets balanced against the willingness to pay. This conclusion was

based on presentations from two speakers about consumer attitudes and the economic behavior of consumers to welfare and pain mitigation. A systematic review of consumer attitudes was not conducted. The information about values and preferences was assessed from the perspectives of the consumers of pork. The result of several US-based voter initiatives were used as evidence of consumer values, whereas the observed low willingness to pay observed in the US or in overseas markets was used as evidence of variations in consumer actions. It was also noted that willingness to pay may be difficult to document in the US market, where there are few niche entry points for pork with differentiated production processes. This situation differs from egg production, where more direct market channels exist for differentiated products such as cage-free eggs.

The recommendations made by the panel were as follows:

Intervention: General anesthesia CO_2/O_2

Few studies were available to assess the efficacy of this intervention; the outcomes assessed did not enable the panel to understand the impacts on pain experienced by the piglets. If animals are properly anesthetized, the expectation was that pain was mitigated during the procedure. It is unclear whether appropriate anesthesia performance or levels can be consistently achieved on-farm. Furthermore, it is not known whether general anesthesia during castration results in a reduction, no change, or increase in pain from 1 to 24 hours after the procedure.

General anesthesia is a complex procedure; clearly the potential exists for under- or overdosing, which would likely result in little to no pain mitigation or increased mortality, respectively. In a production setting with different ages and weights of piglets to process, it is currently unrealistic to expect producers to rapidly, consistently, and safely administer general anesthesia with existing tools. Further, the potential for harm to workers adds an additional concern about the safety of the on-farm use of general anesthetics for pain mitigation. These concerns were major drivers for the strength and direction of the recommendation provided by the panel.

The panel's current position is a strong recommendation against the use of a CO_2/O_2 general anesthesia mixture for pain mitigation during castration in piglets between 1 and 28 days old.

Intervention: Nonsteroidal anti-inflammatory drugs (NSAIDs)

There was an absence of critical outcomes measured for this intervention. This is an intervention designed to mitigate pain from 1 to 24 hours after the procedure. The recommendation means that the panel placed a high value on the cortisol results for this time frame. It was recognized by the panel that cortisol is not a specific indicator of pain and that validated pain assessment measures are needed to more fully assess the benefits of NSAID administration to alleviate pain associated with castration. This is one reason for a weak recommendation. Results from vocalization studies indicated that these strategies do not mitigate the acute pain associated with the procedure. The vocalization results were not unexpected given the mechanism of action of NSAIDs, but they provided another reason why the recommendation was weak rather than strong for these products.

The panel felt that the likely benefits outweighed the harms for NSAIDs. Unlike general anesthesia, the potential for harm to the piglet due to overdose is minimal. Current NSAID products provide a reasonable margin of product safety for published dosing regimens. Additionally, the products are routinely applied via commonly used routes of administration in commercial swine production facilities. There is a limited expectation of benefit for the pain that occurs as the procedure is conducted, as benefits are likely limited to reduction of inflammatory pain after the procedure. Of course, an unresolved issue is the legality of use of NSAIDs in piglets in the US, where no products have been specifically approved to provide analgesia in

livestock. Allowable use of unapproved compounds to provide analgesia in livestock in the US may be regulated under the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994.

The panel's current position is a weak recommendation for the use of NSAIDs for pain mitigation during castration in piglets between 1 and 28 days old.

Intervention: Local anesthesia lidocaine

Lidocaine is an intervention designed to mitigate pain in the short term (i.e., 1–2 hours) after the procedure. There was an absence of information about the *a priori* identified critical outcomes for this intervention. For this intervention, we expect that only acute pain associated with the procedure would be mitigated. Two studies indicated that administration of lidocaine did reduce vocalization, as measured by call energy. However, there was debate among the panel about the value of this outcome; therefore, the evidence base was considered very low quality.

The uncertainty expressed here about the balance of benefits and harms by the panel was related to a failure to document the extent of benefits. The benefit to the piglet is that local anesthetics mitigate pain in the short term. However, the panel proposed that this benefit may not be as great as expected for two reasons. First, in the US production system, there is a reluctance and practical difficulty in taking the extra steps required to administer lidocaine at appropriate time intervals prior to castration. If these steps are not taken, little real benefit for the piglet is realized. Further, based on the mechanism of action rather than empirical studies in piglets, we do not expect pain that occurs after castration to be mitigated by lidocaine. These uncertainties weakened the recommendation. Possible harm to the piglet was thought to be manageable but not negligible. Lidocaine is widely used in human and animal health, and has a reasonable margin of product safety; however, it is theoretically possible that lidocaine could adversely affect wound healing, and administration might be associated with tissue irritation (stinging) that may be painful in testicular tissue.

The panel's current position is a weak recommendation against the use of lidocaine as a pain mitigation strategy for piglets undergoing castration.

EXTENDED SUMMARY OF OBJECTIVE 3- COMPREHENSIVE REPORTING

Comprehensive reporting of research is a fundamental concept in the scientific method and refers to the provision of a publically available, full, and accurate description of research that enables reproduction of the study, assessment of bias, extraction of data from the study, and fulfills the ethical obligation to maximize the utility of the research findings (O'Connor, 2010). Comprehensive reporting is critical to ensure that research information can be utilized after publication. The aim of comprehensive reporting is to decrease the wastage of resources and to increase research utility. The uses of published reports of studies are many and are not limited to those envisioned by the authors. Research results are used to inform next steps in the research continuum, be that decisions about the next research hypothesis to be tested or to make decisions about the adoption of an intervention by an industry. In recent years, the focus on comprehensive reporting has increased in human and veterinary medicine due to consumer concerns about the transparency of research information and the formal integration of scientific information into decision making.

In every area that has been the subject of an assessment of comprehensive reporting, deficiencies have been found (Bhandari et al., 2002; Atkinson, 2003; Anttila et al., 2006; O'Connor et al., 2006; Agha et al., 2007; Armijo-Olivo et al., 2007; Armstrong et al., 2008; Bereza et al., 2008; Al Faleh and Al-Omran, 2009; Bekelman and Yahalom, 2009; Berwanger et al., 2009; Sargeant et al., 2009a; Sargeant et al., 2009b; O'Connor et al., 2010; Sargeant et al., 2011). It should be no surprise then that, in the area of pain mitigation strategies in piglets, reporting has not always been comprehensive. Many of the factors that are routinely expected to be included in a research report were missing. Factors that enabled the review panel to assess the internal and external validity of the results, as well as the actual results, were also missing. Limited

reporting, as opposed to comprehensive reporting, occurs for many reasons, including the difficulties that authors face in anticipating the needs of the end user of a publication, inappropriate corrections from reviewers or editors, and space limitations in publications. Therefore, limited reporting is likely not malicious, and education about what is needed in a comprehensive report is necessary. However, as mentioned, this finding is not unique. All of these topics can and have been addressed in other areas; they are not insurmountable. The EQUATOR[7] network (www.equator-**network**.org) illustrates the current efforts devoted to improving scientific reporting in human medicine.

In several ways, the area of pain mitigation in swine, and potentially swine welfare itself, is ahead of the game, because scrutiny of reporting occurred relatively early in the development of the field. Most areas where reporting has been evaluated have far older, larger bodies of work, and, therefore, many more resources have been devoted to poorly reported research (De Vito et al., 2007; Waddell et al., 2009; Brace et al., 2010; O'Connor, 2010; Sargeant et al., 2010b). Further, because the number of people involved in swine pain research is relatively small, the potential for successful education efforts to redress inadequate reporting are greater. The focus now needs to be on educating everyone involved in the publication process to improve reporting.

Reporting guidelines have been developed for many study designs, and two relate directly to animals: the REFLECT[8] statement (Sargeant et al., 2010a), which focuses on studies that use food animal producing livestock, and the ARRIVE[9] guidelines (Kilkenny et al., 2010), which focus on animals in biomedical research. These statements contain checklists for items to include in publications, the rationale for each checklist item, and examples. These checklists represent a minimum list and more information may be needed. The checklist items are very similar, although the REFLECT statement has a greater focus on clustered populations because those reflect livestock populations. Both statements were created with a consultation process that included input from information scientists, decision makers, research synthesis experts, stakeholders, editors, and researchers. Such diverse consultation is needed to understand end user needs. For example, many researchers question why the methods of allocation should be included in the abstract or title, but this is motivated by information scientists who are tasked with retrieving studies.

The pertinent question now is what actions should come from the findings of the comprehensive reporting assessment. Clearly, increased educational efforts about reporting are needed for authors, editors, reviewers, and granting agencies, which all share some responsibly for comprehensive reporting. Given that reporting guidelines are specific for intervention study design, it does not seem likely that specific reporting guidelines are needed for welfare intervention studies *per se*. However, the panel concluded that what is needed, at a minimum, is a freely available document that illustrates each of the REFLECT items using welfare-oriented examples. Such an approach is provided in the results of objective number three. Distribution of the results of that study and an accompanying document that suggests improvements for reporting at key international welfare conferences and in key welfare publications with concurrent buy-in from influential individuals will be necessary to ensure that such work has an impact. Regrettably, without such an effort, the alternative is that no change will occur in approaches to reporting, reviewing, and editing and, in 5–10 years' time, the same issues will be reidentified. Given awareness of this issue and the use of animals in pain-inducing experiments, to do nothing is indefensible and unethical.

[7]Enhancing the QUAlity and Transparency of health Research
[8]Reporting guidElines For randomized controLled trials for livEstoCk and food safeTy
[9]Animal Research: Reporting *In Vivo* Experiments

Body of Report

1. Introduction

Consumers of animal products are increasingly interested in the ethical and social dimensions of food production (Botonaki et al., 2006; Wright and Middendorf, 2008; FAO, 2009). While most consumers *presume* that producers, especially smaller-scale farmers, take good care of their animals (e.g., by meeting the animals' needs for food, water, and shelter), many are increasingly concerned that, especially in large scale, agribusiness-dominated "industrialized" farming, there is insufficient attention on animals' affective states and quality of life. Both collectively and individually, the American consumer is increasingly becoming more interested in the production history of their food as a form of ethical consumerism (see, for example, Singer and Mason, 2006). Failure or reluctance to address these issues provides public impetus to externally regulate the industry's practices (Rollin, 2004). In the US, recent public concerns regarding the need to protect farm animals has led to increased efforts to employ animal welfare regulation (Rauch and Sharp, 2005). Americans apparently have concerns about farm animal welfare and act on their concerns via voting initiatives and purchasing behaviors (Appleby, 2005; Tonsor and Wolf, 2010). For instance, in November 2006, often with approval rates of 60% or higher, voters overwhelmingly supported animal welfare measures that appeared on state ballots. In 2008, California voters passed Proposition 2, which regulated the housing of gestating sows, egg-laying hens, and veal calves. In 2009, Ohio voters strongly supported the development of a Livestock Care Standards Board to provide oversight of farm animal care practices by passing Issue 2 (Appleby, 2005; Singer and Mason, 2007; Tonsor and Wolf, 2010). These initiatives suggest growing public demand for animal welfare assurance. Despite the animal welfare assurance programs devised by food industry retailers, processors, producer groups, and private organizations, there seems to be a general lack of trust in industry self-regulation and a need for the US animal industries to more demonstrably address animal welfare (Mench, 2003; Swanson, 2008). The public appears to believe that external regulation is still warranted. It is conceivable that the spike in initiatives at the state level may lead to federal legislation of farm animal welfare standards that exceed those outlined by the Animal Welfare Act. Thus, in order for the swine industries to retain their autonomy a clear and coherent plan to address both the scientific and ethical issues that are fundamental to all animal welfare concerns raised by the public and some producer groups is needed. This begins with a critical assessment of its accepted practices as undertaken through this process.

In swine production in the US, castration, tail docking, teeth clipping, and ear notching may be conducted on piglets during the first few days of life. These procedures are considered painful and, in the past decade, the swine industry has funded research into understanding pain in piglets and pain mitigation methods. In this report, we provide a review and summarize the currently available scientific information about pain mitigation strategies for piglets.

The rationale for these procedures differs. Castration reduces boar taint and aggression as males mature to market weights and is used in the majority of pork-producing countries. Male swine in the US, except breeding stock, are most frequently surgically castrated. Alternatives to surgical castration are becoming commercially available. Tail docking is conducted to prevent pigs from biting the tails of their pen mates, resulting in tail infections that can form abscesses and, in severe cases, paralysis or death. Management alternatives to tail docking that provide consistent results are not available. Teeth clipping is conducted to reduce the impact of bites on mammary glands during nursing and on littermates during teat competition. Teeth clipping was a common practice for many years but recently has been reduced. Ear notch identification is recognized as a unique means of individual pig identification for US breed registries and by the US government as an official method for individual animal identification for movement documents. Individual producers may use ear notches to identify date of birth or other management information on individual pigs or groups of pigs.

In the last decade, the swine industry has recognized the importance of high-quality research to inform science-based decisions about animal welfare and pork production. The NPB has funded much of that research. A periodic review of research areas is imperative for determining priorities and ensuring focus on priority areas. Based on that rationale, the goals of this project were to establish the current state of knowledge about pain mitigation strategies in piglets, to provide recommendations about strategies that can be used by producers, and to provide guidance about future directions for research funding. To achieve those goals, the project was partitioned into three objectives. The first objective was to summarize the body of work and identify research gaps (i.e., areas where research is not published). The second objective was to use the available evidence to develop recommendations, where possible. The third objective was to summarize the comprehensiveness of reporting on this topic.

2. Materials and Methods

The methodology employed was a systematic review with a guidelines development process. The systematic review process used was that recommended by the European Food Safety Committee Guidance on Systematic Reviews (EFSA, 2010). The recommended steps for guideline development are described in the literature (Schunemann et al., 2006b; Guyatt et al., 2011g). We report all 21 steps of that recommended process below.

2.1. Priority setting

The Iowa State University (ISU) steering committee did not set the priorities for recommendation development as sometimes occurs (Oxman et al., 2006). Instead, the rationale for reviewing pain mitigation strategies was made by the funding agency. In 2011, the NPB Animal Welfare Committee established a 5-year research strategy to address the US swine industry's animal welfare priorities. Pain management, particularly as it relates to piglet processing procedures (i.e., castration, tail docking, teeth clipping, and ear notching) was identified as a priority for research. The NPB Animal Welfare Committee requested that a literature review workshop focusing on the issue of piglet processing procedures and pain management be conducted.

2.2. Group composition and consultation process

Review participants were divided into two groups (Fretheim et al., 2006a). First, the ISU steering committee was formed during the proposal development phase. The original group contacted by the NPB consisted of Drs. Anna Johnson, Suzanne Millman, and Johann (Hans) Coetzee in July 2012. The original proposal for the project was to hold the review workshop by December 31, 2012, with final project completion by May 1, 2013. Drs. Locke Karriker, James McKean, and Annette O'Connor were added to the committee because they have expertise in swine production practices, techniques for conducting systematic reviews, and a critical appraisal of the scientific literature. Drs. McKean and Johnson were also on the NPB Animal Welfare Committee that requested the review. Also included in the group was NPB program director Sherrie Niekamp; her role was to clarify the purpose of the review for the NPB. The second group comprised the external participants. The ISU steering committee determined the content and methodological expertise needed for recommendation development. The approach to selecting participants followed those proposed previously (Fretheim et al., 2006a) and are summarized here:

- *"Groups that develop guidelines or recommendations should be broadly composed and include important stakeholders such as consumers, health professionals that work within the relevant area, and managers or policy makers. For food production, this is clearly extended to include producers."*

- *"Groups should include or have access to individuals with the necessary technical skills, including information retrieval, systematic reviewing, health economics, group facilitation, project management, writing, and editing."*

- *"Groups should include or have access to content experts."*

- *"To work well, a group needs an effective leader, capable of guiding the group in terms of the task and process, and capable of facilitating collaboration and balanced contribution from all of the group members."*

- *"Because many group members will not be familiar with the methods and processes that are used in developing recommendations, groups should be offered training and support to help ensure understanding and facilitate active participation."*

The ISU steering committee determined that the content expertise necessary for assessment of evidence included expertise in (1) stress physiology, (2) applied ethology or applied animal behavior, (3) pharmacology, (4) swine health and production, (5) food production economics, and (6) animal ethics. Methodological expertise identified included expertise in (1) study design, (2) sources of bias in research, (3) research synthesis, and (4) guideline development. Stakeholder groups identified as important were (1) swine producers, (2) non-government organizations associated with animal welfare groups, and (3) veterinarians. Although recommendations suggest that group size might best be limited to 15 members, the final group recommended included 25 people.

Invitations to participate were distributed on November 5, 2012. Each invited participant was sent a copy of the review protocol to familiarize themselves with the review scope and the approach to be used. Participants were given 2 weeks to review the protocol and were asked to reply to the review coordinator with any comments or suggestions. Although four non-government organizations associated with animal welfare groups were invited to attend, none were able to participate. The final list of participants is reported in Table 1.

2.3. **Managing conflicts of interest**

Conflict of interest statements were not required prior to invitations or participation, but all members provided conflict of interest statements prior to the presentation of the report.

2.4. **Group processes**

The group processes, timing of meeting, voting approach, document preparation, etc., were largely directed by Dr. O'Connor. They were based on GRADE approaches (Fretheim et al., 2006b). Group decisions for the steering committee were made by discussion without formal voting.

At the panel meeting, the approach to decision making was voting using automated clickers. In order for a decision to be made regarding the recommendation of an intervention, a consensus had to be reached. It was decided through voting at the beginning of the panel meeting that consensus would be at least 75% (i.e., 14/18 of the participants had to be in agreement). Voters could not abstain. Several votes were required for assessing the quality of the evidence base for each procedure, intervention, and outcome combination. First, the topic for voting was introduced and explained by the facilitator. Each time, the explanation was shorter. Panel members were required to vote for each bias/outcome/intervention/procedure item. If a consensus was reached, then a vote was taken for the next item. If a consensus was not reached, the panel was informed that a consensus was not reached (numerical results were not reported to avoid bias). The facilitator invited panel members to provide their rationale for a particular vote option or panel members could request further clarification of the items (e.g., what does GRADE mean by "imprecision?"). After

subsequent discussion, another vote would occur. In circumstances where consensus could not be reached within 3–4 votes, additional voting on this item was delayed until other items had been the topic of voting. The process would continue until all bias/outcome/procedure/intervention combinations had been assessed. The process was then repeated for the development of recommendations.

2.5. **Identification of important outcomes**

To identify critical outcomes for guideline development, the outcomes that would be considered important for the assessment of pain mitigation strategies in piglets were assessed. The approach to this step was to ask the ISU steering committee to nominate outcomes considered important prior to conducting the review. After the list of important outcomes had been compiled, based on the opinion of the ISU steering committee, it was shared with the external participants. The external participants were asked to contribute to the list of important outcomes. This list is provided in

Table 2 and

Table 3. The outcomes were divided into four types: behavioral (< 60 minutes), behavioral (1 to 24 hours), non-behavioral (< 60 minutes), and non-behavioral (1 to 24 hours). All members of the panel (steering committee and external participants) were asked to rank the importance of the outcomes using the SurveyMonkey® online survey software and questionnaire tool (SurveyMonkey, Palo Alto, California, USA). Items were ranked 1 through 9 (Guyatt et al., 2011b), and the ISU steering committee and external participants were given the following definitions and explanations as a guide when completing the survey.

Ranks 9 to 7 were defined as **critical outcomes**. Critical outcomes were defined as those essential to the decision-making process. In the context of this review, critical outcomes were those for which it was clear that the outcome was a measure of pain experience rather than other responses, such as stress or fear. Similarly, these may have been outcomes that more directly measured the animals' response in a cascading pathway, that is, cortisol leads to stress leukogram; therefore, cortisol may have been the more direct measure.

Ranks 6 to 4 were defined as **important outcomes**. Important outcomes were not as essential to the decision-making process as described above.

Ranks lower than 4 were defined as **non-important outcomes**. Non-important outcomes were those unlikely to change the decision-making process or inference. Often, these outcomes were measured because of ease of collection or standard practice, yet they were not considered to be truly relevant to the assessment of the intervention and were not included in the guideline development process.

The results of the survey were not evaluated until after the outcome data were extracted from the papers. Data extraction forms were compiled to extract all outcomes, regardless of ranking.

