Prostaglandin E₂ (PGE₂) is an unreliable biomarker for inflammation in castrated piglets: a randomized controlled trial assessing pharmaceutical drug efficiency

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OBJECTIVE
To investigate the effect of intranasal (IN) flunixin meglumine (FM) and intra-inguinal (IG) lidocaine on castration inflammation using prostaglandin E₂ (PGE₂) concentration as a biomarker.

METHODS
This randomized controlled trial was conducted in March 2022. Blood was collected at −24, 1, and 24 hours post-castration for PGE₂ quantification from 195 piglets that received 1 of 8 treatments: (1) saline (1.5 mL) applied IG and IN (0.2 mL) followed by surgical castration (n = 24); (2) saline (1.5 mL) IG and IN (0.2 mL) followed by sham castration (25); (3) lidocaine (20 mg/kg or 1.5 mL) IG followed by surgical castration (24); (4) lidocaine (20 mg/kg or 1.5 mL) IG followed by sham castration (25); (5) FM (2.2 mg/kg) IN followed by surgical castration (25); (6) FM (2.2 mg/kg) IN followed by sham castration (24); (7) lidocaine (20 mg/kg or 1.5 mL) IG and FM (2.2 mg/kg) IN followed by surgical castration (24); and (8) lidocaine (20 mg/kg or 1.5 mL) IG and FM (2.2 mg/kg) IN followed by sham castration (24).

RESULTS
Prostaglandin E₂ concentrations did not increase following the castration procedure and were not an effective biomarker of castration inflammation. Piglets that received lidocaine demonstrated no difference in PGE₂ levels across all time points. Piglets administered FM had lower PGE₂ concentrations at 1 hour and 20 minutes postdrug administration in both the sham and castrated piglets.

CONCLUSIONS
Prostaglandin E₂ was not an effective biomarker to quantify castration inflammation. Flunixin meglumine was able to reduce PGE₂ concentration in piglets regardless of castration procedure, but lidocaine had no impact. Decreased PGE₂ levels in FM-treated pigs are likely associated with the drug’s ability to mitigate a noncastration-associated inflammatory process occurring independent of the castration procedure.

CLINICAL RELEVANCE
Flunixin meglumine reduced circulating PGE₂ concentration in the blood, regardless of the castration procedure, indicating a potential for the drug to mitigate an inflammatory process unrelated to castration.

Keywords: piglets, biomarker, inflammation, castration, prostaglandin

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Castration is a procedure performed in piglets that inflicts tissue damage and inflammation. Even though there are several pharmaceutical options on the market that can be used to mitigate inflammation and pain associated with this common processing procedure, to date, there are no products in the US specifically labeled to manage swine castration.

Methods

This study was part of a larger study conducted at a commercial swine breeding facility located in the southeastern US. This study was approved by the North Carolina State University IACUC (protocol 20-113-01). Before the beginning of the study, surgical castration of the male piglets was routinely performed on farms as a husbandry procedure. Withdrawal periods and drug residues were not a concern in this trial given all piglets enrolled were within 1 week of life and would not be slaughtered for more than 150 days following the trial completion.

Animals, housing, and management

A total of 195 Large White X Duroc cross male piglets from 35 litters were enrolled in this study. Sows and piglets were housed in fully slatted and mechanically ventilated farrowing rooms. Sows were housed in individual farrowing crates (2.5 m X 0.7 m) with additional space for piglets (2.5 m X 1.3 m) surrounding the crates. Lighting was on between 600 and 1630 hours, and sows were provided ad libitum access to water and feed throughout the trial. Piglets had access to the sow at all times.

Study design and treatments

Male piglets were included according to inclusion and exclusion criteria described elsewhere. Selected male piglets were randomly assigned to 1 of 8 treatment regimens: (1) sterile saline (1.5 mL) applied IG and IN (0.2 mL) followed by surgical castration (C; n = 24); (2) sterile saline (1.5 mL) IG and IN (0.2 mL) followed by sham castration (S; 25); (3) lidocaine (20 mg/kg or 1.5 mL) IG followed by surgical castration (CL; 24); (4) lidocaine (20 mg/kg or 1.5 mL) IG followed by sham castration (SL; 24); (5) FM (2.2 mg/kg) IN followed by surgical castration (CF; 25); (6) FM (2.2 mg/kg) IN followed by sham castration (SF; 24); (7) lidocaine (20 mg/kg or 1.5 mL) IG and FM (2.2 mg/kg) IN followed by surgical castration (SLF; 24); or (8) lidocaine (20 mg/kg or 1.5 mL) IG and FM (2.2 mg/kg) IN followed by sham castration (SLF; 24).

Treatment administration

Lidocaine

The administration of treatments is described in detail by Lopez-Soriano et al.

