The castration of male suckling piglets is still an important issue in livestock farming. All over the world castration is commonly practiced primarily ensuring constant meat quality \(^1\) and reducing the risk of boar taint,\(^2\) but also minimizing aggressive and sexual behavior between pigs.\(^3,4\) Some countries in Europe have implemented the use of anesthesia and analgesia during the castration process, as the EU Council Directive 2018/120/EC demands better practices for castrating suckling piglets before 7 days of age. For instance, in Germany, the Animal Protection Law requires pain elimination for surgical piglet castration since 2021. Nevertheless, castration of suckling piglets is still mainly carried out without anesthesia, even though it is commonly accepted as leading to pain and distress and, therefore, reducing animal welfare.\(^5-8\)

Administration of a nonsteroidal anti-inflammatory before the castration of piglets can alleviate pain after but is not able to mitigate acute pain during the procedure.\(^9\) Therefore, methods for controlling acute pain in this context are urgently needed. In addition,
the feasibility for the farmer and the integration into existing workflows are important aspects of the practicability of pain-minimizing measures under field conditions. In Australia and New Zealand, a gel-formulated topical anesthetic (Tri-Solfen®, Medical Ethics Pty Ltd) is used to provide analgesia in lambs and calves undergoing castration, tail docking, and mulesing. It contains a combination of a short-acting (50 g/L Lidocaine hydrochloride) and a long-acting local anesthetic (5 g/L Bupivacaine hydrochloride), as well as 0.048 g/L epinephrine acid tartrate as a vasoconstrictor and 5 g/L Cetrimide as an anti-septic agent. Tri-Solfen® (TS) has been shown to mitigate pain during and after various routine husbandry procedures in farm animals. In lambs, Paul et al.10 documented that administration of TS in the wound together with the intramuscular application of a nonsteroidal anti-inflammatory reduced pain-related behaviors in the first 4 hours after mulesing and peak cortisol concentrations did not differ to control animals. Treatment of 2-month-old calves immediately after dehorning with an adapted TS formula (100 g/L Lidocaine hydrochloride) reduced wound sensitivity for at least 1.5 hours after the procedure, suggesting less postoperative pain.11 Regarding the castration procedure, Lomax et al.12 applied TS into the scrotum of lambs during surgical castration before and after cutting the spermatic cords, accompanied by tail docking. The covering of the spermatic cords with TS led to less pain-related behavior and improved wound healing. Meanwhile, the potential of TS for pain relief during and after the castration of male suckling piglets has also been investigated. Assessing vocalization and defensive movements during the castration procedure, Sheil et al.13 recorded significantly reduced nociceptive motor and vocalization responses in piglets receiving TS 30 seconds before the spermatic cord transection. Additionally, investigations from France, where the castration of suckling piglets was performed using a protocol combining oral sucrose 30%, local instillation of TS, and intramuscular Meloxicam injection, piglets showed lower postoperative pain intensity than piglets of the control group (only treated with Meloxicam).14 In contrast, neither a short- nor a long-acting local anesthetic turned out to be effective at eliminating pain responses to castration as measured by cortisol, hematology, vocalizations, and behavior.15 However, in this study, no waiting time was maintained between the application of the local anesthetic and spermatic cord transection; thus, only conclusions on postoperative analgesia could be drawn.

Nevertheless, all available data regarding the potential of TS to mitigate pain during and after the castration of piglets were collected on awake animals under farm conditions. The assessment of defensive movements during piglet castration has been used in a large number of studies and is considered a reliable and repeatable parameter for documenting pain responses.5,16-18 Nevertheless, there is no feasible differentiation between pain-associated and handling-caused stress responses.

Therefore, the present study was performed using a minimal anesthesia model with a low dose of isoflurane. As isoflurane has no analgesic potential,19 this model provides a light level of general anesthesia without analgesia, enabling differentiation between stress- and nociception-induced responses. This investigation was part of a large-scale study on the efficacy of local anesthesia during surgical castration of piglets. This study part aimed to evaluate whether using a topical anesthetic in piglet castration is a feasible approach to reduce pain during the procedure. Therefore, the hypothesis that TS can minimize variations in heart rate and blood pressure, as well as reduce nociceptive movements during the cutting of the spermatic cords was tested in this study.