2.6. **Explicit definition of the question and eligibility criteria**

Sections 2.6 to 2.9 represent the systematic review methodology. Consistent with systematic review methodology, these steps were all determined *a priori* and reported in a protocol that is available from the review facilitator. The ISU steering committee prepared the protocol.

To address the review, a question was developed that defined the scope of the review in the PICO format: population (P), intervention (I), comparator (C), and outcome (O). The specific review question was:

"In piglets that undergo castration, tail docking, teeth clipping, and/or methods of identification that involve cutting of the ear tissue, such as ear tagging and ear notching (P), what is the effect of pain mitigation (I) compared with no pain mitigation (C) on behavioral (e.g., postures, vocalizations) and non-behavioral (e.g., blood cortisol, norepinephrine, β-endorphin levels) indicators of pain assessed within 60 minutes of the procedure and between 1 and 24 hours of performing the procedure (O)?"

However, further limitations placed on the recommendation development aspect of the project were made by the ISU steering committee. Once the evidence had been obtained, it was presented to the steering committee at a second day-long meeting held on January 14, 2013. The ISU steering committee was asked to decide which information would be assessed as part of the guideline development process. With respect to information that would be assessed, two key decisions were made. First, since only two studies were available regarding pain mitigation for teeth clipping and ear notching, and these were both from the same author and publication, it was decided that this was insufficient information to move forward with an analysis of the data and decision and recommendation making for these procedures. Secondly, only trial arms with a single pain mitigation strategy applied to the piglets would be considered. Therefore, arms where two or more interventions were applied to the piglets in that arm were excluded. For example, if piglets in one arm received both lidocaine (local anesthetic) and meloxicam (NSAID), this arm was

excluded. This decision affected the inclusion of protocols that included ketamine and azaperone together or in combination with other drugs, such as meloxicam.

2.7. Type of study designs for different types of questions

Relevant study designs were parallel trials. The panel allowed non-parallel observational studies to be considered, although none were identified.

2.8. Identification of evidence

2.8.1. The search for evidence

With direction from an information scientist with specialization in the veterinary literature, a search strategy was developed for CAB Abstracts (Thomson Reuters) in September 2012. CAB Abstracts was selected because this database maximizes journal coverage and avoids missing potentially relevant articles in animal health (Grindlay et al., 2012); we inferred the same might be true for animal behavior and welfare. A search strategy was designed to include two concepts from the PICO question: (1) division of the population into searches for piglets and searches for procedures, and (2) the intervention terms, that is, search terms, designed to capture pain mitigation strategies, either pharmacological or non-pharmacological.

The search strategy was reviewed by the ISU steering committee. Although additional search terms were suggested, these did not change the sensitivity of the search. The literature search was conducted in six electronic databases: CAB Abstracts (1910–2012), Biosis (Previews; 1926–2012), Web of Science (1900–2012), PubMed (1965–2012), Agricola (1950–2012), and ProQuest Digital Dissertations (1965–2012). The searches were performed on October 12, 2012 and were repeated on December 13, 2012 to only include citations that may have become available since the first search in October 2012. An example search string with results is provided in Table 4.

2.8.2. Screening for relevant studies

After the search was conducted, the citation titles and abstracts were screened for relevance. We did not screen abstracts reported in foreign languages. If necessary to assess the relevance of the citation to the review, full texts where obtained. If the full text of potentially relevant abstracts were unavailable in English, certified translations were obtained. Translations were performed by Language Scientific Inc. (Medford, MA) and certified as accurate on December 27, 2012. Screening of abstracts was conducted by two independent reviewers. Conflicts were resolved by discussion between the reviewers. Prior to conducting the screening, the reviewers were trained about the review and a test series of abstracts were screened to assess the agreement between the reviewers about relevance of citations. The questions were modified until there was 100% agreement for the 20 abstracts (i.e., κ=1.0). The same reviewers then independently screened all titles and abstracts (if available). Subsequent conflicts were resolved by discussion.

The following screening questions were applied to each citation:

Does the citation describe a primary research study where the study population is pigs less than 28 days of age or described as piglets, suckling, pre-weaned, or of weight consistent with those populations?

Does the citation describe a primary research study where the study population is subjected to castration, tail docking, teeth clipping, ear notching, and/or ear tagging?

Does the citation describe a primary research study where the aim is to assess any intervention designed to mitigate the pain associated with the procedure?

Does the study describe a group of pigs undergoing the same procedure (s) but not receiving the intervention?

Does the study assess outcomes that measure the pain experience during the procedure or within 24 hours of the procedure?

When both reviewers responded "No" to at least one of the above questions, the citation was excluded from the review. When both reviewers responded "Yes" to all of above questions, the citation was included in the review. Conflicts between reviewers were resolved through discussion.

2.8.3. **Extraction of data**

2.8.3.1. Data collection process

Two reviewers (AOC, SG, or RSD) extracted data independently from eligible studies and entered the data on standardized forms designed and stored online in DistillerSR© (Evidence Partners, Canada). After data extraction by the reviewers, conflicts were resolved by one reviewer (RSD). The reviewer went through the answers entered on the separate forms and compared them. If there was a disagreement, the reviewer would re-evaluate the text in the article and then, if necessary, discuss the discrepancy with the other reviewer to resolve the conflict. After this step of conflict resolution, the data extraction forms and publications were made available to the panel members who verified the accuracy of the extracted data. Comments were returned to the primary data extraction team who evaluated each comment and modified the data if required. Each study had only one external reviewer.

Information that was extracted from each study was divided into general study level information, piglet information, procedure information, intervention information, and outcome information**.**

General study level information included the year the study was published, the country in which the study was conducted, the setting for the study (e.g., university-owned farm, laboratory or research facility, privately owned/commercial operation), sow management type (i.e., all-in/all-out, continuous flow), and the number of relevant arms in the trial.

Piglet information collected included the number of piglets enrolled in the study (including non-relevant arms), age at enrollment (reported as range, mean and variation, exact number), weight at the time of the procedure (reported either as range, mean and variation, exact number), and sex.

Procedure information collected included castration and the technique applied (i.e., scrotal incision and cut of spermatic cord removal of testicles, scrotal incision and tear of spermatic cord removal of testicles), tail docking and the technique applied (i.e., hot-docking, cold-docking), teeth resection (i.e., teeth clipped, teeth grinding), and methods of identification that involve cutting of the ear tissue, such as ear tagging and ear notching.

Intervention information was collected at the trial arm level. The information extracted for each arm was the type of treatment [general anesthetic, local anesthetic, nonsteroidal anti-inflammatory drug (NSAID), other or placebo or none], route of administration, time of administration, dosage, frequency, number that received treatment, and number of replicates.

Outcome information was categorized through extraction of behavioral and non-behavioral outcomes as well as adverse events for each relevant arm. For each continuous outcome, we attempted to extract the mean, standard deviation, and/or standard error of the mean, units, p value, and number of piglets in the arm. If data were reported as medians or quartiles, we extracted these data, although they were not included in the meta-analyses. For categorical outcomes, we attempted to extract the proportion with the event (r),

units, p value, and number measured for this outcome. When an outcome was reported as being measured, but no results were reported, we indicated this as "described but not reported."

2.8.3.2. Data manipulations and considerations for data extraction

Data not reported in standard international (SI) units were converted using standard tables. We only extracted measures of variation if the manuscript explicitly stated what the measure of variation was. We used an electronic ruler for data extraction from the figures. When the standard deviation was not reported, this was calculated in RevMan, provided the trial arm mean, the number of animals in the arm, and the standard error were reported. If a study reported the number of animals in the study but not the number per arm, then arm level data were considered missing, as it was not clear that the allocation to the trial arm occurred at a 1:1 ratio.

When studies reported multiple outcomes within the periods of interest, that is, measurements of cortisol at 2, 4, and 24 hours, that all qualified for the 1–24-hour time period, we extracted the time point where the placebo arm level of the outcome was the highest. This approach was applied to all outcomes.

For behavior outcomes, multiple parameters were often reported and not necessarily identified as relevant pain responses. When authors consolidated these pain behaviors into an aggregate pain index score, we preferentially selected the pain index score as the pain behavior outcome. When this was not provided, we randomly selected one behavior outcome within pain-related behaviors associated with avoidance or removal of noxious stimuli. For example, although we recognized that pain can result in general changes in time budgets, we assumed behavior responses directed to the rear quarters (tail flinching, tail wagging, tail rubbing, kicking, scooting, easing the quarters) would be more sensitive measures of pain resulting from castration and tail-docking surgeries, versus behaviors associated with exploratory or feeding motivational systems; hence, we randomly selected a behavior outcome from this group of responses. Similarly, vocalizations are louder, higher in pitch, and greater in number when piglets experience pain, and for studies that reported multiple outcomes for vocalizations, we randomly selected one outcome each for number of calls, call pitch, and call volume.

2.8.3.3. Risk of bias in individual studies

We extracted data that described the use of approaches that increased group exchangeability, such as randomization, blocking, and stratification, to allocate piglets to a group. If authors used the term "random" or "randomly" at any time related to how piglets were assigned to groups, this was considered sufficient. We did not require a description of the randomization approach. We extracted data that described the the use of blinding for outcome assessment, although we did not assess the efficacy of blinding.

2.8.3.4. Assessment of comprehensive reporting

The approach was to assess the comprehensiveness of reporting based on a generic checklist for livestock trials [i.e. "The REFLECT[10] Statement" (Sargeant et al., 2010a)] and to assess the comprehensiveness of the reporting of domain-specific sources of heterogeneity identified as important by the review panel. For the general assessment of comprehensive reporting, we assessed how many select items of the REFLECT guidelines were included in the reports. REFLECT stands for Reporting guidElines For randomized controLled trials for livEstoCk and food safeTy (Sargeant et al., 2010a). We only assessed 17 of the 22 items on the checklist. We did not evaluate the introduction (checklist item 2, with the exception of the last paragraph where the study objectives and/or hypotheses would typically be stated), the discussion (checklist

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item 20), the study's generalizability (checklist item 21), and the overall evidence (checklist item 22), as an evaluation of these items are often subjective and require content expertise.

The selected checklist items assessed included the title and abstract (REFLECT checklist item 1); eligibility criteria for farms and piglets, and the settings and locations where the data were collected (REFLECT checklist item 3); precise details of the interventions for each trial arm, the level at which the intervention was allocated, and how and when the interventions were actually administered (REFLECT checklist item 4); specific objectives and hypothesis (REFLECT checklist item 5); clearly defined primary and secondary outcomes (REFLECT checklist item 6); sample size determination (REFLECT checklist item 7); randomization (sequence generation, allocation concealment, and implementation; REFLECT checklist items 8–10); blinding (REFLECT checklist item 11), statistical analyses (REFLECT checklist item 12), dates defining the periods of piglet recruitment (REFLECT checklist item 14); baseline data (REFLECT checklist item 15); numbers analyzed (REFLECT checklist item 16); outcomes and estimation (REFLECT checklist item 17); ancillary analyses (REFLECT checklist item 18), and adverse events (REFLECT checklist item 19). The rationale for why these items were included in a publication as well as elaborate explanations were provided by Sargeant et al. (Sargeant et al., 2010a).

The following considerations were made. We only extracted the country in which a study was conducted if it was explicitly reported in the article text; no inferences were made from the author information (REFLECT checklist item 3, location). We added one item that assessed whether the studies reported random allocation to a group. This was necessary because the REFLECT statement makes the *a priori* assumption that studies are randomized. REFLECT then asks for information about that allocation approach to assess its validity. However, as is common in many areas of veterinary science and food safety, this *a priori* assumption often does not apply and therefore it is necessary to also ask, "*Does the study describe randomization to group?*"

As with any assessment of comprehensive reporting, we did not make quality assessments. For example, we did not assess whether the approach for allocating piglets to treatment groups reduced bias; rather, we assessed whether allocation was reported. Such information should be reported to enable an informed end user to assess whether the approach controlled for potential biases. This distinction is particularly important when assessing control for methodological heterogeneity, including factors to control for confounding (randomization, blocking, and stratification) and factors to control for misinformation bias [i.e., blinding (REFLECT items 8-11)]. If an article described randomization but failed to describe the approach to randomization, we assumed a formal randomization system was used. If authors reported controlling for a covariate, we assumed it was either by blocking (for continuous outcomes) or stratification (for categorical outcomes), although authors rarely provided this information. Similarly, if authors stated that outcome assessors were unaware of the animal's group status, we did not assess the validity of this statement and assumed it applied to all outcomes.

We assessed the reporting of statistical analyses (REFLECT checklist item 12) according to the guidelines described by Lang and Altman (Lang and Altman, 2013). We considered statistical analyses fully reported if <u>all</u> of the following were provided:

- A full description of the main methods for analyzing the primary and/or secondary objectives of the study;
- Clear methodology used for each analysis, rather than just listing in one place all the statistical methods used;
- A description that data conformed to assumptions of the test used to analyze them. In particular, specifications that 1) skewed data were analyzed with nonparametric tests, 2) paired data were

analyzed with paired tests, and 3) the underlying relationship analyzed with linear regression models was linear;

- Whether and how any allowance or adjustments were made for multiple comparisons (performing multiple hypotheses tests on the same data) when the reported results suggested that such an adjustment was necessary. For example, when studies reported comparisons of multiple time points or trials with more than three arms, we expected a report of the approach to adjusting for such pairwise comparisons, that is, Tukey's, Bonferroni's, etc. If authors did not report the approach but did report that an adjustment was conducted, this was considered full reporting;

- For *t* tests only, whether tests were one- or two-tailed, and justification for the use of one-tailed tests;

- Description of the alpha level (e.g., 0.05) that defined statistical significance;

- The name of the statistical package or program used in the analyses. In this situation, we considered reporting full, even if only the program, rather than the package, was reported; for example, both SAS® and SAS® PROC MIXED were considered full reporting.

If at least one but not all of the above were reported, then we considered statistical analyses partially reported. We did not assess study flow (REFLECT checklist item 13), because we expected that studies will be short in duration and there is unlikely any loss to follow-up that would occur. We however paid special attention to studies that described assessing an adverse event such as wound healing 7, 14, or 21 days beyond a procedural event.

The presence or absence of each REFLECT checklist item was independently evaluated by two reviewers (AOC, SG, or RSD). Conflicts were resolved by one of the reviewers (RSD). Where there was disagreement between reviewers about the presence of a checklist item, the reviewer would re-evaluate the article. If this approach did not resolve the conflict, then the item was discussed with the senior reviewer (AOC). The external review panel and ISU steering committee did not verify the presence of the REFLECT checklist items.

2.8.3.5. Reporting of procedures, trial characteristics, study design features, and summary measures

The procedures of interest were castration, tail docking, teeth clipping, and ear identification methods such as ear notching and ear tagging performed on piglets that were between 1 and 28 days old. As some sources of heterogeneity are domain specific, we also specifically assessed several sources of heterogeneity identified by the ISU steering committee as information that should be extracted from the pain mitigation studies *per se* and that were used in the GRADE process.

Expected sources of clinical heterogeneity captured included country where the study was performed, type of production system (i.e., all-in/all-out, continuous flow, not reported), and facility types where the research was conducted (i.e., university-owned farm, laboratory/research facility, privately owned/commercial operation, not reported). We explicitly evaluated descriptors of animal populations such as age and weight, if authors reported descriptors of outcomes such as number of animals in the trial, number of animals in trial arms, and statistical descriptions of the outcomes such as units for measurement, means, standard deviations, standard errors for continuous outcomes, and comparative characteristics for categorical outcomes expressed as either rates or proportions. Such information would be needed to assess the magnitude of effect so that the balance of benefits and harms could be evaluated (which cannot be evaluated by *p* values) and to enable sample size calculation for prospective study design. The external

review panel and ISU steering committee verified the presence or absence of the study design features, trial characteristics, and descriptors of outcomes.

2.9. **Synthesis and presentation of evidence**

The review protocol proposed that summary effect measures would be calculated and presented where possible. However, the data were very sparse, and the approach to presenting the data analysis for the meeting was limited to descriptive analysis that combined the results into forest plots. Further, although many studies reported measuring outcomes, the data were frequently reported in a manner where extraction of the results was not possible. The approach to data analysis and presentation was as follows:

For castration and tail docking studies, forest plots for 14 possible outcomes for each procedure and intervention type were generated in RevMan. The outcomes were cortisol; norepinephrine; β-endorphins; vocalization [frequency (Hz), energy (dB), rate], and pain-related behaviors, all under 60 minutes and between 1 hour and 24 hours.

Intervention types were very broadly organized as general anesthetics, local anesthetics, and NSAIDs.

Assessment of publication bias would be conducted through generation of funnel plots. However, we did not use tests of funnel plot asymmetry, because the number of studies per intervention was insufficient (< 10) (Sterne et al., 2011). Analyses were conducted in R using the Metan package (Schwarzer, 2012) and RevMan.

Materials available to the panel members included a summary of the literature search, a list of outcomes reported by the papers, a summary of the outcome ranking conducted prior to the meeting, meta-analyses prepared in RevMan software, summary of finding tabs prepared in GRADEpro, and evidence profile tables prepared in GRADEpro to assist them in grading the evidence and making decisions.

2.10. **Specification and integration of values**

With respect to the integration of values, the application of processes that are designed for human health to an animal welfare issue meant that two aspects needed to be clarified: "When assessing benefits and harms, who was the target audience?" and "When assessing values and preferences, who was the target audience?" (Schunemann et al., 2006a). This issue was discussed at a steering committee meeting on January 14, 2013. The steering committee was asked to decide which information would be assessed as part of the recommendation-making process.

With respect to information that would be assessed, two key decisions were made. It was decided that the starting point for discussion would be 1) the balance of benefits and harms related to outcomes measured on the pig and 2) the values and preferences of the public, which include a mix of the consumers of pork and citizens.

At the panel meeting, these ideals were discussed again after two presentations were provided to further help panel members understand this aspect of the process. A presentation was delivered by Dr. Raymond Anthony, entitled, "Integrating Science into Socio-ethical Deliberations on Animal Welfare and Care Policy: How Can Instruments like GRADE Help to Mitigate Pain in Farmed Pigs." This discussion focused on epistemological and normative considerations in relation to US public attitudes toward animal welfare and broader societal concerns (food security, safety, environmental soundness of animal agriculture) and the importance of GRADE in addressing values and preferences in a climate of ethical pluralism, risk, and uncertainty. A second presentation was provided by Dr. Glynn Tonsor of Kansas State University with a focus on the economics of animal welfare issues. This presentation discussed differences between voter (citizen) and consumer preferences, manifest and theoretical willingness to pay, and manifest and

theoretical cost to adopt. Both presentations included discussions of potential biases in the evidence base. A systematic review of values and preferences of consumers or citizens was not conducted.

2.11. **Making judgments about desirable and undesirable effects**

With respect to the balance of benefits and harms, it was clarified at the meeting that, for this meeting, the target audience for values and preferences would be consumers of pork, and for benefits and harms would be outcomes that affect the pig.

2.12. **Taking account of equity**

This component was not explicitly considered in the review, although concerns about the capacities of producers to deliver good welfare were discussed in relation to the role of consumers and supportive prices. The panel agreed that more studies on the relationships between on-farm animal welfare pain assessment, animal welfare guidelines, procedures, and regulations are needed to ensure the burdens and benefits are equitably distributed between producers (of all stripes), agribusinesses, consumers, and livestock animals, and to expose the critical intersections between human and animal welfare.

2.13. **Grading evidence and recommendations**

The approach to grading the quality of the evidence was that recommended by the GRADE working group (Balshem et al., 2011; Guyatt et al., 2011a; Guyatt et al., 2011b; Guyatt et al., 2011c; Guyatt et al., 2011d; Guyatt et al., 2011e; Guyatt et al., 2011f; Guyatt et al., 2011g; Guyatt et al., 2011h; Meerpohl et al., 2012; Andrews et al., 2013a; Andrews et al., 2013b; Brunetti et al., 2013; Guyatt et al., 2013a; Guyatt et al., 2013b; Guyatt et al., 2013c). Briefly, the GRADE system separates the process of grading scientific evidence for making recommendations from the process of making recommendations. Scientific evidence is graded based on the presence or absence of inconsistency, indirectness, imprecision, and risk of bias existing in the evidence base that contributes to each outcome. The evidence base is considered to show evidence of inconsistency if there is a wide variation in point estimates, lack of overlap in confidence intervals, or evidence of heterogeneity among studies. The evidence base is considered to have evidence of indirectness when the study populations, interventions, or outcomes used in the primary research differ from that which where defined in the scope of the review. The evidence base is considered to have evidence of imprecision if the studies have wide confidence intervals, which could result from a sample size that is smaller than the number generated by a conventional sample size calculation for an adequately powered trial. The evidence base is considered to have evidence of risk of bias if the studies in the review fail to report concealment of allocation, blinding, have incomplete accounting of subjects, large loss to follow up, show selective outcome reporting, or other factors such as recruitment bias, stopping early, or using invalidated outcome measures. However, for this project, we limited the inference to bias due to failure to control for confounding. The design tools we expected to be used to ensure exchangeability of the groups were randomization, stratification for categorical factors (such as sow or litter) or blocking for continuous factors (such as weight or age), and blinding.

