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Identification of potential pheromone source in sows

Velliyangiri Silambarasan^a, Govindarajan Deepalakshmi^a, Devaraj Sankarganesh^{b,c},
Varadharaju Nithya^d, Govindaraju Archunan^{a,*}^a Pheromone Technology Laboratory, Department of Animal Science, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India^b Department of Biotechnology, School of Bio and Chemical Engineering, Kalasalingam Academy of Research and Education, Krishnankoil 626126, Tamil Nadu, India^c Department of Microbial Biotechnology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India^d Department of Animal Health and Management, Alagappa University, Karaikudi 630 003, Tamil Nadu, India

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ABSTRACT

Pheromones play a pivotal role in intra-species communication for reproduction and social behavior in a variety of mammals, such as boars. For boars, saliva is a rich source of pheromones, however, the identification of additional sources and relative abundance of pheromones in various body fluids of sows is also essential to understand the reproductive behaviors of pigs. The present study was designed to identify the source(s) of pheromones in sows. We collected urine, feces, saliva and cervical mucus/vaginal wash samples from sows at pre-estrus, estrus and post-estrus phases, and from gilts and exposed boars to each of these potential sources of pheromones. All the boars tested spent more time sniffing and hyper-salivating in response to urine from sows in estrus than that from sows not in estrus. The sniffing behavior of boars towards estrus samples differed from that towards the samples from non-estrus sows ($P < 0.005$) and gilts ($P < 0.001$). Further, hypersalivation behavior of boars differed between estrus samples and gilt samples ($P < 0.05$) and estrus samples compared to pre-estrus samples ($P < 0.05$). This is an indication that pheromones are abundant in the estrus samples. We conclude that urine of estrus sows can be a rich source of pheromones and the same can be used to identify, purify and characterize novel pheromone molecules.

1. Introduction

Animals communicate through chemical signals that are secreted in their body fluids (Seyfarth et al., 2010). Body fluids are free-flowing substances composed of water, salts, electrolytes, proteins, organic compounds, etc. (Waterhouse and Farmery, 2012). The molecules in body fluids such as volatile organic compounds, fatty acids, and proteins serve as chemical signals. Pheromones are used by animals to advertise the social status to conspecifics (Archunan et al., 2014).

We and others have reported the presence of pheromones in various body fluids of livestock animals such as cow (Klemm et al., 1987; Sankar et al., 2007; Sankar and Archunan, 2008, 2011), buffalo (Rajananarayanan and Archunan, 2011; Karthikeyan and Archunan, 2013; Karthikeyan et al., 2013), goat (Sankarganesh et al., 2014, 2019), sheep (Signoret, 1991), and horse (Mozuraitis et al., 2012).

In boars, saliva is the major source of the pheromones androstenone and androstenol. These pheromones were partially successful in eliciting mating stance behavior in sows; a test useful to detect estrus (Melrose et al., 1971). Recently, one more pheromone (quinoline) was identified in boar saliva, which - together with androstenone and

androstenol - elicited sexual behaviors in sows (Devaraj et al., 2018).

While improving reproductive success in pigs requires knowledge of both sow and boar pheromones, studies performed hitherto have focused only on boars. Therefore, in this study, we asked whether the body fluids of sows contain any pheromone molecules that coordinate sexual activities in boars. In view of this, the present study was aimed to identify the source(s) of pheromones in sows in different phases of estrous cycle. In this regard, we collected urine, feces, saliva and cervical mucus/vaginal wash from estrus and non-estrus sows, and gilts. The reproductive behaviors (sniffing and hypersalivation) in boars were recorded after exposure to the body fluids.

2. Materials and methods

2.1. Animal selection

In this study, we used well-nourished large white Yorkshire pigs from Veterinary College and Research Institute (VCRI), (Namakkal & Orathanadu, Tamilnadu) and commercial farms in Tiruchirappalli, Tamilnadu. Sows ($n = 20$), gilts ($n = 7$) and boars ($n = 7$) were housed

* Corresponding author.

E-mail addresses: garchu56@yahoo.co.in, archunan@bdu.ac.in (G. Archunan).

