

Effects of prostaglandins and prostaglandin synthesis inhibitors on sexual behavior in boars

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SUMMARY

Experiments were conducted investigating the effects of prostaglandins and prostaglandin synthesis inhibitors on libido in boars. In Experiment 1, two prostaglandin products were compared with regard to expediting the training of boars for semen collection. On each of five consecutive days, boars received i.m. treatment with saline, dinoprost tromethamine or cloprostenol sodium (n=12/group). On each of day 1 (p=0.06), day 2 (p<0.05), and day 3 (p<0.05), but not on day 4 or 5 (p>0.1), the percentage of boars collected after dinoprost tromethamine, but not cloprostenol sodium, was greater than controls. In Experiments 2 and 3, libido in boars that were trained previously for semen collection was assessed after treatment with prostaglandin synthesis inhibitors, testing the hypothesis that endogenous release of prostaglandin is necessary for expression of sexual behaviors. In Experiment 2, boars treated with flunixin meglumine (n=12) had suppressed (p<0.01) levels of 15-ketodihydro-prostaglandin-F_{2α} (PGFM) in serum but characteristics of libido were similar (p>0.1) to

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controls (n=12). In Experiment 3, boars were administered indomethacin orally (n=12) or served as untreated controls (n=12). Indomethacin decreased ($p<0.01$) serum levels of PGFM, increased ($p<0.05$) the number of false mounts (mounting artificial sow but dismounting before an ejaculate was collected), and tended ($p=0.09$) to lengthen the interval between entering the collection pen and the start of ejaculation. These results suggest that prostaglandin synthesis and release is necessary for the complete display of normal sexual behaviors in boars. *Reproductive Biology* 2007 7 2:163–175.

Key words: boar, semen collection, sexual behavior, prostaglandin

INTRODUCTION

Efficient operation of a commercial stud is dependent on a battery of trained boars that consistently and rapidly mount an artificial sow and subsequently allow semen collection via the gloved hand technique. Work conducted in our laboratory demonstrated that exogenous treatment with prostaglandin- $F_{2\alpha}$ ($PGF_{2\alpha}$) enhances sexual behavior to varying degrees in boars [5, 6, 9]. For example, in sexually active boars (i.e., boars experienced with natural mating), $PGF_{2\alpha}$ decreased the number of sessions required for training, the number of false mounts (mounting artificial sow but dismounting before a complete ejaculate was collected), and elapsed time between entering the semen collection pen and the start of ejaculation [5]. In a subsequent experiment, Kozink et al. [9] reported that treatment with $PGF_{2\alpha}$ increased sexual behavior scores (1 to 5 scale; 1=no interest in artificial sow; 5=mounted artificial sow and allowed semen collection), although the number of boars trained for semen collection was similar between treated and control groups. In that study, young boars lacking experience with natural mating were employed.

In our previous experiments [5, 6, 9] the prostaglandin product employed was dinoprost tromethamine (Lutalyse; Pfizer Animal Health, New York, NY, USA). In a clinical trial conducted under commercial

conditions, Szurop et al. [13] reported that treatment with a synthetic prostaglandin (cloprostenol sodium; Enzaprost-F; Chinoin, Budapest, Hungary) stimulated mounting behavior and allowed collection of semen from both young boars without previous sexual experience and older, previously trained boars displaying a loss of libido. Given these results, we hypothesized that there may be differences in the efficacy of different prostaglandin products in terms of stimulating sexual behavior.

Thus, the objective of the first experiment was to extend our previous findings [5, 9] and compare two commercially available prostaglandin products with regard to expediting the training of boars for semen collection. Young boars without previous experience with natural mating were utilized. In the second and third experiments, sexual behavior in mature boars that were trained previously for semen collection was assessed after treatment with different prostaglandin synthesis inhibitors in order to test the hypothesis that endogenous release of prostaglandin is necessary for the expression of sexual behaviors. We selected flunixin meglumine and indomethacin because these non-steroidal anti-inflammatory drugs have been used in previous experiments with swine [10, 12].