For each procedure and intervention combination, the quality of work for each available outcome was graded. The categories considered were risk of bias, indirectness, inconsistency, imprecision, and publication bias. When any of these factors were considered to be present, the body of evidence was downgraded. Factors that upgraded the body of evidence were direction of confounding, a large effect, and evidence of a dose response relationship.

The panel members were required to vote for each section. If a consensus was reached, the group voted on the next item. If a consensus was not reached, the panel was informed that consensus was not reached and discussion continued. The facilitator would often invite people to provide their rationales for a particular vote option or to discuss the GRADE term further. After discussion, another vote would occur. In circumstances where consensus could not be reached, the vote was delayed until other issues had been the

topic of voting. The process would continue until all outcomes for each procedure and intervention combination had been assessed, and then the panel used this information in the development of recommendations.

All participants were required to vote on the following domains with respect to each outcome for each intervention and procedure:

- Risk of bias (Low or Serious or Very Serious)
- Indirectness (Low or Serious or Very Serious)
- Inconsistency (Low or Serious or Very Serious)
- Imprecision (Low or Serious or Very Serious)
- Evidence of publication bias (Undetected or Strongly Suspected)

With respect to grading the evidence, it was not possible for the panel to reach a consensus on numerous occasions about the quality "imprecision." A large amount of time was spent trying to understand the definition of imprecision. The facilitator suggested that imprecision should be based on the number of studies and the width of the confidence interval. In particular, if the range of the confidence interval suggested different clinical implications, this would be considered evidence of imprecision. The solution to this impasse was to include the two rankings. For example, if the panel could not reach consensus and the votes were split between serious and very serious, we would consider that uncertainty in the next voting stage. All participants were then required to vote about the entire body of work, each intervention, and procedure:

- Quality of body of work (Very low or Low or Moderate or High)
- Absence of high-quality evidence (Yes or No)
- Uncertainty about the balance of benefits and harms for the piglet (Yes or No)
- Uncertainty about the values and preferences of the consumers of pork (Yes or No)

Finally, after having established these concepts, the panel was asked to vote on the following:

- For or against the intervention (For or Against), and then
- The strength of the recommendation (Weak or Strong).

2.14. **Taking account of costs and resources**

Panel members were asked to incorporate economics into the consideration of values and preferences. As the costs of interventions must be absorbed by either producers or consumers, these costs affect willingness to pay and willingness to adopt. The process involved both weighing costs and benefits and weighing the costs against each other. With respect to resources, panelists were asked to not incorporate the availability of products into the consideration of values and preferences. Instead, resource implications were added to the judgment. Largely, this discussion was limited to the factors affecting the legal availability of products, as some products included in the review were not registered for use for pain mitigation in the US, although they are registered in Canada and in many countries in the European Union.

2.15. **Applicability, transferability, and adaptation of guidelines**

Our goal was to provide the evidence and protocol in a format that would enable others to use the evidence. We provided the protocol to CAMRADES for publishing and intend to publish the review of evidence and results of grading as different publications to clearly differentiate between the two aspects and enable others to use the evidence to develop their own guidelines based on local values and preferences.

2.16. **Training in GRADE and systematic reviews for panel participants**

As indicated in prior publications, it is important that panel members are familiar with evidence synthesis approaches, which include systematic review and the GRADE process. Opportunities to learn about the systematic review and guideline development were provided several times prior to the panel meeting and are summarized below:

- The review facilitator provided the ISU steering committee with a presentation about research synthesis and recommendation making at each full-day planning meeting. The ISU steering committee was already very familiar with systematic review processes, so this topic was not the focus.

- The protocol outlining the review process was distributed, and the participants were asked to comment. This step introduced a formal approach to defining the scope and review process.

- Participants were asked to rank outcomes in a formal survey. This step illustrated that multiple outcomes would be assessed.

- The ISU steering committee and the external panel members, other than the economist and producers, were required to verify the validity of the extracted data for several papers included in the review. As well as providing an opportunity for data validation, this process enabled panelists to become familiar with the formal approach to data extraction used in the systematic reviews.

- A video produced by the facilitator describing the data extraction software and the approach was provided. This process enabled panelists to become familiar with the formal approach to data extraction.

- Several 5- to 15-minute videos were created by the facilitator and made available 7 days prior to the panel meeting to introduce the GRADE approach and the rationale behind panel member selection. The videos were available to the ISU steering committee and the external panel members via YouTube. This process enabled panelists to become familiar with the formal approach to guideline development.

- The first half of the panel meeting was devoted to discussing evidence synthesis and recommendation development. Topics covered in these sections included identification of sources of bias in scientific research; how to read a meta-analysis forest plot; and the domains for assessing quality of research: bias, consistency, imprecision, indirectness, publication bias, magnitude of effect, the potential direction of confounding bias, and evidence of a dose-response effect. The discussion also covered the domains for recommendations: quality of evidence, values and preferences, balance of benefits and harms to the individual, and resource implications. This process enabled panelists to become familiar with the formal approach to guideline development.

- Presentations about values and preferences were included in the meeting. This process enabled panelists to become familiar with the formal approach to guideline development.

2.17. Other aspects

The structure of the reports, methods of peer review, planned methods of dissemination, and implementation agreed upon were as follows: The review team would create a draft of the report with three distinct sections describing research gaps, guideline development, and translations issues. All members of the ISU steering committee were responsible for commenting on and correcting this draft. This draft would then be shared on-line for comment from the external panel. After the comment period had ended, the ISU steering committee met again to discuss the comments, and then ISU steering committee members were assigned aspects of the review to finalize: research gaps, guideline development, and translation issues. The review team would then combine the three sections into a final report and share it with the external panel members for comment.

2.18. The panel meeting

The panel meeting was held at the Renaissance Hotel in Des Moines, IA, US, from February 12 to 14, 2013. The meeting was chaired by the review coordinator, Dr. Annette O'Connor. February 12th and 13th were full days, and the meeting concluded at noon on February 14, 2013.

3. Results

3.1. Participants: Review team, ISU steering committee, and external participants

Twenty-one people were present at the meeting. The list of participants, including the expertise to which they were allocated, is listed in Table 1. Clearly, some participants had multiple fields of expertise, whereas others did not indicate a field of expertise. All 21 people were present on February 12 and 13, 2013. One participant (Dr. Johann Coetzee) was unable to be present on the final day, February 14, 2013. Three people (AOC, RSD, and SG) were not required to vote.

3.2. Research gaps: Identifying the scope of scientific information currently available on pain mitigation strategies in piglets and research gaps

3.2.1. Summary of procedures studied

The frequency count of studies identified for the procedures of interest are shown in

Table 5. In 40 studies, piglets underwent castration only. In seven studies, piglets underwent tail docking only. In one study, piglets underwent teeth clipping only, and in one study, piglets underwent ear notching only. There was an overlap in three studies. In one study, piglets underwent three procedures: tail docking, ear notching, and teeth clipping. In two studies, piglets underwent both castration and tail docking (see Figure 1 for study flow diagram).

3.2.2. **Summary of outcomes studied**

The frequency counts for the number of studies that reported the outcomes of interest are provided in

Table 2. Many outcomes identified as potentially of interest were not reported. The results of the ranking of relevant outcomes important to the review are shown in

Table 3. Only electroencephalogram readings (EEG; < 60 minutes), haptoglobin (1 to 24 hours), substance P (< 60 minutes, and 1 to 24 hours), playing activity (1 to 24 hours), feeding and nursing (1 to 24 hours), and vocalization: peak amplitude (< 60 minutes; dB) and peak frequency (< 60 minutes; Hz) were considered critical. Cortisol was considered an important non-behavioral outcome under 60 minutes. For the 1–24-hour period, the vote was split evenly between critical (3 of 9, 33%), important (3 of 9, 33%), and unimportant (3 of 9, 33%). However, cortisol was the most reported outcome in the scientific literature. Of the non-behavioral outcomes that were considered critical, substance P was reported in one study, whereas haptoglobin was not reported in any study. EEG readings were reported in one study, but the outcome failed to meet the eligibility criteria. Of the behavioral outcomes voted critical, playing and nursing/suckling were measured in three studies.

For some outcomes, the units of measurement varied. Vocalizations were frequently reported as a measure of acute pain, especially in piglets that were being castrated. However, there were considerable variations in the methods of assessment of vocalization, descriptions of vocalization measures, and the units used to report vocalization. For example, (Courboulay et al., 2010) used a sonometer to record the "intensity of cries" whereas (Marx et al., 2003) used a microphone with digital software to record the maximum amplitude of a call. Both outcomes shared the same units (dB); however, the latter mean was reported as a negative value. In another example, (Rittershaus et al., 2009) reported an increase in sounds per second while(Kluivers-Poodt et al., 2012) reported the number of calls per second. (Marx et al., 2003) compared the number of grunts and the number of cries between treatment and control groups. The conclusion was to only extract vocalization data in which hertz (Hz) units were described, signifying a measure of vocalization frequency; decibels (dB), signifying a measure of vocalization energy; or a measure of vocalization rate, such as squeals per second.

3.2.3. **Summary of interventions studied**

For the intervention report, the unit of concern was the study arm, so the count refers to the number of arms in which a particular type of intervention was studied. Sometimes within a study, factors that differentiated arms were minimal. For example, one study assessed the differences between the same anesthetic agent delivered while the animal was in ventral or dorsal recumbency (Zimmermann et al., 2011); this was still considered to be a study with two arms.

The interventions assessed were largely pharmacological, classified as general anesthetics using inhalants, tranquilization, or sedation using a combination of products; local and topical anesthetics; and NSAIDs. The results of the research gaps for pain mitigation strategies comprise the interventions by procedure: general anesthesia for castration (Table 6), local anesthesia for castration and tail docking (Table 7), and NSAIDs for castration and tail docking (

Table 8). General anesthetic interventions assessed more than once were CO_2/O_2 mix (six trial arms), 100% CO_2 (five trial arms), isoflurane mixtures (three trial arms), ketamine mixtures (three trial arms), and halothane mixtures (two trials arms). The outcomes assessed in these trial arms often differed. NSAID protocols assessed more than once were meloxicam (14 trial arms), flunixin meglumine (eight trial arms), and ketoprofen (three trial arms). Local and topical anesthetic interventions assessed more than once were lidocaine (14 trail arms), procaine (two trial arms), and butanilicaine phosphate (two trial arms).

Not all interventions assessed were registered or legal for use in the US, such as metimazole used by Langhoff et al. (2009). Two non-pharmacological interventions were also identified. One publication (with four studies) assessed the impact of oral 12% sucrose (Rand et al., 2002), and another study assessed the impact of prenatal stress by mingling groups of multiparous sows twice during gestation (Rutherford et al., 2009).

3.3. Reviewing the state of scientific evidence and developing recommendations about pain mitigation strategies in piglets undergoing routine processing procedures

Based on the criterion that more than one study from two or more authors had to be available to make a judgment about an intervention, it was only possible to assess interventions applied to piglets that underwent castration, but not tail docking. The 14 possible outcomes for each intervention type were cortisol; norepinephrine; β-endorphins; vocalization [frequency (Hz), energy (dB), rate]; and pain-related behaviors, all within 60 minutes, and between 1 and 24 hours. The interventions available for grading were general anesthesia (mixtures of CO_2/O_2), local anesthetics, and NSAIDs. Not all outcomes were reported for the interventions were available for grading. The individual results of the panel votes are shown in Table 9.

3.3.1. Intervention: General anesthesia - CO_2/O_2

The only inhalation anesthetic protocol that met the selection criteria was carbon dioxide mixed with oxygen to provide CO_2/O_2 anesthesia. A decision was made at the panel meeting to combine the recommendation about CO_2/O_2 anesthesia regardless of differences in application, doses, and flow rates. The GRADE summary of findings that summarizes the changes in outcomes associated with the intervention is provided in Table 10.

The panel assessment of the quality of the body of work is reported in the evidence profile (Table 11). **The panel's current position is a strong recommendation against the use of a CO_2/O_2 general anesthesia mixture for pain mitigation during castration in piglets between 1 and 28 days old.** The recommendation, rationale, and proposal for review are included in Table 12.

The outcomes reported that were available for grading regarding CO_2/O_2 anesthesia were cortisol (within 60 minutes, and between 1 and 24 hours) and β-endorphins (within 60 minutes). For β-endorphins, the panel could not reach a consensus about imprecision.

3.3.2. Intervention: Non-steroidal anti-inflammatory drugs

Several NSAIDs contributed to this body of work. These included meloxicam, flunixin meglumine, and carprofen. The outcomes available for voting were cortisol (under 60 minutes, and between 1 and 24 hours), vocalization [energy that had decibels (dB) as units and pain-like behaviors between 1 and 24 hours]. The GRADE summary of findings that summarizes the changes in outcomes associated with the intervention is provided in Table 13. The panel assessment of the quality of the body of work is reported in the evidence profile (Table 14). **The panel's current position is a weak recommendation for the use of NSAIDs for pain mitigation during castration in piglets between 1 and 28 days old.** The recommendation, rationale, and proposal for review of the NSAID recommendations are included in Table 15.

Indirectness was not voted upon for this intervention, because it was generally agreed that no serious indirectness of evidence was demonstrated. The very narrow target population, and votes of "low" applied across all outcomes and all interventions were factors in this decision. No consensus was reached about imprecision for either vocalization measures.

3.3.3. **Intervention: Local anesthesia - lidocaine**

Lidocaine was the local anesthesia pain mitigation strategy reported. The only outcome available for voting was vocalization [energy reported as decibels (dB) units]. The results of the panel votes are shown in Table 9. Indirectness was not voted upon under this intervention, as it was generally agreed that no serious indirectness of evidence was presented for this question. The consensus (75%) was that there was evidence of low risk of bias among the studies that reported this outcome. There was also consensus (75%) that the evidence demonstrated a low inconsistency. The panelists agreed (94%) that the overall quality of the body of work was very low. **Based on the reviewed evidence, the panel presented a weak recommendation against the use of lidocaine as a pain mitigation strategy for 1–28-day-old piglets undergoing castration.** The summary of findings, evidence profile, and recommendation are provided in Table 16, Table 17, and Table 18.

3.4. **Other findings: Comprehensive reporting**

Fifty-two studies (from 40 full articles) were assessed for comprehensive reporting. The unit of concern for reporting was a study. Two or more studies were occasionally reported in one article.

3.4.1. **Reporting consistent with REFLECT guidelines**

As described above, we used the REFLECT statement to assess the comprehensiveness of reporting. A summary of the reported, partially reported, or not reported items on the REFLECT checklist are shown in Table 19, Table 20, Table 21, and Table 22. Below, we provide a short narrative, and examples when the item was reported, using papers that may have been included in the review.

3.4.1.1. Abstract or title items

Abstract or title: Item 1: Allocation of study units (i.e., piglets) to treatment groups was reported in five studies from two articles (Rand et al., 2002; Hansson et al., 2011), although only the data from Hansson et al. (2011) was included in the meta-analysis because Rand et al. (2002) used sucrose, which was not an NSAID, general anesthetic, or local anesthetic. Descriptions were made in the abstracts only.

*"Piglets were **randomly** assigned to receive 1.0 ml of a 12% sucrose solution (treatment group) or a placebo (1.0 ml of air) administered via syringe in the mouth, 60 s before commencement of one of the management procedures."* (Rand et al., 2002)

*"Four male piglets in each of 141 litters in five herds were **randomly** assigned to one of four treatments: castration without local anaesthesia or analgesia (C, controls), analgesia (M, meloxicam), local anaesthesia (L, lidocaine), or both local anaesthesia and analgesia (LM). Lidocaine (L, LM) was injected at least three minutes before castration and meloxicam (M, LM) was injected after castration. During castration, vocalisation was measured and resistance movements judged."* (Hansson et al., 2011)

A descriptor in the title or abstract will "allow easy identification of this study design for people conducting electronic data searches to identify evidence for the efficacy of interventions, and for those conducting systematic review." (Sargeant et al., 2010a). Failure to control for confounding increases the potential for bias in a study. A title example not part of this review is shown below.

"Study design: Prospective, randomized study" (Hodgson, 2006)

3.4.1.2. Introduction items

Items 1 and 2 of the REFLECT statement were not assessed.

3.4.1.3. Methods and Materials items

Participants: Item 3: The eligibility criteria, settings, and locations of the studies were seldom reported. We considered this item partially reported if at least one but not all of these items were reported. No study described eligibility criteria for the piglets. Twenty-three studies explicitly reported the country where the study was conducted as the United States (2), Australia (4), Germany (8), France (3), Belgium (1), Brazil (1), Switzerland (2), or Sweden (1). Thirty studies did not explicitly report the country in which the study was conducted. Examples of locations reported include the following:

"Sows were housed in commercial farrowing crates on a commercial farm in Saxony-Anhalt, Germany." (Marx et al., 2003)

"The investigation was conducted at the Futterkmap teaching and research center of the Agricultural Commission of Schleswig-Holstein..." (Muhlbauer et al., 2009)

"An experiment was conducted on a commercial farm in the municipality of Holambra, São Paulo State." (Cordeiro et al., 2012)

Study setting was described in less than one-half of the studies: university-owned farm (7), laboratory/research facility (6), privately-owned/commercial operation (5), or not reported (35). No single article reported all criteria.

Interventions: Item 4: As expected, most interventions were pharmacological in nature. Two articles reported non-pharmacological interventions, that is, the use of sucrose (Rand et al., 2002) and stressing of multiparous sows (Rutherford et al., 2009). For pharmacological interventions, we extracted the name, concentration, and dose of the drug, as well as the volume, route, and frequency of the administration of the drug if available. If at least one but not all of the above were not described, we considered the intervention information partially reported. Details of the intervention used in the treatment arms were adequately reported in 21 studies. For studies that assessed NSAIDs, it was occasionally difficult to determine the actual dose given to the piglets, especially when the piglet weights were inadequately described (Table 21). Many studies described the time at which an intervention was given. For example:

"Two groups were treated with Flunixin (5 mg); the group termed Flu-30 received an i.m. injection of Flunixin 30 min before castration and of 0.1 ml NaCl (0.9%) immediately before castration, the group termed Flu-0 received 0.1 ml NaCl (0.9%) 30 min before castration and Flunixin immediately before castration." (Reiner et al., 2012)

Often the number of doses was not explicitly described in a study. It is assumed that interventions were given once, mainly because of the short duration between the administration of the intervention, occurrence of the procedure, and collection of the outcome of interest.

Objectives: Item 5: An attempt was made by each author to describe the objective of the study, and sometimes a secondary objective was listed. Very few studies translated the objective into a testable hypothesis. Knowing the exact metric that will be tested is important, because some outcomes are considered critical, important, and not important; therefore, it is helpful if the metric is included in the hypothesis. For example, the objective may be to assess the impact of the intervention on pain mitigation, whereas a hypothesis translates this objective to clarifying that the impact will be measured using the metric energy, that is, the mean energy (Hz) of vocalizations was equal in piglets receiving the intervention

compared to the mean energy of vocalizations in piglets without the anesthetic: H_0 = mean1 - mean2 = 0. Clarification of the hypothesis ensures that the end user knows the metric being used to assess the objective and should identify the primary outcome if the sample size rationale is not provided.

We were unable to determine the objective in a few studies, because they were either not clearly stated or they might have been available in the last paragraph of the introduction of an article that was not translated. To be considered fully reported, objectives had to be associated with a hypothesis that related to the outcomes. An example of a well-described objective and hypothesis is shown below.

