

Impact of a Novel Boar Pheromone on Weaned Sow Behavior and Reproduction

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Research

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Abstract

Background

Reproductive performance on commercial farms depends on the ability to identify sexual behavior and breed females during estrus. The presence of a boar is essential in determining sow sexual behaviors and is greatly mediated by the adult boar odor. This study sought to examine the effects of a novel boar salivary pheromone on weaned sow sexual receptivity and subsequent reproductive performance.

Methods

One hundred weaned sows were randomly assigned to either a control treatment where sows were exposed during heat detection to a live mature boar (BG) or to BOARBETTER® (Vétoquinol, Lure, France), given as a single spray while a boar grunting audio file was played (PG). Sexual behaviors were assessed twice daily during a Back-Pressure Test (BPT). Sows expressing behavioral estrus were mated. Ultrasound was used to assess ovarian activity from weaning until the end of ovulation. Reproductive and prolificacy data were collected until farrowing.

Results

Twelve sows were eliminated from the study because of early ovulation, no ovulation, or infectious disease. Breeding rate did not differ between the two treatments (BG: 100% vs PG: 91%). Sows in the BG had a shorter wean-to-estrus interval (97 vs 108 h) and longer estrus duration than PG sows (57 vs 39 h; $p < 0.05$). Wean-to-ovulation interval did not differ but onset of estrus to ovulation interval was longer in PG compared with BG sows (40 vs 30 h, $p < 0.01$). Follicle size at the onset of estrus was larger among PG than BG sows (5.76 vs 5.43 mm, $p = 0.04$). Timing of ovulation relative to insemination did not differ between the two treatment groups. Pregnancy rates were identical or similar for sows in the two treatment groups (93%) as was number of pigs born per litter.

Conclusions

Exposing weaning sows to the novel pheromone and the boar grunting sound achieved high estrus detection rate. Estrus was delayed and shortened but sows were successfully bred and were farrowed. Despite the absence of teaser boar, the procedure incorporating the novel pheromone and the boar grunting sound was shown to be efficient for sow breeding.

Introduction

Behavioral estrus is the period in the estrous cycle where sows are receptive to boars and thus allow copulation [1]. In modern pig farming, successful breeding requires that all sows in estrus be identified. To induce behaviors associated with estrus in sows, a live boar is walked in front of weaned sows while a person applies pressure on sow's back (Back Pressure Test – BPT). The boar provides a combination of visual, olfactory, tactile and auditory stimuli that together with the tactile stimulation of the BPT induces a

sexual behavioral response in estrus sows including a standing motionless with rigid limbs in a state called the standing reflex. The occurrence of the standing reflex is the best indicator that a sow is in estrus and can be bred.

Detecting gilts and sows in estrus is critical to reproductive success. Live boar stimulation plays a major role in the detection of the standing reflex on many commercial farms. About 48 to 60 % of sows in estrus will show the standing reflex in response to the BPT alone [1-6]. This percentage may increase as estrus progresses and approaches the time of ovulation [4,6]. Signoret [6] found that 60 % of estrus gilts did not showed standing reflex in response to the BPT alone while 90% of estrus gilts showed the standing reflex with the odor of a boar and an audio of boar grunts. When preputial fluid or boar-specific steroids such as androstenone and androstenol were sprayed on sow snout during the BPT [6,7,8], these salivary molecules were not quite as good as a live boar at indication of the full behavioral response. Thus, while pheromones add to the sows that respond to BPT, the boar effect on sow sexual behavior was thought to be greatly, but not entirely, mediated by the reported boar pheromone (androstenone) secreted by the submaxillary glands of an adult boar [9].

McGlone [10] found that not one, but three boar-specific volatile molecules were unique in boar saliva (androstenol, androstenone, and quinoline) compared to the saliva of sows. The combination of these three boar-specific molecules induced more complete sow sexual behavioral expressions, than the mixture of only androstenol and androstenone [11]. A patented boar pheromone mixture called BOARBETTER® (BB) has been developed and is now commercially available [11]. Since BB was shown to induce more complete sexual behaviors in estrous sows than androstenone alone [12], it was hypothesized that BB could be used as a natural, more complete olfactory stimulation to facilitate and improve the detection of sows in estrus without a boar. The use of BB might be a natural way to improve sow reproductive performance without hormonal treatment particularly in situations where boar stimulation and access is limited. The sow's estrus behavioral response was reported to be longer in duration (by several hours) when a boar was present compared with no boar exposure [2,3]. Prior to this study, we did not know how BB might change sow estrus timing and the timing of ovulation. Thus, the objective of this study was to compare weaned sow reproductive parameters and ovulation time when sows were heat checked with BB or a boar.