**Methods**

The study was performed in accordance with the EU Directive 2010/63/EU, the German Animal Welfare Act (2018), and the ARRIVE guidelines. All procedures were approved by the Ethical Committee for Animal Experiments of the Government of Upper Bavaria, Munich, Germany (Reference Number ROB-55.2-2532.Vet_02-19-11).

**Animals**

Eighteen clinically healthy male German Landrace/German large white X Piétrain piglets from 3 litters were included in this study, meeting the following criteria: minimum body weight of 1.4 kg, aged 3 to 7 days of life and no evidence of hernia or cryptorchidism. Neither teeth clipping, ear tagging, nor tail docking was applied to the piglets. All piglets received iron orally (1 mL per piglet; Ursoferran 150 mg/mL, Serumwerk Bernburg AG) during the first 10 hours of life. Sows and piglets were housed in the animal husbandry unit of the Clinic for Swine (Oberschleissheim, Germany). Housing was in accordance with the German Order for the Keeping of Productive Animals. Included piglets were randomized and distributed to 2 experimental groups. Piglets of 1 group were receiving Tri-Solfen (TS), and piglets of the second group a Placebo (P). All participants involved were blinded regarding the allocation of the piglets to the treatment groups.

**Anesthesia**

To measure nociception during castration, a minimal anesthesia protocol with isoflurane (Isoflurane Baxter vet., Baxter Deutschland GmbH) was used aiming to keep the piglets in a hypnotic state during the procedure to preclude interfering factors such as fear and stress. During anesthesia, animals were breathing spontaneously using a circle rebreathing circuit with an oxygen carrier gas flow rate of 3 L/min. Isoflurane was inhaled via a mask and the concentration was monitored by a connected anesthetic gas monitor (Vamos® plus, Dräger Medical Deutschland GmbH). For achieving and maintaining light anesthesia in accordance with Guedel scheme III.1,20 the minimum alveolar concentration of isoflurane was determined for every single piglet.
individually: for the induction of anesthesia 5% isoflurane in oxygen was administered, thereafter concentration was reduced while placing measurements devices to reach the appropriate isoflurane dose. For sustaining light anesthesia, the anesthetic depth was evaluated via interdigital pinches. For this purpose, a pen clamp was closed in the interdigital space of the posterior claw for a maximum of 5 seconds to a maximum of the first locking position. A single pelvic limb movement with immediate calming was considered as correct anesthetic depth and isoflurane concentration was maintained. Isoflurane concentration was further reduced by 0.2% if no reaction after the interdigital pinch was observed. In case of excessive response to the interdigital pinch, the isoflurane concentration was increased by 0.2%. After a stabilization period of 3 minutes, the reaction to a further interdigital pinch was assessed and isoflurane concentration was maintained or in-/decreased again depending on the piglets’ movement intensity. Twenty minutes after adjusting the depth of anesthesia the castration process was started with the application of a vapocoolant spray onto the scrotal skin. This time period was chosen because the present study was part of a comprehensive study and comparability with other parts of the study had to be ensured. Afterward, skin incisions were made, TS was applied in both created wound gaps and subsequently, the spermatic cords were severed. Anesthesia was maintained for another 90 minutes after castration before piglets were euthanized intravenous with overdosed pentobarbital (Euthadorm 500 mg/mL; Injektionslösung, Hugo Sachs Elektronik—Harvard Apparatus GmbH). After implementing all measurement devices and adjusting anesthesia according to Guedel scheme III.1, the castration procedure was started. A vapocoolant spray (PreOp PLUS, chilled antiseptic for animals, Medical Ethics Pty Ltd) that is comprising a hydrocarbon propellant in an aerosol canister and induces in this way a temperature reduction through evaporation of the volatile liquid spray from the skin surface, was used to anesthetize the skin. It was applied 3 times to the scrotum and the residue was wiped off every time after 15 seconds. Immediately thereafter, 2 vertical scrotal incisions through the skin and the processus vaginals were made using a scalpel. Thereupon, the topical anesthetic (Tri-Solfen, Medical Ethics Pty Ltd) or P (Placebo, Medical Ethics Pty Ltd) containing the same amount of epinephrine was administered using an irrigation cannula and the appropriate applicator depending on body weight: piglets less than 2 kg body weight received a total of 0.4 mL TS or P in each wound cavity, heavier animals 0.8 mL per side. After a waiting period of 30 seconds, both spermatic cords were severed individually using an emasculator. This was followed by an application of TS/P to the wound edges (0.1 mL or 0.2 mL per side, respectively).