"The objective of this study was to evaluate the effect of providing CO_2 anesthesia before castration on the behavior of piglets for up to 8 d after castration in comparison with piglets castrated without anesthesia…The hypothesis of this study is that piglets will experience less pain and discomfort after castration when anesthetized with CO_2 before castration, thus improving their overall welfare." (Beirendonck et al., 2011)

Most studies assessed more than one outcome as a measure of piglet "pain and discomfort." Clearly defining objectives and hypotheses, and linking them to outcomes was considered important in the decision-making process.

Outcomes: Item 6: We were able to identify the outcomes of interest in the studies included in the review. However, a clear description of which outcomes were primary or secondary was never explicitly reported by authors that assessed multiple outcomes. For studies that assessed only one outcome, we assumed that this was the primary outcome. For example, expression of c-Fos-positive neurons was the only outcome assessed by Nyborg et al. (Nyborg et al., 2000). Some studies that reported behavioral outcomes reported the observation technique. For example:

"Pens were observed at random within a sequence according to a scan sampling procedure. The duration of each observation period (i.e., 10 min multiplied by the number of pens) was considered adequate to achieve the objective of the present study." (Beirendonck et al., 2011)

"... and the behavior of each individual pig was recorded using 1 min scan samples (direct observations) for 120 min." (Sutherland et al., 2011)

Knowledge of the primary outcome is necessary to assess the power of the study. Unless explicitly declaring that a study is a pilot or is designed to generate a hypothesis, assessments of interventions should be hypothesis driven. The hypothesis should be specific enough to enable determination that the number of animals enrolled should be sufficient to enable detection of a clinically meaningful difference in the outcome. Researchers therefore should prospectively design and justify the sample size, which requires knowledge of the primary outcome. Further, the primary outcome should be directly linked to the objective and the null hypothesis. If authors do not have an *a priori* hypothesis about a set outcome, the potential to "data mine" for statistically significant differences and selective reporting bias is high. As no studies in the review explicitly identified the primary outcome, the example below is from a different topic area.

"The primary outcome was IBK cumulative incidence over the study period. The secondary outcome was weaning weight." (Funk et al., 2009)

Sample size: Item 7: No studies reported the rationale for the sample size. This was surprising, as all studies seemed to purposefully assess the effect of an intervention on an outcome; therefore, the number of animals needed to detect the magnitude of effect of interest is a prerequisite step in study design. The example below is not from the group of studies reviewed.

"Prior to conducting the study, it was determined that twelve animals per group were required to obtain 80% power to detect a 60% difference in IBK risk between groups based on an expected 10% IBK risk in controls and at least 70% IBK risk in inoculated animals. The test was based on a one sided difference in proportions test for independent binomial data with significance level 0.05. Thus, our aim was to enroll 36 animals. No stopping rules or interim analyses were planned or conducted." (Gould et al., 2013)

Randomization: Items 8–10: Each of these items relates to the approach to allocation based on the assumption that the study is randomized. A description of the method of developing the randomization (sequence generation, allocation concealment, implementation) was never provided in any study. An example of descriptions of these items can be found in the article by Sargeant et al. (Sargeant et al., 2010a). However, 33 of 52 studies used the term "randomly," "randomized," or "random" in their description of piglet allocation to a treatment group. Occasionally, it was unclear whether the approach used was truly random, despite a description as such. For example, Langhoff et al. (Langhoff et al., 2009) described randomly assigning 245 clinically healthy piglets to one of 12 experimental groups. However, the sample sizes in each of the seven relevant arms were quite different. Moreover, it appeared as if there were actually two studies reported, one in which cortisol was assayed and one in which piglet behavior was assessed, as shown below:

1. For piglets that had cortisol assessed after undergoing castration:
   Group castration NaCl, n=28
   Group castration meloxicam, n=25
   Group castration flunixin, n=26
   Group castration carprofen, n=15

2. For piglets that had behavior assessed after castration:
   Group castration NaCl, n=10
   Group castration meloxicam, n=10
   Group castration flunixin, n=10

Blinding: Item 11: Blinding (whether for allocation of treatments or interventions or assessment) was infrequently reported by authors. Of the 52 studies, 18 explicitly reported whether blinding was incorporated; however, none provided a full description of the approach used to blind the study or what task was blinded. The examples below are illustrative of the value of comprehensive reporting. Because the authors provided this information, end users can assess the impact of bias, which is far preferable to having no information about potential bias.

"Two technicians, who were not blind to the treatments due to practical reasons, performed all measurements. The measurements were split between the two technicians with each technician performing the same measurements in all herds." (Hansson et al., 2011)

"The study was performed "blindly", only the operator performing the injection being able to associate the S, C, M and T treatments to the color codes." (Wavreille et al., 2012)

Statistical methods: Item 12: An effort was made by many authors to report statistical analyses. Statistical methods were not reported in eight studies. In the remaining 44 studies, statistical methods were considered partially reported, because they failed to meet <u>all</u> the criteria described above. An assessment of comprehensive reporting of statistical methods is very difficult, as the measure of comprehensiveness is that a reasonably informed individual would be able to assess the validity. Some examples are shown below.

"Least square mean estimates for each treatment group and the corresponding estimated SE are reported. Pairwise comparisons were conducted using Bonferroni's method to adjust for multiple

comparisons and avoid inflation of Type I error rate. Statistical significance for these multiple comparisons was designated a priori as a P-value ≤ 0.05" (Coetzee et al., 2012)

"A condition of the applied statistical model is that the performance of the empirical estimation passes the convergence test (SAS Inst. Inc.). Hence, lateral lying, ventral lying, and sleeping were grouped under 'lying,'; teat seeking, suckling, and udder massage were grouped under 'udder activity;' huddled up, trembling, spasms, scratching, and tail wagging were grouped under 'pain related behaviors;' nosing, chewing, licking, playing, and aggression were grouped under 'interaction behaviors;' walking and running were grouped under 'walking;' and sitting, standing, and kneeling were grouped under 'postures'..." (Beirendonck et al., 2011)

"Data were not normally distributed and were dichotomized using the median as cut-off value. The binary data were analyzed using the logistic mixed model, with fixed effects being treatment, observation period and the interaction between treatment and observation period, as well as piglet BW and age at castration, with the piglet as random effect. Random effects accounted for the variability between the piglets within and between litters. The applied procedure made it possible to allocate a random effect to a variable (SAS Inst. Inc.), so that piglets could be regarded as the experimental units." (Beirendonck et al., 2011)

"For physiological measures, the main fixed effects were treatment and time. Litter was a random effect. The interactions between treatment by time and treatment by litter were included in the model." (Sutherland et al., 2012)

3.4.1.4. Results items

Recruitment: Item 14: Dates relevant to the study performance were described in six studies. Such information is useful for understanding the impact of season or year on an outcome. Although it is difficult to envision how year or season could affect the response of swine to pain mitigation, such information is very relevant for other topics. It is important to report both dates and study location (i.e., country, region), since season and environmental changes may influence the results. Examples from the articles include the following:

"This study was carried out on a 600-sow commercial swine operation between May and November 2011." (Tenbergen, 2012)

*"The study was conducted between October 2009 and February 2010 in five piglet-producing herds in the south-central part of Sweden." (*Hansson *et al., 2011)*

"The studies were conducted in two piglet breeding operations (Unit A 550 breeding sows, two-week production cycle; Unit B 560 sows, four-week production cycle) from February 2003 to May 2003" (Lahrmann et al., 2006)

Baseline data: Item 15: Baseline demographics and clinical characteristics of each group were generally poorly reported. Where weight and age information was presented as a summary measure, we considered this to be partially reported. Interestingly, this information was frequently reported in the Methods section and not explicitly in the Results section. REFLECT and other statements make the distinction that the Materials and Methods could, and potentially should, be written before the study is started, therefore the demographic information of the study groups such as the mean age (and standard deviations) and mean weight are results and should be presented in the Results section. See Figure 2 from Rutherford et al. (2009).

Numbers analyzed: Item 16: The actual number of piglets that contributed to the data analysis was

frequently not reported. Presumably, authors felt that reporting the number of enrolled animals would suffice; however, this is often insufficient. It requires an assumption that no losses to follow up occurred, something rarely stated explicitly. Further, sometimes the unit of analysis is not the same as the number of animals in the study. This was particularly important for the behavior data that could be reported as the number of pigs that demonstrated an activity or the number of time periods where an event was observed. These clearly have different denominators. Similarly, some outcomes appeared to be measured only on a subset of enrolled animals, perhaps because testing all animals was time consuming or expensive. An example where numbers analyzed was well reported is provided by Tenbergen et al. in Table 4.2 (Tenbergen, 2012). In this study, a subset was selected for analysis of cortisol. It was still unclear how this subset was selected, given the different numbers in each group (i.e., randomly, systematically), or criteria used to select the piglets. See Figure 3 and Figure 4 from Sutherland et al. (2012).

Outcomes and estimation: Item 17: Effect measures regarding outcomes were often poorly reported. It is important to know the magnitude of the effect when decisions must be made about the balance of benefits and harms. If only the p value is reported, it is not even possible to know the direction of the effect (i.e., whether the intervention increased or decreased the outcome). Further, measures of variation were often not reported or not reported clearly, especially in figures where it was not always possible to discern if the bar was an SEM, an SD, or a confidence interval. In studies that used random effects variables to control for clustering, the variance components were never reported, despite their importance for future study design and interpretation.

Ancillary analyses: Item 18: Ancillary analyses were not reported in any study, as primary and secondary outcomes were not reported.

Adverse events: Item 19: The adverse events relevant to the review were herniations, wound healing, morbidity, and mortality. We were able to extract at least one of these adverse events from 15 of 52 studies. These studies were all related to castration. There were no adverse events reported for the studies in which piglets' tails were docked. Only one study described herniation; in this study, a single pig died as a result of an inguinal hernia (Schwab et al., 2012). Seven studies reported mortality, five studies reported morbidity, and seven studies reported wound healing assessed every day for 7, 14, or 21 days. The significance of adverse events was often unclear in these studies. We could not determine if the observed events were due to the intervention or the procedure. Where mortality was described, no information was given regarding historical values of the facility, if applicable. Research gaps associated with poor reporting of summary measures were consistent among studies that reported adverse events. Sometimes adverse events were reported in a way that we could not extract the group to which the animals that experienced the intervention were allocated, as shown below.

"There was not a significant difference between treatment groups with respect to mortality rate. Piglets receiving meloxicam had a mortality rate of 3.18% and piglets receiving the placebo had a mortality rate of 3.84% (P=0.33). Piglets receiving ketoprofen had a mortality rate of 2.91% whereas piglets receiving the placebo had a mortality rate of 3.94% (P=0.27)." (Tenbergen, 2012)

3.4.2. **Reporting of procedures in the literature**

Forty of 52 studies described piglets that underwent castration only. Seven of 52 studies described piglets that underwent tail docking only. One of 52 studies described piglets that underwent ear notching only, and one of 52 studies described piglets that underwent teeth clipping only. In one study, piglets underwent three procedures: tail docking, ear notching, and teeth clipping. In two studies, piglets underwent both castration and tail docking.

Of the 42 studies that described piglets undergoing castration, 26 studies reported the technique of castration performed as either incision of the scrotal sac with a *cut* of the spermatic cord or a *tear* of the spermatic cord. Sixteen of the 42 castration studies did not report the castration technique. All 10 studies that described piglets undergoing tail docking reported the approach to tail docking as the use of side cutters (7), a cauterizing blade (2), or surgical cutters (1). The two studies that assessed teeth clipping and methods of identification that involved resection of ear tissues were from one article (Rand et al., 2002). Both studies that described teeth clipping reported the approach to teeth removal as clipping, rather than grinding or not reported. Similarly, both articles that described resection of ear tissue reported the approach as ear notching rather than ear tagging or not reported.

3.4.2.1. Reporting of trial characteristics

The following trial characteristics were collected on standard forms: year the study was published, country in which the study was conducted, sow management types (i.e., all-in/all-out, continuous flow) and setting for the study (e.g., university-owned farm, laboratory or research facility, privately owned/commercial operation).

Three studies reported the production system in which the piglets were raised as all in/all out compared to 49 studies that did not report the production system. Sow management systems were reported as farrowing crates (14), farrowing pens (1), individual farrowing pens (1), or not reported (37).

No studies described the source population or the study population as selected from the source population. For example, if a large farm had 1,000 sows, no study described the rationale of selection of the particular population of sows included in the study, that is, whether sick sows were excluded. Similarly, no studies explicitly discussed exclusion criteria for piglets, such as weight or health parameters.

3.4.2.2. Reporting of study design features

Issues pertaining to randomization and blinding are described above as part of the REFLECT research gaps. The issue of blocking of study populations as a study design tool for the control for confounding is described here. Blocking was reported in 39 of 52 studies. Covariates controlled were either blocking by weight; litter; weight and litter; sow or weight, litter, and adoption. No study that controlled for weight using blocking explicitly reported the size of the block.

Of the studies that were included in the GRADE guideline development, the prevalence of randomization, blocking, and stratification are included in the GRADE tables (Tables 8–16). In this subset of studies, few studies reported these features. For example, of the three studies that provided data for cortisol under 60 minutes for piglets that underwent castration, two reported randomization, none reported blinding, and one reported blocking.

No studies specifically addressed the issue of parallel design, and it was assumed that most trials were conducted in parallel. Sometimes, the description of the trial clearly indicated that the arms were conducted in parallel. For example, when authors reported "each treatment occurred one in each arm," we assumed these must be parallel. However, on occasion, it was unclear whether studies combined in single analyses were conducted in parallel. For example, two studies separately reported the following trial arms:

Publication 1: (Tenbergen, 2012)

> *"Group 1: males receiving an IM injection of 0.4 mg/kg of body weight of meloxicam, n=743*
> *Group 2: males receiving an IM injection of 0.4 mg/kg of body weight of a placebo, n=756*
> *Group 3: females receiving an IM injection of 0.4 mg/kg of body weight of meloxicam, n=684*
> *Group 4: females receiving an IM injection of 0.4 mg/kg of body weight of a placebo, n=705."*
> The authors reported that all treatments were represented in each litter.

Publication 2: (Tenbergen, 2012)

> *"The two studies involved a combined total of 2,990 male piglets from 997 litters (study 1: 1,499 piglets from 407 litters; study 2: 1,491 piglets from 590 litters). In both studies, piglets were randomly allocated to receive an IM injection of either the analgesic meloxicam (study 1; Metacam®, Boehringer Ingelheim Ltd., Burlington, ON: 0.4 mg/kg) or ketoprofen (study 2; Anafen®, Merial Canada Inc., Baie D'Urfé, QC: 3 mg/kg) or a placebo at least 30 minutes prior to castration. Both treatments were represented in each litter."*

When presented, the results of groups 1 and 2 in Publication 1 were exactly the same as the results of study 1 in Publication 2. It was clear that within a study, the arms were conducted in parallel; however, it was not clear if the two studies in Publication 2 were conducted in parallel. Information about the dates on which the studies were conducted was not included in either publication.

3.4.2.3. Reporting of summary measures

The comprehensiveness of reporting summary measures was generally inadequate for measures such as means, SDs, SEMs, or arm sample sizes. The omission of at least one of these parameters led to the exclusion of many trial arms that had the potential to provide data toward the meta-analysis and evidence analysis. Table 19 shows the frequency of not reporting complete arm data among studies that contributed to the meta-analysis. For example, of the eight relevant study arms that reported assessing cortisol under 60 minutes, two studies did not report means and two did not report SDs or have sufficient information to be able to calculate the SDs in RevMan. It is possible to extract SDs from p values, and indeed these were occasionally reported, but sometimes these would compare multiple arms, some of which were not relevant to the review (e.g., sham arms, arms in which multiple interventions had been applied). It was decided by the ISU steering committee not to extract p values.

Occasionally, studies would describe summary data for one arm and not the comparison arm, or report data on figures. Figures are often considered a useful way to convey information; however, important details were frequently missing from figures. For example, axes were unlabeled or the measure for the error bars was not discernible as the SEM, SD, or 95% confidence interval. For example, of the 15 studies that assessed NSAIDs for castration and reported the outcome for cortisol within 60 minutes, 10 of these reported data with figures only. We used a digital ruler to extract data from figures. For some studies, it was unclear if error bars were SEMs or SDs, since this was not reported in the text. Sometimes error bars were uneven on either end of the mean, making it difficult to have confidence in such a measure, or error bars were stacked on top of each other, making it difficult to measure the size of a single error bar. When authors reported medians and quartiles, these could not be included in the meta-analysis, although the reporting was comprehensive.

4. Discussion

4.1. Discussion about research gaps

Upon completion of the evaluation of the scope of scientific studies pertaining to neonatal management practices that cause pain in piglets, it is evident that the number of studies exploring this research question is sparse, particularly for studies that show an intervention with positive effects on pain mitigation. The lack of scientific evidence, validated methods, and harmonization among researchers in the field makes it very challenging for stakeholders to respond to societal concerns with science-based recommendations for reducing the pain caused during castration, tail docking, teeth clipping, and identification. It would be prudent to rank these management procedures for establishing funding priorities in the US, in regard to the number of animals affected by these procedures, as well as the severity and duration of pain involved. It is

the opinion of the steering committee that since the majority of male piglets are castrated, and the majority of both male and female piglets have their tails docked, funding should be directed toward these procedures first. Teeth clipping and ear notching are less commonly practiced and, hence, lower in priority.

With respect to outcomes measured in studies that assessed pain, many outcomes considered important are frequently not reported. This provides an opportunity for future studies. We are unaware of other reports in which a group of researchers collectively identified outcomes that are relevant to assessment of pain in piglets. Future researchers should consider this list of outcomes when designing studies. It should not be surprising that some outcomes have few or no reports. The body of work is small, and the number of researchers capable of conducting these studies is limited. Further, researchers with expertise in recording of pharmacological indicators may differ from those collecting behavior data, and therefore, the number of outcomes assessed by each group may be limited by areas of expertise. Although promising pain outcomes and associated techniques for measurement are emerging, specific expertise is often restricted to a single or few research teams without replication of studies between institutions using standardized methods, as is the mainstay of scientific inquiry. Pain research, particularly in regard to alleviating distress in livestock, is an emerging field of inquiry. While it is a young discipline and consensus has yet to be established on how to measure pain compared to more established disciplines, such as microbiology, reproductive physiology, or nutrition, increased attention on understanding the mechanisms of pain in the animal science and veterinary medicine communities is a sign of the seriousness to address the welfare concerns associated with contemporary standard on-farm procedures. The concept of animal welfare that is adopted, however, that is, whether the emphasis is on standard veterinary measures of good health, normal growth, and reproduction; on positive and negative affective states; or on the ability to lead natural lives (Sandoe and Simonsen, 1992; Fraser et al., 1997) will influence the nature of how pain research is carried out. Regardless of which concept of welfare is assumed or prioritized (Haynes, 2008), the challenge for contemporary swine production is to clearly articulate what constitutes acceptable quality of life for farmed pigs. Doing so requires being mindful that both members of the public and producers alike may have varying understandings and emphases, and that these should not be summarily dismissed as irrelevant or unimportant. To a large extent, producers and the concerned public must attend to the following concerns (Sandoe et al., 2003): "What is a good life for an animal? What is the relationship between dignity and welfare? What are acceptable compromises?" However, animal scientists and veterinarians have their work cut out for them. Here, the challenge, as noted by Professor Marion Dawkins, one of the pioneers of animal welfare science, is that animal welfare researchers are typically caught in a two-stage process, particularly when addressing the welfare of farm animals: to validate fundamental research measures or responses and, before being in a position to use this knowledge in applied research, to address the original concerns, particularly when addressing the welfare of farm animals (Dawkins, 1997).

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage." Hence, pain is by definition an affective state, only known by the individual experiencing it, and can only be measured indirectly in humans and animals. Verbal self-reporting is a common approach to pain assessment in human medicine, whereas behavioral, endocrine, and neurophysiological techniques for assessing pain in non-verbal humans or animals are in their infancy. The body of scientific evidence for pain mitigation interventions during piglet processing collected in our systematic review is characteristic of pain research, namely, several of the studies were designed to explore the fundamental research question, "What are reliable and sensitive measures of pain?" In this context, there is an *a priori* assumption that the procedure (e.g., castration) produces pain; hence, candidate outcome variables should be present when piglets receive the procedure and absent when they do not. Similarly, there is an assumption that these pain outcomes would be reduced or absent when analgesia or anesthesia is provided. In the absence of the fundamental science informing us about the sensitivity and specificity of the neurophysiological, endocrine, or behavioral outcomes associated with pain, we are restricted in our ability to compile a reliable database for efficacy and reliability of pain-mitigating interventions.