MATERIALS AND METHODS

The research was conducted at the Tidewater Agricultural Research and Extension Center in Suffolk, VA, USA and an experimental protocol was approved by the Animal Care Committee of Virginia Polytechnic Institute and State University. The building was curtain-sided and serviced by propane gas heaters and a thermostatically-controlled sprinkler system. Yorkshire×Landrace boars were individually housed in pens (4.5 m²) that had a combination of solid concrete and solid steel rod flooring and were each equipped with a nipple drinker. A separate pen (5.8 m²) contained an artificial sow (Minitube of America; Minneapolis, MN, USA) that facilitated semen collection *via* the gloved hand technique. The same trained technician performed all semen collections. Boars were fed daily

2.3 to 2.7 kg of a fortified, corn and soybean meal-based diet that met or exceeded the recommendations for the various nutrients [11].

Characteristics of sexual behavior were assessed as previously described [5, 9] and included: reaction time (the interval between entering the collection pen and first interaction with artificial sow), the interval between entering the collection pen and the start of ejaculation, duration of ejaculation, and number of false mounts (mounting artificial sow but dismounting before allowing a complete collection of semen). Boars were also assigned a sexual behavior score (1 to 5) based on the following system: 1-displayed no interest in the artificial sow, 2-displayed slight interest in artificial sow but did not attempt to mount, 3-mounted the artificial sow but did not display an erection, 4-mounted the artificial sow and displayed an erection, but did not allow semen collection, and 5-mounted the artificial sow and allowed semen collection.

For Experiments 2 and 3, blood samples were collected *via* jugular venipuncture while boars were restrained with a metal snare. Blood was allowed to clot overnight at 4°C and serum was harvested following centrifugation. Serum concentrations of 15-ketodihydro-prostaglandin-F_{2α} (PGFM), a metabolite of PGF_{2α}, were determined using a commercially available enzyme-linked immunosorbent assay kit (Neogen Corporation, Lexington, KY, USA). Assay sensitivity was 20 pg/ml. Intra- and inter-assay coefficients of variation averaged 7.9% and 9.0%, respectively.

Experiment 1: Effect of prostaglandin treatment on sexual behavior in boars being trained to mount an artificial sow for semen collection

Boars (n=36; 298±3 days of age and 155±2 kg body weight) were moved on five consecutive days to the semen collection pen. Approximately one minute before entering the pen each day, boars received i.m. treatment with either 0.9% saline (controls), Lutalyse (10 mg) or cloprostenol sodium (250 µg; Estrumate; Schering-Plough Animal Health, Kenilworth, NJ, USA) (n=12/group). Boars remained in the collection pen for a maximum of thirty minutes each day. The percentage of boars allowing semen collection on each day was recorded and sexual behavior was evaluated as described above.

Experiment 2: Effect of flunixin meglumine on sexual behavior in boars trained to mount an artificial sow and allow semen collection

Mature boars (533.9 ± 31.0 days of age) on a routine, once weekly semen collection frequency, were moved to the semen collection pen 30 minutes after i.m. treatment with 500 mg flunixin meglumine (Flunixinamine; Fort Dodge Animal Health, Fort Dodge, IA, USA) ($n=6$) or 10 ml 0.9% saline ($n=6$). One week later, the experiment was repeated, but boars that previously received flunixin meglumine were treated with saline and vice versa. Boars remained in the semen collection pen for a maximum of 30 minutes and sexual behavior was evaluated as described above. The dose of flunixin meglumine utilized and timing of treatment relative to observations of sexual behavior were based on a previous report in the literature [12]. After leaving the collection pen, blood samples were collected, serum was harvested, and serum PGFM concentrations determined as previously described.