Preparation nociceptive measurements
After the induction of anesthesia, the setup for measurement devices and preparation for the experimental procedure was performed as previously described in detail.21,22 All animals were assumed to experience similar stress from the basic experimental setup. Piglets were placed in a supine position and fixated in this position with warm water bottles that helped to maintain a physiological body temperature. Twenty minutes before general anesthesia local anesthetic cream (Emla®, AstraZeneca GmbH) was applied to the skin of the jugular groove. Eye ointment (Bepanthen Augen- und Nasensalbe, Bayer Vital GmbH) was administered, and cotton wool was placed in the external auditory canal to avoid the impact of background noises on measurement results. The local anesthetic Lidocaine (lidocaine 2%, Bela-pharm Arzneimittelfabrik) was infiltrated subcutaneous in a maximum dosage of 0.3 mL in the region for the vascular access (jugular groove) before skin incision. The left carotid artery was visualized by preparation and a microtip catheter (FISO-LS Fiber Optic Pressure Catheter, FOP-LS-ZFR-10, FISO Technologies Inc) was inserted for invasive systolic, diastolic, and mean arterial blood pressure measurement (PLUGSYS module, EIM-B, EIM-A, heart rate module, HAEMODYN software, Hugo Sachs Elektronik—Harvard Apparatus GmbH; FFP-LS and Evolution Software, FISO Technologies Inc). Heart rate was determined by electrocardiography (PLUGSYS module, Transducer Amplifier module TAM, heart rate module, HAEMODYN software, Hugo Sachs Elektronik—Harvard Apparatus GmbH). Additionally, oxygen saturation was measured via a pulse oximeter (2500A VET, Nonin Medical Inc) placed at the base of the piglet’s tail. Body temperature (PLUGSYS Thermocouple Amplifier Module (TCAM), HAEMODYN software, Hugo Sachs Elektronik—Harvard Apparatus GmbH), respiratory frequency, and end-tidal CO₂ were monitored (Vamos® plus, Dräger Medical Deutschland GmbH) throughout the anesthesia period.

Local anesthesia and castration
After implementing all measurement devices and adjusting anesthesia according to Guedel scheme III.1, the castration procedure was started. A vapocoolant spray (PreOp PLUS, chilled antiseptic for animals, Medical Ethics Pty Ltd) that is comprising a hydrocarbon propellant in an aerosol canister and induces in this way a temperature reduction through evaporation of the volatile liquid spray from the skin surface, was used to anesthetize the skin. It was applied 3 times to the scrotum and the residue was wiped off every time after 15 seconds. Immediately thereafter, 2 vertical scrotal incisions through the skin and the processus vaginals were made using a scalpel. Thereupon, the topical anesthetic (Tri-Solfen, Medical Ethics Pty Ltd) or P (Placebo, Medical Ethics Pty Ltd) containing the same amount of epinephrine was administered using an irrigation cannula and the appropriate applicator depending on body weight: piglets less than 2 kg body weight received a total of 0.4 mL TS or P in each wound cavity, heavier animals 0.8 mL per side. After a waiting period of 30 seconds, both spermatic cords were severed individually using an emasculator. This was followed by an application of TS/P to the wound edges (0.1 mL or 0.2 mL per side, respectively).

Heart rate and blood pressure measurements
During the entire procedure systolic, diastolic, and mean arterial blood pressure (MAP) were recorded using the microtip catheter in the left carotid artery and heart rate (HR) by using an ECG. One minute before the “events” [(1) application of vapocoolant, (2) skin incision, and (3) cutting of the spermatic cords] mean baseline values of MAP and HR were calculated. Subsequently, the maximum deviation, as well as the percent deviation from baseline were determined for 1 minute after the event.