Methods

2.1 Animals and Housing

The work was performed on a commercial farm, not associated with an institution that has an ethics committee. However, this work followed accepted industry and welfare standards of the EU and Spain. The commercial farm was located near Arbeca (Lleida), Spain.

Two consecutive weaning groups of 50 Landrace x Large White sows were used in this study (N = 100 weaned sows). After weaning, sows were housed in conventional breeding crates (2 m x 0.6 m) and were

fed 2.8 kg of a commercial sow diet once a day. All sows had free access to water. Sows used in this study were selected based on the criteria of parity (1 – 7), acceptable body condition score (8 mm minimum P2 backfat thickness), previous lactation length (26.3 ± 1.6), and recent prolificacy (litter size = 14.35 ± 0.35). Unhealthy sows, sows with corpus hemorrhagicum (CH) or corpus luteum (CL) by the second day post-weaning, and sows that showed no ovulation after 192 hours post-weaning were excluded from the study. No hormonal or other treatments were used during this study to induce estrus or ovulation. To avoid cross-contamination between the different stimulation methods, all sows within the same weaning group were allocated to the same treatment group which then was changed randomly over time.

2.2 Estrus detection

Sows were checked for estrus behaviors daily at 09:00 and 15:00 starting from the first day post-weaning until sows ceased showing the standing reflex. Boar contact was performed with a trained, adult boar for the live-boar control group (BG). Estrus detection was conducted by a worker leading a boar along the corridor in front of the sows. The boar stood a few minutes in front of a group of 3 sows ensuring nose to nose contact. At the same time, another worker was doing the BPT. The BPT consisted of applying pressure to the back of the sows trying to mimic the tactile stimuli of a boar and evoke the standing reflex [3].

Estrus detection for the PG was conducted by spraying 4 mL of BB on the snout of each sow and conducting the BPT from 15 seconds after spraying. Three sows were sprayed, then each sow in this group was tested one by one. An audio record of boar courting grunts was played from the beginning of the estrus detection session to stimulate all sows to stand up as well as until the end of the estrus detection session. The live mature boar was not present during the BPT in this group.

The standing reflex was defined as when a sow was motionless with rigid limbs during the BPT [10]. A sow was considered in estrus if she showed the standing reflex during the BPT regardless of the presence of other signs of estrus such as vulva reddening and swelling or mucus discharge. The onset of estrus was defined as the time between two consecutive BPT where sows showed the standing reflex on the second but not the first BPT. The end of estrus was defined as the time between two consecutive BPT where sows showed the standing reflex on the first but not the second BPT. The wean-to-estrus interval (WEI) was the time in hours from weaning until the onset of estrus. Estrus duration in hours was calculated by subtracting the WEI from the end of estrus.

2.4 Ovulation

Transrectal ultrasonography was used to determine the time of ovulation. A portable ultrasound (Esaote Mylab Delta, Chagrin Falls, OH, USA) coupled with a microconvex probe was used. Ultrasound images were collected at a frequency of 8.6 MHz. Data interpretation was conducted following Kauffold [13] methods. The first ultrasound image was collected at 48 hours post-weaning to assess sow reproductive tract and detect a possible lactational estrus. Sows with visible CH or CL at this time were excluded from

the study. After 48 h post-weaning, transrectal ultrasonography was performed on each sow every 24 hours until 96 hours post-weaning then every 12 hours until 192 hours post-weaning until detection of ovulation. Ovulation was determined by the presence of CH or CL in the presence or absence of pre-ovulatory follicles. The ovulation time was defined as the time between two transrectal ultrasonography where the first one showed only preovulatory follicles (> 6 mm) and the second one showed CH with no more than one pre-ovulatory follicle. In case that more than one preovulatory follicles were visible with a CH, the ovulation time was the time the image was taken. Follicles were measured vertical to the screen. The weaning to ovulation interval (WOI) was defined as the time in hours from weaning to ovulation. The estrus to ovulation interval (EOI) was defined as the time in hours from the onset of estrus to ovulation. Follicular size at onset of estrus was estimated by linear regression.

2.5 Insemination, reproductive and prolificacy performance

The breeding protocol used in this study was a non-conventional method adapted to the farm needs. Table 1 shows the breeding protocol followed in relative to the onset of estrus. This variable time of insemination was based on the results of previous studies [5, 14, 15] to ensure sows were inseminated 0 to 24 hours before ovulation. The technique used was a conventional insemination with a dose of 80 ml and a minimum of 27.5 million spermatozoid per milliliter provided by the local insemination center. At 25 days post insemination, ultrasound was performed on each sow to confirm pregnancy. Pregnancy was confirmed by the presence of embryonic vesicles. The potential breeding rate was calculated by dividing the number of sows bred over the number of ovulating sows. The percentage of sows inseminated twice was calculated by dividing the number of sows that received two inseminations by the total number of sows bred. The pregnancy rate was defined as the number of sows found pregnant by ultrasound at 25 d over the number of sows bred [12]. Litter size, number of piglets born alive, and stillborn were recorded at farrowing.