Experiment 3: Effect of indomethacin on sexual behavior in boars trained to mount an artificial sow and allow semen collection

Mature boars (631.2 ± 2.0 days of age) on a routine, once weekly semen collection frequency, were used. Boars were paired so that boars in the pair had a history of displaying similar sexual behavior characteristics. On the day of the experiment, boars were fed the daily ration (controls; $n=12$) or the daily ration top-dressed with 2.73 g of indomethacin (Sigma-Aldrich Co., St. Louis, MO, USA) ($n=12$). Boars were moved to the semen collection pen approximately 30 minutes after complete consumption of feed. Sexual behavior was evaluated as described above and after leaving the semen collection pen, blood samples were collected, serum was harvested, and serum PGFM concentrations determined. The dose of indomethacin utilized and timing of treatment relative to observations of sexual behavior were based on a previous report in the literature [10].

Statistical Analyses

Data were analyzed using SAS (SAS Institute, Inc., Cary, NC, USA). Analyses included chi squares procedures or ANOVA using the GLM

procedure. Individual means were compared using the PDIFF option of the GLM procedure. For Experiment 1, the percentages of boars from which semen was collected on each day were compared using chi square procedures. Sexual behavior scores were subjected to repeated measures ANOVA using a model that included treatment, day and the treatment by day interaction as possible sources of variation. Characteristics of sexual behavior for boars from which semen was collected were evaluated using ANOVA with treatment as the main effect.

For Experiment 2, serum concentrations of PGFM and characteristics of sexual behavior were subjected to ANOVA. The model included sequence (either flunixin meglumine then saline, or saline then flunixin meglumine), boar within sequence, and treatment as possible sources of variation. For Experiment 3, concentrations of PGFM in serum and sexual behavior characteristics were compared by ANOVA using a model that included pair and treatment as main effects.

RESULTS

Experiment 1: Effect of prostaglandin treatment on sexual behavior in boars being trained to mount an artificial sow for semen collection

There was an effect of treatment on the percentage of boars mounting the artificial sow and allowing semen collection on day 1 ($p < 0.05$), and tendencies for treatment effects on day 2 ($p = 0.07$) and day 3 ($p = 0.09$). On each of day 1 ($p = 0.06$), day 2 ($p < 0.05$), and day 3 ($p < 0.05$), the percentage of boars collected after treatment with dinoprost, but not cloprostenol, was greater than controls. There were no effects of treatment on the percentage of boars collected on day 4 ($p = 0.35$) and day 5 ($p = 0.44$; fig. 1).

There were effects of treatment ($p < 0.05$) and day ($p < 0.01$), but no treatment by day interaction ($p = 0.92$) on sexual behavior scores. Sexual behavior scores for boars treated with dinoprost were greater ($p < 0.01$) than for cloprostenol-treated boars or controls (fig. 2). The number of ejaculates collected from dinoprost-treated boars tended to be greater ($p = 0.07$) than the other two groups, but other characteristics of sexual behavior for boars

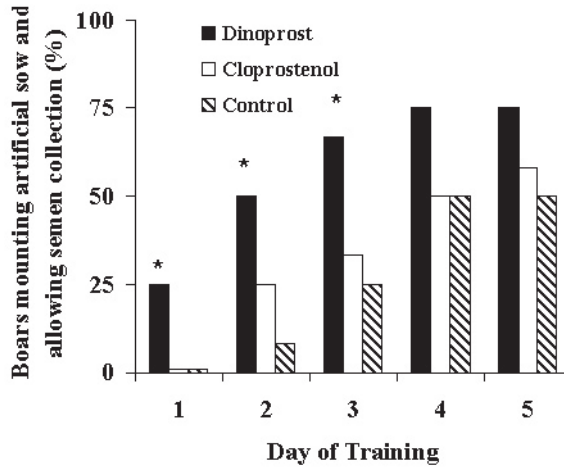


Figure 1. Boars mounting artificial sow and allowing semen collection after receiving dinoprost (10 mg), cloprostenol (250 μ g), or 0.9% saline (controls) (n=12/group) daily during a 5-day training period. Data were subjected to chi square analyses and the percentages of boars successfully collected after dinoprost treatment differed from controls on day 1 ($p=0.06$), day 2 ($p<0.05$), and day 3 ($p<0.05$) and are marked with an asterisk