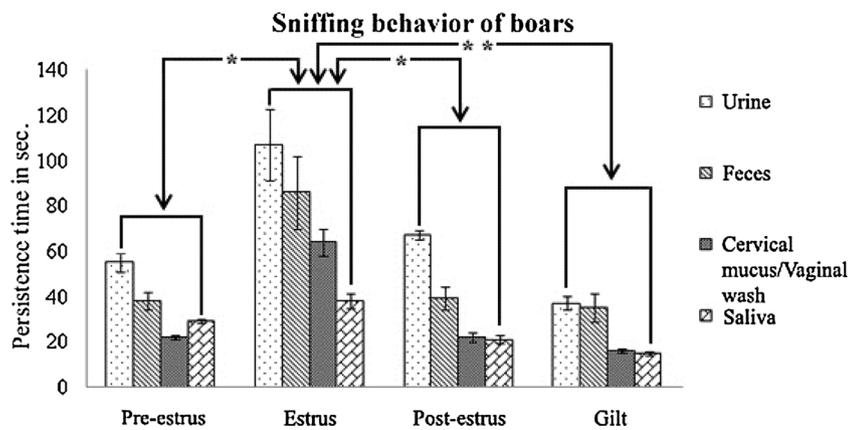


Fig. 1. Sniffing behavior of boars (in sec.) toward different body secretions collected from pre-estrus, estrus, post-estrus sows, and gilts. Bars represent SEM (7 observations among 7 boars). Difference of means (post-hoc analysis) between phases (Estrus vs. Pre-estrus, Estrus vs. Post-estrus, and Estrus vs. Gilt), and between sources (Urine vs. Feces, Urine vs. Cervical mucus/Vaginal wash, and Urine vs. Saliva) was compared by Tukey's test. Labels indicate (*) $P < 0.005$, and (**) $P < 0.001$ as level of significance.

separately in clean concrete paddocks. Each paddock was equipped with fans and water sprayers to help the pigs keep cool. Each sow was housed in a paddock that was 7×2 feet (length by width), and each boar was housed in a paddock that was 7×8 feet. The animals were fed with powdered nutrient diet three times a day and water *ad libitum*. This study did not cause any distress to the animals and samples were collected by non-invasive approach, therefore, ethical clearance is not mandatory for this study.

2.2. Estrus detection and samples collection

We assessed estrus-specific signs and behaviors and applied back pressure test in the presence of a live boar to confirm estrus in sows (Soede et al., 2011). The back pressure test is sensitive and specific enough to detect estrus precisely in sows. During estrus, all the sows expressed lordosis and rigid standing reflex behaviors, and were immobile; however other behavioral signs were varied. The urine, feces, saliva and cervical mucus/vaginal wash were collected in sterile glass container during pre-estrus, estrus, and post-estrus phases of sows, and from gilts. Urine (midstream) was collected (50–250 ml) by free-catch method, and feces was sampled during defecation. Saliva was collected by swabbing the lower buccal cavity of the sows using a cotton ball and the cotton was squeezed to collect the saliva samples. The cervical mucus was collected as thick secretions in estrus sows. The non-estrus sows and gilts apparently had no thick mucus, so we sprayed 1 mL of sterile water over the vagina and collected the water (vaginal wash). The samples were labelled appropriately, transported on ice to the laboratory, and stored at -20°C for a maximum of one week.

2.3. Exposure of samples

We placed each sample into a petri dish. This petri dish was presented to a boar in his paddock. We measured hypersalivation behavior by calculating the duration of salivation. We used 2 mL of urine, 10 gm of feces, 1 mL of saliva and 1 mL of cervical mucus/vaginal wash in behavior assay after standardization of quantity of each stimulus. The urine sample was soaked in cotton, whereas feces, saliva, and cervical mucus/vaginal wash were kept as such. Each day, the behavior evaluation was started at 7 AM without affecting the feeding time of boars. We allowed the boars to investigate the samples for 20 min, but the investigation time and response of the boars lasted up to two minutes for any source/sample. The boars were allowed to rest for 30–60 min between each sample exposure. A boar was tested with the same type of sample collected from different phases of different sows with a maximum number of 4 samples /day. The paddock was cleaned during the interval and ensured not to carry-over odor of previous sample. The samples collected from 20 sows and 7 gilts were tested randomly with 7 boars and the behavioral responses were recorded (in seconds) in real-time and ensured that all type of samples were exposed to all the boars.

The tests were replicated for 7 times, by exposing the same sample to 7 different boars. The boars had no prior experience of the donor sows, from which the samples were collected.

2.4. Statistical analysis

The data were compiled using SigmaPlot (v. 12.0, Systat Software Inc.), and analysed through two-way ANOVA. The difference of means was compared with post hoc analysis using Tukey's test to reveal the statistical significance, and the level of significance was set at $P < 0.05$. The factors used for post hoc analysis included phases (i.e. estrus vs. gilt, estrus vs. pre-estrus and estrus vs. post-estrus) and samples (i.e. urine vs. feces, urine vs. cervical mucus/vaginal wash, and urine vs. saliva).