2.5 Statistical analysis

The experimental design was a completely random design with two treatments (BG vs PG) with 100 weaned sows enrolled in the study. Each measure was evaluated for adherence to the assumptions of parametric analyses. Non-parametric statistics were used when ANOVA assumptions were not met. The Fisher's exact test was conducted to evaluate differences in ovulation rate, breeding rate, number of sows inseminated twice, and pregnancy rate. The Mann-Whitney test was used to determine differences in sow reproductive parameters between the BG and the PG. All statistical analyses were conducted in SAS statistical software (SAS version 9.4; SAS Inst., Inc., Cary, NC, USA). A statistically significant difference was declared at a p -value ≤ 0.05 .

Results

The cumulative percentages of ovulating sows over time are given for the two groups in Figure 1. Two of the 50 sows weaned in the BG were removed from the study: one sow had CL at 48 hours post-weaning, and another was excluded because of an infectious disease. Three sows did not show estrus before 192

hours post-weaning. Estrus was detected in the remaining 45 sows in the BG and they all received two inseminations. Of the 50 sows in the PG, one sow was removed from the study due to lameness and two additional sows did not ovulate before 192 hours post-weaning. Four out of the remaining 47 sows in the PG ovulated but did not show behavioral estrus when heat checked. Six out of the 43 sows bred in the PG, received only one insemination. Neither the ovulatory rates (93.8 % vs 95.9 %; $p = 0.68$) nor the breeding rates (100 % vs 91.5 %; $p = 0.12$) were significantly different between treatment groups.

Table 2 shows the reproductive parameters evaluated in this study for the BG and the PG. BG sows had shorter WEI (97 h vs 108 h, $p < 0.01$) and longer estrus duration (57.4 h vs 39.3 h, $p < 0.01$) than sows in the PG. Ovulation timelines were between 120-192 hours post-weaning with no noticeable differences over time between treatment groups (Figure 1). Average WOI was not different between groups (137 vs 138 h $p > 0.05$). Because BG sows showed an earlier onset of estrus than sows in the PG, EOI was longer in BG compared to sows in the PG (40.1 vs 29.7 h, $p < 0.01$). Ovulation occurred at $70 \pm 20\%$ and $76 \pm 20\%$ of estrus in the BG and the PG group respectively. From 48 to 120 hours post-wean, an increase in the follicle size overtime was observed in each group with no significant difference between groups whatever the measurement day. Then, in accordance with later onset of estrus in PG group, follicle size at onset of estrus was significantly larger in PG as group (5.43 vs 5.76 mm; $p = 0.04$).

The estrus to first and second insemination interval was greater for BG sows than for sows in the PG (respectively 26.2 vs 17.9 h, $p < 0.01$ and 34.6 vs 29.0 h, $p < 0.01$). Timing of inseminations relatively to ovulation did not differ between groups (for 1st AI: 13.9 vs 11.8 h; $p = 0.49$ and for 2nd IA; 5.5 vs 3.5 h; $p = 0.21$). A total of 72% and 71% of the inseminations occurred in the 24 h period before ovulation respectively in BG and in PG (Figure 2). The percentage of sows inseminated twice (100 % vs 86.0 %; $P = 0.01$) was lower for the PG than the BG.

There was no statistically significant difference in the pregnancy rate between groups (93.3 % vs 93.0 %: $p > 0.10$). All pregnant sows farrowed. There was no difference in litter characteristics (Total born, live born, dead born) between groups ($p > 0.10$).

Discussion

Overall, combining back pressure test with boar pheromones and boar grunts instead of a live mature live boar yielded similar breeding and prolificacy results. Follicular development was not affected, resulting in a similar ovulation rate and similar ovulation timeline. Estrus was nevertheless detected later, closer to ovulation and lasted less time. In accordance with later onset of estrus, insemination occurred later after weaning but timings of inseminations relative to ovulation were similarly matched in PG inseminations than BG inseminations.