To complement the focus on interventional pain mitigation strategies at the time of castration, researchers must begin ranking animal-based outcomes that show an application to pain that the animal is experiencing both at the time of the procedure and during the period afterwards. Upon reflecting on outcomes cited in the literature it became apparent that, although some measures have been used in other species subjected to pain or painful procedures, and the measures are biologically sensible, little to no piglet-specific benchmarking of these measures has occurred. Consequently, there are barriers for interpreting the data with regard to pain experienced by the piglet. For example, β-endorphin was a unit of measure ranked as important by the steering committee because β-endorphins have analgesic properties in the body to mitigate or dull pain. β-endorphins bind to and activate opioid receptors. Similarly, substance P (SP), an 11-amino acid prototypic neuropeptide of more than 50 known neuroactive molecules, including calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), and endothelin (ET), regulates the excitability of dorsal horn nociceptive neurons and is present in areas of the neuroaxis involved in the integration of pain, stress, and anxiety. The absence of SP and CGRP in the dorsal root ganglia of naked mole rats has been shown to selectively reduce responses to painful stimuli. Plasma SP concentrations have been used to evaluate muscle pain, spinal disease, soft tissue injury, and the efficacy of therapeutic agents for osteoarthritis, headache, and fibromyalgia in human medicine. Onuaha and Alpar (2001) (Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)(Onuoha and Alpar, 2001)demonstrated that plasma SP levels were up to 27-fold greater in human patients with soft tissue injury compared to healthy controls, whereas CGRP levels increased 4-fold; plasma NPY and ET concentrations were unchanged. In veterinary species, Zetner et al. (Zetner et al., 1996) found that cortisol levels decreased significantly after handling and periodontal treatment in cats, whereas plasma SP levels remained elevated. These data suggest that plasma SP measurements may discriminate between a stressful event, culminating in elevated plasma cortisol concentrations, and more specific nociceptive stimuli, resulting in increased plasma SP concentrations. However, inherently important questions remain unanswered. For example, what are normal and abnormal levels of β-endorphins and SP in the piglet? Are the modes of action in the piglet similar to other species? What levels of β-endorphins and SP indicate a welfare challenge to the piglet? In addition, studies that reported on β-endorphins did not run the measure using a porcine-specific assay calibrated directly to the piglet, which raises concerns about the accuracy of the data reported. These research gaps are more "basic" than applied. However, the paucity of information in this area suggests that monies be placed into funding basic biological research to determine whether β-endorphins and other physiologically based measures are useful to indicate pain in the piglet. This is a fundamental and conceptual change in funding for the NPB, which historically might fund research in which many welfare measures are collected because it seems "complete," but in which interpretation will be difficult at the conclusion of the study.

The steering committee ranked several animal-based outcomes as "critical" before reviewing the literature. However, once the screening had occurred, it became apparent that our ranking of critical outcomes and data available in the literature did not match well. For example, collection, reporting, and interpretation of vocalization data were not standardized, partly because of the emergence of new technologies. In some cases, an objective of the research was to validate these technologies. Hence, in many castration studies, piglet calls were reported, yet researchers had different ways of recording the call, units for reporting their data, and the conclusions drawn. Even within our panel there was disagreement on which measures of vocalizations are robust indicators of piglet pain [several examples of measures, including number of calls, peak frequency (Hz), duration of calls, and numerous others, exist]. Another intriguing behavioral area was the activity state of "playing." Although ranked as a critical outcome by the group, because the absence of playing has been shown to be a sensitive indicator of surgical pain in the calf (Mintline et al., 2013), the information related to piglets and pain was sparse. After discussion as a group, it became apparent that play outcomes may not be useful in the piglet, since play-related activity is rarely performed at an age when most processing occurs in the US (<7 days of age). Similarly, other outcome measures were ranked as

"important" by the group in regards to other species and their relation to pain would be very useful to pursue, such as EEG and orbital infrared thermal imaging.

Other outcomes had conflicting rankings; for example, feeding events (defined as teat seeking/udder mouthing), heart rate variability, and norepinephrine were ranked as "important/not important." Conditioned responses of the animals were ranked "critical/not important." This simply shows uncertainty in these areas. Similar uncertainty occurred with assessing the practicalities, and hence the reliability of the data, for quantifying β-endorphins and heart rate variability in field studies. The reason for the uncertainty of rankings is unclear for outcomes. For example, the outcome may inherently vary a great deal, the expert's knowledge of the outcome may vary, or the evidence base for the outcome may be minimal.

There was also an interesting divide within the group in regard to how "objective, repeatable, and therefore meaningful" behavioral measures were in regard to grading pain in the neonatal piglet. Numerous comments were made about the "subjective" nature and "lack of calibration" that behavioral measures had compared to physiological measures. As discussed above, applied ethology is a relatively new discipline and has been making important and necessary strides in regard to the collection and reporting of behavioral measures. However, the lack of thorough reporting within journal articles often question how carefully the data were collected and the subsequent data interpretation with respect to the research question. Similar weaknesses were observed in the reporting of physiological outcomes, and general familiarity of most panel members with endocrine assays relative to techniques and experimental designs in applied ethology may have overemphasized the criticisms of the behavior outcomes. It is therefore suggested that the NPB spends time thinking through what steps they would like to see in future animal welfare research reports and publications to ensure that the methods and results are transparent. For pain studies in general, researchers should minimally report the *a priori* calculated sample size for the outcome(s) of interest, randomization and blinding, experimental units used in statistical analyses for each outcome, equipment and assays used, and whether these methods are validated for piglets, units of measurements, and descriptions of population means and distribution. For behavior studies specifically, it is suggested that the following be presented: a complete ethogram describing the behaviors/motor patterns quantified, how individual pigs were identified, sampling procedures over time (scans, continuous) and over individual pigs, type of equipment used for behavioral observation (live vs. digital), and whether data are reported as states, events, or bouts.

Research should not be limited to products that are currently legal to use in the US. The intent of this discussion was considered with NSAIDs. At this time, NSAIDs are not approved by the US FDA for pain mitigation in pigs that are destined for human consumption (pork). However, this limitation should not limit the funding of basic questions on the pharmacokinetic properties of NSAIDs. Allowing researchers to obtain data to rank the effectiveness of NSAIDs to provide pain relief to neonatal piglets at the time of castration is necessary. This in turn can be incorporated back into pain mitigation studies that use ranked animal-based behavioral and physiological outcomes to determine efficacy for pain relief. Hence, basic research is needed to provide data that can be presented to the government when needed. In conjunction with pain mitigation studies, a holistic approach to the implications for the stockperson, drug withdrawal requirements, food safety, and cost must also be considered.

To summarize, with limited funding options and to have the most meaningful impact on neonatal piglet welfare, funding should focus on castration and tail docking of piglets. The NPB should rank critical animal-based measures that have been collected or warrant further study, with the basis of sound biological reasoning as predictors of pain in the piglet, and support research in these areas. Funds are needed for basic scientific pursuits for neurophysiological and behavioral indicators of pain in the piglet. Once these animal-based measures have been validated in the laboratory, on-farm applied research can occur.

Finally, animal welfare science is a new discipline in which novel techniques are being developed to quantify physiological and behavioral indicators of pain and distress. Consequently, the lack of

standardization in methodology should be expected at this stage of inquiry. The NPB should insist on superior reporting of their funded pain-related research, including clearly explained protocols that can be replicated, adequate experimental design including controls and blinding, and results that communicate population probability distribution (means or medians) and estimates of variance (SD, SEM, or confidence intervals). The REFLECT statement for reporting randomized controlled trials for livestock and food safety provides useful guidelines that could be modified for animal welfare research applications (Sargeant et al., 2010a).

Unlike in Europe, there are currently no analgesic compounds approved for the alleviation of pain in food animals in the US (American Veterinary Medical Association, 2012). In accordance with FDA Guidance Document 123, validated methods of pain assessment must be used to prove that a pharmaceutical is efficacious before it can be labeled as an analgesic (Food and Drug Administration, 2006). This point supports basic research on pain markers in piglets, conducted in a controlled environment. In fact, normal data (e.g., unstimulated piglets) for some of the critical outcomes reported in the literature are still missing. Without a gold standard method to objectively recognize and quantify pain perception in animals, addressing questions about animal welfare become more difficult (Anil et al., 2005). Given the changes in EEGs specifically associated with human experiences of pain (Chen et al., 1989; Bromm and Lorenz, 1998; Chen, 2001), similar features of EEGs that allow detection of noxious stimuli have now been validated in calves during castration (Bergamasco et al., 2011). The same considerations should be done for SP evaluation and vocalizations. Vocalization outcome measures should utilize a rigorous approach in terms of focusing on more standardized and objective variables (e.g., peak amplitude and frequency rather than main frequency). Special attention to protocol selection and study design should validate these measures under controlled circumstances. After achieving the basic knowledge and values of the critical outcomes, the "field setting" approach can be used. During this step, pain mitigation strategies can be assessed to test the most effective/feasible/cost-effective pharmacological intervention(s) for pain mitigation in routine piglet processing procedures.

4.2. **Discussion about recommendations**

Surgery-induced pain consists of two phases: an immediate incisional phase and a prolonged inflammatory phase that arises primarily due to tissue damage. The incisional phase is primarily neurally mediated and the inflammatory phase is primarily mediated by prostaglandin synthesis and cytokine release. The goal of administering analgesic compounds prior to castration is to mitigate both the incisional (general anesthesia, local anesthesia) and inflammatory (NSAIDs) phases of the pain response. Among the interventions evaluated here, local and general anesthesia would be expected to act on the incisional phase, and NSAIDs would be expected to suppress inflammatory responses. Effective analgesia may require a multimodal approach using compounds that act on different receptor targets along the nociceptive pathway. This result may be achieved through a combination of local anesthesia, NSAIDs, and/or general anesthesia.

The use of general inhalation anesthesia has been projected as a method to reduce pain manifestations associated with castration in piglets between 1 and 28 days old. Limited studies measuring cortisol at 60 minutes and 24 hours, and β-endorphins at 60 minutes were assessable during this process (Table 8). The panel's strong recommendation against the use of CO_2/O_2 for pain mitigation during castration of 1–28–day-old piglets was based, in part, on the paucity of reviewable studies and the lack of demonstrable pain mitigation after anesthesia recovery in those reviewed. The quality of information was graded low to moderate for evaluation of cortisol and β-endorphin levels following anesthesia. The risk:reward ratio was judged to be poor because of the hurdles to successful anesthetic application at the field level, which were caused by normal body weight and age ranges, lack of standardized equipment or protocols, and variable applicator administration skills versus the potential for increased mortality resulting from anesthetic overdose or ineffectiveness with dosing. The likelihood that these risk:reward considerations could be overcome by additional research was judged to be low.

The use of local anesthesia produced by the introduction of lidocaine for pain mitigation during castration in piglets between 1 and 28 days of age was considered. Efficacy of pain relief was measured by cortisol levels 60 minutes after surgery. Only two studies were included to measure effects, but both demonstrated lower cortisol levels in treatment pigs, enabling the panel to assign a moderate quality score to this research (Table 14). Vocalization scores indicated that pain occurring at the time of castration was not mitigated by lidocaine anesthesia. The quality of evidence to support this intervention was judged very low. A risk:reward ratio was not attainable based on the available evidence, but with minimal rewards reported, the likelihood of a positive ratio was greatly diminished. Therefore the panel's current recommendation is weak against the use of local anesthesia for pain mitigation during castration. The paucity of studies, but with those presented graded as moderate, caused the panel to indicate that this intervention should receive encouragement for additional research that measures a range of critical outcomes and assesses adverse events potentials. Results of these studies should be re-evaluated in several years to determine whether local anesthesia should be considered a pain mitigation strategy for castration of 1–28–day-old piglets.

NSAIDs reviewed for pain mitigation during castration were primarily meloxicam studies. Based on the current understanding of the mechanism of action for NSAIDs, they would not be expected to mitigate incisional pain transmission via nerves, which generally transfer stimuli as electric pulses. These transmissions are rapid and start almost immediately after castration. NSAIDs are more likely to impact inflammatory pain stimuli that can be transmitted as a consequence of the production of cytokines and prostaglandins when cell walls are damaged and subsequently metabolized. NSAIDs interfere with the enzymes involved in the synthesis and possibly the metabolism of prostaglandins and change the ratio of various cytokine levels. Since specific cytokines are capable of producing pain with different efficacies, the final ratio influences the overall perception of pain. This understanding is consistent with the findings for NSAIDs (Table 11). Mean cortisol levels were lower at 60 minutes and 24 hours after castration, but vocalization energy was higher at the time of castration. Pain-like behaviors were observed more in the intervention group at 24 hours. While a delay in the start of cytokine-mediated pain is expected, the relative rate at which each subsides is unknown. Pain-like behaviors observed at 24 hours may not be a response specifically related to cytokines or an accurate assessment of the activity of NSAIDs.

While the quality of studies evaluated for the NSAID intervention was high, the panel felt that there was a lack of outcomes evaluated for this intervention, with decisions relying primarily on cortisol as an outcome. Among the 14 outcomes determined by the panel to be important, only data on cortisol and vocalization were evaluated. Consequently, the overall quality of the evidence for this intervention was judged to be low. Additional research and assessment of NSAIDs as an intervention using these additional critical outcomes would potentially strengthen the evidence for recommendations regarding NSAIDs. The potential for benefits was judged to outweigh the potential for harms in terms of physiological impact. However, from a resource perspective, the lack of an FDA-approved product is a major barrier to implementation. The panel's current recommendation is a weak recommendation for the use of NSAIDs for pain mitigation during castration in piglets between 1 and 28 days of age.

4.3. **Discussion about comprehensive reporting**

Reports of research trials performed are published to inform persons interested in a specific subject domain. With the widespread availability of electronic resources accessible through the World Wide Web, publications are now easily accessible compared to a decade ago. Ideally, research eventually informs policy that influences actions or processes. It is imperative that reports of research be comprehensive and reported in a manner that enables translation and reproduction. A reader should be able to assess the internal and external validity of a study.

The body of work we assessed was larger than expected because of the inclusion of non-English publications. Our judgments were based solely on published material, because this is what is available to

producers, veterinarians, and other stakeholders. No efforts were made to contact authors or sponsors. Many authors did not report important study design features, especially pertaining to randomization, blinding, and blocking. It was difficult to assess confounding in these studies. Without a complete description of the formal process of randomization, blinding, and blocking, the internal validity of the study is questionable.

Missing or inadequately reported summary measures limited the ability of the GRADE participants to collect and analyze data as well as to make informed decisions regarding pain mitigation strategies for piglets undergoing castration, tail docking, teeth clipping, and ear notching. GRADE recommends assessing five to seven outcomes. Our topic had 14 outcomes, increasing the number of possible outcomes we could assess. However, even with this increase in assessable outcomes, there were still few studies for each outcome.

It is unlikely that research can be performed on a large scale, because the cost and performance would be prohibitive. Influences such as setting and country are important, because it is through this information that research can be inferred. Reporting the setting of a given study is essential, because it affects the ability of stakeholders to make inferences, as results in more controlled settings, such as a laboratory or university farm, may differ from results in a commercial setting. The direction of bias has not been empirically described in animal welfare, but we hypothesize that pain mitigation strategies likely work better in research settings, as more management and research factors are controlled.

It was clear from the evidence that much research has been performed on pain mitigation for castration. The paucity of information available for other procedures, that is, tail docking, teeth clipping, and ear notching, made it difficult for reviewers to assess the efficacy of pain mitigation using these approaches. Much research has already been conducted on whether these procedures are painful, and all were assumed painful for this review. Pain mitigation is expected to become a higher priority area for consumers and retailers, as attention moves beyond gestation sow housing. Since these procedures are performed routinely, they are likely to receive greater attention. The absence of sufficient studies that report pain mitigation for teeth clipping and ear notching led to exclusion.

With respect to interventions, we were not surprised that many studies assessed NSAIDs. This is likely the more practical approach, especially in commercial settings that have many piglets, because it involves less handling of an animal compared to protocols that involve the use of local anesthesia. NSAIDs also use less equipment and have a greater margin of safety than general anesthetics. NSAIDs are not ideal for incisional pain but are preferable for inflammatory pain.

In conclusion, for publications that report pain mitigation during routine management procedures, we advocate that authors use available reporting guidelines, such as the REFLECT or CONSORT statements, and adhere to them to decrease obvious reporting errors and increase the reliability and usefulness of their publications.

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[1] NPB grant #.

[2] Author(s) names if applicable.

[3] Acknowledgements:

Table 1: Expertise sections identified as necessary for the GRADE guidelines and the ISU steering committee and external participant member categorization.

Expertise	Participant
Stress physiology	Sherrie Niekamp – National Pork Board, USA Luciana Bergamasco – Virginia Polytechnic Institute and State University, USA Mhairi Sutherland – Innovative Farm Systems, New Zealand Eberhard von Borell – Martin Luther University Halle-Wittenberg, Germany
Applied ethology and behavior	Anna Johnson – Iowa State University, USA Suzanne Millman – Iowa State University, USA Jeremy Marchant-Forde- USDA-ARS, USA Ed Pajor – University of Calgary, Canada Kenny Rutherford - Scottish Agricultural College, UK
Pharmacology	Johann (Hans) Coetzee – Iowa State University, USA Kip Lemke – University of Prince Edwards Island, Canada
Animal health	Locke Karriker – Iowa State University, USA Jim McKean – Iowa State University, USA Guy Martineau – Ecole Nationale Veterinaire de Toulouse, France
Study design	Annette O'Connor – Iowa State University, USA Rungano Dzikamunhenga - Iowa State University, USA Locke Karriker – Iowa State University, USA Hans Coetzee – Iowa State University, USA
Animal welfare NGO	Not represented – an invitation was extended to four individuals; however, all declined to participate
Swine veterinarians	Michelle Sprague – American Association of Swine Veterinarians, USA Locke Karriker – Iowa State University, USA James McKean – Iowa State University, USA
Swine producers	Gene Nome – Murphy Brown Inc., USA Brent Scholl – Scholl Farms, USA
Agricultural economics	Glynn Tonsor – Kansas State University, USA
Animal ethicist	Raymond Anthony – University of Alaska Anchorage, USA

Table 2: List of outcomes of interest identified by the ISU steering committee and the external participants, and the frequency of reporting in studies identified in the review.

	Castration		Tail docking		Ear notching		Teeth clipping	
	0–60 minutes	1–24 hours	0–60 minutes	1–24 hours	0–60 minutes	1–24 hours	0–60 minutes	1–24 hours
Adrenocorticotropic hormone (ACTH)	5	0	1	0	0	0	0	0
β-endorphins	6	2	0	0	0	0	0	0
Body temperature	1	2	0	0	0	0	0	0
Cortisol	16	13	3	4	0	0	0	0
Electrocardiogram (ECG)	0	0	0	0	0	0	0	0
Electroencephalogram (EEG)	0	0	0	0	0	0	0	0
Epinephrine	4	0	0	0	0	0	0	0
Haptoglobin	0	0	0	0	0	0	0	0
Heart rate	4	0	0	0	0	0	0	0
Norepinephrine	4	0	0	0	0	0	0	0
Respiratory rate	4	0	0	0	0	0	0	0
Substance P	1	1	0	0	0	0	0	0
Vocalization								
Call duration	3	0	0	0	0	0	0	0
Call rate	2	0	0	0	0	0	0	0
Main frequency	4	0	1	0	0	0	0	0
Peak amplitude	2	0	0	0	0	0	0	0
Peak frequency	3	0	0	0	0	0	0	0
Activity event								
Defecation	0	0	0	0	0	0	0	0
Escape attempts	1	0	0	0	0	0	0	0
Urination	0	0	0	0	0	0	0	0
Activity state								
Lying	5	7	3	2	0	0	0	0
Playing	3	4	0	0	0	0	0	0
Running	2	2	0	0	0	0	0	0
Sitting	3	4	2	1	0	0	0	0
Aggression	2	2	0	0	0	0	0	0
Avoidance	3	3	2	0	0	0	0	0
Body movement								
Ear flicking	0	0	0	0	0	0	0	0
Head shaking	0	0	2	0	2	0	2	0
Rear end movement	7	3	3	0	2	0	2	0
Feeding event								
Suckling/nursing	3	5	0	0	0	0	0	0
Teat seeking/udder movement	2	3	1	1	0	0	0	0
Teeth champing/chewing	0	0	2	0	2	0	2	0
Feeding state								

Suckling/nursing	2	4	0	0	0	0	0	0
Teat seeking/udder movement	0	1	2	1	0	0	0	0
Teeth champing/chewing	0	0	0	0	0	0	0	0

Table 3: The number of participants voting and the percentage of votes for the outcomes ranked as critical, important, and unimportant. The ranking with the most votes is indicated in the final column.

