**Supplementary Table S1:** Characteristics of 52 studies evaluated in 40 articles

| Study characteristics | Castration | Tail Docking | Ear Notching | Teeth Clipping |
| --- | --- | --- | --- | --- |
| Experiments | 42 | 10 | 2 | 2 |
| Technique | 0 | 0 | 0 | 0 |
| Castration – cut | 21 | 0 | 0 | 0 |
| Castration – tear | 5 | 0 | 0 | 0 |
| Castration – not reported | 16 | 0 | 0 | 0 |
| Tail docking – side cutters | 0 | 7 | 0 | 0 |
| Tail docking – blade | 0 | 2 | 0 | 0 |
| Tail docking – surgical cutters | 0 | 1 | 0 | 0 |
| Ear ID – notching | 0 | 0 | 2 | 0 |
| Teeth trimming – clip | 0 | 0 | 0 | 2 |
| Language |  |  |  |  |
| English | 24 | 8 | 2 | 2 |
| German | 17 | 0 | 0 | 0 |
| French | 2 | 2 | 0 | 0 |
| Danish | 1 | 0 | 0 | 0 |
| Type of Publication |  |  |  |  |
| Published article | 38 | 7 | 2 | 2 |
| Conference proceeding | 2 | 0 | 0 | 0 |
| Thesis | 2 | 3 | 0 | 0 |
| Report | 1 | 0 | 0 | 0 |
| Not Reported  | 1 | 0 | 0 | 0 |
| Country |  |  |  |  |
| Australia | 0 | 2 | 2 | 2 |
| Belgium | 1 | 0 | 0 | 0 |
| Brazil | 1 | 0 | 0 | 0 |
| Canada | 0 | 0 | 0 | 0 |
| France | 1 | 2 | 0 | 0 |
| Germany | 8 | 0 | 0 | 0 |
| Sweden | 1 | 0 | 0 | 0 |
| Switzerland | 2 | 0 | 0 | 0 |
| United States | 2 | 1 | 0 | 0 |
| Not reported | 28 | 5 | 0 | 0 |
| Setting |  |  |  |  |
| University farm | 3 | 2 | 2 | 2 |
| Private farm | 4 | 3 | 0 | 0 |
| Laboratory facility | 4 | 2 | 0 | 0 |
| Not reported  | 33 | 3 | 0 | 0 |
| Production system |  |  |  |  |
| All in/all out | 3 | 3 | 0 | 0 |
| Not reported | 41 | 7 | 2 | 2 |
| Management system |  |  |  |  |
| Farrowing crates | 10 | 6 | 0 | 0 |
| Farrowing pens | 1 | 0 | 0 | 0 |
| Not reported | 32 | 4 | 2 | 2 |
| Number of animals  | 9766 | 5065 | 115 | 117 |
| Not reported  | 2 | 0 | 0 | 0 |
| Age  |  |  |  |  |
| Range | 1–28 days | 18 hours–7 days | 0 | 0 |
| Min | 1 day | 18 hours | 0 | 0 |
| Max | 28 days | 7 days | 3 days | 3 days |
| Not reported | 1 | 0 | 0 | 0 |
| Weight (kg) |  |  |  |  |
| Range | 1–10.6  | 0 | 0 | 0 |
| Min | 1 | 0 | 0 | 0 |
| Max | 10.6 | 0 | 0 | 0 |
| Mean |  | 2.2  |  |  |
| Not reported | 31 | 8 | 2 | 2 |
| Breed |  |  |  |  |
| Meat breed | 31 | 9 | 2 | 2 |
| Not reported | 12 | 1 | 0 | 0 |
| Other | 1 | 0 | 0 | 0 |
| Number of relevant arms in study |  |  |  |  |
| 2 | 23 | 8 | 2 | 2 |
| 3 | 11 | 0 | 0 | 0 |
| 4 | 7 | 0 | 0 | 0 |
| 5 | 2 | 2 | 0 | 0 |
| 6 | 1 | 0 | 0 | 0 |
| Intervention |  |  |  |  |
| Pharmacological | 39 | 5 | 0 | 0 |
| Non-pharmacological | 6 (1 says both) | 7 (2 say both) | 2 | 2 |

**Supplementary Table S2:** Reporting of compound, dose and time of administration for studies that used non-steroidal anti-inflammatory drug (NSAID)