Estrus is defined as the period during which the sow shows receptive behaviors, i.e. a 'standing reflex', in reaction to boar stimuli. The courtship behaviors of the boar involve olfactory (boar odor), visual (presence of the boar), auditory, and tactile (rubbing of back and flanks) [6]. Application of back-pressure

mimics boar tactile stimulation and has been shown to trigger a standing reflex in 49% of estrus females [6] in the absence of a boar. The responsiveness depends on the time relative to the beginning of estrus. Maximal behavioral responsiveness (of 59% of sows) was observed 24 to 36 hours after the beginning of estrus. Adding olfactory stimuli to the detection procedure improved responsiveness to BPT: over 60% of estrus gilts, could exhibit a standing reaction in presence of boar odor. Signoret & Du Mesnil Du Buisson [16] showed that olfactory and auditory stimuli appear to be most important; 90% of the estrous gilts responded in the presence of these stimuli only. Several 16-androstenes isolated from boar saliva [17] have been demonstrated to facilitate the display of standing reflex in estrus sows [5, 8, 18]. Androstenone was considered the most effective compound, eliciting a standing reflex [7,8]. Melrose [8] reported that 59% of sows express estrus behaviors with no boar stimulation, 78% of sows expressed estrus with an androstenone spray, but 97% of sows expressed estrus with fence-line contact with an adult boar. In our study, 91% of sows in estrus expressed sexual behaviors in response to BPT, the BB pheromone and the boar grunting audio. This is not statistically different than when a live boar caused 100% of weaned sows to express sexual behaviors. This result was in line with McGlone [10] who showed under experimental conditions that sow sexual responsive to BPT could be increased from 73% to 86% by spraying the BB pheromone studied in the present work before BPT while androstenone only numerically increased sow sexual responsiveness to 78% of sows tested. The addition of the auditory cues could have helped to increase the detection rate as well. Interestingly, our results are line with observations from Signoret and Du Mesnil reported above who used natural boar odor and sound stimulation for estrus detection [16].

This study did not include a group of sows kept without any stimulation (neither pheromone nor mature live boar) and where estrus would have been assessed only using BPT because the study was performed under commercial conditions. Previous works had shown that the estrus detection rate could plateau at 60% 24 to 36 hours after the onset of estrus [6], thus missing the fertile window for too many sows.

Depending on the stimulus or combination of stimuli used to evoke estrous behavior, phases of estrus can be defined [2]. Willemse and Boender [19] defined the period of estrus which the sow showed receptive behavior in response to BPT. This phase covered the middle two-thirds of a longer phase during which the sow was responsive to the same BPT, but in the presence of a mature boar; called the 'boar period'. Langedijk studied the impact of different stimuli combinations and intensities as well as the impact of housing conditions on estrus phases of weaned sows regularly exposed to boars [3]. They reported that when no boar was used, the onset of estrus was delayed by 10 h and estrus duration was shortened by approximately 20 hours compared to the boar estrus. In the present study, detection of estrus combining BPT and pheromones compared to BPT in presence of a fence-line mature boar resulted in later onset of estrus (weaning-to-estrus interval respectively of 108 h and 97 h) and shorter duration of estrus (39 vs 57 h). Our study confirmed that reducing live boar stimulation of the sow, delayed the onset of estrus and shortened its duration.

A critical role of estrus detection is to more accurately predict ovulation timelines. The consistency of estrus to ovulation duration at approximately two-thirds of the time in estrus on average as well as its heterogeneity between individuals were previously largely reported in sows exposed to mature boars [13,

15, 20, 21,22]. Heat detection procedures where sows were exposed to lower levels of sexual stimuli yielded similar observations [3]. Our data aligned with these findings.

Under commercial conditions, artificial insemination strategies depend on a number of factors. Among them, the estrus detection time relative to ovulation plays a major role as successful fertilization when a single insemination is performed during a 24 h period before ovulation [13,15,21] When the BB pheromones and auditory cues were used for estrus detection, estrus was detected later while ovulation was maintained in time resulting in a shorter estrus-ovulation interval. No negative impact on insemination timelines and reproductive results was observed by having a pheromone-induced shorter estrus duration because the insemination strategy was based on twice daily heat detection routine. Frequent estrus detection indeed increases the probability to detect estrus and then to inseminate sows during their best fertilization window. The earlier and longer duration of estrus by several hours reported when a boar was present was associated with extra periods of time in which effective fertilization of oocytes is less likely. The shorter estrus duration in sows not exposed to the live mature boar was associated a higher prevalence of sows inseminated only once. No negative impact of single insemination was observed since insemination happened for these sows during the best fertilization and all these sows ultimately farrowed. The routine farm insemination protocol was not modified or adapted before the beginning of the study. Despite onset of estrus was delayed when a boar was not present, the timings of inseminations relatively to ovulation were not impacted, suggesting the relevance of the insemination protocol in force on the farm as well to cope with this situation.

