

Administering tolfenamic acid to gilts and sows before artificial insemination did not improve litter size but tended to reduce post-service serum prostaglandin F2 α levels

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Abstract

This study examined the impact of intramuscular administration of tolfenamic acid 10 min before artificial insemination (AI) on serum PGF2 α levels and reproductive performance in gilts and sows. In Experiment I, 20 gilts were divided into two groups: control (n = 10) and treatment (n = 10). Serum samples were collected 30 min before and after insemination to measure PGF2 α levels. In Experiment II, 175 gilts and sows were divided into control (25 gilts and 56 sows) and treatment (16 gilts and 78 sows) groups. In both experiments, the treatment group received an intramuscular injection of 2 mg/kg tolfenamic acid 10 min before AI, while the control group received no injection. The reproductive performance of the gilts and sows was compared between the groups. In Experiment I, the serum PGF2 α level in the treatment group tended to be lower after insemination compared to before AI (652.5 ± 233.9 vs. 1335.4 ± 237.9 pg/ml, respectively, $P = 0.063$), while no significant difference was observed in the control group (556.3 ± 206.0 vs. 653.1 ± 218.8 pg/ml, $P = 0.314$). Notably, gilts with serum PGF2 α levels above 750 pg/ml after insemination had significantly fewer piglets born alive per litter compared to those with PGF2 α levels below 750 pg/ml (5.2 ± 1.9 vs. 10.2 ± 1.2 , $P = 0.044$). In Experiment II, no significant differences in litter traits were observed between the control and treatment groups ($P > 0.05$). In conclusion, administering tolfenamic acid 10 min before AI did not improve litter size at birth in either gilts or sows. However, in gilts, tolfenamic acid administration tended to reduce serum PGF2 α levels. Furthermore, higher serum PGF2 α levels post-insemination was associated with fewer piglets born alive per litter. This suggests that post-insemination serum PGF2 α levels may affect litter size in gilts. Further research is needed to explore the factors influencing PGF2 α levels after AI and to develop optimal management strategies.

Keywords: artificial insemination, NSAIDs, reproduction, prostaglandin, swine

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Introduction

Artificial insemination (AI) is currently a common mating technique used in pig production worldwide (Knox, 2016; Tummaruk *et al.*, 2022). Moreover, with post-cervical AI, the semen dosage can be reduced from 3 billion to 1 billion sperm per dose without negatively affecting litter size, farrowing rate, or the number of sperm within the sperm reservoir (Watson and Behan, 2002; Sumransap *et al.*, 2007). After semen is deposited into the uterus, there is a significant increase in the defensive immune response, including the migration of polymorphonuclear leukocytes, followed by phagocytosis and the secretion of proinflammatory mediators (Katila, 2012; Svensson *et al.*, 2021). The proinflammatory mediators chemotactically attract white blood cells to the inflamed tissue (Kaplanski *et al.*, 2003; Svensson *et al.*, 2021) and initiate the release of arachidonic acid from the plasma membrane through enzymatic catalysis by phospholipase (Smith *et al.*, 2000). Arachidonic acid is then metabolized by two cyclooxygenases (COX) isoenzymes (COX-1 and COX-2), leading to the subsequent formation of prostaglandins (Smith *et al.*, 2000). Although COX-1 and COX-2 isoforms exhibit slight variations in their physiological roles, both enzymes contribute to the production and release of prostaglandins during inflammation (Ricciotti and FitzGerald, 2011).

Prostaglandin F_{2α} (PGF_{2α}) is one of the well-known prostaglandins, mainly derived from the female reproductive organs (Ricciotti and FitzGerald, 2011). PGF_{2α} plays significant roles in reproductive physiology, including follicular growth, ovulation, luteolysis, fertility, and implantation (Weems *et al.*, 2006; Leonardi *et al.*, 2012), and also serves as a proinflammatory mediator in the inflammatory response (Zhang *et al.*, 2010). In sows, plasma PGF_{2α} metabolite concentration is normally maintained at about 200–300 pmol/ml (i.e., 70.9 to 106.35 pg/ml) after natural mating (Norrby *et al.*, 2007). In contrast, the concentration of plasma PGF_{2α} metabolite in sows inseminated using AI was increased by 2.5 times compared to that observed after natural mating (Norrby *et al.*, 2007). Furthermore, AI using an intrauterine catheter showed an earlier increase in the level of prostaglandin metabolite compared to using a conventional intracervical AI catheter (Norrby *et al.*, 2007). These data indicate that the AI process in pigs still needs further investigation to understand the cause and consequence of increased serum PGF_{2α} metabolite after AI in sows.