Outcomes	n	Critical	Important	Not important	Plurality ranking
Behavioral outcomes (< 60 minutes)					
Vocalization – call duration	11	27%	55%	18%	Important
Vocalization – call rate	10	10%	60%	30%	Important
Vocalization – main frequency (pitch)	11	45%	45%	9%	Critical/important
Vocalization – peak amplitude	10	60%	30%	10%	Critical
Vocalization – peak frequency (pitch)	11	64%	27%	9%	Critical
Activity event – defecation	9	0%	33%	67%	Not important
Activity event – escape attempts	11	36%	36%	27%	Critical/important
Activity event – urination	9	0%	22%	78%	Not important
Activity state – lying	9	0%	56%	44%	Important
Activity state – playing	10	30%	30%	40%	Not important
Activity state – running	9	0%	33%	67%	Not important
Activity state – sitting	9	0%	33%	67%	Not important
Aggression event	9	0%	44%	56%	Not important
Conditioned avoidance testing	9	0%	11%	89%	Not important
Body movement event – ear flicking	11	27%	55%	18%	Important
Body movement event – head shaking	11	27%	55%	18%	Important
Body movement event – rear end movement such as kicking, scratching	11	27%	64%	9%	Important

Feeding event – suckling/nursing	10	10%	30%	60%	Not important
Feeding event – teat seeking/udder mouthing	9	11%	44%	44%	Important/not important
Feeding event – teeth champing/chewing	9	22%	44%	33%	Important
Non-behavioral outcomes (< 60 minutes)					
Adrenocorticotrophic hormone (ACTH)	8	13%	50%	38%	Important
B-endorphins	7	29%	57%	14%	Important
Body temperature	7	29%	57%	14%	Important
Cortisol	9	11%	56%	33%	Important
Electrocardiograph readings (ECG)	8	25%	63%	13%	Important
Electroencephalogram readings (EEG)	8	50%	38%	13%	Critical
Epinephrine	8	25%	50%	25%	Important
Haptoglobin	8	0%	25%	75%	Not important
Heart rate	9	33%	44%	22%	Important
Norepinephrine	8	38%	50%	13%	Important
Respiratory rate	9	22%	67%	11%	Important
Substance P	8	50%	13%	38%	Critical
Behavioral outcomes (from 1–24 hours)					
Vocalization – call duration	9	0%	22%	78%	Not important
Vocalization – call rate	9	11%	11%	78%	Not important
Vocalization – main frequency (pitch)	9	11%	22%	67%	Not important
Vocalization – peak amplitude	9	11%	22%	67%	Not important

Vocalization – peak frequency (pitch)	9	11%	11%	78%	Not important
Activity event – defecation	10	0%	30%	70%	Not important
Activity event – escape attempts	9	0%	22%	78%	Not important
Activity event – urination	10	0%	20%	80%	Not important
Activity state – lying	11	27%	45%	27%	Important
Activity state – playing	11	55%	36%	9%	Critical
Activity state – running	11	36%	55%	9%	Important
Activity state – sitting	11	27%	45%	27%	Important
Aggression event	9	0%	22%	78%	Not important
Conditioned avoidance	10	40%	20%	40%	Critical / not important
Body movement event – ear flicking	10	40%	40%	20%	Critical / not important
Body movement event – head shaking	11	36%	36%	27%	Critical / not important
Body movement event – rear end movement such as kicking, scratching	11	36%	45%	18%	Important
Feeding event – suckling/nursing	11	45%	36%	18%	Critical
Feeding event – teat seeking/udder mouthing	11	27%	55%	18%	Important
Feeding event – teeth champing/chewing	10	20%	60%	20%	Important
Non-behavioral outcomes (from 1–24 hours)					
Adrenocorticotrophic hormone (ACTH)	8	25%	63%	13%	Important
B-endorphins	7	29%	57%	14%	Important

Body temperature	7	29%	57%	14%	Important
Cortisol	9	33%	33%	33%	All
Electrocardiograph readings (ECG)	8	13%	50%	38%	Important
Electroencephalogram readings (EEG)	8	13%	50%	38%	Important
Epinephrine	7	14%	29%	57%	Not important
Haptoglobin	6	50%	33%	17%	Critical
Heart rate	7	14%	43%	43%	Important/not important
Norepinephrine	7	14%	43%	43%	Important/not important
Respiratory rate	6	0%	67%	33%	Important
Substance P	7	43%	29%	29%	Critical

Table 4: Search terms applied in CABI: CAB Abstracts (Web of Knowledge, Thomson Reuters) on October 14, 2012.

Line	Search terms	Number of citations
#1	Pigs OR pig OR swine OR hogs OR hog OR piglets OR piglet (topic search)	267, 866
#2	Pain OR stress* OR well-being OR welfare OR anaesthesia OR anesthesia OR anesthetic OR anaesthetic OR analgesia OR analgesic (topic search)	337,757
#3	Tail docking OR tail resection OR docking OR castration OR castrating OR castrated OR orchiectomy OR teeth clipping OR tooth resection OR teeth resection OR tooth clipping OR tooth OR teeth OR tooth grinding OR clipping OR ear notching OR notching OR ear-notching OR ear tagging OR ear-tagging (topic search)	54,340
	#1 and #2 and #3	614

Table 5: Frequency count of studies identified for the main management procedures of interest to the review.

Technique	Castration*	Tail docking*	Ear notching¶	Teeth clipping¶
Castration – cut	21	-	-	-
Castration – tear	5	-	-	-
Castration – not reported	16+	-	-	-
Tail docking – side cutters	-	7	-	-
Tail docking – cauterizing blade	-	2	-	-
Tail docking – surgical cutters	-	1	-	-
Ear notching	-	-	2	-
Teeth clipping	-	-	-	2
Number of studies (n=52)	42	10	2	2

*Includes one study that used castration and tail docking on the same animals.
¶Includes one study that used teeth trim, ear notching, and tail docking on the same animals.
+Includes one study that described scrotal incision but did not report whether the spermatic cord was cut or torn.

Cut: Describes castration technique where scrotal incision was made and spermatic cord was *cut*.
Tear: Describes castration technique where scrotal incision was made and spermatic cord was *torn*.

NB: In 40 studies, piglets underwent castration only. In seven studies, piglets underwent tail docking only. In one study, piglets underwent teeth clipping only, and in one study piglets underwent ear notching only. Total studies for single procedures is 49. There was an overlap in three studies. In one study, piglets underwent three procedures, that is, tail docking, ear notching, and teeth clipping. In two studies, piglets underwent both castration and tail docking.

Table 6: General anesthesia protocols used in the studies that assessed castration.

Author	General anesthesia protocols used in the studies that assessed castration*
(Zimmermann et al., 2011)	Agent: CO_2/O_2 Dose: 70% CO_2/30% O_2 Route: inhalation Duration of administration: 75 seconds
(Zimmermann et al., 2011)	Agent: CO_2/O_2 Dose: 70% CO_2/30% O_2 Route: inhalation Duration of administration: 75 seconds
(Schulz et al., 2007a)	Agent: isoflurane Dose: 5% volume with gas flow rate of 6 l/min Route: inhalation Duration of administration: 90 seconds
(Waldmann et al., 1994)	Agent: thiopental sodium Dose: 20– 30 mg/kg Route: intra-abdominal
(McGlone et al., 1987)	Agent: mixture of xylazine, ketamine, and 5% glyceryl guaiacolate Dose: 2.2 ml/kg of 500 mg xylazine, 500 mg ketamine, and 500 ml 5% glyceryl guaiacolate Route: intravenous in the anterior vena cava
(Beirendonck et al., 2011)	Agent: CO_2 Dose: 100% CO_2 Route: inhalation via a face mask Duration of administration: 25 seconds before the procedure
(Muhlbauer et al., 2009)	Agent: CO_2/O_2 Dose: 70% CO_2/30%O_2 Route: inhalation Duration of administration: 45 seconds
(Schulz et al., 2007b)	Agent: isoflurane Dose: 5% volume Route: inhalation, 6 l/min fresh gas flow rate (atmospheric oxygen) Duration of administration: 90 seconds prior to the procedure
(Lahrmann et al., 2006)	Agent: ketamine/azaperone combination Dose: 25 mg/kg ketamine; 2 mg/kg azaperone Route: intramuscularly
(Schonreiter et al., 2000)	Agent: CO_2/O_2 Dose: 60% CO_2 and 40% O_2 Route: inhalation Duration of administration: 30 seconds
(Jaggin et al., 2001)	Agent: CO_2/O_2 Dose: 60% CO_2/40% O_2 Route: inhalation but pig outside the box
(Mauch and Bilkei, 2004)	Agent: acepromazine and ketamine (10%) Dose: 2 mg/kg acepromazine and 10 mg/kg ketamine Route: intramuscularly

(Jaggin et al., 2001)	Agent: halothane in pure oxygen Dose: 5% halothane with 2 l pure oxygen Route: inhalation Duration of administration: 120 seconds
(Schmidt et al., 2012)	Agent 1: ketamine Dose: 25 mg/kg Route: intramuscularly behind base of ear Time of administration: 10 minutes before the procedure Agent 2: azaperone Dose: 2 mg/kg Route: intramuscularly behind base of ear Time of administration: 10 minutes before the procedure
(Walker et al., 2004)	Agent : isoflurane/O_2 Dose : 5% isoflurane/95% O_2 Route : inhalation Duration of administration: mean 128 seconds
(Jaggin et al., 2001)	Agent : CO_2/O_2 Dose : 80% CO_2/20% O_2 Route : mask induction via inhalation with 10 l oxygen/minute flow rate Duration of administration: 30 seconds
(Sutherland et al., 2012)	Agent: CO_2 Dose: 100% CO_2 Route: inhalation Duration of administration: 30 seconds
(Rault and Lay, 2011)	Agent: nitrous oxide (N_2O)/O_2 combination Dose: 70% N_2O/30% O_2 Route: small-sized mask connected to anesthesia machine, rate of 1 l/min Duration of administration: at least 150 seconds
(Jaggin et al., 2001)	Agent: halothane Dose: 5% halothane Route: inhalation, 2L pure oxygen Duration of administration: 120 seconds,
(Sutherland et al., 2012)	Agent: CO_2 Dose: 100% CO_2 Route: surgical gas mask on snout that covered the whole mouth Duration of administration: 30 seconds
(Sutherland et al., 2011)	Agent: CO_2 Dose: 100% CO_2 Route: inhalation Duration of administration: 30 seconds
(Sutherland et al., 2011)	Agent: CO_2 Dose: 100% CO_2 Route: inhalation Duration of administration: 30 seconds

*Agent, dose, route of administration, and duration of administration is described for each general anesthesia protocol where information was explicitly provided in the study.

Table 7: Local anesthesia protocols used in the studies that assessed castration and tail docking.

Author	Local anesthesia protocols used in the studies that assessed castration and tail docking
(Waldmann et al., 1994)	Agent: butanilicaine phosphate Dose: 3 to 5 ml Route: subcutaneous infiltration of the incision line and spermatic cord
(Waldmann et al., 1994)	Agent: butanilicaine phosphate Dose: 1 ml Route: intratesticularly
(Jaggin et al., 2001)	Agent: lidocaine Dose: 1.2 ml Route: intratesticularly Frequency and time of administration: once into each testicle, 10 minutes apart; then waited 10 minutes before castration
(Courboulay et al., 2010)	Agent: lidocaine Dose: 1 ml of 2 g lidocaine Route: one-half distributed between the testicle and the left scrotal pouches, and the other half administered to the right side areas
(Rittershaus et al., 2009)	Agent: chlorethyl cooling spray on skin and spermatic cord Route: spray on skin and spermatic cord
(Rittershaus et al., 2009)	Agent: combination of chlorethyl for skin anaesthesia with lidocaine spray, which was applied to the spermatic cord Route: topical, sprayed on skin
(Rittershaus et al., 2009)	Agent: skin anesthetic (EMLA-cream), which is established in human medicine Route: topical
(Nyborg et al., 2000)	Agent: bupivacaine (with noradrenaline) Dose: 0.25% (total of 2.8 ml) Route: 1 ml intrafunicularly into each spermatic and 0.8 ml subcutaneously
(Horn et al., 1999)	Agent: 2% lidocaine Dose: 0.5 ml Route: intratesticularly Time of administration: 2.5 minutes before castration
(Horn et al., 1999)	Agent: 2% lidocaine Dose: 0.7 ml Route: 0.5 ml given intratesticularly and 0.2 ml given subcutaneously Time of administration: 2.5 minutes before castration
(Cordeiro et al., 2012)	Agent: 20% lidocaine Time of administration: 10 minutes before testes incision
(Schulz et al., 2007a)	Agent: 2% procaine hydrochloride Dose: 10 mg (0.5 ml) Route: intratesticularly Time of administration: 15 minutes before castration
(Marx et al., 2003)	Agent: 2% lidocaine Dose: 0.5 ml per testis Route: intratesticularly Time of administration: 2 minutes before castration

(Kluivers-Poodt et al., 2012)	Agent: lidocaine Dose: 1 ml Route: two injections of 1.0 ml lidocaine, 0.8 ml was injected into the testicle, and the remaining 0.2 ml subcutaneously during withdrawal of the needle from the testicle Time of administration: 15 minutes before the procedure
(Kluivers-Poodt et al., 2012)	Agent: lidocaine Dose: 1 ml Route: two injections of 1.0 ml of lidocaine, 0.8 ml was injected into the testicle, and the remaining 0.2 ml subcutaneously during withdrawal of the needle from the testicle Time of administration: 15 minutes before the procedure
(Leidig et al., 2009)	Agent: procaine Dose: 10 mg Route: intratesticularly Time of administration: 5 minutes prior to the procedure
(White et al., 1995)	Agent: lidocaine hydrochloride 2% diluted in sterile saline to 1% Dose: 1.5 ml pigs < 8 days, otherwise 2 ml Route: one-half subcutaneously, other one-half not reported Time of administration: 3 minutes before the procedure
(Hansson et al., 2011)	Agent: lidocaine Dose: 0.5 ml in each testicle Route: intratesticularly and subcutaneously, into the scrotum
(Hansson et al., 2011)	Agent: lidocaine Dose: 0.5 ml in each testicle Route: intratesticularly
(Sutherland et al., 2010)	Agent: cetacaine (14% benzaine, 2% butamben, and 2% tetracaine hydrochloride) Route: topically onto the spermatic cords before the cords were cut and onto the skin at the edge of the castration wound
(Sutherland et al., 2010)	Agent: tri-solfen (40.6 g/l lignocaine, 4.5 g/l bupivacaine, 24.8 mg/l adrenaline, and 5.0 g/l cetrimide) Dose: 0.5 ml Route: topically onto the spermatic cords before being cut and onto edge of skin of the castration wound
(Sutherland et al., 2011)	Agent: 2% lidocaine Dose: 0.5 ml Route: subcutaneously at the base of the tail ~2 cm from the point where the tail was cut Time of administration: immediately before tail docking
(Sutherland et al., 2011)	Agent: cetacaine Route: topical Time of administration: 2 seconds onto the tail wound, after tail docking
(Sutherland et al., 2011)	Agent: tri-solfen Route: topical Frequency of administration: once after tail docking
(Sutherland et al., 2010)	Agent: cetacaine (14% benzaine, 2% butamben, and 2% tetracaine hydrochloride) Route: topically onto the spermatic cords before the cords were cut and onto the skin at the edge of the castration wound
(Sutherland et al., 2010)	Agent: tri-solfen (40.6 g/l lignocaine, 4.5 g/l bupivacaine, 24.8 mg/l adrenaline, and 5.0 g/l cetrimide) Dose: 0.5 ml Route: directly onto each spermatic cord

(Sutherland et al., 2011)	Agent: 2% lidocaine Dose: 0.5 ml Route: subcutaneously at the base of the tail (2 cm from the point where the tail was cut) Frequency of administration: once
(Sutherland et al., 2011)	Agent: cetacaine Route: topically Frequency of administration: once for 2 seconds
(Sutherland et al., 2011)	Agent: tri-solfen Route: topically after tail docking
(Prunier et al., 2001)	Agent: vaporized refrigerant

*Agent, dose, route of administration, frequency, and time of administration is described for each local anesthesia protocol where information was explicitly provided in the study.

Table 8: Nonsteroidal anti-inflammatory protocols used in trial arms that assessed castration and tail docking.

Author	NSAID protocols used in the studies that assessed castration and tail docking
(Langhoff et al., 2009)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly Time of administration: 15–30 minutes before the procedure
(Langhoff et al., 2009)	Agent: flunixin meglumine Dose: 2.2 mg/kg Route: intramuscularly Time of administration: 15–30 minutes before the procedure
(Schulz et al., 2007b)	Agent: meloxicam Dose: 20 mg/ml Route: intramuscularly
(Zoels et al., 2006)	Agent: meloxicam (20 mg/ml) Dose: 0.4 mg/kg Route: intramuscularly Time of administration: 15 minutes before castration
(Wavreille et al., 2012)	Agent: tolfenamic acid Dose: 2 mg/kg (0.08–0.18 ml) Route: intramuscularly Time of administration: 1 hour prior to castration
(Wavreille et al., 2012)	Agent: meloxicam Dose: 0.4 mg/kg (0.13–0.29 ml) Route: intramuscularly Frequency of administration: 1 hour prior to castration
(Schwab et al., 2012)	Agent: ketoprofen Dose: 0.03 mg/kg Route: intramuscularly Time of administration: 10–30 minutes before castration
(Tenbergen, 2012)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly Time of administration: 30 minutes before the procedure
(Courboulay et al., 2010)	Agent: 1 % ketoprofen Dose: 0.7 5ml per piglet Route: intramuscularly
(Kluivers-Poodt et al., 2012)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly in the neck Time of administration: 15minutes before the procedure
(Kluivers-Poodt et al., 2012)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly in the neck Time of administration: 15 minutes before the procedure

(Schmidt et al., 2012)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly behind the base of ear Time of administration: 10 minutes before the procedure
(Schmidt et al., 2012)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly behind the base of ear Time of administration: 10 minutes before the procedure
(Hansson et al., 2011)	Agent: meloxicam Dose: 0.2 ml of 5 mg/kg stock Route: intramuscularly Frequency of administration: once
(Hansson et al., 2011)	Agent: meloxicam Dose: 0.2 ml Route: intramuscularly Frequency of administration: once
(Sutherland et al., 2012)	Agent: flunixin meglumine Route: intramuscularly
(Sutherland et al., 2012)	Agent: flunixin meglumine Route: intramuscularly
(Reiner et al., 2012)	Agent: flunixin Dose: 5 mg Route: intramuscularly Time of administration: 30 minutes before castration
(Reiner et al., 2012)	Agent: flunixin Dose: 5 mg Route: intramuscularly Time of administration: immediately before castration
(Reiner et al., 2012)	Agent: meloxicam Dose: 2 mg or 2 mg/kg Route: intramuscularly Time of administration : immediately before castration
(Sutherland et al., 2012)	Agent: flunixin meglumine Route: intramuscularly
(Sutherland et al., 2012)	Agent: flunixin meglumine Route: intramuscularly
(Langhoff et al., 2009)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly Time of administration: 15–30 minutes before the procedure
(Langhoff et al., 2009)	Agent: flunixin meglumine Dose: 2.2 mg/kg Route: intramuscularly Time of administration: 15–30 minutes before the procedure
(Langhoff et al., 2009)	Agent: metimazole sodium Dose: 50 mg/kg BW Route: intramuscularly Time of administration: 15–30 min before castration

(Langhoff et al., 2009)	Agent: carprofen Dose: 0.4 mg/kg Route: subcutaneously Time of administration: 15–30 minutes before the procedure
(Tenbergen, 2012)	Agent: meloxicam Dose: 0.4 mg/kg Route: intramuscularly Time of administration: 30 minutes prior to the procedure
(Tenbergen, 2012)	Agent: ketoprofen Dose: 3 mg/kg Route: intramuscularly Time of administration: 30 minutes before the procedure

*Agent, dose, route of administration, frequency, and time of administration is described for each NSAID protocol where information was explicitly provided in the study.