Conclusions

In conclusion and under the conditions of the study, the current data demonstrate that exposing weaning sows to the novel pheromone and the boar grunting sound achieved high estrus detection rate. Estrus was delayed and shortened but sows were successfully bred and farrowed. Despite the absence of teaser boar, the procedure incorporating the novel pheromone and the boar grunting sound was shown to be efficient for sow breeding.

Abbreviations

BPT: Back-pressure test; BB: BOARBETTER®; BG: Boar treatment group; PG: BB pheromone treatment group. CH: corpus hemorrhagicum; CL: or corpus luteum. WEI: wean-to-estrus interval, in hours. WOI: weaning to ovulation interval, in hours. EOI: estrus to ovulation interval, in hours.

Declarations

Acknowledgements

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Author's contributions

The study was conducted by AVB in Spain. LLR assisted in data collection. Statistical analyses and editing the manuscript were performed by AVB, DR and OM. All authors reviewed and approved the final version of the manuscript prior to submission.

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Availability of data

Data will be freely and the boar grunt audio file will be provided to any qualified, interested party.

Ethics approval and consent to participate

The animals in this study were on a commercial farm that followed European and Spanish guidelines for animal care. While procedures may have been uncomfortable, there was no pain or other distress in the animals during this study. Informed consent was obtained prior to the study from the farm owner after the principal investigator explained all procedures and methods in the study.

Consent for publication

Not applicable

Competing interests

Vetoquinol funded this study and licensed the technology for international marketing.

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Tables

Table 1. Breeding protocol used and definitions.		
Onset of estrus, h	Estrus-1 st AI, h	Estrus-2 nd AI, h
≤ 96	24	36**
102-128	12*	24
≥ 144	Immediately during BPT	-

Onset of estrus = Time in hours from weaning to the first observation of estrus

Estrus-1stAI = Time in hours from the onset of estrus to the first insemination

Estrus-2ndAI = Time in hours from the onset of estrus to the second insemination

BPT: back pressure test

*Insemination planned 12 h after the onset of estrus was performed 6 or 18 hours after the onset of estrus depending on working hours.

**Insemination planned 36 h after the onset of estrus were performed 30 or 42 hours after the onset of estrus depending on working hours.

Table 2. Reproductive and prolificacy parameters of weaned sows heat checked with a live boar (BG) or the boar pheromone (PG).

Parameters	Boar Group (BG) n = 45	Pheromone Group (PG) n = 43	SE	<i>P</i> value
Estrus and ovulation				
WEI, h	97.1	108.0	2.24	< 0.01
End of estrus, h	154	147	1.84	< 0.01
Estrus duration, h	57.4	39.3	1.71	< 0.01
WOI, h	137	138	2.12	0.67
EOI, h	40.1	29.7	1.78	< 0.01
Follicle size, mm				
48 h Post-weaning	4.32	4.37	8.06	0.27
72 h Post-weaning	4.82	4.69	1.31	0.81
96 h Post-weaning	5.49	5.70	1.08	0.09
120 h Post-weaning	6.15	6.35	1.21	0.20
Follicle size at onset of estrus (mm)	5.43	5.76	1.17	0.04
Insemination				
1 st AI, h	123	126	1.60	0.14
Estrus-1 st AI, h	26.2	17.9	1.07	< 0.01
1 st AI-Ov, h	-13.9	-11.8	1.48	0.49
2 nd AI, h	132	136	1.91	0.43
Estrus-2 nd AI, h	34.6	29.0	0.99	< 0.001
2 nd AI-Ov, h	-5.5	-3.5	1.82	0.21
Prolificacy				
Total born	15.0	15.0	0.38	0.63
Born alive	14.1	14.5	0.37	0.24
Born dead	0.85	0.50	0.24	0.45
SE = Largest standard error of the LSmeans				
WEI= Weaning to estrus interval				
WOI = Weaning to ovulation interval				