The release of prostaglandins after insemination may result from the immune response to semen antigens following artificial insemination (Katila, 2012) and from some degree of trauma to the female reproductive tract due to the AI process (Heuwieser *et al.*, 2011). In cows, increased uterine PGF_{2α} concentrations are associated with higher embryonic mortality and lower pregnancy rates (Scenna *et al.*, 2005). Tolfenamic acid, a non-steroidal anti-inflammatory drug (NSAID) like carprofen, ibuprofen, flunixin, meloxicam, and phenylbutazone, is commonly used in domestic animals for its anti-inflammatory, antipyretic, and analgesic properties

(Tummaruk and Sang-Gassanee *et al.*, 2013; Virginia, 2015). These NSAIDs effectively inhibit prostaglandin production by targeting COX enzymes (Radi, 2009). Tolfenamic acid, in terms of pharmacological activity, is typically classified as a nonselective COX inhibitor, capable of inhibiting both COX-1 and COX-2 (Lees *et al.*, 2004). NSAIDs are used not only for their anti-inflammatory properties but also for their antiluteolytic effects to reduce early embryonic loss in cattle (Jaśkowski *et al.*, 2021). However, the fertility rates of cows treated with carprofen during AI did not differ from those of untreated control cows (Heuwieser *et al.*, 2011). Conversely, cows administered ibuprofen or flunixin meglumine before embryo transfer showed pregnancy rates 5% to 26% higher than control cows (Elli *et al.*, 2001). These differences in pregnancy rates may be influenced by factors such as dosage, administration method, and cow characteristics like parity and breed (Spencer *et al.*, 2020). Nevertheless, these data indicate that some NSAIDs could be used to reduce post-service inflammation in pigs and hence improve their subsequent reproductive performance. In the field of pig reproduction, research on the effects of NSAIDs on plasma prostaglandin concentration and reproductive performance following AI is currently limited. Therefore, this study aims to evaluate serum PGF_{2α} levels and subsequent reproductive performance in gilts and sows treated with tolfenamic acid prior to artificial insemination.

Materials and Methods

The experiments were conducted in accordance with the procedures approved and licensed by the Animal Care and Use Committee of the School of Agricultural Resources, Chulalongkorn University (protocol number IACUC 002/01/2566), following the Ethical Principles and Guidelines for the Use of Animals established by the National Research Council of Thailand.

Experimental design: Experiment I assessed the effect of administering tolfenamic acid 10 min before insemination on serum PGF_{2α} levels. The gilts were divided into two groups: control (n = 10) and treatment (n = 10). The treatment group received intramuscular injections of 2 mg/kg tolfenamic acid (Tolfedine®CS, Vêtoquinol, Québec, Canada) 10 min prior to insemination, while the control group did not receive any injection. Serum PGF_{2α} levels before and after insemination were analyzed and compared between the control and treatment groups.

Experiment II examined the effects of tolfenamic acid treatment on reproductive performance in gilts and sows. Experiment II was conducted during the hot (April to May) and rainy (June to July) seasons. A total of 175 females, consisting of 41 gilts and 134 sows (parity 1–6), were selected from both seasons (hot, n = 79; rainy, n = 96) and divided into a control group (gilts, n = 25; sows, n = 56) and a treatment group (gilts, n = 16; sows, n = 78). Both groups received tolfenamic acid treatment as outlined in Experiment I. The reproductive performance of the gilts and sows, including farrowing rate and litter traits, was

subsequently analyzed and compared between the control and treatment groups.