Table 9: GRADE voting results.

	CO_2/O_2 general anesthesia			Local anesthesia	NSAID			
	Cortisol 0–60 minutes	Cortisol 1–24 hours	β-endorphins	Energy 0–60 minutes	Cortisol 0–60 minutes	Cortisol 1–24 hours	Energy 0–60 minutes	Rate 0–60 minutes
Risk of bias								
Low	17	11	11	75	100	NC	82	94
Serious	83	83	89	25	0	NC	12	6
Very serious	0	6	0	0	0	NC	6	0
Indirectness								
Low	83	83	-	-	-	-	-	-
Serious	6	17	-	-	-	-	-	-
Very serious	11	0	-	-	-	-	-	-
Inconsistency								
Low	6	83	83	75	94	83	83	83
Serious	89	17	17	19	6	11	17	11
Very serious	6	0	0	6	0	6	0	6
Imprecision								
Low	11	17	NC	NC	83	94	NC	NC
Serious	89	83	NC	NC	17	6	NC	NC
Very Serious	0	0	NC	NC	0	0	NC	NC
Publication bias								
Undetected	83	94	-	-	89	100	83	89
Strongly	17	6	-	-	11	0	17	11
Body of work								
Very Low	94	-	NC	94	6	-	-	-
Low	0	-	NC	6	89	-	-	-
Moderate	6	-	NC	0	6	-	-	-
High	0	-	NC	0	0	-	-	-
Absence of high-quality evidence								
Yes	100	-	-	-	88	-	-	-
No	0	-	-	-	12	-	-	-
Benefits and harms								
Yes	76	-	-	81	12	-	-	-

No	24	-	-	19	88	-	-	-
Values and preferences								
Yes	100	-	-	Yes?	88	-	-	-
No	0	-	-		12	-	-	-
Recommendation								
For	0	-	-	25	82	-	-	-
Against	100	-	-	75	18	-	-	-
Recommendation strength								
Weak	6	-	-	88	82	-	-	-
Strong	94	-	-	12	18	-	-	-

NC: No consensus.

Table 10: Summary of findings for CO_2/O_2 anesthesia for castration.

CO_2/O_2 general anesthesia mixture for pain mitigation during castration in piglets between 1 and 28 days old

Population: Pain mitigation during castration in piglets between 1 and 28 days old
Settings: Commercial swine production facilities
Intervention: General anesthesia

Outcomes	Illustrative comparative difference in outcomes* (95% CI)	No of animals and studies reporting the outcome	No of animals and studies included in in the meta-analysis	Quality of the evidence (GRADE)
Cortisol 60 minutes	The mean cortisol 60 minutes - CO_2/O_2 in the intervention groups was 33.97 higher (57.41 lower to 125.35 higher)	240 (five studies[1,2,3,4,5])	208 (three studies [1,2,3])	⊕⊖⊖⊖ very low[6]
Cortisol 24 hours	The mean cortisol 24 hours - CO_2/O_2O_2 in the intervention groups was 59.97 lower (92.78 to 27.17 lower)	220 (four studies[1,3,4,5])	188 (two studies[1,3])	⊕⊕⊖⊖ moderate/ low[7]
β-endorphin 60 minutes	The mean β-endorphin 60 minutes - CO_2/O_2O_2 in the intervention groups was 1.06 higher (0.66 lower to 2.78 higher)	115 (four studies[2,3,4,5])	115 (two studies[2,3])	⊕⊕⊖⊖ moderate/low[7]

CI: Confidence interval.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

MA: Data included in meta-analysis for calculation of the comparative levels.

[1]Randomized, nonblinded, and blocked.

[2]Randomized, nonblinded, and nonblocked.

[3]Nonrandomized, nonblinded, and nonblocked.

[4]Nonrandomized, nonblinded, and nonblocked.

[5]Nonrandomized, nonblinded, and nonblocked.

[6]One study showed a positive effect, favoring the intervention; the other point estimate was positive, but the 95% interval included the null value 0.

[7]Concern about the width of the interval.

Table 11: Evidence provided for CO_2/O_2 anesthesia for castration.

Quality assessment							No of patients		Effect	Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	General anesthesia	Control	Absolute		
Cortisol 60 minutes - CO_2/O_2 (Better indicated by lower values)											
3	Randomized trials[1,2,3]	Serious	Serious[6]	No serious indirectness	Serious	None	107	101	MD 33.97 higher (57.41 lower to 125.35 higher)	⊕⊖⊖⊖ VERY LOW	IMPORTANT
Cortisol 24 hours - CO_2/O_2 (Better indicated by lower values)											
2	Randomized trials[1,3,]	Serious	No serious inconsistency	No serious indirectness	Serious[7]/ very serious	None	97	91	MD 59.97 lower (92.78 to 27.17 lower)	⊕⊕⊖⊖ MODERATE/LOW	IMPORTANT
β-endorphin 60 minutes - CO_2/O_2 (Better indicated by lower values)											
2	Randomized trials[2,3,]	Serious	No serious inconsistency	No serious indirectness	Serious[7]/ very serious	None	60	55	MD 1.06 higher (0.66 lower to 2.78 higher)	⊕⊕⊖⊖ MODERATE/LOW	IMPORTANT

[1]Randomized, nonblinded, and blocked.
[2]Randomized, nonblinded, and nonblocked.
[3]Nonrandomized, nonblinded, and nonblocked.
[4]Nonrandomized, nonblinded, and nonblocked.
[5]Nonrandomized, nonblinded, and nonblocked.
[6]One study showed a positive effect, favoring the intervention; the other point estimate was positive, but the 95% interval included the null value 0.
[7]Concern about the width of the interval.

Table 12: Recommendation for use of CO_2/O_2 general anesthesia mixture for pain mitigation during castration in piglets between 1 and 28 days old.

Recommendation: The panel's current recommendation is a strong recommendation against the use of a CO_2/O_2 general anesthesia mixture for pain mitigation during castration in piglets between 1 and 28 days old.

We propose that this recommendation about the use of general anesthesia as an approach to castration could be revisited in 3–5 years if new high-quality research that assessed critical outcomes, replicated the speed of castration as occurs on-farm, replicated the spectrum of piglet's weights castrated in US production, and assessed adverse events was available.

Animals: Piglets undergoing castration
Setting: Commercial swine production facilities
Intervention: General anesthesia

Factor	Decision	Explanation
Quality of evidence	Very low	Few studies were available to assess the efficacy of this intervention; the outcomes assessed did not enable the panel to understand the impact of the pain experience on the animals. If animals are properly anesthetized, the expectation was that pain was mitigated during the procedure. It is unclear if appropriate anesthesia levels can be consistently achieved on-farm. Further, it is not known if general anesthesia during castration results in no change, reduced, or increased pain manifestations 1–24 hours after the procedure.
Balance of benefits and harms	Potential for harms outweighs the potential benefits	General anesthesia is a complex procedure, and clearly the potential exists for under- or overdosing that would result in either no effect or increased mortality. In a production setting with different ages and weights of piglets to process, it is currently unrealistic to expect producers to rapidly, consistently, and safely administer general anesthesia with existing tools. Further, the potential for harm to workers adds an additional concern about the safety of the on-farm use of general anesthetics for pain mitigation. These concerns were major drivers for the strength and direction of the recommendation provided by the panel.

Values and preferences	Major variation in values and preferences present	The panel felt that there was evidence of large variations in consumer values and preferences. The information about values and preferences was assessed from the perspective of the consumers of pork. Large variations in how consumers and citizens value pain mitigation are expected. The results of several US-based voter initiatives were used as evidence of citizen values, whereas the observed low willingness to pay observed in the US and overseas markets was used as evidence for variations in consumer actions. It was also noted that willingness to pay may be difficult to document in the US market, where there are few niche entry points for pork with differentiated production processes. This situation differs from egg production, where more direct market channels exist for products such as cage-free eggs. Given the current difficulties with implementing general anesthesia on-farm, it is unlikely that more consistency in values and preferences would change the panel's recommendation.
Resources		The panel did not include a vote on the impact of resources; however, comment about the issues is warranted. Documentation that administration equipment is affordable, reproducible in effect, rapid, applicable to the production site, and safe for animals and workers would be required for this to be considered a practical intervention. Coupled with NSAID application, general anesthesia may fit into a pain mitigation strategy for castration.

Table 13: Summary of findings of NSAIDs for castration.

NSAIDs for pain mitigation during castration in piglets between 1 and 28 days old.

Population: Pain mitigation during castration in piglets between 1 and 28 days old.
Setting: Commercial swine production facilities
Intervention: NSAIDs

Outcomes	Illustrative comparative risks* (95% CI)		No of participants (studies)	No of participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			
	Control	NSAIDs			
Cortisol 60 minutes	The mean cortisol 60 minutes in the intervention groups was 93.59 lower (138.44 to 48.74 lower)		634 (14 studies [1,2,3,4,5,6,7,8,9,10,11,12,13,14])	488 (11 studies [1,2,3,4,5,7,8,9,10,11,12])	⊕⊕⊕⊕ high[15]
Cortisol 24 hours	The mean cortisol 24 hours in the intervention groups was 39.17 lower (51.87 to 26.47 lower)		441 (nine studies [1,2,6,7,9,10,11,12,14])	295 (seven studies [1,2,7,9,10,11,12])	⊕⊕⊕⊖ moderate[16]
Vocalization - Energy (dB) 60 minutes	The mean vocalization - energy (dB) 60 minutes in the intervention groups was 47.4 higher (54.03 lower to 148.82 higher)		357 (five studies[1,6,7,8,13])	342 (two studies[7,8])	⊕⊕⊕⊕ high[15]
Pain-like behaviors 24 hours	The mean pain-like behaviors 24 hours in the intervention groups was 0.30 standard deviations higher (0 to 0.59 higher)		280 (five studies[1,2,3,7,13])	180 (three studies[1,2,3])	⊕⊕⊕⊖ moderate[17]

*The basis for the **assumed risk** (e.g., the median control group risk across studies) is provided in the footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1]Randomized, and blinded, and blocked.
[2]Randomized, blinded, and blocked.
[3]Randomized, blinded, and blocked.
[4]Randomized, blinded, and blocked.
[5]Randomized, blinded, and blocked.

[6]Randomized, blinded, and nonblocked.
[7]Randomized, non-blinded, and blocked.
[8]Randomized, nonblinded, and blocked.
[9]Randomized, nonblinded, and nonblocked.
[10]Randomized, nonblinded, and nonblocked.
[11]Randomized, nonblinded, and nonblocked.
[12]Randomized, nonblinded, and nonblocked.
[13]Nonrandomized, nonblinded, and blocked.
[14]Nonrandomized, nonblinded, and nonblocked.
[15]No explanation was provided.
[16]Approximately 50% failed to include information about controlling for important confounders and blinding.
[17]In the absence of being able to reach consensus, this would be either high or moderate; it is currently indicated as moderate so the table is complete; in the final version, we will have it as high/moderate.

Table 14: Evidence profiles of NSAIDs for castration.

Quality assessment							No of patients		Effect	Quality	Importance
No of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**NSAID**	**Control**	**Absolute**		
Cortisol 60 minutes (better indicated by lower values)											
11	Randomized trials[1,2,3,4,5,7,8,9,10,11,12]	No serious risk of bias[15]	No serious inconsistency	No serious indirectness	No serious imprecision	None	233	255	MD 93.59 lower (138.44 to 48.74 lower)	⊕⊕⊕⊕ HIGH	
Cortisol 24 hours (better indicated by lower values)											
7	Randomized trials[1,2,7,9,10,11,12]	Serious[16]	No serious inconsistency	No serious indirectness	No serious imprecision	None	138	157	MD 39.17 lower (51.87 to 26.47 lower)	⊕⊕⊕⊖ MODERATE	
Vocalization - Energy (dB) 60 minutes (better indicated by lower values)											
2	Randomized trials[7,8]	No serious risk of bias	No serious inconsistency	No serious indirectness	No serious imprecision[15]	None	171	171	MD 47.4 higher (54.03 lower to 148.82 higher)	⊕⊕⊕⊕ HIGH	
Pain-like behaviours 24 hours (better indicated by lower values)											
3	Randomized trials[1,2,3]	No serious risk of bias	No serious inconsistency	No serious indirectness	Serious[17]	None	90	90	SMD 0.30 higher (0 to 0.59 higher)	⊕⊕⊕⊖ MODERATE	

[1]Randomized, blinded, and blocked.
[2]Randomized, blinded, and blocked.
[3]Randomized, blinded, and blocked.
[4]Randomized, blinded, and blocked.
[5]Randomized, blinded, and blocked.
[6]Randomized, blinded, and nonblocked.
[7]Randomized, nonblinded, and blocked.
[8]Randomized, nonblinded, and blocked.
[9]Randomized, nonblinded, and nonblocked.

[10]Randomized, nonblinded, and nonblocked.
[11]Randomized, nonblinded, and nonblocked.
[12]Randomized, nonblinded, and nonblocked.
[13]Nonrandomized, nonblinded, and blocked.
[14]Nonrandomized, nonblinded, and nonblocked.
[15]No explanation was provided.
[16]Approximately 50% failed to include information about controlling for important confounders and blinding.
[17]In the absence of being able to reach consensus, this would be either high or moderate; it is currently indicated as moderate so the table is complete; in the final version, we will have it as high/moderate.

Table 15: Recommendation for use of NSAIDs for castration.

Recommendation: The panel's current recommendation is a weak recommendation for the use of NSAIDS for pain mitigation during castration in piglets between 1 and 28 days old.

We propose that this recommendation about the use of NSAIDS as an approach to castration could be revisited in 1–2 years if more products become available on the US market and outcomes considered critical to long-term pain mitigation are included in the studies.

Animals: Piglets undergoing castration
Setting: Commercial swine production facilities
Intervention: NSAIDs

Factor	Decision	Explanation
Quality of evidence	Low	There is an absence of critical outcomes measured for this intervention. This is an intervention designed to mitigate pain 1–24 hours after the procedure. The recommendation means the panel placed high value on the cortisol results for this time frame. It was recognized by the panel that cortisol is not a specific indicator of pain, and validated pain assessment measures are needed to more fully assess the benefits of NSAID administration to alleviate the pain associated with castration. The vocalization results indicate that these strategies do not mitigate the acute pain associated with the procedure. The vocalization results were not unexpected, given the mechanism of action of these products but provided another reason that the recommendation was weak rather than strong for these products.
Balance of benefits and harms	The potential for benefits outweighs the potential harms	The panel felt that the likely benefits outweighed the harms for NSAIDs. Unlike general anesthesia, the potential for overdose is minimal. Current NSAID products provide a reasonable margin of product safety for published dose regimens. Additionally, the products are routinely applied via commonly used routes of administration in commercial swine production facilities. There is a limited expectation of benefit for incisional pain with benefits limited to the reduction of inflammatory pain after the procedure.

Values and preferences	Major variations in values and preferences present	The panel felt that there was evidence of large variations in consumer values and preferences. The information about values and preferences was assessed from the perspective of the consumers of pork. Large variations in how consumers and citizens value pain mitigation are expected. The results of several US-based voter initiatives were used as evidence of citizen values, whereas the observed low willingness to pay observed in the US and overseas markets was used as evidence of variations in consumer actions. It was also noted that willingness to pay may be difficult to document in the US market, where there are few niche entry points for pork with differentiated production processes. This situation differs from egg production, where more direct market channels do exist for products, such as cage-free eggs. Given the current difficulties with implementing general anesthesia on-farm, it is unlikely that more consistency in values and preferences would change the panel's recommendation.
Resources		Currently, the absence of an FDA-registered product for pain mitigation is a major barrier that must be resolved. The primary impediment to regulatory approval for pain indications is the absence of validated methods for pain assessment in swine. Similarly, several of the products under consideration are considered prescription drugs. Such a designation makes their widespread use in production settings more difficult and expensive to manage.

Table 16: Summary of findings of local anesthesia for castration.

Local anesthesia for pain mitigation during castration in piglets between 1 and 28 days old.

Population: Pain mitigation during castration in piglets between 1 and28 days old
Setting: Commercial swine production facilities
Intervention: Local anesthesia

Outcomes	Illustrative comparative risks* (95% CI) Corresponding risk Local anesthesia	No of participants (studies)	No of participants in the meta-analysis (studies)	Quality of the evidence (GRADE)	Comments
Energy 60 minutes - Lidocaine	The mean energy 60 minutes - lidocaine in the intervention groups was 8.8 lower (10.86 to 6.74 lower)	342 (four studies[1,2,3,4])	342 (two studies[1,2])	⊕⊕⊕⊖ MODERATE	

*The basis for the **assumed risk** (e.g., the median control group risk across studies) is provided in the footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1]Randomized, nonblinded, and blocked.
[2]Randomized, nonblinded, and blocked.
[3]Nonrandomized, nonblinded, and blocked.
[4]Nonrandomized, nonblinded, and blocked.

Table 17: Evidence profile for local anesthesia for castration.

Author(s):
Date: 2013-02-05
Question: Should local anesthesia be used for pain mitigation during castration in piglets between 1 and 28 days old?
Setting: Commercial swine production facilities.
Bibliography:

Quality assessment							**No of patients**		**Effect**		**Quality**	**Importance**
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Local anesthesia	Control	Relative (95% CI)	Absolute		
Energy 60 minutes - Lidocaine (better indicated by lower values)												
2	Randomized trials[1,2]	No serious risk of bias	No serious inconsistency	No serious indirectness	Serious	None	171	171	-	MD 8.8 lower (10.86 to 6.74 lower)	⊕⊕⊕⊖ MODERATE	

[1]Randomized, nonblinded, and blocked.
[2]Randomized, nonblinded, and blocked.
[3]Nonrandomized, nonblinded, and blocked.
[4]Nonrandomized, nonblinded, and blocked.

Table 18: Recommendation for lidocaine for castration.

Recommendation: The panel's current recommendation is a weak recommendation against the use of lidocaine for pain mitigation during castration in piglets between 1 and 28 days old.

We propose that this recommendation about the use of local anesthetic as an approach to castration could be revisited in 1–2 years if new high-quality studies that assessed critical outcomes; replicated the on-farm speed of castration; approximated the spectrum of piglet weights to be castrated in the US production; and assessed possible adverse events were available, and information needed for appropriate off-label use or registration for use to mitigate pain in piglets was available.

Animals: Piglets undergoing castration
Setting: Commercial swine production facilities
Intervention: General anesthesia

Factor	Decision	Explanation
Quality of evidence	Very Low	Lidocaine is an intervention designed to mitigate pain in the short term, that is, 1–2 hours after the procedure. There was an absence of information about the *a priori* identified critical outcomes for this intervention. For this intervention, we would expect that only incisional pain associated with the procedure would be mitigated. Two studies did indicate that administration of lidocaine did reduce vocalization, as measured by call energy. However, there was debate among the panel about the value of this outcome; therefore, the evidence base was considered very low.

Balance of benefits and harms	Uncertainty that the potential benefits were greater than the harms	The uncertainty expressed here about the balance of benefits and harms by the panel was related to a failure to document the extent of benefits. The benefit to the piglet is that, provided the extra application steps necessary to utilize this procedure where utilized, local anesthetic would mitigate pain in the short term. However, for two reasons, the panel proposed that those benefits may not be as great as expected. First, in the US production system, there is a reluctance and practical difficulties to taking the extra steps to administer lidocaine before the procedure. If these steps are not taken, little real benefit for the piglet is realized. Further, based on the mechanism of action rather than on empirical studies in piglets, we would not expect that inflammatory pain associated with castration to be mitigated by lidocaine. This uncertainty weakened any recommendations. Possible harms to the piglet were thought to be minimal, as lidocaine is widely used in human and animal health, and has a reasonable margin of product safety; therefore, the harms that would occur are minimal. It is also theoretically possible that lidocaine could adversely affect wound healing.
Values and preferences	Major variation in values and preferences	The information about values and preferences was assessed from the perceived perspective of the consumers of pork and by citizens generally. It was considered that there were large variations in how the consumers and citizens value pain mitigation but this was not known with uncertainty. In making this assessment, the results from voter initiatives were used as evidence of citizen values, whereas the observed low willingness to pay scores observed in the US and overseas markets provided dichotomous evidence for consumer valuations. No direct data about pain mitigation in piglets or consumer preferences was used. It was also noted that willingness to pay may be difficult to document in the US market, where there are few unique entry points for pork with differentiated production processes. This situation differs from egg production, where more direct market channels exist for differentiated products, such as cage-free eggs.