EOI = Estrus to ovulation interval

1st AI = Time of the first artificial insemination after weaning

Estrus-1stAI = Interval from the onset of estrus to first insemination

2nd AI = Time of the second artificial insemination after weaning

Estrus-2ndAI = Interval from the onset of estrus to the second insemination

1st AI-Ov = First artificial insemination to ovulation interval

2nd AI-Ov = Second artificial insemination to ovulation interval

Figures

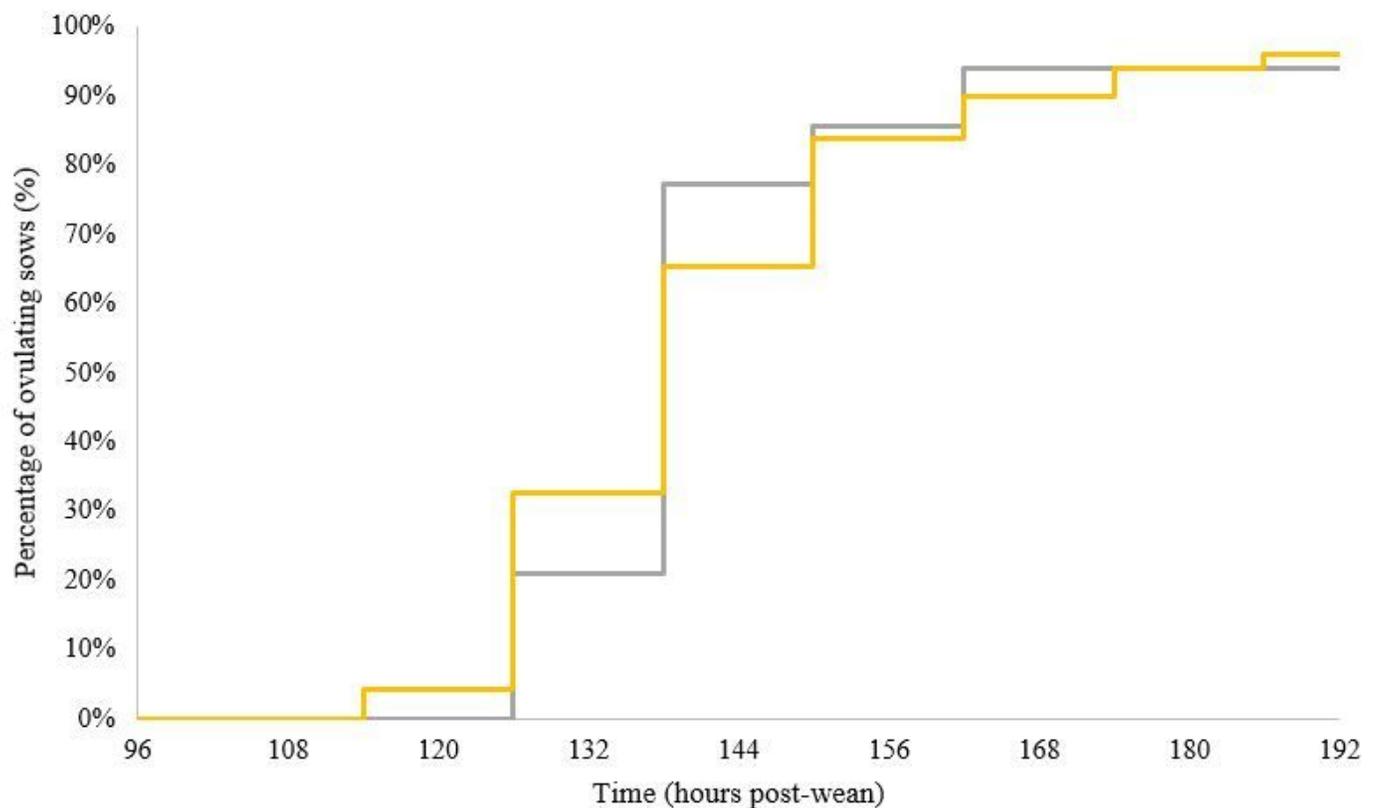


Figure 1

Cumulative percentage of ovulating sows either exposed to a live mature boar (BG) or to boar pheromones only (PG) in relation to time from weaning. Fifty sows were assigned to each treatment. Two sows were excluded in BG and one sows was excluded in PG.