General management: The study was conducted on a commercial swine herd in Thailand, consisting of 500 Landrace × Yorkshire crossbred sows managed under a batch weaning system. The females were housed in a closed facility equipped with an evaporative cooling system. Replacement gilts, aged seven months, were sourced from a commercial breeder. Gilts that displayed their first standing estrus in the gilt pool, along with weaned sows on day 24 of lactation, were moved to the mating house and housed individually in crates (0.6 × 2.0 m) on a concrete slatted floor. To facilitate batch AI management, replacement gilts were inseminated following the detection of synchronized standing estrus, while weaned sows were inseminated after the detection of natural standing estrus. The gestating females were fed a commercial diet containing 3.0 Mcal of energy/kg and 15.0% crude protein at a rate of 2.0–3.0 kg per day. The feed was gradually increased to 3.0 kg per day from the 11th to 15th week of gestation and then gradually decreased during the last week of gestation. Water was provided on demand through drinking nipples. Seven days before the expected date of parturition, the gestating females were moved to the farrowing unit, where they were housed individually in 0.6 × 2.4 m crates placed in the center of 2.0 × 2.4 m farrowing pens. On the day of the farrowing, the females were carefully monitored from a distance, with intervention and assistance provided when dystocia was identified (defined as an interval of more than 30 min between the births of piglets).

Estrus synchronization and artificial insemination: Estrus synchronization in the gilts was achieved by supplementing their feed with 20 mg of altrenogest (Virbages[®], Virbac, Carros Cedex, France) per animal daily for 18 consecutive days. Estrus detection in gilts began on the final day of altrenogest supplementation, while for weaned sows, it started the day after weaning. Estrus detection was conducted twice daily, from 6:00–7:00 am and 5:00–6:00 pm, using the back pressure test in the presence of a mature boar. Females were considered to be in estrus when they exhibited the standing reflex. Both gilts and sows were inseminated 2–3 times during the estrus period. In gilts, AI was performed at the onset of detected estrus and repeated every 12 h. For sows, AI was conducted 12 h after the onset of estrus and then repeated at 12-h intervals. Both the control and treatment groups were inseminated using an intra-uterine AI catheter. The study utilized commercial pooled semen from Duroc boars at a concentration of 30×10^6 sperm/ml, with motility above 60%, in an 80 ml dose.

Blood collection and PGF2 α assay: In Experiment I, blood samples were collected from gilts in both the control group (n = 10) and the treatment group (n = 10). The blood was drawn 30 min before and after the first insemination. The collected blood was stored in clot activator tubes at room temperature for 1 h. Following this, the samples were centrifuged at 2000 × g for 10 min to separate the serum, which was then immediately

stored at -20°C until PGF2 α assay. The qualitative determination of PGF2 α in the samples was performed using a competitive immunoassay kit (PGF2 α ELISA Kit, Abnova, Heidelberg, Germany). Briefly, the serum samples were diluted with assay buffer at a 1:10 v/v ratio. Next, 100 μ l of the diluted sample was mixed with 50 μ l of alkaline phosphatase-conjugated PGF2 α . Then, 50 μ l of polyclonal antibody to PGF2 α was added to the mixture. The samples were placed on a plate shaker for 2 h at room temperature. After incubation, the wells containing the shaken solution were emptied and rinsed three times with 400 μ l of tris-buffered saline. After the final wash, 5 μ l of alkaline phosphatase-conjugated solution and 200 μ l of p-nitrophenyl phosphate substrate solution were added to the wells. The solution was incubated at room temperature for 45 min, followed by the addition of 50 μ l of stop solution. The resulting yellow color was measured using a microplate reader set to 405 nm. The PGF2 α concentration in the samples was approximately quantified using the standard curve method. Standard solutions were prepared by diluting the stock PGF2 α solution with assay buffer to achieve concentrations of 3.0, 12.2, 48.8, 195.3, 781.2, 3125.0, and 12500.0 pg/ml, with the assay buffer serving as the negative control.

Data: The interval from the end of estrus synchronization to service in gilts and the interval from weaning to service in sows were recorded. In gilts, this interval was defined as the period from the last day of altrenogest supplementation to service. Females returning to estrus between 18–24 days post-service were categorized as having a regular return to estrus. The regular return rate was calculated by dividing the number of females with regular returns by the total number of inseminated females, then multiplying by 100. The farrowing rate was defined as the number of females that farrowed divided by the number of inseminated females multiplied by 100. Throughout gestation, factors contributing to reproductive failure, including irregular returns to estrus, abortion, and other reasons for culling, were also recorded. On the farrowing day, the females were closely monitored, and data were collected on the number of piglets born alive, stillborn piglets per litter, mummified fetuses per litter, and the total number of piglets born per litter.