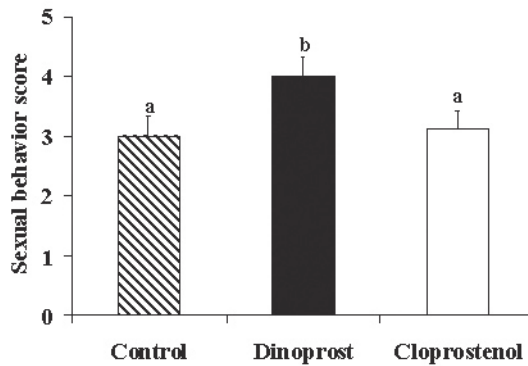


Figure 2. Sexual behavior scores (1 to 5 scale; 1=displayed no interest in the artificial sow, 5=mounted the artificial sow and allowed semen collection) during a 5-day semen collection training period in boars receiving dinoprost, cloprostenol, or 0.9% saline (controls; n=12/group). Bars marked with different letters differ ($p<0.01$). Data were analyzed using ANOVA for a repeated measures design and values are LSMean \pm SEM

from which semen was collected during the course of the experiment were not affected by treatment ($p>0.1$; tab. 1).

Table 1. Sexual behaviors in boars, semen from which was collected after treatment with dinoprost (10 mg) or cloprostenol (250 μ g)

Item	Dinoprost	Cloprostenol	Control	SE ⁴
No. boars	9	7	6	---
No. ejaculates/boar	3.9 ^a	2.9 ^b	2.7 ^b	0.4
Reaction time ¹ (s)	13.5	7.9	18.4	9.5
Interval to ejaculation ² (s)	568.9	694.3	669.5	147.0
Duration of ejaculation (s)	369.0	392.7	483.8	46.7
False mounts ³	3.3	4.7	4.1	1.4

¹interval between entering collection pen and first interaction with artificial sow; ²interval between entering collection pen and start of ejaculation; ³mounting artificial sow but dismounting before allowing a complete collection of semen; ⁴data were analyzed using ANOVA with treatment as the main effect, and SE were generated during analysis; ^{a,b}within a row means without a common superscript tended to differ ($p=0.07$)

Experiment 2: Effect of flunixin meglumine on sexual behavior in boars trained to mount an artificial sow and allow semen collection

Compared with controls (230.2 ± 14.4 pg/ml), concentrations of PGFM in serum were lower ($p<0.01$) for boars treated with flunixin meglumine (65.7 ± 14.4 pg/ml). Characteristics of sexual behavior were not affected ($p>0.1$) by treatment (tab. 2).

Experiment 3: Effect of indomethacin on sexual behavior in boars trained to mount an artificial sow and allow semen collection

Treatment with indomethacin (52.5 ± 10.7 pg/ml) resulted in lower ($p<0.01$) serum concentrations of PGFM compared with controls (122.2 ± 10.7 pg/ml). Indomethacin-treated boars displayed more false mounts ($p<0.03$) and tended to have a greater interval to ejaculation ($p=0.09$). There were no effects of treatment ($p>0.1$) on other characteristics of sexual behavior (tab. 3).

Table 2. Effects of i.m. flunixin meglumine (500 mg) on characteristics of sexual behavior in boars exposed to an artificial sow

Item	Flunixin meglumine	Control	SE ⁵
No. Observations	12	12	---
Reaction time ¹ (s)	18.5	13.3	2.8
Interval to ejaculation ² (s)	225.5	294.0	73.5
Duration of ejaculation (s)	393.7	350.9	34.1
False mounts ³	1.5	1.0	0.6
Sexual behavior score ⁴	4.3	4.5	0.1