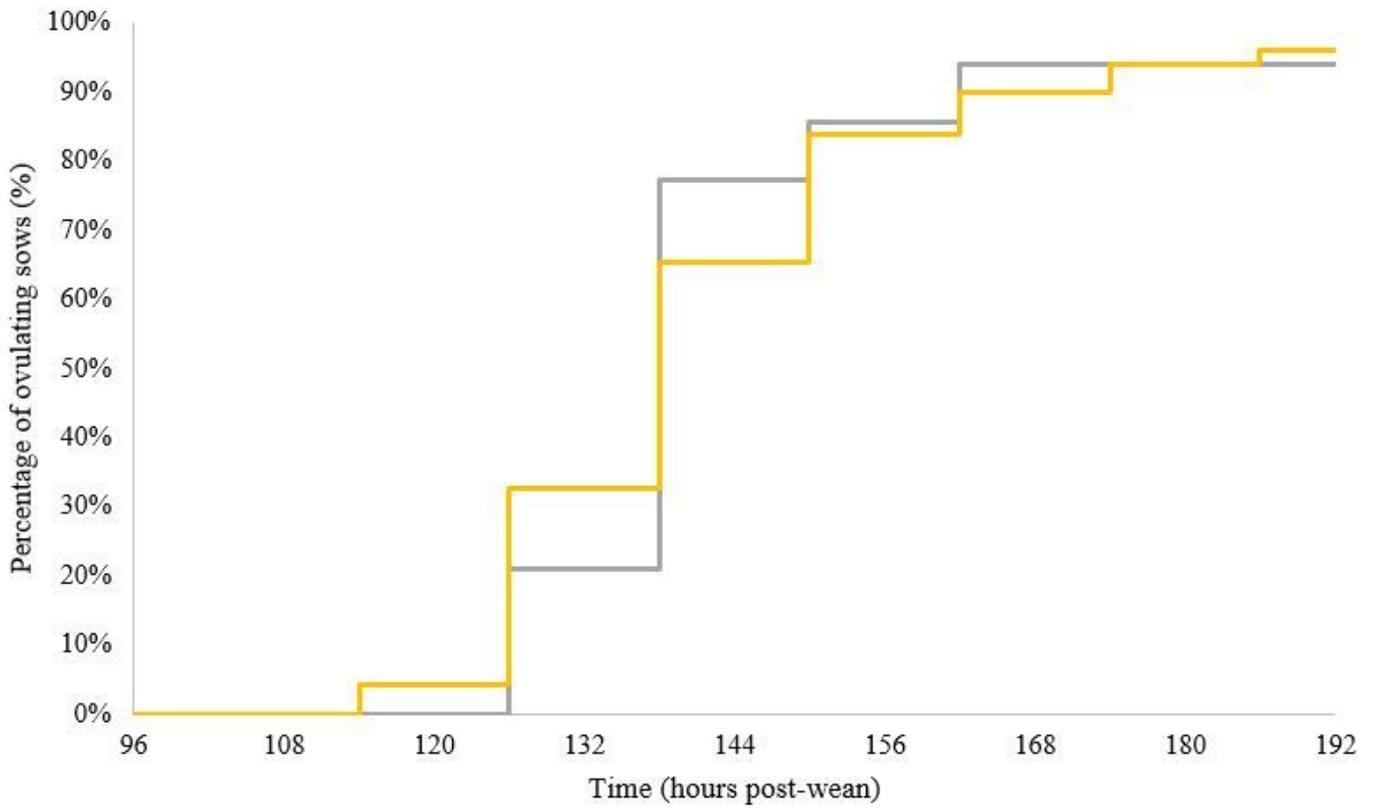


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Cumulative percentage of ovulating sows either exposed to a live mature boar (BG) or to boar pheromones only (PG) in relation to time from weaning. Fifty sows were assigned to each treatment. Two sows were excluded in BG and one sows was excluded in PG.