Statistical analysis: Statistical analyses were performed using SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA). Continuous variables were analyzed using the MEANS procedure, while the FREQ procedure was used for categorical variables. Univariate analysis assessed the normality of continuous variables. In Experiment I, PGF2 α concentrations before and after AI were compared using a paired t-test. To evaluate the impact of toltenamic acid treatment and serum PGF2 α levels on gilts reproductive performance, independent variables included treatment groups (control and treatment) and PGF2 α levels (≥ 750 pg/ml and < 750 pg/ml). Dependent variables, such as farrowing rate, number of piglets born alive, stillborn piglets per litter, mummified fetuses per litter, and the total number of piglets born per litter, were analyzed using one-way analysis of variance with the GLM procedure. Least-

squares means $\pm$ SEM were calculated for each variable and compared using the least significant differences (LSD) test. The farrowing rate was assessed with Fisher's exact tests. In Experiment II, the main effect was the treatment groups (control and treatment). The intervals from estrus synchronization to service in gilts (≤ 7 days and > 7 days), from weaning to service in sows (3–5, 6–7, and > 7 days), and the seasons (hot and rainy) were included as covariates. Dependent variables included litter traits, regular return-to-estrus rate, and farrowing rate. The statistical models considered the treatment group and its two-way interaction with each covariate. The impact of continuous independent variables on litter traits was analyzed using the GLM procedure. Categorical data, including regular return rate, farrowing rate, and total born classes (≤ 12 , 13–16, > 16), were compared between treatment groups using logistic regression with the generalized linear mixed model (GLIMMIX) procedure. Least-squares means were calculated for each variable class and compared using the least significant difference test. A significance level of $P < 0.05$ was used for all analyses.

Results

Experiment I: The concentration of PGF2 α in the serum of gilts after insemination ranged from 104.3 to 2062.3

Table 1 Effect of tolafenamic acid treatment on reproductive performance and serum PGF2 α concentration in gilts after insemination (least-squares means $\pm$ SEM)

Parameters	Control	Treatment	P value
Number of gilts	10	10	-
Farrowing rate (%)	100.0	80.0	0.136
Number of piglets born alive per litter	9.3 $\pm$ 1.6	8.3 $\pm$ 1.7	0.661
Number of stillborn piglet per litter	1.0 $\pm$ 0.8	3.3 $\pm$ 0.8	0.063
Number of mummified fetuses per litter	6.3 $\pm$ 2.0	4.5 $\pm$ 2.3	0.559
Total number of piglets born per litter	16.5 $\pm$ 1.2	16.0 $\pm$ 1.3	0.784
Serum PGF2 α			
Before insemination (pg/ml)	556.3 $\pm$ 206.0	1335.4 $\pm$ 237.9	0.029
After insemination (pg/ml)	653.1 $\pm$ 218.8	652.5 $\pm$ 233.9	0.998