Briefly, lidocaine was buffered by mixing 2 mL of 8.4% sodium bicarbonate with 20 mL of 2% lidocaine hydrochloride injectable solution (20 mg/mL), resulting in a final mixture of 22 mL as previously described. To the best of the authors’ knowledge, there are no studies establishing the effective dose concentration of lidocaine in neonatal pigs. Extrapolating dose concentrations from other species, such as ruminants, is deemed inappropriate due to distinct physiological variations across species. Consequently, the dose was determined by the co-author (MSL), a veterinarian with extensive clinical experience in implementing pain management strategies during piglet castration. As of the present, this dose of local anesthetic has been administered to over 40,000 piglets as part of an on-farm protocol, with no adverse effects attributed to the drug concentration. Piglets enrolled in lidocaine treatment groups (CL, SL, CLF, and SLF) were injected with buffered lidocaine (20 mg/kg IG) or 1.5 mL into each inguinal canal (left and right) approximately 20 minutes before the castration procedure. Piglets enrolled in the control treatment (C, S, CF, and SF) were handled in an identical manner.

Flunixin meglumine

Immediately following IG injection, piglets enrolled in the FM treatment groups (CLF, SLF, CF, and SF) received 2.2 mg/kg of FM (Banamine-S; Merck Animal Health) in one nostril using a nasal IN mucosal atomization device attached to a 0.5-mL bottle mount vaccinator. The dose of FM used in this study has been recently validated. Piglets in
the control group were handled in the same manner, and 0.2 mL of sterile saline was administered as described above.

Castration procedure
Castration was performed by the same trained caretaker from the farm. Piglets undergoing surgical castration were placed in an inverted position, and 2 vertical incisions were made using a scalpel blade. Following this, testicles were exposed, spermatic cords cut, and testicles were physically removed by traction. Piglets assigned to the sham castration groups were handled in a similar manner to castrated piglets (ie, picking up 1 individual pig from the crate, holding the pig in an inverted position against the body of the castrator and applying pressure to the scrotal area).

Blood sampling
Blood was sampled from all piglets at −24, 1, and 24 hours postcastration procedure. Blood samples were collected by puncture of the orbital sinus using a disposable 20-gauge, 1.0-inch (2.5-cm) needle and deposited into 4-mL red evacuated glass tubes. Piglets were collected by puncture of the orbital sinus using a disposable 20-gauge, 1.0-inch (2.5-cm) needle and deposited into 4-mL red evacuated glass tubes. Blood samples were maintained in a cooler and centrifuged for 30 minutes and centrifuged at 3,000 X g for 5 minutes. Serum was stored in 1.5-mL microcentrifuge tubes at −80 °C, and assays were performed 2 months later.

Prostaglandin E₂ quantification
Prostaglandin E₂ concentration was measured through a commercial enzyme-linked immunosorbent assay kit (catalog no. 514531; Cayman Chemical) as previously described. This assay has a range from 0.39 to 50 pg/mL and a sensitivity of approximately 2 pg/mL. Briefly, samples were purified by mixing 4 times the volume of ice-cold acetone added to the serum aliquot. The volume was adjusted based on the amount of samples available. Then, samples were maintained at −20 °C for 30 minutes and centrifuged at 3,000 X g for 5 minutes. The supernatant was then relocated to 13 X 100-mm glass tubes, evaporated using a concentrator, and reconstituted to the initial serum volume with the kit buffer. An aliquot of the reconstituted sample was derivatized with adjusted kit components, and the manufacturer’s protocol was then followed. Samples were diluted into 1:5 and 1:15 proportions and ran in duplicates. The absorbance was measured at 405 nm following 60 minutes of development. The PGE₂ concentration of the sample used to evaluate repeatability among plates averaged 12.5 pg/mL.

Statistical analysis
All data were analyzed using SAS (version 9.4; SAS Institute Inc). A multilevel linear model was used to compare the repeated measures of PGE₂ concentrations (continuous dependent variable) over time points (−24, 1, and 24 hours postcastration) and between the 8 treatments. The model structure was chosen according to the smallest Bayesian information criterion. Treatments, time points, and the interaction between treatments and time points were included as independent variables into fixed effects, while piglets were included as random effects. The underlying relationship analyzed with linear regression models was linear and assumptions of the underlying relationship analyzed with linear regression were 8.95 and 12.51%, respectively. There was a treatment by time point (P < .001), treatment (P < .001), and time point effect (P < .001) on serum PGE₂ concentrations (Table 1). The PGE₂ concentrations did not differ between treatments at −24 hours (P ≥ .16). However, at 1 hour postcastration, piglets in

