Behavioural alterations in piglets after surgical castration: Effects of analgesia and anaesthesia

Jinhyeon Yun[a,*], Anna Ollila[b,*], Anna Valros[a], Paula Larenza-Menzies[c], Mari Heinonen[b], Claudio Oliviero[b], Olli Peltoniemi[b]

[a]Research Centre for Animal Welfare, Department of Production Animal Medicine, P.O. Box 57, 00014 University of Helsinki, Finland

[b]Production Animal Hospital, Department of Production Animal Medicine, P.O. Box 66, 00014 University of Helsinki, Finland

[c]Department of Anaesthesiology and Perioperative Intensive-Care Medicine, University of Veterinary Medicine, Vienna, Austria

*Equal contribution and corresponding author. Email: jinhyeon.yun@helsinki.fi; ell.anna.ollila@gmail.com

Abstract

The present study aimed to use behavioural measures to assess pain induced by surgical castration of piglets, and evaluate the efficacy of pain-relief medications. In total, 143 male piglets from 29 sows were used. The treatments included: 1) non-castration (NC; n = 28), 2) castration without medication (SC; n = 29), 3) castration with meloxicam injection 0.4 mg/kg i.m. (ME; n = 28), 4) castration with 0.5 ml of 2% lidocaine in each testicle (LA; n = 29), and 5) castration with general inhalation anaesthesia using isoflurane (1.5%) and meloxicam injection (GA; n = 29). Behaviour was monitored continuously for a ten minute period one hour prior to castration (-1 h), as well as immediately (0 h), one hour (1 h), and two hours (2 h) after castration. Behaviour was also monitored twice (08:00 and 20:00) during the following day. Compared to -1 h, castration induced changes in several behavioural measures in SC piglets at 0 h, suggesting that castration was painful. Furthermore, inactive standing or sitting, tail wagging and aggressive behaviour differed between SC and NC piglets at 0 h. ME and LA piglets spent less time standing or sitting inactively, and LA and GA piglets showed more tail wagging than SC piglets at 0 h ($P < 0.05$ for all). No other behavioural measures differed among the various groups of castrated piglets. In conclusion, the results indicate that surgical castration is indeed painful. However, the efficacy of various pain-relief protocols in piglets shortly after castration was not verified.

Keywords: piglet castration; pain assessment; pain behaviour; social contagion; anaesthesia; analgesia

1. Introduction

In modern swine production, castration of male piglets is a standard practice, mainly to reduce the risk of unfavourable odour (boar taint) in the meat. However, surgical castration without pain mitigation causes welfare problems for piglets because the procedure is painful (O'Connor et al., 2014; Prunier et al., 2006; Von Borell et al., 2009). To reduce pain induced by surgical castration, many European countries advise the use of anti-inflammatory pain treatment in pig production (European Commission, 2010). However, proof of the efficacy of various treatments has not been consistent (O'Connor et al., 2014; Prunier et al., 2006).

Currently, non-steroidal anti-inflammatory drugs (NSAIDs), as well as local and general anaesthetics, are the most frequently studied treatments for mitigating the pain induced by surgical castration of piglets (De Briyne et al., 2016; O'Connor et al., 2014). However, their efficacy in reducing pain remains controversial. It is presumed that NSAIDs are more efficient for the inflammatory component of pain rather than for acute incisional pain, which is mediated by several other compounds and receptors (Coetzee, 2013a; O'Connor et al., 2014). Thus, the efficacy of NSAIDs as the sole treatment of castration-related pain cannot be taken for granted. On the other hand, the efficacy of local anaesthesia alone in the relief of castration-related pain in piglets also varies, depending on the technique (O'Connor et al., 2014; Sutherland et al., 2010) and method of pain evaluation (e.g. electroencephalography, vocalization tests, cortisol levels, pain-related behaviour). Similarly, numerous studies have shown inconsistent results regarding the use of general anaesthesia alone, particularly volatile agents that may remove conscious perception of pain during the castration procedure, but may not reduce postoperative pain (Axiak et al., 2007; O'Connor et al., 2014; Walker et al., 2004).