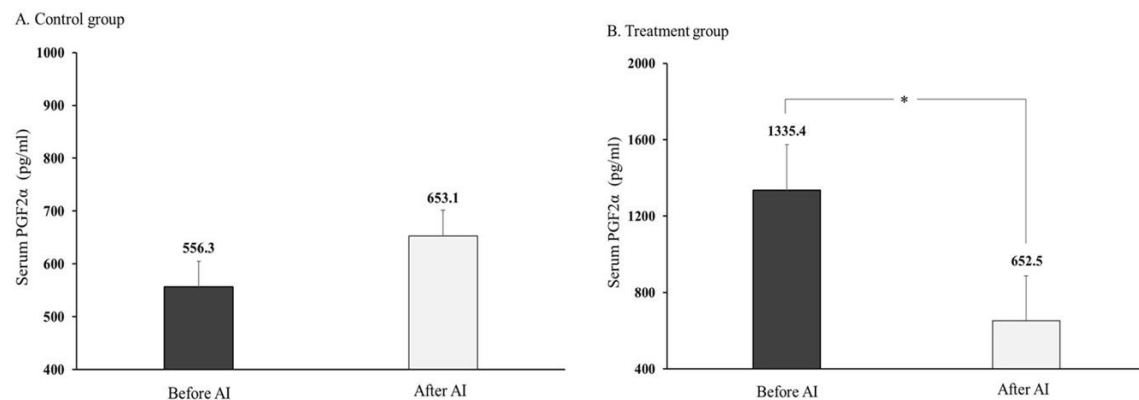


Figure 1 Serum PGF2 α concentration at 30 min before and after insemination of the gilts within control and treatment groups, * $P = 0.063$

pg/ml. Before AI, the serum PGF2 α level in the treatment group was higher than that in the control group (1335.4 $\pm$ 237.9 vs. 556.3 $\pm$ 206.0, $P = 0.029$; Table 1). However, after AI, the serum PGF2 α levels in both the treatment and control groups were similar (652.5 $\pm$ 233.9 vs. 653.1 $\pm$ 218.8, $P = 0.998$; Table 1). In the control group, the serum PGF2 α level in gilts after insemination remained stable compared to the level before insemination (653.1 $\pm$ 218.8 vs. 556.3 $\pm$ 206.0, $P = 0.314$, Fig. 1A). Conversely, in the treatment group, the serum PGF2 α level after insemination tended to be lower than that before AI (652.5 $\pm$ 233.9 vs. 1335.4 $\pm$ 237.9, $P = 0.063$, Fig. 1B). Administration of tolafenamic acid 10 min before AI did not affect reproductive performance in gilts ($P > 0.05$; Table 1). However, PGF2 α levels after AI influenced the number of piglets born alive per litter ($P < 0.05$, Fig. 2A). Females with serum PGF2 α levels greater than 750 pg/ml after insemination had fewer piglets born alive per litter compared to those with levels below 750 pg/ml (5.2 $\pm$ 1.9 vs. 10.2 $\pm$ 1.2, $P = 0.044$, Fig. 2A). No effects of PGF2 α levels after insemination were found on total births, stillborns, or mummified fetuses ($P > 0.05$, Fig. 2A and 2B).

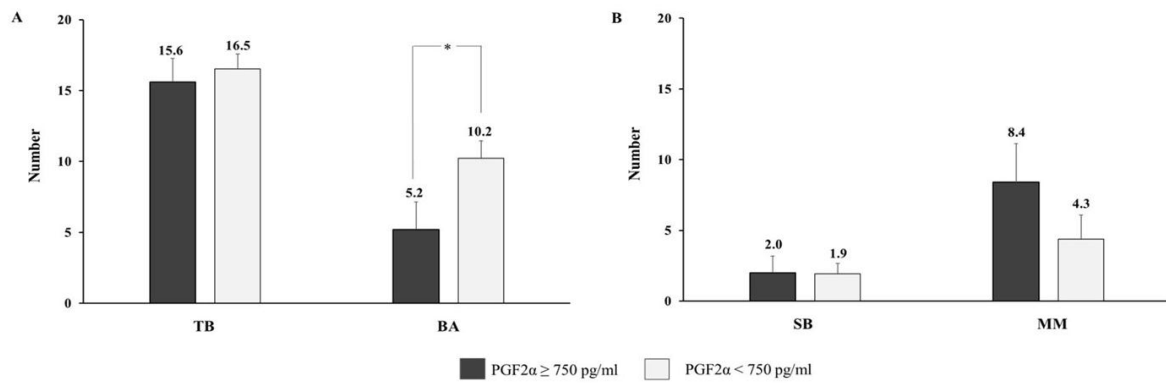


Figure 2 Relationship between serum PGF2α concentration at 30 min after insemination and reproductive performance of the gilts, * $P = 0.044$