Nocifensive movements
During the application of vapocoolant, skin incision, and cutting of spermatic cords each animal was immobilized by the same person and nocifensive movements were assessed generating a nocifensive score. This restraining person was blinded to the treatment and assessed every movement of the 4 limbs and the back. Additionally, each event was...
recorded to confirm the assessed movements afterward. The scoring of nocifensive movements was adopted from Saller et al. and assessed for the left and right testicles individually in terms of frequency and intensity of movements of all 4 legs and the back. A maximum score of 28 points (14 per testicle) could be assigned at each event (Table 1).

**Table 1**—Assessment of the nocifensive score.

<table>
<thead>
<tr>
<th>Leg movements score (each leg separately)</th>
<th>Number of movements</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No movement</td>
</tr>
<tr>
<td>1</td>
<td>One movement</td>
</tr>
<tr>
<td>2</td>
<td>Two or 3 movements</td>
</tr>
<tr>
<td>3</td>
<td>More than 3 or long-lasting movements</td>
</tr>
</tbody>
</table>

Maximum leg movement score 12

<table>
<thead>
<tr>
<th>Back movements score</th>
<th>Number of movements</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No movement</td>
</tr>
<tr>
<td>1</td>
<td>Muscle contraction</td>
</tr>
<tr>
<td>2</td>
<td>Movements</td>
</tr>
</tbody>
</table>

Maximum back movement score 2

Maximum score per testicle 14

Maximum score per event 28

Scoring system adopted by Saller et al. to assess nocifensive movements of legs and back during castration of piglets at the 3 defined events “application of vapocoolant spray,” “skin incision,” and “cutting of spermatic cords.”

**Statistics**

The target value for sample size calculation was HR immediately after castration. To demonstrate a difference between the groups as statistically significant, a group size of 9 animals was determined necessary to prove a statistically significant difference in heart rate between the groups, assuming a dispersion of 90 beats/min. Because a dropout rate of about 10% is to be expected, 1 reserve animal was required. Statistical significance was considered at P < .05. These statistical analyses were performed using R statistical software version 3.6.1 and IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp, Armonk). For the statistical analysis metric variables were tested for normal distribution via the Shapiro-Wilk normality test. In normally distributed data (nocifensive movements), the Mann-Whitney U test was used. Dichotomous variables were analyzed for associations by χ²-test (nocifensive movements yes/no to the 3 events). The results are presented as violin plots.

**Results**

On the day of castration, piglets had an average age of 4.6 ± 1.0 days (range = 3–6). There was no significant difference between the 2 groups. The mean body weight was 2.1 ± 0.3 kg (min = 1.7, max = 2.6) in the TS group and differed significantly (P < .05) from the mean body weight in the P group (mean = 1.8 ± 0.3 kg, min = 1.4, max = 2.4). No piglet had to be excluded from the data evaluation.

From the time of induction of anesthesia, until the castration process was started (beginning with the application of the vapocoolant spray), an average of 60.7 minutes (min = 48, max = 86; SD = 11.6) was needed to set all measurement devices and find the required anesthetic depth. On average, the isoflurane concentration had to be adjusted 0.8 times per animal (min = 0, max = 2; SD = 0.9) to set the correct anesthetic depth. The mean required end-tidal isoflurane concentration for achieving anesthesia stage III.1 of the Guedel scheme was 1.4 ± 0.2% with no differences between piglets of the TS and the P group. For the performance of the entire castration procedure (including the application of vapocoolant, skin incisions, application of TS/P, cutting of spermatic cords, and second application of TS/P) a mean total time of 2.65 ± 0.13 min was required. The entire anesthesia protocol lasted on average 154.2 (min = 141, max = 179; SD = 11.5) minutes before the animals were euthanized for spinal cord collection.

**Mean arterial blood pressure (MAP) and HR**

Raised mean MAP and mean HR measurements were summarized (Table 2). The baseline mean MAP for all piglets was 53 ± 16 mmHg and the mean HR