Surgical castration causes behavioural changes in piglets due to pain (and pain-related distress). Hence, behavioural responses have been widely used as a tool for assessing pain (Gottardo et al., 2016; Hay et al., 2003; Llamas Moya et al., 2008; Sutherland et al., 2010). Therefore, the first aim of this study was to confirm results from previous studies showing that surgical castration is painful. Specifically, behavioural responses of piglets castrated without the administration of pain-relief were compared to those of their non-castrated littermates before and after the castration procedure. The second aim was to evaluate the efficacy of some commonly used pain-relief protocols by observing piglet behaviour within the first two hours after castration, and again on the following day. We hypothesized that surgical castration without analgesia or anaesthesia would induce behavioural changes indicative of severe pain, and that these changes could be mitigated by different pain-relief treatments during castration.

2. Materials and methods

All experimental procedures performed in this study were approved by the Ethical Committee for International Animal Use and Care of the University of Helsinki, Helsinki, Finland (permission ESAVI/3331/04.10.03/2011).

2.1. Animals and management

The trial was conducted on a commercial pig herd in southern Finland in 2015. The herd housed approximately 170 sows (Finnish Landrace × Yorkshire). The sows were moved to a farrowing unit seven days prior to parturition. The farrowing pen (2.4 × 2.0 m) consisted of a conventional steel farrowing crate (2.1 × 0.8 m) on a 1/5 slatted concrete floor. The sows were kept in the crate until weaning (i.e. approximately four weeks postpartum). A creep area for piglets was situated in one corner of each pen, with a heat lamp and a bucket of sawdust provided twice daily on the solid concrete floor. The sows were allowed ad libitum access to water from a nipple drinker, and were fed a standard lactation diet three times per day via an automatic liquid feeding system. On the first day postpartum, litter sizes were equalized and piglets were orally supplemented with iron. Their teeth and tails were left intact. Piglets that were splay-legged or deformed, that received medications or were born to a sow that was medicated, or that weighed less than 1000 g were excluded from the study and moved to a separate area. The resulting average number of piglets per litter was 11.4 (± 1.8), with 6.7 (± 1.5) male piglets.

The experiment was carried out in three batches, each with nine or ten sows resulting in a total of 145 male piglets from 29 sows (5 male piglets per litter). On the fifth day postpartum, five male piglets from each litter were randomly allocated to the five different pain-relief treatments, respectively, during castration.

2.2. Experimental design and treatments

On the day of castration, all piglets selected for the trial were first moved into a trolley by the researcher. Before castration began, each piglet received an individual marking pattern on their back for recognition during the video monitoring. The treatments were: 1) non-castration, but staying on the trolley while other littermates were castrated (NC; n = 29), 2) surgical castration without medication (SC; n = 29), 3) surgical castration with meloxicam injection (0.4 mg/kg i.m.) 10 min prior to castration (ME; n = 29), 4) surgical castration with local anaesthesia (0.5 ml of 2% lidocaine in each testicle) 10 min prior to castration (LA; n = 29), and 5) surgical castration with general inhalation anaesthesia using isoflurane (1.5%) and meloxicam injection (0.4 mg/kg i.m.) 10 min prior to castration (GA; n = 29). Surgical castration was performed by a trained veterinary surgeon using an open technique approach (i.e. cutting the *tunica vaginalis* to expose the testicle). The piglets stayed on the trolley while the castration procedures of the other littermates were completed, then returned to the farrowing pen together immediately after castration.

2.3. Behavioural observations

Internet protocol (IP) cameras (Niceview NICECAM420WL, Niceview Corp.) were mounted in each pen. The video sequence output was recorded using IP-camera software (Blue Iris v.2.64, Perspective Software Corp.). The display resolution was 640 × 480 pixels, and the frame rate was 2 FPS. The CowLog v.2.0 (Hänninen and Pastell, 2009) behavioural recording program was used for data analyses. The researchers involved in behavioural observations and statistical analyses were unaware of which piglets were allocated to each of the different treatments during the castration procedure.

Piglets were video-recorded from one hour before the treatment/castration until 20:00 the next day. Behavioural observations were performed according to ethogram (Table 1), which was determined based on previous studies of castration in piglets (Hay et al., 2003; Llamas Moya et al., 2008; McGlone et al., 1993; Mellor et al., 2000), and lambs (Molony and Kent, 1997). Behavioural observations of the focal piglets were monitored continuously for a 10 min period at one hour before all the piglets were lifted for the castration procedure (-1 h), and then immediately (0 h), one hour (1 h), and two hours (2 h) after all the piglets were returned to the pen, and at 08:00 and 20:00 the following day. If more than half of a litter was sleeping or nursing during the monitoring periods, the behavioural observations for the 10 min period continued immediately after sleeping or nursing had finished. This procedure was used to reduce potential missing values in the monitoring periods. In the meantime, isolation and desynchronization were recorded regardless of whether the piglets were sleeping or nursing. Head shaking, leg crossing or shaking, rump rubbing, abnormal walking, tail wagging, and aggressiveness were recorded as events. A new event was counted whenever the behaviour had ceased for longer than one second. During each 10 min observation period, the total duration of inactive standing or sitting, isolation and desynchronization was recorded.