¹interval between entering collection pen and first interaction with artificial sow; ²interval between entering collection pen and start of ejaculation; ³mounting artificial sow but dismounting before allowing a complete collection of semen; ⁴sexual behavior score: 1-displayed no interest in artificial sow, 2-displayed slight interest in artificial sow but did not attempt to mount, 3-mounted the artificial sow but did not display an erection, 4-mounted the artificial sow and displayed an erection, but did not allow semen collection, and 5-mounted the artificial sow and allowed semen collection; ⁵data were subjected to ANOVA and SE were generated during analysis

Table 3. Effects of orally administered indomethacin (2.73 g) on characteristics of sexual behavior in boars exposed to an artificial sow

Item	Indomethacin	Control	SE ⁵
No. Observations	12	12	---
Reaction time ¹ (s)	13.8	10.2	3.7
Interval to ejaculation ² (s)	426.8	251.0	66.9
Duration of ejaculation (s)	323.7	293.9	22.1
False mounts ³	4.7	1.8	0.9
Sexual behavior score ⁴	5.0	5.0	---

¹interval between entering collection pen and first interaction with artificial sow; ²interval between entering collection pen and start of ejaculation; ³mounting artificial sow but dismounting before allowing a complete collection of semen; ⁴sexual behavior score: 1-displayed no interest in artificial sow, 2-displayed slight interest in artificial sow but did not attempt to mount, 3-mounted the artificial sow but did not display an erection, 4-mounted the artificial sow and displayed an erection, but did not allow semen collection, and 5-mounted the artificial sow and allowed semen collection; ⁵data were subjected to ANOVA and SE were generated during analysis

DISCUSSION

We previously demonstrated that i.m. treatment of sexually active boars (i.e., boars experienced with natural mating) with 10 mg dinoprost enhanced

sexual behavior and decreased the number of sessions required for training for semen collection [5]. Subsequently, Kozink et al. [9] treated sexually inexperienced boars with dinoprost at doses of 5, 10, or 20 mg. Boars given 10 mg dinoprost had significantly greater libido scores than controls, but there was no effect of the prostaglandin product on the percentage of boars successfully trained for semen collection.

In the current investigation (Experiment 1), sexually inexperienced boars were employed and in contrast to our previous work [9], dinoprost treatment decreased the time necessary to successfully train boars for semen collection. The equivocal results could perhaps be explained by differences between studies in the age, weight or genetics of the experimental animals, or the intensity of dinoprost treatment and exposure to the artificial sow. In the current study, Yorkshire×Landrace, maternal-line boars were employed whereas Kozink et al. [9] used lean-type, terminal-line boars from a commercial source. Boars used in the experiment reported herein were also older (298 days of age) and heavier (155 kg body weight) than boars employed by Kozink et al. [9] (177 days of age and 113 kg body weight). Age- and weight-related differences in libido were reported by Esbenshade et al. [4], and in that study, boars did not exhibit sexual motivation combined with ejaculation until between 150 and 270 days of age. Finally, in our previous study [9], boars were moved to the semen collection pen twice weekly for six weeks compared to once daily for 5 days in the current study. Perhaps more intense exposure to an artificial sow combined with frequent treatment with dinoprost is a better regimen to employ when training boars for semen collection.

Szurop et al. [13] reported that a prostaglandin analogue (cloprostenol sodium) stimulated sexual behavior and allowed collection of semen from both young boars (210 to 225 days of age) without previous sexual experience, and older boars displaying a loss of libido. In all of our previous studies [5, 6, 9] we used dinoprost tromethamine (Lutalyse) in attempts to alter sexual behavior. The current study (Experiment 1) compared dinoprost and a cloprostenol sodium product with regard to enhancing libido. Dinoprost tromethamine (2-amino-2-[hydroxymethyl]propane-1,3-diol;7-[3,5-dihydroxy-2-(3-hydroxyoct-1-enyl)cyclopentyl]hept-5-enoic

acid) is the tromethamine (THAM) salt of the naturally occurring $\text{PGF}_{2\alpha}$. Cloprostenol (7-[2-beta-(4-(3-chlorophenoxy)-3-hydroxyl-1-butenyl)-3,5 dihydroxycyclopentyl]-5-heptenoic acid) is a synthetic $\text{PGF}_{2\alpha}$ analogue. Dinoprost, but not cloprostenol, accelerated the successful training of boars for semen collection. Differences in action of drugs on sexual behavior in boars may depend on differences in pharmacological or biological activity and/or affinity for receptors.