Experiment II: The reproductive performance at farrowing between gilts and sows showed no significant differences between the control and treatment groups ($P > 0.05$, Table 2). The influence of covariates and their interactions on the reproductive performance of gilts and sows was observed and is detailed in Tables 3 and 4, respectively. In gilts, the number of piglets born alive per litter and the occurrence of mummified fetuses in both the control and treatment groups were affected by the seasons (Table 3). Gilts inseminated in the hot season had fewer piglets born alive per litter compared to those inseminated in the rainy season (9.0 ± 0.9 vs. 12.4 ± 1.2 , $P = 0.034$). Similarly, gilts inseminated in the hot season had more mummified fetuses than those inseminated in the rainy season (4.9 ± 1.1 vs. 0.3 ± 1.6 , $P = 0.024$). In sows, reproductive performance in both control and treatment groups was influenced by seasons, weaning-to-service interval (WSI), and their interactions with

the treatment group (Table 4). Sows inseminated in the hot season had more piglets born alive per litter than those inseminated in the rainy season (13.2 ± 0.4 vs. 11.5 ± 0.5 , $P = 0.019$). Additionally, sows with a WSI greater than 7 days had fewer stillborn piglets than those with a WSI of 3–5 days (0.8 ± 0.3 vs. 1.9 ± 0.3 , $P = 0.032$). The interaction between the groups and seasons led to different numbers of piglets born alive per litter in treatment sows inseminated in the hot season compared to the rainy season (11.2 ± 0.6 vs. 13.0 ± 0.5 , respectively, $P = 0.048$). The interaction between treatment and WSI showed that the number of piglets born alive per litter in treatment sows with a WSI of 3–5 days (11.4 ± 0.6) was lower than in treatment sows with a WSI of 6–7 days (13.7 ± 0.6 , $P = 0.017$). The number of piglets born alive per litter varied among treatment sows ($P < 0.05$) but not among control sows ($P > 0.05$) with different WSIs.

Table 2 Effects of tolfenamic acid treatment on the reproductive performance of gilts and sows (least-squares means $\pm$ SEM)

Parameters	Control	Treatment	P value
Gilts			
Number of gilts	25	16	-
Regular return-to-estrus rate (%)	12.0	18.7	0.550
Farrowing rate (%)	88.0	75.0	0.280
Number of piglets born alive per litter	10.9 ± 0.9	9.7 ± 1.3	0.497
Number of stillborn piglets per litter	0.9 ± 0.4	2.3 ± 0.6	0.077
Number of mummified fetuses per litter	3.0 ± 1.1	3.1 ± 1.5	0.965
Total number of piglets born per litter	14.7 ± 0.8	15.1 ± 1.1	0.804
- ≤ 12 (%)	27.2	33.3	0.710
- 13–16 (%)	45.4	25.0	0.240
- > 16 (%)	27.2	41.6	0.391
Sows			
Number of sows	56	78	-
Regular return-to-estrus rate (%)	3.6	6.4	0.466
Farrowing rate (%)	83.9	91.0	0.211
Number of piglets born alive per litter	12.8 ± 0.5	12.2 ± 0.4	0.429
Number of stillborn piglets per litter	1.0 ± 0.3	1.5 ± 0.2	0.145
Number of mummified fetuses per litter	0.8 ± 0.3	1.0 ± 0.2	0.563
Total number of piglets born per litter	14.6 ± 0.5	14.8 ± 0.4	0.842
- ≤ 12 (%)	41.0	28.2	0.119
- 13–16 (%)	30.3	39.7	0.263
- > 16 (%)	28.5	32.0	0.660

Table 3 Effects of tolfenamic acid treatment groups and seasons on the reproductive performance of gilts.

Variables	Covariates	P value	Categories of covariates	Least-squares means $\pm$ SEM
Live born	seasons	0.034	hot	9.0 $\pm$ 0.9 ^a
			rainy	12.4 $\pm$ 1.2 ^b
Mummified fetuses	seasons	0.024	hot	4.9 $\pm$ 1.1 ^a
			rainy	0.3 $\pm$ 1.6 ^b
	groups $\times$ seasons	0.030	control, hot	5.7 $\pm$ 1.5 ^a
			control, rainy	0.3 $\pm$ 1.5 ^b
			treatment, hot	4.0 $\pm$ 1.6 ^{ab}
			treatment, rainy	0.3 $\pm$ 1.8 ^b

^{a, b} within categories of covariates differs significantly ($P < 0.05$). Nonsignificant effects are not shown in the table.