2.4. Statistical analysis

SAS v.9.4 (SAS Institute Inc., NC, USA, 2012) was used for statistical processing of all the data. The piglet was the experimental unit, and the sow nested within the batch was used as a random effect. The UNIVARIATE procedure with the Shapiro-Wilk test was used to test normality. None of the variables were normally distributed. Thus, the GLIMMIX procedure was used to analyse all behaviour data. A Poisson distribution with a log link function was fitted to the model. The data are presented as LS means ± SEMs on the original scale.

Behavioural changes of SC and NC according to the observation periods were analysed using repeated measures with a ‘first order ante-dependence’ structure. Castration procedure (SC and NC) and its interaction with the observation periods were included as fixed effects. Post-hoc contrast analyses were performed to compare behavioural observations between SC and NC piglets 1) in each monitoring period, and 2) before (-1 h) and after (0 h, 1 h, 2h, and at 08:00 and 20:00 the following day) castration.

The behavioural effects of the treatments after castration were analysed by repeated measures with a ‘compound symmetric covariance’ structure. The data were analysed separately for 1) shortly after castration (i.e. 0 h, 1 h, and 2 h), and 2) one day after castration (i.e. at 08:00 and 20:00 the following day). The pre-treatment data (i.e. -1 h) was fitted to the repeated measure test as a baseline measurement. The effect of the treatments on the frequencies of tail wagging and aggressive behaviour before and after castration were analysed using repeated measures with a ‘first order ante-dependence’ structure. Differences between the treatments were analysed with an *F* test in each observation period using the slice statement. Multiple comparisons were performed by the Tukey-Kramer procedure.

3. Results

Data from one NC and one ME piglet were omitted from behavioural observations because both had a seizure during the monitoring periods.

3.1. Behavioural changes before and after castration in NC and SC piglets

Among the SC piglets, the analysis of contrasts revealed a higher frequency of head shaking ($F_{1,56} = 4.22$, $P < 0.05$), leg crossing or shaking ($F_{1,56} = 5.74$, $P < 0.05$), abnormal walking behaviour ($F_{1,56} = 6.33$, $P < 0.05$), and longer duration of inactive standing or sitting ($F_{1,56} = 10.39$, $P < 0.01$) at 0 h, when compared with those at -1 h. Tail wagging ($F_{1,56}$ = 3.59, P = 0.06), rump rubbing ($F_{1,56}$ = 3.81, P = 0.06), isolating ($F_{1,56}$ = 3.35, P = 0.07), and desynchronizing ($F_{1,56}$ = 3.95, P = 0.05) behaviours also had a tendency to differ between -1 h and 0 h, although not significantly. At 1 h, the occurrences of these behaviours decreased to levels similar to those at -1 h, except for abnormal walking (Table 2).

NC piglets showed higher frequencies of tail wagging ($F_{1,54}$ = 4.35, P < 0.05), abnormal walking ($F_{1,54}$ = 7.34, P < 0.01) and aggressive behaviour ($F_{1,54}$ = 8.21, P < 0.01) at 0 h, when compared with -1 h. The frequency of head shaking also tended to be higher at 0 h ($F_{1,54}$ = 2.95, P = 0.09).

SC piglets spent longer times standing or sitting inactively than NC piglets at 0 h ($F_{1,109}$ = 10.67, P < 0.01; Table 2). NC piglets showed higher frequencies of tail wagging ($F_{1,109}$ = 6.27, P < 0.05) and aggressive behaviour ($F_{1,109}$ = 13.63, P < 0.001) than SC piglets at 0 h (Table 2). However, other behaviours, including head shaking, leg crossing or shaking, rump rubbing, abnormal walking, isolation and desynchronization, did not differ between SC and NC piglets at 0 h or on the following day (Table 2).