Resources		As with NSAIDS, the absence of FDA-registered products for local anesthesia to reduce pain in swine is a major barrier that must be resolved if local anesthetic products are to be adopted. Lidocaine is a prescription drug requiring regulation at point of use to address untoward effects from its use. This is not a trivial barrier to adoption, because extra label use of products in the US falls under the jurisdiction of AMDUCA. Among the processes required by AMDUCA, veterinarians must provide producers with withdrawal times for meat production. Such information is difficult to obtain, as methods of determining meat withdrawals are not harmonized across countries, and withholding times used in swine production elsewhere cannot be guaranteed to meet FDA requirements. For producers, extra-label drug use requires the maintenance of records that indicate the animals treated and the dose. Such a designation makes the widespread use of lidocaine in US production settings difficult and expensive to manage for the producer and legally difficult for veterinarians. The failure to document great benefit combined with resource issues lead to a weak recommendation against the use of local anesthetic.

Table 19: Reporting of trial level sources of clinical heterogeneity – all not reported.

Data not reported	Castration	Tail docking	Ear notching	Teeth clipping
Setting	33	3	-	-
Production system	41	7	2	2
Management system	32	4	2	2
Age	1	-	-	-
Weight	31	8	2	2
Breed	12	1	-	-
Total experiments	44	10	2	2

Table 20: Frequency of not reporting summary measures in studies of castration.

	Number of relevant study arms	Number of arms for which data was extracted from figures	Number of arms that had missing summary features	Description of missing summary measures
General anesthesia (CO_2/O2)				
Cortisol 60 minutes	8	4	3	2 means, 3 SDs
Cortisol 24 hours	6	2	3	2 means, 3 SDs
β-endorphins 60 minutes	9	2	2	2 means, 2 SDs
β-endorphins 24 hours	3	1	2	2 means, 2 SDs, 2 arm sample size
Norepinephrine 60 minutes	2	1	1	Arm sample size
Pain-like behaviors 60 minutes	8	4	2	1 mean and 2 SDs
Local anesthesia (Lidocaine)				

Cortisol 60 minutes	8	7	7	6 SDs and 1 arm sample size
Cortisol 24 hours	6	6	6	6 SDs and 3 arm sample size
Norepinephrine 60 minutes	1	-	1	1 mean, 1 SD and 1 arm sample size
Frequency 60 minutes	4	-	3	3 SDs and 1 arm sample size
Energy 60 minutes	4	2	2	1 SD and 2 arm sample size
Rate 60 minutes	8	-	7	7 SDs and 3 arm sample size
Pain-like behaviors 60 minutes	3	-	2	1 mean, 2 SDs and 2 arm sample size
Pain-like behaviors 24 hours	1	-	1	mean, SD, arm sample size
NSAIDs (all except tolfenamic acid)				
Cortisol 60 minutes	15	10	2	1 mean, 2 SDs and 1 arm sample size
Cortisol 24 hours	10	4	3	2 means, 3 SDs
Energy 60 minutes	5	1	3	1 mean, 1 SD and 3 totals
Pain-like behaviors 60 minutes	2	-	2	1 SD and 1 arm sample size
Pain-like behaviors 24 hours	5	-	2	1 SD and 1 arm sample size

SD: Standard deviation.

Table 21: Reporting of piglet weight, NSAIDs, dose, and frequency of administration.

Study	Reported weight of piglets	Drugs/intervention	Dose reported	Frequency of administration
(Langhoff et al., 2009)	Birth weight: at least 1 kg	Meloxicam	0.4 mg/kg	15–30 minutes before procedure
		Flunixin Meglumine	2.2 mg/kg	
(Zoels et al., 2006)	Not reported	Meloxicam	0.4 mg/kg	15 minutes before castration
(Wavreille et al., 2012)	2.6±0.4 kg	Tolfenamic acid	2 mg/kg (0.08–0.18 ml)	1 hour prior to castration
		Meloxicam	0.4 mg/kg (0.13–0.29ml)	1 hour prior to castration
(Schwab et al., 2012)	Not reported	Ketoprofen	0.03 mg/kg	10–30minutes before castration
(Tenbergen, 2012)	Not reported	Meloxicam	0.4 mg/kg	30 minutes prior to procedure
(Courboulay et al., 2010)	Not reported	Ketoprofen	0.75 ml per piglet	Not reported
(Kluivers-Poodt et al., 2012)	Not reported	Meloxicam	0.4 mg/kg	15 minutes before procedure
(Schmidt et al., 2012)	>2 kg	Meloxicam	0.4 mg/kg	10 minutes before procedure
(Hansson et al., 2011)	2.2±0.5 kg	Meloxicam	0.2 ml of 5 mg/kg	Not reported
(Sutherland et al., 2012)	2.1 kg	Flunixin meglumine	Not reported	Not reported
(Reiner et al., 2012)	2.1±0.076 kg	Flunixin	5 mg	30 minutes before castration
(Reiner et al., 2012)	2.1±0.076 kg	Flunixin	5 mg	Immediately before castration
(Reiner et al., 2012)	2.1±0.076 kg	Meloxicam	2 mg or 2 mg/kg	Immediately before castration
(Sutherland et al., 2012)	2.0±0.6 kg	Flunixin meglumine	Not reported	Not reported
(Langhoff et al., 2009)	Birth weight: at least 1kg	Meloxicam	0.4 mg/kg	15–30 minutes before procedure
(Langhoff et al., 2009)	Birth weight: at least 1kg	Flunixin meglumine	2.2 mg/kg	15–30 minutes before procedure
(Langhoff et al., 2009)	Birth weight: at least 1kg	Carprofen	1.4 mg/kg	15–30 minutes before procedure
(Tenbergen, 2012)	Not reported	Ketoprofen	3 mg/kg	30 minutes before procedure

Table 22: Reporting of selected REFLECT items.

REFLECT checklist descriptor	Reported	Partially reported	Not reported
How **study units** were allocated to interventions (i.e., "random allocation," "randomized," or "randomly assigned," or "weight matched") in abstract or title	5	-	47
Eligibility criteria for owner/managers and study units at each level of the organizational structure, and the settings and locations where the data were collected.	-	10	42
Precise details of the interventions intended for each group, **the level at which the intervention was allocated,** and how and when interventions were actually administered.	21	31	-
Specific objectives and hypotheses.	7	8	37
Clearly defined primary and secondary outcome measures and the levels at which they were measured, and, when applicable, any methods used to enhance the quality of measurements (e.g., multiple observations, training of assessors).	6	5	41
How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	-	-	52
Randomization – Sequence generation.	-	-	52
Randomization – Allocation concealment.	-	-	52
Randomization – Implementation.	-	-	52
Random/randomized/randomly/random order.	33	-	
Blinding (masking).	-	18	34
Statistical methods.	-	44	8
Dates defining the periods of recruitment and follow-up.	6	-	46
Baseline demographic and clinical characteristics of each group.	-	5	47

Numbers analyzed.	1	7	44
Outcomes and estimation.	-	11	41
Ancillary analyses.	-	-	52
Adverse events.	-	22	30

Figure 1: Study flow diagram

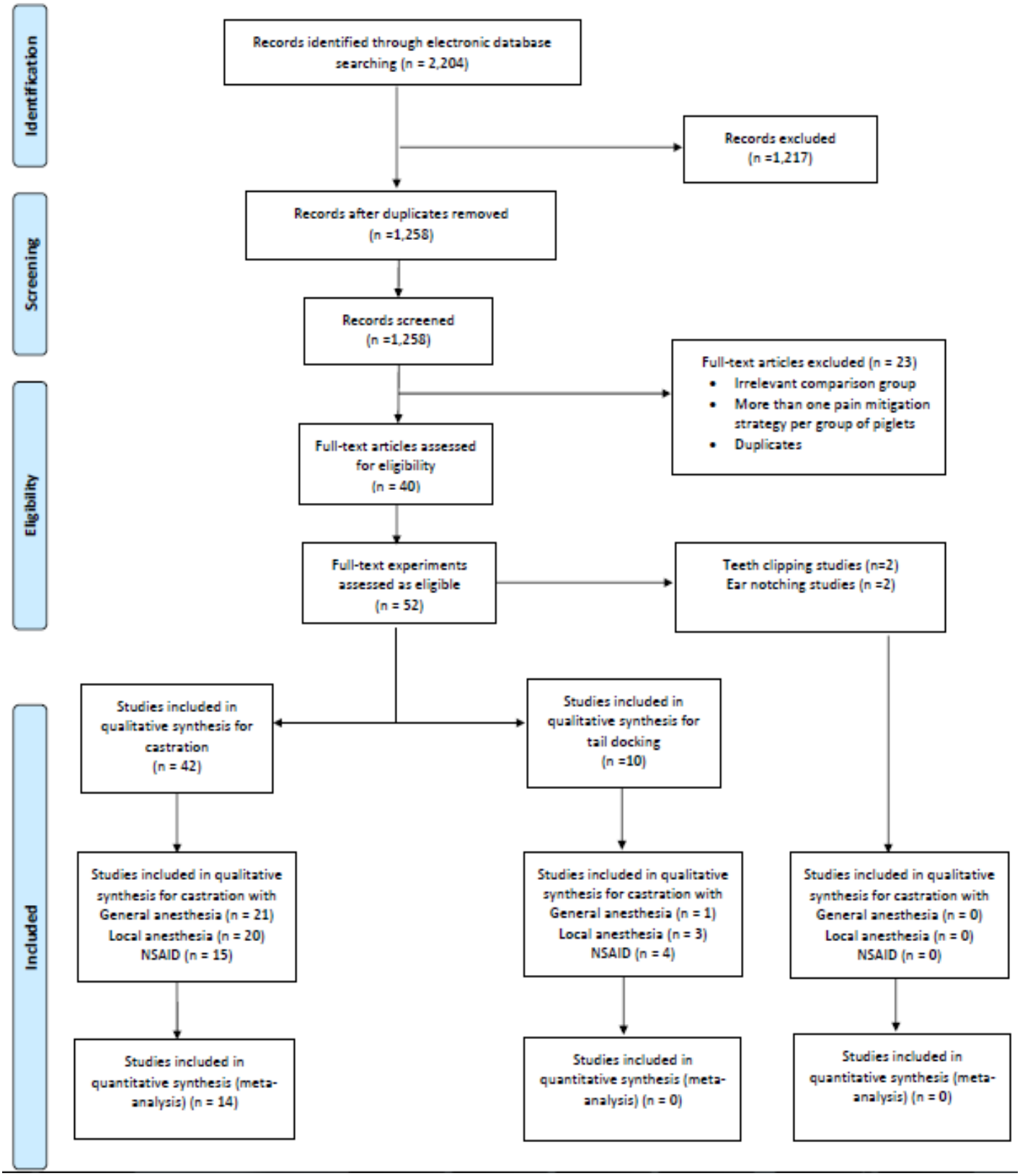

Figure 2: Table 1 from "Pre-natal stress amplifies the immediate behavioural responses to acute pain in piglets" (Rutherford et al., 2009).

Permission must be obtain before public release

Table 1. Summary statistics (mean ± s.e.) for mixed or control sows and their litters.

variable	mixed	control	statistics
sows			
weight gain (kg) over mix period	-0.53 ± 2.46	13.95 ± 2.27	$W = 10.31$, $p = 0.031$
body lesions at end of mix period	83.6 ± 9.6	14.2 ± 2.8	$W = 22.22$, $p = 0.007$
behaviour (% of daytime in bedded area) during mix period	15.3 ± 3.7	76.4 ± 3.5	$W = 32.69$, $p = 0.004$
salivary cortisol (ng ml^{-1})			
mix 1, day 1	5.21 ± 0.52	2.76 ± 0.36	$W = 12.48$, $p = 0.002$
mix 2, day 1	4.99 ± 0.53	2.32 ± 0.20	$W = 15.99$, $p < 0.001$
piglets			
litter size	12.1 ± 0.81	11.5 ± 0.98	$W = 0.28$, $p = 0.62$
day 1 weight (kg)	1.45 ± 0.04	1.49 ± 0.07	$W = 0.47$, $p = 0.53$
day 1 ponderal index	72.9 ± 2.07	72.5 ± 2.96	$W = 0.01$, $p = 0.92$

Figure 3: Table 2 from "The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia" (Sutherland et al., 2012).

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Table 2. Cortisol concentrations (ng/mL) in pigs (least squares means ± SE) in response to control handling or castration with or without pain relief (n = 10/treatment)

	Treatment[1]							
Time	CON	CO2	CON+NSAID	CAS	CAS+CO2	CAS+NSAID	CAS+BOTH	SE[2]
0 min	10.0	8.9	11.5	7.8	7.4	9.6	4.2	11.8
30 min	24.2^{a}	26.7^{a}	20.6^{a}	70.2^{b}	51.7^{b}	49.3^{b}	63.8^{b}	11.6
60 min	30.6^{a}	39.3^{a}	34.5^{abc}	98.6^{d}	49.4^{abcd}	79.9^{c}	43.9^{abcd}	11.8
120 min	6.3^{a}	12.3^{a}	23.3^{abc}	46.0^{c}	14.8^{ac}	54.5^{dc}	41.9^{c}	11.9
180 min	12.7	10.4	11.2	11.6	6.3	20.4	33.0	11.7
24 h	11.6	10.8	13.1	18.8	16.7	24.5	8.8	11.5
3 d	11.3^{ab}	10.8^{ab}	29.8^{ab}	7.3^{a}	8.2^{a}	22.9^{b}	27.6^{b}	11.5

[1]Treatments: sham castration (CON); sham castration while the pig was anesthetized with carbon dioxide (CO2); sham castration plus non-steroidal anti-inflammatory drug (NSAID) administered at the time of handling (CON+NSAID); castration (CAS); castration while the pig was anesthetized with carbon dioxide (CAS+CO2); castration plus NSAID administered at the time of castration (CAS+NSAID); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH).

[2]Pooled SE.

[a-d] Within a row, means without a common superscript differ ($P < 0.05$).

Figure 4: Table 4 from "The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia" (Sutherland et al., 2012).

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Table 4. The percentage of time pigs spent performing behaviors and postures (least squares means ± SE) after control handling or castration with or without pain relief (n = 10/treatment)

	Treatments[1]								*P*-value		
Behavior[2]	CON	CO2	CON+ NSAID	CAS	CAS+ CO2	CAS+ NSAID	CAS+ BOTH	SE[3]	Treatment	Period	Treatment × Period
Lying without contact	2.1[a]	2.5[ab]	1.4[a]	4.1[bc]	4.9[c]	1.1[a]	1.1[a]	0.8	< 0.0001	0.066	0.001
Lying with contact	38.9	39.7	38.9	34.4	35.7	41.0	38.0	2.8	0.141	0.000	0.891
Nursing	12.1	12.4	14.4	14.4	12.3	13.1	13.7	2.5	0.908	0.235	0.775
Sitting	0.7	0.9	0.9	1.0	0.7	0.7	0.6	0.3	0.860	0.001	0.335
Standing	7.0	5.7	5.3	6.8	6.8	5.1	7.9	1.5	0.302	< 0.0001	0.875
Walking	1.5	1.1	1.4	1.6	1.2	1.4	0.7	0.7	0.741	< 0.0001	0.805
Pain-like behaviors	0.9	1.0	0.9	1.2	1.4	0.7	1.0	0.4	0.744	< 0.0001	0.007

[1]Treatments: sham castration (CON); sham castration while the pig was anesthetized with carbon dioxide (CO2); sham castration plus NSAID administered at the time of handling (CON+NSAID); castration (CAS); castration while the pig was anesthetized with carbon dioxide (CAS+CO2); castration plus NSAID administered at the time of castration (CAS+NSAID); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH).

[2]Behaviors are described in Table 1.

[3]Pooled SE.

[a-c] Within a row, least square means without a common superscript differ ($P < 0.05$).

Declaration of interest statements

Dr. Anthony is the ethics advisor for American Veterinary Medicine Association's Animal Welfare Committee and currently receives funding from USDA.

Dr. Bergamasco has been a consultant for Hormel Foods Corporation, co-investigator in funding from NPB.

Dr. Coetzee has been a consultant for Intervet-Schering Plough Animal Health (Now Merck), Boehringer-Ingelheim Vetmedica, Zoetis Animal Health and Norbrook Laboratories Ltd. He has also addressed the Staff College at the US Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) and has received funding from USDA- CSREES, Animal Protection (Animal Well-being), NRI Grant # 2008-35204-19238 and 2009-65120-05729.

Dr. Dzikamunhenga was supported as a postdoctoral fellowship funded by the National Pork Board.

Ms. Gould was partially supported by the National Pork Board.

Dr. Johnson serves on the Animal Welfare Committee for the NPB, and the Animal Well-being committee for IPPA, and receives complimentary travel and lodging to attend meetings. She currently consults for Bob Evans, Elanco, McDonalds and Murphy-Browns. In the past she has consulted for Kroger's. Funding has been received through the following pharmacology agencies Boehringer-Ingelheim Vetmedica, Elanco and Pfizer. Dr. Johnson has received funding for welfare related research from NPB.

Dr. Locke Karriker has been a consultant. He consults for Boehringer Ingelheim Vetmedica, McDonald's, Safeway Inc and Bayer Animal Health. He currently has active research funding from the National Pork Board, the Iowa Pork Producers Association, the USDA Higher Education Challenge Competitive Grants Program, and Boehringer Ingelheim Vetmedica. He is the Director of the Swine Medicine Education Center at Iowa State University.

Dr. Marchant-Forde has received research funding from, and been a research grant reviewer for NPB. Funding has been received through Elanco for projects unrelated to pain interventions. As a Federal Government employee, he has had no financial compensation for consulting activities from any commercial company

Dr. Martineau has received research funding from Bayer, Boehringer-Ingelheim, MSD, Novartis, VIRBAC, Zoetis. None of these researches were related on drugs related to pain. He is also regularly consultant for major swine Coops in France as well as "Institut Technique du Porc" similar to NPB.

Dr. Millman current serves on the Animal Well-being Committee for Iowa Pork Producers Association, and consults for McDonalds Corporation, Humane Farm Animal Care and HyLine International (poultry). Her pain related research has been funded by Agriculture and Food Research Initiative competitive grants from the USDA National Institute of Food and Agriculture (grant #2012-67021-19363 and #2011-67021-30369), Natural Sciences and Engineering Research Council of Canada, Canadian Foundation for Innovation, National Pork Board, Iowa Pork Producers, Ontario Pork, Boehringer-Ingelheim Vetmedica (bovine only), Novartis Animal Health (bovine), Intervet-Schering Plough Animal Health (bovine), Pfizer Animal Health (bovine), Merck-Merial (bovine). She has received complimentary travel and lodging to attend meetings from Boehringer-Ingelheim Vetmedica, Inc., Bayer Animal Health.

Dr. McKean serves on the NPB Animal Welfare Committee and has served on the IPPA Swine Health and Welfare Committee and as such received complimentary travel and lodging to attend meetings. Mc Kean

has received research grants from the NPB. He does not solicit animal welfare funding or consultation arrangements. Dr. McKean has received funding for food safety research from NPB and the current project related to animal welfare.

Dr. O'Connor serves on the food safety committee for the NPB and receives complimentary travel and lodging to attend meetings. Dr. O'Connor has received funding for food safety research from NPB and the current project related to animal welfare. Dr. O'Connor has been funded for projects by Pfizer Animal Health unrelated to pain interventions or swine welfare. Dr. O'Connor teaches clinical trial design for swine producers for Boehringer-Ingelheim Vetmedica.

Dr. Pajor serves on the NPB Animal Welfare committee and has received funding from for animal welfare research from NPB and USDA- CSREES, Animal Protection (Animal Well-being), NRI Grant # 2009-65120-05729.

Dr. Sprague has received complimentary travel and lodging to attend meetings from Boehringer-Ingelheim Vetmedica, Inc., Zoetis and Novartis Animal Health.

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