**Table 1**—Mean (SD) prostaglandin E₂ concentrations (pg/mL) in male piglets assigned to 1 of 8 treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>−24 hours</th>
<th>1 hour</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>72.90 (1.17)</td>
<td>68.91 (1.00)</td>
<td>65.22 (1.16)</td>
</tr>
<tr>
<td>CF</td>
<td>49.92 (1.11)</td>
<td>27.89 (0.96)</td>
<td>53.91 (1.16)</td>
</tr>
<tr>
<td>CL</td>
<td>53.55 (1.17)</td>
<td>76.27 (1.00)</td>
<td>55.36 (1.16)</td>
</tr>
<tr>
<td>CLF</td>
<td>58.07 (1.19)</td>
<td>33.43 (1.00)</td>
<td>52.56 (1.16)</td>
</tr>
<tr>
<td>S</td>
<td>56.65 (1.14)</td>
<td>49.22 (0.96)</td>
<td>52.65 (1.14)</td>
</tr>
<tr>
<td>SF</td>
<td>57.09 (1.19)</td>
<td>26.14 (1.00)</td>
<td>37.61 (1.16)</td>
</tr>
<tr>
<td>SL</td>
<td>57.06 (1.14)</td>
<td>54.88 (0.96)</td>
<td>62.54 (1.11)</td>
</tr>
<tr>
<td>SLF</td>
<td>54.34 (1.19)</td>
<td>26.48 (1.00)</td>
<td>42.37 (1.16)</td>
</tr>
</tbody>
</table>

The 8 treatments were (1) sterile saline applied intra-inguinal (IG) and intranasally (IN) followed by surgical castration (C; n = 24); (2) sterile saline IG and IN followed by sham castration (S; 25); (3) lidocaine IG followed by surgical castration (CL; 24); (4) lidocaine IG followed by sham castration (SL; 25); (5) flunixin meglumine (FM) IN followed by surgical castration (CF; 25); (6) FM IN followed by sham castration (SF; 24); (7) lidocaine IG and FM IN followed by surgical castration (CLF; 24); or (8) lidocaine IG and FM IN followed by sham castration (SLF; 24) and the pairwise comparisons of all treatments at −24, 1, and 24 hours relative to castration procedure in a 2022 study.

Within a column, least squares means without a common superscript differ (P ≤ .05) due treatment X time point interaction.

Within a row, least squares means without a common superscript differ (P ≤ .05) due treatment X time point interaction.

Final data collection included data from 195 male piglets over 35 litters (9.0 ± 1.1 days of age, 3.20 ± 0.70 kg body weight, and 5.6 ± 1.7 piglets enrolled per litter). An acceptable tissue withholding time was observed before the barrows entered the food chain. The intra-and interassay coefficients of variation were 8.95 and 12.51%, respectively. There was a treatment by time point (P < .001), treatment (P < .001), and time point effect (P < .001) on serum PGE₂ concentrations (Table 1). The PGE₂ concentrations did not differ between treatments at −24 hours (P ≥ .16). However, at 1 hour postcastration, piglets in
treatments that received IN FM demonstrated lower PGE$_2$ concentrations ($P \leq 0.09$) than piglets that did not receive IN FM (C vs SF; CL vs SF, CL vs CLF, S vs SF, SL vs SF, and SL vs SLF). Furthermore, at 24 hours postcastration, sham-castrated piglets that received lidocaine had ($P = 0.02$) greater PGE$_2$ concentrations than sham-castrated piglets that received IN FM (SL vs SF; Table 1). Piglets in the CF, SF, and SLF groups had ($P \leq 0.05$) lower PGE$_2$ concentrations at 1 and 24 hours postprocedure in comparison with their respective baselines. Finally, IG lidocaine did not lower PGE$_2$ concentrations at any time point.

**Discussion**

This is the first study investigating the effects of IN FM and IG lidocaine application on PGE$_2$ production in piglets following surgical castration. Results from this study demonstrated that PGE$_2$ concentrations did not increase following castration, suggesting that PGE$_2$ is not an effective biomarker in quantifying castration inflammation. In humans, inhibiting PGE$_2$ synthesis has been an important anti-inflammatory strategy for over a century and the mechanism of prostaglandin synthesis and its effects are well established. In humans, PGE$_2$ has been shown to induce homeostatic, inflammatory, and even anti-inflammatory effects. In swine, however, there are no studies validating the use of PGE$_2$ concentrations as a proxy for inflammation caused by castration. In fact, the use of PGE$_2$ concentrations as a biomarker in swine has been contradictory. Previous research in castrated piglets, for example, did not demonstrate any difference in PGE$_2$ metabolites in piglets that were castrated versus piglets that were sexually intact (control group). Other research found that meloxicam administered to the sow was effective at decreasing PGE$_2$ concentrations in castrated piglets for up to 90 hours after drug administration. Further research in castrated piglets found that flunixin was able to lower PGE$_2$ concentrations in the sham-castrated group but not in the surgically castrated group. Therefore, it is possible that PGE$_2$ concentration is simply not a reliable biomarker to measure inflammation in piglets undergoing castration.