3.2. Effects of pain relief treatments on behavioural changes after castration

Two hours after the castration procedure, frequencies of leg crossing or shaking behaviour in ME, LA, and GA piglets were higher than those in NC piglets (P < 0.01 for all; Table 3), but did not differ from those in SC piglets. ME piglets tended to show more rump rubbing encounters than NC piglets (P = 0.05), but there were no differences comparing to SC, LA or GA piglets (Table 3). The frequency of abnormal walking was not differed between NC and ME piglets, but higher in SC, LA, and GA piglets (P < 0.05 for all; Table 3). SC piglets spent longer times standing or sitting inactively than NC and ME piglets (P < 0.05 for both), but there were no differences in LA and GA piglets two hours after the castration procedure (Table 3).

At 0 h, the frequency of tail wagging behaviour in NC, LA, and GA were higher than that in SC piglets (P < 0.05 for all; Fig 1). At 2 h, GA piglets showed higher frequencies of tail wagging behaviour than NC (P < 0.05; Fig 1). During the day after the procedure, behavioural observations did not differ among treatments (Table 3). At 2 h, NC piglets showed more frequent aggressive behaviour than SC piglets (P < 0.01), but ME, LA, and GA piglets did not differ from NC or SC piglets (Table 3). At 0 h, NC piglets showed the highest number of aggressive encounters of all treatments (P < 0.001; Fig 1). No differences among treatments were observed at 1 h, 2 h or during the following day (Fig 1). The observations for isolating and desynchronizing piglets did not differ between treatments during the two hours immediately after the procedure or during the following day (Table 3).

4. Discussion

The current findings obtained from comparisons of behavioural differences pre- and post-castration support the results from previous studies, which show behavioural alterations that suggest castration is painful in pigs (Gottardo et al., 2016; Hay et al., 2003; Llamas Moya et al., 2008; Sutherland et al., 2010). However, this study reports only a few differences in the behaviour of non-castrated piglets as compared to piglets castrated with no pain relief, immediately after the castration procedure: Only tail wagging, inactive standing or sitting, and aggressive behaviour differed between these treatments. Furthermore, the current results failed to verify the efficacy of pain-relief medications shortly after castration when different treatments are applied within the same litter.

To the best of our knowledge, this is the first study to compare behavioural differences pre- and post-castration. Other studies have evaluated pain in castrated piglets by comparing behaviours of non-castrated littermates only after the procedure. However, it has been suggested that behavioural reactions to stress can also be altered by emotional contagion through littermates (Boissy et al., 2007; Langford et al., 2006; Reimert et al., 2013; Špinka, 2012). Hence, the emotional contagion of behavioural responses between littermates receiving different treatments may limit the interpretation of the results of the present study. Furthermore, we cannot preclude that the emotional state of the non-castrated piglets who received the same general handling procedure while their littermates were castrated could also be changed in the current study.

Previous studies have shown that castrated piglets displayed more isolation and desynchronization than their non-castrated littermates (Hay et al., 2003; Llamas Moya et al., 2008). However, Gottardo et al. (2016) did not observe any differences in isolation behaviour between castrated piglets without pain-relief medications and those treated with topical anaesthetics prior to castration, although other pain-related behaviours did differ between the two groups in the first 30 min after castration. Similarly, we did not observe differences in isolation and desynchronization of piglets between the treatments shortly after castration. This might be a result of the higher density of castrated piglets (at least four castrated piglets with or without pain-relief medications), whereas the previous studies included only two or three castrated piglets (Hay et al., 2003; Llamas Moya et al., 2008). When more than half of the littermates were simultaneously suffering due to castration with or without pain-relief medications, which is probable in the current study, the outcomes of observations in isolation and desynchronization would be hampered. Alternatively, transmission of emotions, as discussed above, could affect the social cohesion of castrated piglets. Indeed, a study by Reimert et al. (2013) demonstrated that negative emotional state assessed by behavioural changes was evident both in pigs suffering from the aversive event and in their control pen mates. It is known that negative emotion can spread more strongly than positive emotion because animals generally respond more rapidly to threats (for a review, see Špinka, 2012). This could also explain the increased number of abnormal walking events documented for non-castrated piglets when they returned to the pen with their castrated littermates. Consequently, comparing behavioural changes between castrated and non-castrated piglets by measuring pain immediately after castration should be more carefully considered when pen space is shared, since such behaviour could be affected by social contagion among littermates.