Table 4 Effect of weaning-to-service interval and season of treatment on reproductive performance of the sows.

Variables	Covariates	P value	Categories of covariates	Least-squares means $\pm$ SEM
Live born	groups $\times$ WSI	0.023	control, 3–5 days	12.3 $\pm$ 1.1 ^{ab}
			control, 6–7 days	12.1 $\pm$ 0.9 ^{ab}
			control, > 7 days	13.9 $\pm$ 0.8 ^a
			treatment, 3–5 days	11.4 $\pm$ 0.6 ^b
			treatment, 6–7 days	13.7 $\pm$ 0.6 ^a
			treatment, > 7 days	11.2 $\pm$ 1.1 ^{ab}
	seasons	0.019	hot	11.5 $\pm$ 0.5 ^a
			rainy	13.2 $\pm$ 0.4 ^b
	groups $\times$ seasons	0.048	control, hot	11.8 $\pm$ 0.9 ^{ab}
			control, rainy	13.4 $\pm$ 0.6 ^b
			treatment, hot	11.2 $\pm$ 0.6 ^a
			treatment, rainy	13.0 $\pm$ 0.5 ^b
Stillborn	WSI	0.032	3–5 days	1.9 $\pm$ 0.3 ^a
			6–7 days	1.2 $\pm$ 0.3 ^{ab}
			> 7 days	0.8 $\pm$ 0.3 ^b
	groups $\times$ WSI	0.047	control, 3–5 days	2.0 $\pm$ 0.6 ^b
			control, 6–7 days	0.9 $\pm$ 0.5 ^{ab}
			control, > 7 days	0.6 $\pm$ 0.4 ^a
			treatment, 3–5 days	1. $\pm$ 0.31 ^b
			treatment, 6–7 days	1.5 $\pm$ 0.3 ^{ab}
			treatment, > 7 days	1.1 $\pm$ 0.5 ^{ab}
Total born	groups $\times$ WSI	0.046	control, 3–5 days	14.9 $\pm$ 1.2 ^{ab}
			control, 6–7 days	14.3 $\pm$ 1.0 ^{ab}
			control, > 7 days	15.0 $\pm$ 0.8 ^{ab}
			treatment, 3–5 days	14.1 $\pm$ 0.6 ^a
			treatment, 6–7 days	16.2 $\pm$ 0.7 ^b
			treatment, > 7 days	13.4 $\pm$ 1.8 ^{ab}

Different superscripts ^{a, b} within categories of covariates differ significantly ($P < 0.05$). Nonsignificant effects are not shown in the table.

Discussion

To our knowledge, no previous studies have investigated the impact of tolfenamic acid or other NSAIDs on serum PGF $_{2\alpha}$ levels in pigs. In dogs, administering tolfenamic acid at 4–8 mg/kg body weight can reduce PGE $_2$ levels in exudate within 20 min (McKellar *et al.*, 1994). In cattle, both oral and intravenous administration of flunixin meglumine effectively suppressed PGF $_{2\alpha}$ biosynthesis (Odensvik, 1995). Calves receiving 2.2 mg/kg of intravenous flunixin during surgery showed decreased serum PGE $_2$ levels up to 12 h post-administration (Fraccaro *et al.*, 2013). Similarly, piglets given flunixin before castration had lower PGE $_2$ levels than control piglets (Merenda *et al.*, 2022). In the current study, administering tolfenamic acid prior to insemination may reduce serum PGF $_{2\alpha}$ levels, indicating that when excessive inflammation occurs due to insemination, the use of tolfenamic acid may help minimize the degree of inflammation.

Following insemination, PGF $_{2\alpha}$ may influence porcine fertility both positively and negatively, depending on the stage of gestation. In this study, elevated levels of PGF $_{2\alpha}$ after insemination are linked to a decrease in the number of piglets born alive per litter. Prostaglandins, including PGE $_2$, can be converted into PGF $_{2\alpha}$ through the activity of PGF synthase and carbonyl reductase 1 enzymes (Waclawik and Ziecik, 2007; Saint-Dizier *et al.*, 2019). It is possible that the increased PGF $_{2\alpha}$ levels post-insemination result from an excessive rise in PGE $_2$, which may be induced by inflammatory responses triggered by trauma or pathogens in the uterus during insemination. This rise in PGF $_{2\alpha}$ could potentially contribute to the observed decrease in the number of piglets born alive.