**Table 2**—Mean values and SD of mean arterial blood pressure (MAP) and heart rate (HR) of baseline measurements before and to the time point of each defined event (“interdigital pinch,” “application of vapocoolant,” “skin incision,” and “cutting of spermatic cords”) in the 2 study groups Tri-Solfen and Placebo.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Interdigital pinch</th>
<th>Application of vapocoolant</th>
<th>Castration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BL</td>
<td>Event</td>
<td>BL</td>
</tr>
<tr>
<td>Tri-Solfen</td>
<td>MAP (mmHg)</td>
<td>56 ± 18</td>
<td>56 ± 18</td>
<td>55 ± 18</td>
</tr>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>170 ± 34</td>
<td>170 ± 33</td>
<td>169 ± 30</td>
</tr>
<tr>
<td>Placebo</td>
<td>MAP (mmHg)</td>
<td>48 ± 10</td>
<td>48 ± 11</td>
<td>47 ± 10</td>
</tr>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>172 ± 27</td>
<td>174 ± 27</td>
<td>175 ± 27</td>
</tr>
</tbody>
</table>

BL = Baseline.
was 172 ± 32 bpm and did not differ between the 2 study groups for both parameters. Regarding the interdigital pinch for adjusting a light isoflurane anesthesia in accordance with Guedel scheme III.1, no differences were observed between the groups in changes of MAP and HR.

Percent changes of MAP and BP from baseline in piglets at the 2 defined events application of vapocoolant and skin incision were assessed for all animals (n = 18) together, as the procedure was identical in both groups. The application of vapocoolant led to a mean percentual change in MAP and HR of 5.0 ± 2.4% (min = 0.9, max = 11.1) and 1.9 ± 1.2% (min = 0.6, max = 4.6), respectively. Performing skin incisions provoked a mean percentual change of 14.0 ± 6.7% (min = 5.5, max = 28.2) in MAP and 3.1 ± 1.9% (min = 0.6, max = 7.0) in HR.

For the event cutting of spermatic cords the percent changes of MAP and BP from baseline were evaluated separately in piglets of the TS and P group (Figure 1). The MAP differed significantly (P < .000) between the 2 groups: the TS group showed significantly lower maximum MAP changes (mean = 13.6%, min = 8.8, max = 20.1; SD = 4.1) than the P group (mean = 35.6%, min = 24.5, max = 46.6; SD = 7.8). No significant differences in HR deviation between the TS and P group were detected.

**Discussion**

The present investigation on a topical anesthetic is a small part of a large-scale study on the use of local anesthesia for pain relief in piglet castration. There have been studies on the effectiveness of TS in reducing pain during and after castration, albeit generally carried out on awake animals mostly using defensive movements and vocalization to assess the perception of pain.13,14,24 In the present study physiological parameters were recorded and evaluated in addition to nocifensive movements. As these parameters are easily influenced by other factors such as stress and fear,25 a minimal anesthesia model was chosen to be able to measure nociception by itself.

To minimize litter effects and to maintain the blinding of the study-performing persons, piglets were allocated randomly to the 2 study groups. Although no difference in age was apparent between the groups, piglets belonging to the P-group had significantly lower mean body weight than those of the TS group. It can be assumed that this was a result of the small number of animals and the resulting randomization, which could not take weight into account. Because only clinically healthy animals were included in the study and the amount of the applied TS or P was adjusted to the weight, the different weight distribution is considered nonrelevant to the results of the study.

Haga et al26 evaluated changes in the MAP as the most sensitive indicator of nociception under isoflurane anesthesia. Accordingly, using TS for local anesthesia, our results showed significantly decreased changes in MAP during the cutting of the spermatic cords compared with the administration of P. This is in line with the results of Saller et al21 using the same

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**Figure 1**—Visualization of percent deviations of mean (red dot) arterial blood pressure (A) and heart rate (B) from baseline as well as nocifensive scores (C) at the event “cutting of spermatic cords” in the experimental groups Placebo and Tri-Solfen as violin plots. For each box-and-whisker plot, the solid line within the box represents the median. The lower and upper limits of the box represent the interquartile (25th and 75th percentiles) range, respectively. The whiskers delimit the range; green and orange dots represent each individual piglet.
minimum anesthesia model but utilizing different local anesthetics for piglet castration. They analyzed mean percent changes in MAP of 45% following the spermatic cord transection after injection of natrium-chloride and significantly fewer deviations from the baseline of MAP with previous application of local anesthesia. In our study, MAP differed from the mean baseline measurement by 13.6 ± 4.1% in the TS group, which is slightly higher than the mean change in MAP (6.7%) in piglets that were only handled in the study of Salier et al.21 However, this is below the 20% deviation that is assumed as an indicative sign of relevant pain during a surgical procedure by Bova et al.27 This confirms, that after an exposure time of 30 seconds, TS reduced effectively nocifensive reactions while cutting the spermatic cords, as previously proposed by Sheil et al24 in conscious piglets.