Administration of meloxicam, which is an NSAID with a half-life of 15 to 20 h, has been recommended for mitigating pain induced by surgical castration of pigs. Gottardo et al. (2016) demonstrated that in the first 30 min after castration, piglets who received injectable meloxicam prior to castration displayed similar pain-related behaviour compared to non-castrated piglets. It has been suggested that NSAIDs play a role in inhibiting the production of prostaglandins and cytokines that are involved in cell signalling, and altering cytokine ratios that could affect pain sensitivity (Coetzee, 2013a; Fraccaro et al., 2013; Ochroch et al., 2003). However, pain stimulation induced by castration is known to be transmitted immediately via nerves as an electric pulse (Coetzee, 2013b). Consequently, a meloxicam injection for alleviating castration-induced pain in piglets has been reported to be only effective on the first day post procedure (Hansson et al., 2011; Keita et al., 2010; Kluivers-Poodt et al., 2013), whereas the effect was not evident during castration (Kluivers-Poodt et al., 2012). The current study appears to support the finding that meloxicam administration does not alleviate pain shortly after castration.

According to various physiological and behavioural assessments, administration of lidocaine, a commonly used local anaesthetic, into the testicles and scrotum of piglets prior to castration was suggested to be effective in reducing castration-related pain (for reviews see De Briyne et al., 2016; Prunier et al., 2006; Von Borell et al., 2009). In studies of lambs, the beneficial effects in reducing pain induced by castration and tail docking were demonstrated when these local anaesthetics were administered in spray or gel form before the procedures (Lomax et al., 2008; Paull et al., 2009). The study by Gottardo et al. (2016) also revealed that one hour after castration, blood cortisol levels of castrated piglets that received topical administration of tetracaine were lower than those that did not receive pain-relief medications. However, in line with other research (reviewed by O'Connor et al., 2014), our current findings indicated that local anaesthesia might not be effective in relieving short-term pain induced by castration. This suggestion arises from comparison of behavioural changes between piglets castrated with and without pain-relief medications. On the other hand, Young (2007) suggested that local anaesthesia could be effective in mitigating inflammatory pain caused by open wounds in young children. Similar to the efficacy of NSAIDs, it can therefore be speculated that administration of local anaesthetics might be more effective for relieving mid- to long-term pain related to inflammation induced by an incisional wound, rather than acute pain after castration. Further studies are needed to verify the long-term efficiencies of local anaesthetics in pain alleviation in piglets after castration.

Of the various general anaesthetic methods of inhalation or injection, isoflurane anaesthesia has been commonly used for castration in pigs, because of its relatively rapid processing time and safety for both workers and animals (De Briyne et al., 2016; Hodgson, 2007; Von Borell et al., 2009; Walker et al., 2004). In addition, Schulz et al. (2007) demonstrated that the isoflurane anaesthesia method for piglets did not induce marked stress during the procedure. However, its role in relieving pain during and after castration remains uncertain (O`Conner et al., 2014; Prunier et al., 2006; Schulz et al., 2007; Walker et al., 2004). In this study, NSAIDs were supplemented with isoflurane anaesthesia because general anaesthesia could be effective in reducing pain when used with analgesics (Prunier et al., 2006). Nevertheless, the current findings support those of previous studies that have also indicated that isoflurane anaesthesia may not in fact be effective in mitigating pain in piglets immediately or shortly after castration. However, it needs to be considered that the use of behavioural alterations to verify the efficacy of anti-inflammatory pain treatments is not straightforward in the current study design. Specifically, we did not exclude the potential influences of emotional contagion between experimental animals, as discussed above.

Previous studies categorizing tail wagging as a pain-related behaviour have reported that it occurred more often in castrated piglets compared with non-castrated piglets, for several days post-procedure (Hay et al., 2003; Prunier et al., 2006). In contrast, however, the present results demonstrated that the piglets castrated without pain medication showed less frequent tail wagging than the non-castrated piglets. The non-treated piglets also showed less frequent tail wagging in comparison to the castrated piglets under local or general anaesthesia immediately after castration. The current study further revealed that tail wagging was more frequently observed in the non-castrated piglets when they returned to the litter with their castrated littermates after the procedure. These findings may support the suggestion that tail wagging in pigs can also be interpreted as an indication of positive emotional states, for instance, compensation (Reimert et al., 2013), familiarity (Terlouw and Porcher, 2005), and playfulness (Newberry and Wood-Gush, 1988). However, the reason for the higher frequency of tail wagging two hours post-procedure in the piglets castrated under general anaesthesia was not determined in the current study. This highlights the need for further studies of tail wagging behaviour: is it a measure of a negative emotional state, such as pain; does it indicate a positive state; is it simply an indicator of general emotional arousal?