| Study | Drugs/intervention | Dose reported | Time of administration |
| --- | --- | --- | --- |
| ([Courboulay *et al.*, 2010](#_ENREF_3)) | 1% Ketoprofen | 0.75 ml per piglet | Not reported |
| ([Hansson *et al.*, 2011](#_ENREF_4)) | Meloxicam | 0.2 ml of 5 mg/kg BW | Not reported |
| ([Kluivers-Poodt *et al.*, 2012](#_ENREF_5)) | Meloxicam | 0.4 mg/kg | 15 minutes before castration |
| ([Langhoff *et al.*, 2009](#_ENREF_6)) | Meloxicam | 0.4 mg/kg BW | 15–30 minutes before castration |
| Flunixin meglumine | 2.2 mg/kg BW | 15–30 minutes before castration |
| Carprofen | 1.4 mg/kg BW | 15–30 minutes before castration |
| ([Reiner *et al.*, 2012](#_ENREF_8)) | Flunixin | 5 mg | 30 minutes before castration |
|  | Flunixin | 5 mg | Immediately before castration |
|  | Meloxicam | 2 mg or 2 mg/kg | Immediately before castration |
| ([Schmidt *et al.*, 2012](#_ENREF_10)) | Meloxicam | 0.4 mg/kg | 10 minutes before castration |
| ([Schwab *et al.*, 2012](#_ENREF_11)) | Ketoprofen | 0.03 mg/kg | 10–30 minutes before castration |
| ([Sutherland *et al.*, 2012](#_ENREF_13)) | Flunixin meglumine | Not reported | At the time of castration  |
| ([Sutherland *et al.*, 2012](#_ENREF_13)) | Flunixin meglumine | Not reported | At the time of castration |
| ([Tenbergen, 2012](#_ENREF_14)) | Meloxicam | 0.4 mg/kg | 30 min prior to procedure |
| ([Tenbergen, 2012](#_ENREF_14)) | Ketoprofen | 3 mg/kg | 30 min before procedure |
| ([Wavreille *et al.*, 2012](#_ENREF_15)) | Tolfenamic acid | 2 mg/kg (0.08–0.18 ml) | 1 h prior to castration |
| Meloxicam | 0.4 mg/kg (0.13–0.29 ml) | 1 h prior to castration |
| ([Zoels *et al.*, 2006](#_ENREF_16)) | Meloxicam 20 mg/ml | 0.4 mg/kg | 15 min before castration |

**Supplementary Table S3**: Examples of check items from the REFLECT statement sections related to the introduction and discussion from the reviewed trials reported

| Paper sectionand topic | Item | Example from review studies |
| --- | --- | --- |
| Title & Abstract | 1 | “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](#_ENREF_7)). |
| Introduction -- Background | 2 | “Carbon dioxide gas is currently being used in several countries to stun pigs before exsanguination during slaughter. Carbon dioxide gas is usually administered at concentrations greater than 80% to minimize the aversion period experienced by the animal and reduce the risk of inadequate anesthesia (Nowak et al., 2007). Kohler et al. (1998) concluded that CO2 anesthesia with a concentration of 80% can be induced safely and rapidly in pigs and that castration can be performed without any reaction, but stress induced by handling and manipulation before castration is not reduced. Administering CO2 can cause behavioral signs of aversion in pigs. Svendsen (2006), on the other hand, stated that aversion before losing consciousness is compensated for by the fact that piglets experience complete anesthesia and analgesia during castration. Gerritzen et al. (2008) observed that heavy breathing was the only typical behavior that piglets exhibited when exposed to a mixture of 70% CO2 and 30% O2 . Gerritzen et al. (2008) also concluded that the period of unconsciousness and analgesia achieved by this gas mixture was long enough to castrate pigs without them experiencing pain. Trembling behavior has been observed in piglets, lambs, and dogs for several days after castration, which is indicative that castration causes pain (Morton and Griffiths, 1985; Wemelsfelder and van Putten, 1985; Molony et al., 1997). Other pain-specific behaviors observed in piglets after castration include spasms, tail wagging, scratching, and huddling up, as well as changes in lying and suckling behavior (McGlone et al., 1993; Taylor et al., 2001; Hay et al., 2003; Llamas Moya et al., 2008). However, the effect of CO2 anesthesia before castration on pain-related behaviors displayed by piglets after castration has not yet been studied. The hypothesis of this study is that piglets will experience less pain and discomfort after castration when anesthetized with CO2 before castration, thus improving their overall welfare.” ([Beirendonck *et al.*, 2011](#_ENREF_1)) |
| Discussion --Interpretation | 20 | “In our study, it was shown that the required current of 1.25 A stunned a vast majority (92%) of the animals correctly. When using a lower current (0.6 A), however, stun quality results were poor. Furthermore, both trials reported here generally support earlier findings that tongs’ position influences stun quality, where a frontal position (as required by Swedish animal welfare legislation) is more efficient than other positions (reference in original text), although these results were only statistically significant in one of the two trials included”([Berg *et al.*, 2012](#_ENREF_2)).\* |
| Generalizability | 21 | “The results presented here represent some preliminary evidence that the immediate responses to acute pain are increased as a consequence of maternal pregnancy stress. Pain perception is dynamic and labile and is open to manipulation by the social and physical environment throughout life. The extent to which prenatally induced changes might persist throughout an individual’s lifetime remains to be determined. However, in rats, PNS increased the severity of the spontaneous behavioural response to formalin through to adulthood” ([Rutherford *et al.*, 2009](#_ENREF_9)).  |
| Overall evidence | 22 | “Although the interquartile range was lowest in the SHAM group, the CAST group was also low and did not differ significantly from the SHAM group. SHAM and LIDO piglets had significantly lower entropy than CAST piglets, with the other groups in between. This is quite opposite to the findings of Puppe et al., 2005 (reference in original text), but the direction of differences fits with what we found in the other parameters. Taken together, the vocal parameters are indicative of a pain reducing effect of lidocaine. It is also apparent, however, that not all pain is relieved. This could be due to the fact that intratesticularly applied local anaesthesia probably does not provide full anaesthesia of the cremaster muscle, scrotal ligament and intra-abdominal part of the spermatic cord” ([Kluivers-Poodt *et al.*, 2012](#_ENREF_5)). |
|  |  |  |