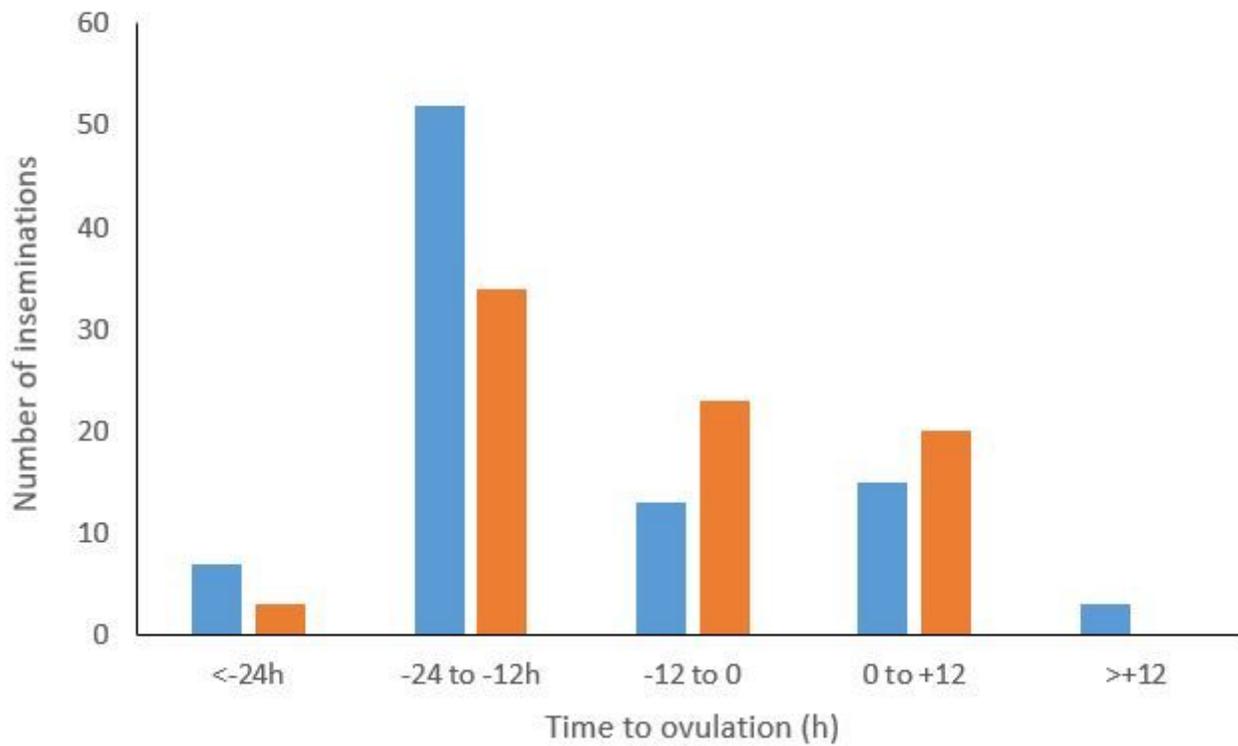


Figure 2

Distribution of artificial inseminations of weaned sows ovulating either exposed to a live mature boar (BG) or to boar pheromones only (PG) in relation to time from ovulation. Forty five sows and forty three sows were inseminated respectively in BG and PG.

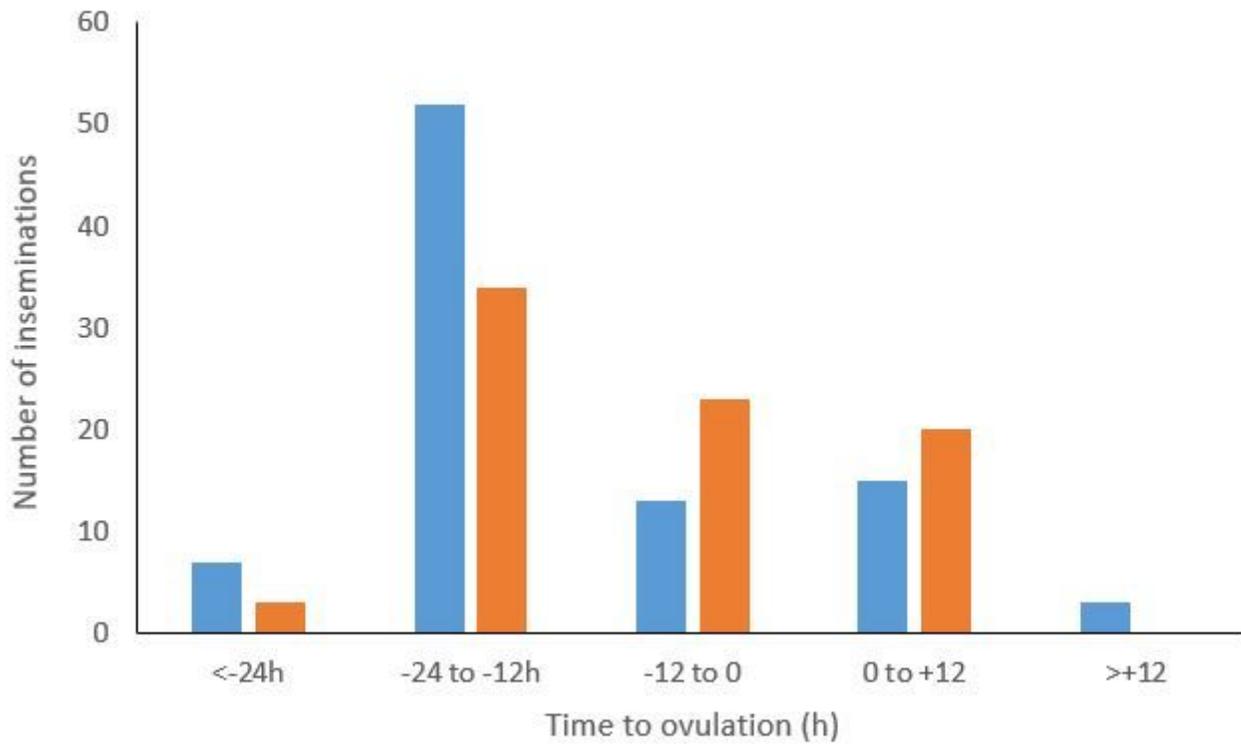


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