Previous studies have failed to determine an effect of castration on aggressive behaviour in piglets (Hay et al., 2003; O'Connor et al., 2014; Sutherland et al., 2010). Our study, however, demonstrated that the castration procedure - irrespective of the pain-relief medication administered - appeared to be associated with a reduction of aggressive behaviour immediately after the procedure. Castrated piglets have been reported to be less active and in less physical contact with their littermates than non-castrated pigs (Llamas Moya et al., 2008; Mellor et al., 2000), possibly because active or social behaviour could increase pain perception due to movements of the injured tissues (Hay et al., 2003; Llamas Moya et al., 2008; Mellor et al., 2000). The studies by Hay et al. (2013) and Llamas Moya et al. (2008) furthermore suggested that the castrated piglets displayed more inactive standing or sitting as a way to avoid inflicting more pain. In the current study, we found that the castrated piglets that did not receive any pain-relief treatment were less aggressive and spent more time standing or sitting inactively in comparison with their non-castrated littermates. As aggression usually results in vigorous movements, it can be speculated that the castrated piglets might be reluctant to show aggressive behaviour immediately after the procedure.

5. Conclusions

This study supports previous findings that surgical castration of piglets without administration of pain-relief medication changes their behaviour in a way that is indicative of pain immediately after the procedure. However, behavioural alterations of non-castrated littermates can also occur, most likely as a result of emotional contagion of the littermates, or changes in their emotional state due to the general handling procedure. Hence, the identification of true pain in castrated piglets can become complicated by these other factors. We therefore suggest that assessment of behavioural alterations pre- and post-castration would be more appropriate for evaluating pain induced by castration in piglets. We furthermore suggest that the current results cannot prove that pain-relief medications, such as those used in the present study, would be effective in mitigating pain in piglets immediately or shortly after castration.

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Table 1. Description of behaviour and parameters examined.

Behaviour	Description
Head shaking[1]	Shaking head vigorously from side to side.
Leg crossing or shaking[1]	Crossing or shaking hind legs while standing or sitting. Scratching the body or ear with the legs is not included.
Rump rubbing[1]	Moving rump up and down or side to side against the floor, pen wall, farrowing crate, or any surfaces of the pen.
Abnormal walking[1]	Walking with a limp, back arch or hind leg stiffness. Flopping down on the ground while walking.
Tail wagging[1]	Actively wagging tail in any direction, but mostly from side to side, when the piglet is lying, sitting or standing.
Inactive standing/sitting[2]	Sitting or standing without any motion, with the head down (lower than shoulder level), while at least half of the littermates are awake. Duration of less than 20 seconds is not included.
Aggression[1]	Forceful pushing or hitting with the head or biting behaviour toward littermates.
Isolation[2]	Complete physical separation from littermates. The distance is at least 40 cm to the nearest piglet or group of piglets. Duration of less than 20 seconds is not included.
Desynchronization[2]	Different activities than the majority of the littermates (e.g. the piglet is sleeping while most of the others are suckling). Duration of less than 20 seconds is not included.

[1]The number of encounters in 10 min periods was recorded.

[2]Total duration in 10 min periods was recorded.

Table 2. Effects of castration without pain relief medications before and after the procedure on behavioural changes in five-day-old piglets.

Observations[1]	Trt[3]	Time[2] -1 h	0 h	1 h	2 h	08:00	20:00	*P* value Trt	*Trt ×Time*
Head shaking	SC	0.3 ± 0.2	0.8 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.27	0.51
	NC	0.1 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1		
Leg crossing	SC	0.1 ± 0.1	0.8 ± 0.3	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.02	0.32
	NC	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1		
Rump rubbing	SC	0.0 ± 0.0	0.4 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.22	0.31
	NC	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0		
Abnormal walking	SC	0.0 ± 0.0	2.2 ± 0.6	1.0 ± 0.3 *	0.3 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.04	0.26
	NC	0.3 ± 0.1	1.4 ± 0.4	0.3 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.1		
Tail wagging	SC	0.1 ± 0.0	0.3 ± 0.1 *	0.4 ± 0.2	0.5 ± 0.3	1.2 ± 0.7	0.3 ± 0.1	0.45	0.05
	NC	0.3 ± 0.2	1.0 ± 0.4	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1		
Inactive standing or sitting, sec	SC	3 ± 1.9	102 ± 25.3 ***	39 ± 17.3 *	27 ± 8.3	13 ± 7.3	12 ± 6.2	0.01	<.0001
	NC	14 ± 6.9	24 ± 10.0	9 ± 5.0	28 ± 13.1	28 ± 11.2	6 ± 3.2		
Aggression	SC	0.1 ± 0.1	0.0 ± 0.0 ***	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.2	0.1 ± 0.1	0.03	<.01
	NC	0.2 ± 0.1	1.3 ± 0.4	0.0 ± 0.0	0.1 ± 0.1	1.0 ± 0.5	0.2 ± 0.1		
Isolation, sec	SC	4 ± 2.5	31 ± 14.9	25 ± 12.3	27 ± 10.6	20 ± 12.7	34 ± 17.5	0.96	0.35
	NC	30 ± 18.5	19 ± 9.7	9 ± 4.3	41 ± 17.0	31 ± 12.4	14 ± 5.8		