In the present study, we employed a dose of dinoprost used in our previous experiments with boars [5, 6, 7, 9] and that induces parturition in sows [3]. We cannot discount the possibility that a different dose of cloprostenol may have had positive effects on sexual behavior. However, the dose employed (250 μg) is biologically active, at least in female swine, as it induces parturition [8]. Moreover, treatment of boars with 500 μg cloprostenol did not affect sexual behavior but caused emesis (Estienne et al., unpublished observation). Perhaps cloprostenol, at some dose less than 250 μg or between 250 and 500 μg enhances sexual behavior.

Although at least some exogenously administered prostaglandin products enhance sexual behavior, it is not known if endogenous release of prostaglandin is necessary for normal display of libido in boars. We tested this hypothesis in Experiments 2 and 3, by assessing sexual behavior in boars after pharmacologically blocking production of prostaglandin-endoperoxide synthase (PTGS)-dependent metabolites of arachidonic acid.

Mounting behavior and ejaculation were not abolished even though prostaglandin synthesis was inhibited as evidenced by a dramatic decrease in circulating PGFM concentrations occasioned by treatment with flunixin meglumine (Experiment 2) and indomethacin (Experiment 3). Indomethacin is a classical, non-selective PTGS inhibitor. Flunixin meglumine is a PTGS inhibitor, but also has very strong glucocorticoid activity and may act through steroid receptors. It is possible that although synthesis was greatly suppressed, a minimal level of prostaglandin was available to stimulate mounting behavior. Moreover, prostaglandin-induced sexual behavior may be manifested by mechanisms working within the central nervous system. Burne et al. [2] demonstrated that dinoprost injections induced nest-building behavior in sows and c-fos mRNA expression in

various regions of the brain. Treatment of boars with flunixin meglumine resulted in suppressed circulating levels of PGFM but the ability of this compound to cross the blood brain barrier and affect central synthesis of prostaglandins is unknown. On the other hand, indomethacin has been demonstrated to cross the blood brain barrier [1]. In Experiment 3 of the current report, treatment of boars with indomethacin suppressed circulating concentrations of PGFM. We cannot ascertain the effects of treatment on prostaglandin release within the brain, but it is reasonable to assume that central prostaglandin synthesis was at least partially inhibited. Although boars fed indomethacin still mounted the artificial sow and ejaculated, subtle effects on sexual behavior, including a trend for an increased interval to ejaculation and an increase in the number of false mounts were observed in treated individuals. These results are consistent with the hypothesis that central prostaglandin synthesis and release is necessary for the complete display of normal sexual behaviors in boars.

Whether effects of indomethacin were due specifically to decreases in concentrations of $\text{PGF}_{2\alpha}$ is not known. Indeed indomethacin treatment would be expected to suppress synthesis of other eicosanoids such as prostaglandin E. Finally, concentrations of PGFM for control boars in Experiment 1 were approximately 88% higher than levels displayed by controls in Experiment 2, a difference perhaps related to differences in age of subjects (534 and 631 days of age for Experiments 1 and 2, respectively).

In summary, treatment with dinoprost, but not cloprostenol, stimulated sexual behavior and accelerated the training of boars for semen collection. Treatment of boars previously trained for semen collection with indomethacin, but not flunixin meglumine, had subtle effects on sexual behavior including a trend for an increase in the interval from entering the collection pen to the start of ejaculation and an increase in the number of false mounts. The role of prostaglandins in controlling sexual behavior in boars warrants further scientific evaluation.

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