Supporting the results of the invasive blood pressure measurements, we detected significantly fewer nocifensive movements after the administration of TS than after the instillation of P while cutting the spermatic cords. Defensive movements were already accepted as valid pain parameters.28 It should be noted that in the present study, the movements of piglets were observed under isoflurane anesthesia. This might also affect the appearance, duration, and intensity of these nocifensive movements. Nevertheless, the influence is classified as negligible, as for one a minimal anesthesia model with low-dosed isoflurane was used and for another isoflurane was shown to have little effect on cardiovascular response to noxious stimuli.29 Thus, as these results are in line with findings of previous study parts that demonstrated nocifensive movements appropriate for assessing pain perception, they confirm the effectiveness of TS in reducing pain during spermatic cord transection.21,22

In contrast to the results of Salier et al,21 no significant alteration in HR was observed between the 2 study groups while cutting the spermatic cords. In earlier studies on pain elimination during castration under isoflurane anesthesia, a significant change in HR was noted in the control group during the painful procedure.30 The sample size in the present study was calculated based on these values, which might be a limitation of this study. However, in accordance with our data, Werner et al22 were also unable to detect any significant differences in HR deviation between the treatment and control group after cutting the spermatic cord using a minimum anesthesia model likewise. Nevertheless, in the present study, as in the 2 previous parts of the study,21,22 there was always a significant difference between the control and the group under local anesthesia concerning MAP deviations during the cutting of the spermatic cords. Similarly, in studies of Haga et al,26 changes in MAP in comparison to EEG and HR were found to be the most sensitive parameter for determining nociception in 19–29 kg pigs under isoflurane anesthesia. Thus, heart rate measurements should be considered a less sensitive cardiovascular parameter than blood pressure changes for detecting painful stimuli during the castration procedure.

Although cutting the spermatic cords is considered the most painful part of castration,6,18 skin incision for protrusion of the testicles causes pain as well. To reduce the distress caused by skin incision a vapocoolant spray was used to anesthetize the skin. Nevertheless, mean nocifensive movements were higher during the scrotal skin incision with vapocoolant spray of all study animals than during cutting spermatic cords of piglets belonging to the TS group. Unfortunately, a control group without treatment for scrotal incisions is missing. Vapocoolant was applied in both study groups (TS and P) to create uniform conditions for the subsequent cutting of spermatic cords as its effectiveness was not the main focus of this study. Although this is limiting the results, it should be mentioned that these findings are not in accordance with the study by Lomax et al.31 They were able to reduce behavioral nociceptive responses during ear notching in piglets using the same vapocoolant in comparison with untreated control animals. As the scrotum is higher perfused than the mainly cartilaginous tissue of the ear it seems likely, that a temperature of 10 degrees, which is considered to be the threshold for an effective nerve conduction blockade,32 was not or only reached for a short time and therefore the vapocoolant was not able to achieve a sufficient effect in this region.

Performing castration with TS, a period of 30 seconds has to be maintained after the instillation of the gel formulation before the onset of effect, which means a prolonged fixation time. As it is commonly accepted that handling and restraining cause stress to the animals,16 this has to be assessed as a limitation of the method, as additional stress is caused by prolonged handling.

Conclusively, under standardized conditions, TS provided a significant reduction of nocifensive reactions while cutting the spermatic cords. In the minimal anesthesia model, the administration of TS reduces pain-associated parameters significantly while cutting the spermatic cords. Nevertheless, as TS has to be administered in the wounds and not on the skin surface to reach its efficacy, the skin incisions in advance of cutting the spermatic cords are still a painful procedure. Therefore, future research should focus on skin anesthesia as the use of vapocoolant in the present study was not promising. Additionally, the postulated waiting period of 30 seconds between TS application and the cutting of the spermatic cord requires a prolonged fixation, which means a higher level of fear and stress. Therefore, there should be a re-examination of additional value versus additional stress using TS for castration in conscious piglets.

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data analysis, and interpretation, or writing and publication of the manuscript.

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