Desynchronization, sec	SC	6 ± 3.7	30 ± 11.0	21 ± 11.8	18 ± 6.4 *	8 ± 6.0	48 ± 18.4	0.73	0.13
	NC	32 ± 22.6	17 ± 10.0	5 ± 2.8	55 ± 25.3	13 ± 5.8	24 ± 10.6		

[1]The values represent LS means ± SEMs of frequency or duration (seconds) of behavioural observations.

[2]Behavioural observations were monitored for the first 10 minutes at one hour before all the piglets were lifted for the castration procedure (-1 h), and immediately (0 h), one hour (1 h), and two hours (2 h) after all the piglets were returned to the pen, and at 08:00 and 20:00 the following day.

[3]Treatments: 1) SC (n = 29): surgical castration without medication, 2) NC (n = 28): non-castration

*** Values are significantly different between treatments within periods, *; $P < 0.05$, ***; $P < 0.001$.

Table 3. Effects of five different castration procedures for five-day-old piglets on behavioural observations during the first two hours and the day following the procedure.

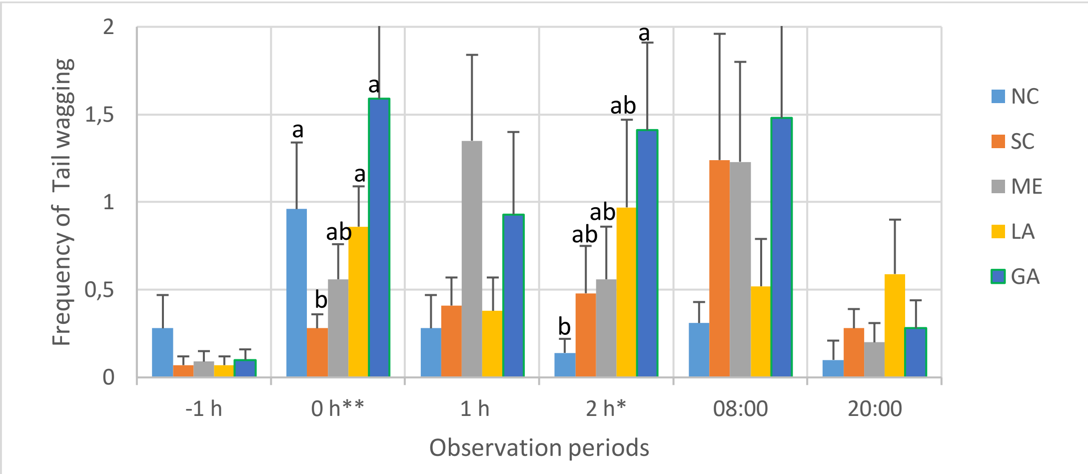
Observations[1]	Time[2]	Treatments[3]					P value
		NC	SC	ME	LA	GA	*P* value
Head shaking	*0-2h*	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.57
	1 d	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.64
Leg crossing	*0-2h*	0.2 ± 0.1[b]	0.5 ± 0.1[ab]	0.8 ± 0.2[a]	0.8 ± 0.2[a]	0.8 ± 0.3[a]	<.001
	1 d	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.53
Rump rubbing	*0-2h*	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.11
	1 d	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.65
Abnormal walking	*0-2h*	0.6 ± 0.1	1.2 ± 0.3	0.9 ± 0.2	1.2 ± 0.3	1.3 ± 0.3	0.09
	1 d	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.74
Tail wagging	*0-2h*	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	1.3 ± 0.4	0.13
	1 d	0.2 ± 0.1	0.8 ± 0.4	0.7 ± 0.3	0.5 ± 0.2	0.9 ± 0.4	0.13
Inactive standing or sitting, sec	*0-2h*	21 ± 5.8	55 ± 12.6	25 ± 7.3	33 ± 8.0	42 ± 11.3	0.10
	1 d	16 ± 5.8	13 ± 5.8	17 ± 6.8	14 ± 7.1	17 ± 8.4	0.99
Aggression	*0-2h*	0.5 ± 0.1[a]	0.1 ± 0.0[b]	0.3 ± 0.2[ab]	0.3 ± 0.1[ab]	0.3 ± 0.1[ab]	0.02
	1 d	0.6 ± 0.3	0.4 ± 0.1	0.7 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.88
Isolation, sec	*0-2h*	20 ± 6.4	30 ± 7.7	21 ± 5.6	27 ± 7.5	38 ± 10.9	0.59
	1 d	23 ± 6.7	27 ± 14.6	27 ± 9.0	24 ± 7.8	30 ± 9.7	0.98
Desynchronization, sec	*0-2h*	21 ± 7.6	26 ± 5.3	16 ± 4.6	18 ± 5.1	31 ± 10.0	0.50
	1 d	19 ± 6.0	28 ± 10.4	13 ± 5.9	9 ± 5.1	22 ± 8.4	0.46