Although PGE$_2$ levels were not impacted by the castration procedures, FM-treated piglets did demonstrate decreased PGE$_2$ concentrations following the procedure. Piglet immune response may often be compromised if the piglet was exposed to disease in utero or shortly after birth. Possible exposure to disease or enhanced microbial load at the time of the trial may have triggered an inflammatory response in piglets independent of the castration procedure. The production of the proinflammatory cytokines resulting PGE$_2$ production was mitigated by the administration of FM. In this study, IN FM at a dose of 2.2 mg/kg effectively lowered PGE$_2$ production by 1 hour and 20 minutes postdrug administration. This is similar to the results from a previous study that found that serum PGE$_2$ concentrations did not differ between castrated piglets receiving transdermal flunixin or saline solution but sham castrated that received flunixin had lower PGE$_2$ concentrations than sham-castrated piglets treated with saline. Results from this study in 2022 reinforce the conclusion that flunixin has the properties to mitigate PGE$_2$ concentrations but PGE$_2$ concentrations are unrelated to inflammation induced by castration.

In this study, castrated and noncastrated piglets that received FM IN demonstrated lower serum PGE$_2$ concentrations at 1 hour postcastration than piglets that did not receive the drug. Results in the present study are supported by Nixon and colleagues, who found that FM was the most effective drug at reducing piglets’ PGE$_2$ concentrations immediately postcastration. Nonetheless, it must be noted that even though FM decreased PGE$_2$ immediately after castration in the current study, at 24 hours postprocedure, only sham-castrated piglets that received IN FM had lower PGE$_2$ than sham-castrated piglets that received lidocaine. This finding contradicts the results from Nixon et al who demonstrated that FM maintained PGE$_2$ inhibition beyond 24 hours postadministration in castrated and tail-docked piglets. In this regard, the results from Nixon et al should be compared with caution, given that the sampling site (ie, interstitial fluid), procedures (ie, castration and tail docking), and the administration route (ie, IN) were different from those in the present study (ie, IM).

In the present study, at 1 hour and 20 minutes postdrug administration, piglets that received both lidocaine and FM combined had lower PGE$_2$ concentrations than piglets that only received lidocaine (CLF vs CL and SLF vs SL). In addition, although not significant, piglets that received the combined treatment also had numerically higher PGE$_2$ concentrations than piglets that only received flunixin (CF and SF). These findings suggest that FM by itself is able to reduce PGE$_2$ concentrations whereas lidocaine might actually have induced prostaglandin synthesis. Our findings are corroborated by the larger study that showed that FM was effective in mitigating physiological responses in castrated piglets whereas lidocaine was ineffective. More specifically, piglets treated with IN FM had lower cortisol levels immediately postcastration whereas piglets treated with lidocaine had the highest concentrations of cortisol at 1 hour postcastration.

In this study, IG lidocaine did not mitigate inflammation at any time point. While buffering lidocaine may have lowered pain associated with the substance injection, possible damage to the spermatic cord or other tissues during injection might have been responsible for the increased inflammation observed in this study, as previously suggested. Another explanation is that lidocaine may be associated with an increase in PGE$_2$ as previous research in neurons has reported intracellular calcium release postlidocaine administration, which might have been responsible for initiating enhanced electrical responsiveness by the cell resulting in transient nerve irritation. This hypothesis is reinforced by the fact that, at 24 hours postdrug administration, noncastrated piglets that received lidocaine maintained greater PGE$_2$ concentrations.
One limitation of this study is the fact that castration did not increase PGE2 concentration as was hypothesized, therefore significantly limiting PGE2 use as a biomarker for surgical inflammation in swine. Another limitation is the fact that it is unclear whether the transient reduction in prostaglandin production reported here is biologically significant as to the authors’ knowledge there are no established laboratory ranges for PGE2 in swine.

Results from the current study suggest that IN FM is effective in lowering PGE2 levels 1 hour and 20 minutes following drug administration. However, a single IN FM administration was not effective in keeping the lower PGE2 concentration 24 hours following drug administration when compared to the other treatments. Future research should consider administration frequency and dosing of IN FM to control pain for a longer period postcastration.

In conclusion, the castration procedure did not increase PGE2 concentration in piglets. Intranasally applied FM at 2.2 mg/kg was effective in decreasing PGE2 concentrations in piglets 1 hour and 20 minutes post-drug administration. However, this effect was not sustained long term. The administration of lidocaine IG was not able to lower PGE2 production reported here is biologically significant as to the authors’ knowledge there are no established laboratory ranges for PGE2 in swine.

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References