3. Results

3.1. Sniffing behavior of the boars

Boars differed in the amount of time they investigated (sniffing) urine, saliva, and cervical mucus /vaginal wash of pre-estrus, estrus and post-estrus sows and gilts. The boars sniffed the estrus samples for significantly higher time than the respective samples of non-estrus and gilts. The post hoc comparison revealed that the sniffing by boars towards estrus samples was highly significantly different from gilts' samples ($P < 0.001$), and significantly different from pre-estrus ($P < 0.005$) and post-estrus ($P < 0.005$) samples. Among the sources tested, urine elicited a greater response in the boars as evidenced by the significantly increased time in sniffing toward urine than other sources. Notably, the sniffing time of boars towards urine was significantly different from saliva ($P < 0.005$) and cervical mucus/vaginal wash ($P < 0.005$). However, the sniffing by boars did not differ significantly between urine and feces samples (Fig. 1).

3.2. Hypersalivation behavior of the boars

The boars' hypersalivation behavior differed when they were exposed to samples of estrus and non-estrus sows, and gilts. The duration of hypersalivation was higher for urine (91 ± 9 s.) and feces (91 ± 20 s.) of estrus sows than other samples. The post hoc comparison revealed that hypersalivation behavior of boars toward estrus samples was significantly different from samples of gilts ($P < 0.05$) and pre-estrus sows ($P < 0.05$), but not with samples of post-estrus sows. However, hypersalivation behavior of boars did not differ significantly between urine and other samples (saliva, feces, and cervical mucus/vaginal wash). Among the comparisons, the least difference was found with urine vs. saliva followed by urine vs. feces (Fig. 2).

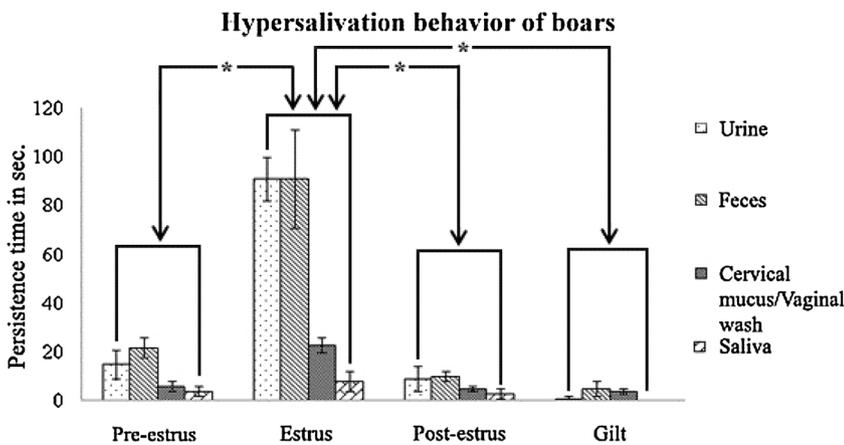


Fig. 2. Hypersalivation behavior of boars (in sec.) toward different body secretions collected from pre-estrus, estrus, post-estrus sows, and gilts. Bars represent SEM (7 observations among 7 boars). Difference of means (post-hoc analysis) between phases (Estrus vs. Pre-estrus, Estrus vs. Post-estrus, and Estrus vs. Gilt), and between sources (Urine vs. Feces, Urine vs. Cervical mucus/Vaginal wash, and Urine vs. Saliva) were compared by Tukey's test. Labels indicate (*) $P < 0.05$ as level of significance.

4. Discussion

In natural mating system, boar expresses courtship behavior when it sniffs the sow along the side of the nose, flanks and vulva, which was referred as sniffing behavior (Hemsworth and Tilbrook, 2007). The boars also express courtship behavior by secreting high amount of saliva and makes gentle jaw movements, which is referred as hypersalivation behavior (Fraser, 1984). In the present study, we found significant difference in sniffing and hypersalivation behaviors of boars between samples of estrus and non-estrus sows, and gilts. The sniffing behavior was significantly different for urine vs. saliva and urine vs. cervical mucus/vaginal wash. In addition, secretions of estrus sows are more attractive to the boars than non-estrus sows and gilts. Our study suggests that estrus urine may be a primary source of pheromones in sows.

We also found that sniffing and hypersalivation behaviors of boars did not differ between urine and feces, which imply feces as a secondary source of pheromones in sows. This observation is in agreement with earlier studies, wherein estrus urine (Rivard and Klemm, 1989; Rajanarayanan and Archunan, 2011; Mozuraitis et al., 2012) and estrus feces (Karthikeyan et al., 2013; SankarGanesh et al., 2014) were found to be rich sources of pheromones. Interestingly, the lack of differences of hypersalivation behavior of boars with urine vs. saliva and cervical mucus/vaginal wash implies that these samples could also be considered as additional sources of pheromones in sows.

5. Conclusion

We conclude from the present study that secretions of estrus sows are more attractive to the boars than those from non-estrus sows and gilts. Our study indicates estrus urine as a prominent pheromone source in sows. This will form a basis for future investigation with urine as a rich source to identify, purify and characterize pheromones in sows.

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