[1]The values represent LS means ± SEMs of frequency or duration (seconds) of behavioural observations.

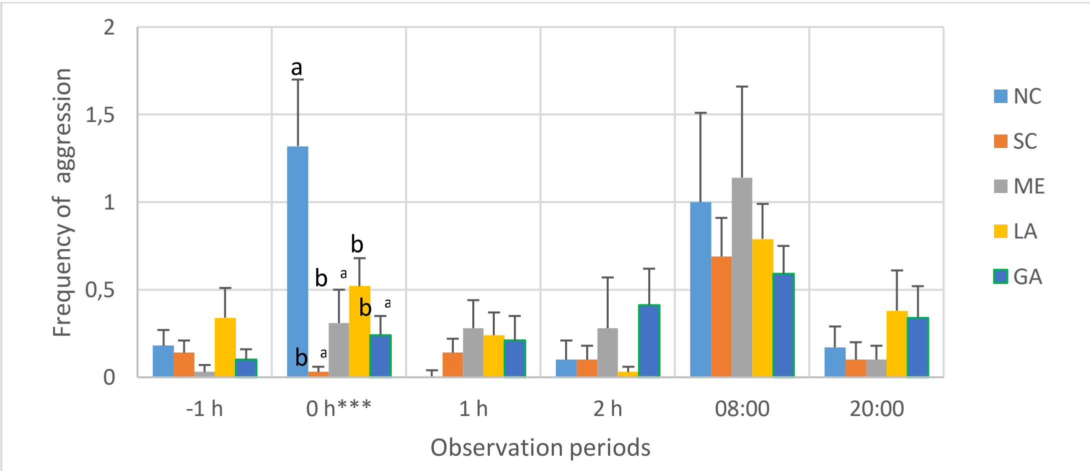
[2] Repeated measures carried out on the values, monitoring continuously for each 10 minute period with two set times: 1) *0-2h*: immediately, one hour, and two hours after all the piglets were returned to the pen, and 2) *1 d*: at 08:00 and 20:00 the following day.

[3]Treatments: 1) NC (n = 28): non-castration, 2) SC (n = 29): surgical castration without medication, 3) ME (n = 28): surgical castration with meloxicam injection, 4) LA (n = 29): surgical castration with local anaesthesia with lidocaine (2%), and 5) GA (n = 29): surgical castration with general inhalation anaesthesia using isoflurane (1.5%) and meloxicam injection.

[ab] Different letters indicate that the values differ within variables ($P < 0.05$).



(A)



(B)

Fig 1. The effect of treatment on frequencies (means with SEM) of tail wagging (A) and aggressive (B) behaviour for the first 10 minutes at one hour before all the piglets were lifted for the castration procedure (-1 h), and immediately (0 h), one hour (1 h), and two hours (2 h) after all the piglets were returned to the pen, and at 08:00 and 20:00 the following day. Treatments are 1) NC (n = 28): non-castration, 2) SC (n = 29): surgical castration without medication, 3) ME (n = 28): surgical castration with meloxicam injection, 4) LA (n = 29): surgical castration with local anaesthesia with lidocaine (2%), and 5) GA (n = 29): surgical castration with general inhalation anaesthesia using isoflurane (1.5%) and meloxicam injection. Asterisks indicate significant differences between the treatments at the observation periods (* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001). Different letters (a, b) without a common superscript (ab) indicate that the means differ (*P* < 0.05).