\*Example not selected from the study set

**Supplementary Figure S1:** An example of an approach to reporting comparisons in an intervention trial. The authors provided the number of animals in each group, magnitude of effect in each group, a measure of precision, and a descriptor of the probability that the result was due to chance under the null hypothesis i.e., the p value ([Sutherland *et al.*, 2011](#_ENREF_12)).

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**References**

Beirendonck Sv, Driessen B, Verbeke G and Geers R 2011. Behavior of piglets after castration with or without carbon dioxide anesthesia. Journal of Animal Science 89, 3310-3317.

Berg C, Nordensten C, Hultgren J and Algers B 2012. The effect of stun duration and level of applied current on stun and meat quality of electrically stunned lambs under commercial conditions. Animal Welfare 21, 131-138.

Courboulay V, Hemonic A, Gadonna M and Prunier A 2010. Effect of local anesthesia or anti-inflammatory treatment on pain associated with piglet castration and on labour demand Castration avec anesthesie locale ou traitement anti-inflammatoire: quel impact sur la douleur des porcelets et quelles consequences sur le travail en elevage? Acta Veterinaria Scandinavica 42, 27-34.

Hansson M, Lundeheim N, Nyman G and Johansson G 2011. Effect of local anaesthesia and/or analgesia on pain responses induced by piglet castration. Acta Veterinaria Scandinavica 53, 34.

Kluivers-Poodt M, Houx BB, Robben SR, Koop G, Lambooij E and Hellebrekers LJ 2012. Effects of a local anaesthetic and NSAID in castration of piglets, on the acute pain responses, growth and mortality. Animal 6, 1469-1475.

Langhoff R, Zoels S, Barz A, Palzer A, Ritzmann M and Heinritzi K 2009. Investigation about the use of analgesics for the reduction of castration-induced pain in suckling piglets. Berliner Munchener Tierarztliche Wochenschrift 122, 325-332.

Rand JS, Ainscow J, Blackshaw JK, Noonan GJ and Priest J 2002. Oral administration of a 12% sucrose solution did not decrease behavioural indicators of distress in piglets undergoing tail docking, teeth clipping and ear notching. Animal Welfare 11, 395-404.

Reiner G, Schollasch F, Hillen S, Willems H, Piechotta M and Failing K 2012. Effects of meloxicam and flunixin on pain, stress and discomfort in male piglets during and after surgical castration. Berliner und Munchener Tierarztliche Wochenschrift 125, 305-314.

Rutherford KM, Robson SK, Donald RD, Jarvis S, Sandercock DA, Scott EM, Nolan AM and Lawrence AB 2009. Pre-natal stress amplifies the immediate behavioural responses to acute pain in piglets. Biology Letters 5, 452-454.

Schmidt T, König A and von Borell E 2012. Impact of general injection anaesthesia and analgesia on post-castration behaviour and teat order of piglets. Animal 6, 1998-2002.

Schwab S, Follrich B, Kurtev V and Keita A 2012. Ketoprofen - practical use and efficacy for post-surgical analgesia in piglet castration Ketoprofen - Einsatz und Wirksamkeit zur post-operativen Analgesie bei der Ferkelkastration. Tierarztliche Umschau 67, 207-213.

Sutherland MA, Davis BL and McGlone JJ 2011. The effect of local or general anesthesia on the physiology and behavior of tail docked pigs. Animal 5, 1237-1246.

Sutherland MA, Davis BL, Brooks TA and Coetzee JF 2012. The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia. Journal of Animal Science 90, 2211-2221.

Tenbergen R 2012. Investigation of the use of analgesics at the time of castration and tail-docking and following parturition for improving performance and reducing pain in pigs. MSc Thesis. University of Guelph, Guelph, Ontario, Canada.

Wavreille J, Danard M, Servais V, Art T, Nicks B and Laitat M 2012. Effect of preoperative meloxicam or tolfenamic acid administration on stress and pain induced by surgical castration in piglets Analgesie preemptive du porcelet avant la castration: comparaison de l'effet de l'acide tolfenamique et du meloxicam sur la douleur et le stress. Journees de la Recherche Porcine en France 44, 275-276.

Zoels S, Ritzmann M and Heinritzi K 2006. Effect of analgesics on castration of male piglets. Berliner Munchener Tierarztliche Wochenschrift 119, 193-196.