One week after ovulation, the corpora lutea (CL) reaches full maturity and begins secreting progesterone, which is essential for establishing and maintaining pregnancy (Likso *et al.*, 2021). The CL becomes sensitive to PGF $_{2\alpha}$ around 12 to 13 days post-

ovulation, with luteolysis typically occurring around day 15 (Soede *et al.*, 2011). Consequently, in this study, the reduced number of piglets born alive per litter may not be directly associated with CL regression due to elevated PGF2 α levels after artificial insemination. However, the prolonged presence of PGF2 α beyond day 15 of pregnancy could potentially impact reproductive performance through its luteolytic effects.

After insemination, PGF2 α induced by seminal plasma in the porcine oviduct was essential for transporting gametes and embryos (Saint-Dizier *et al.*, 2019). Supplementing boar semen with PGF2 α during insemination enhances myometrial contractility, facilitating sperm transport and leading to higher farrowing rates and larger litter sizes in females inseminated with PGF2 α -supplemented semen (Kos and Bilkei, 2004). Recent studies have demonstrated that during porcine conceptus implantation, PGF2 α promotes the secretion of vascular endothelial growth factor, angiopoietins, and fibroblast growth factor from the trophoblast. These factors support the formation of capillary-like structures in the endometrial endothelium and its network between days 15 and 20 of pregnancy (Kaczynski *et al.*, 2020). Additionally, PGF2 α released from the conceptus and endometrium during implantation stimulates the synthesis of mitogen-activated protein kinase and focal adhesion kinase, which are involved in trophoblast cell proliferation and adhesion (Kaczynski *et al.*, 2018). These findings suggest that PGF2 α plays a beneficial role in establishing porcine pregnancy from insemination to implantation. However, the early detection of PGF2 α after insemination may not be a positive indicator for fertilization and early embryonic development. The mechanism by which inflammatory processes affect early embryonic development in pigs remains unclear and may require further investigation.

The present findings emphasize the influence of seasons on the reproductive performance of both gilts and sows. Although there are no previous reports on the seasonal effects on prostaglandin levels or the reproductive performance of gilts and sows treated with NSAIDs before insemination, it is well-documented that hot climates negatively impact female fertility (Tummaruk *et al.*, 2010). Sows inseminated during the hot season exhibited lower breeding success compared to those inseminated during the rainy season (Tummaruk *et al.*, 2004) or the cool season (Pearodwong *et al.*, 2020a). Up to date, in swine, the study on the effect of heat stress on the post-service inflammatory response has been investigated before. In cattle, an increased incidence of uterine disease or postpartum inflammation has been reported to be associated with heat stress exposure of the cows during late pregnancy (Molinari *et al.*, 2022). Therefore, when considering the use of tolafenamic acid post-insemination to improve female fertility, potential confounding factors due to the seasonal variation must be carefully evaluated. PGF2 α and PGE2 are involved in stimulating the synthesis of theca cell collagenase, which plays a role in ovulation (Murdoch and McCormick, 1993; Abdulrahman and Fair, 2019). Additionally, previous research has shown that PGF2 α influences estrus expression post-weaning and

ovulation (Ziecik *et al.*, 2011; Katila, 2012; Roongsitthichai *et al.*, 2015). The size of the preovulatory follicle and the interval between weaning and ovulation can be affected by the timing between weaning and estrus in sows (Pearodwong *et al.*, 2020b). A prolonged weaning-to-estrus interval also results in a shorter estrus-to-ovulation interval (Kemp and Soede, 1996).

In conclusion, administering 2 mg/kg of tolafenamic acid via intramuscular injection 10 min before insemination tended to reduce serum PGF2 α levels. Additionally, a negative correlation was observed between post-insemination serum PGF2 α levels and the number of piglets born alive per litter. However, tolafenamic acid treatment did not have a significant effect on reproductive performance. Factors such as seasons and weaning-to-service intervals were found to influence the reproductive performance of gilts and sows.

Conflicts of interest: There were no conflicts of interest that may have biased the work reported in this study.

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