Steroidome and metabolome analysis in saliva from immature to pubertal gilts to identify potential biomarkers of receptivity to boar effect

Ghylène Goudet

Steroidome and metabolome analysis in saliva from immature to pubertal gilts to identify potential biomarkers of receptivity to boar effect

Ghylène Goudet, Philippe Liere, Lydie Nadal-Desbarats, Antoine Pianos, Doryan Grivault, Cécile Douet, Christophe Staub, Eric Venturi, Jonathan Savoie, Stéphane Ferchaud, Florence Maupertuis, Antoine Roinsard, Sylviane Boulot, Armelle Prunier

Abstract
Our objective was to develop alternatives to hormones for estrus synchronization in gilts. Gilts exhibit a pre-puberty period with high urinary estrone concentration during which boar exposure could induce the first ovulation. We searched for salivary biomarkers of this period. Urine and saliva were collected on six 140-day-old gilts until puberty for estrone assay, metabolome and steroidome analysis. We identified 23 metabolites and 28 steroids in saliva. The concentration of 8 of them showed significant variations at the pre-puberty period, they were candidate biomarkers. Saliva was collected from 30 gilts exposed to a boar and subjected to estrus detection from 150 to 175 days of age. Metabolome and steroidome analyses allowed the identification of 33 metabolites and 29 steroids in saliva. Their concentrations were not significantly different between receptive and non-receptive gilts. Thus, we could not identify salivary biomarkers of the period of receptivity to the boar effect.

Introduction
Effective methods for synchronizing estrus in gilts are important for batch management. Batches enables all-in/all-out systems that allows a better disease control, a tight control at the crucial stages of production and a more efficient use of time and materials. Hormonal treatments, such as Altrenogest®, are used in conventional pig farms, but are not allowed in organic farms. Our objective was to develop a strategy to stimulate first estrus of gilts on a date compatible with the sow management. Before puberty, gilts exhibit a pre-puberty period during which boar exposure could induce and synchronize first ovulation (Camous et al., 1985). During this period, urinary estrone levels are high, but urine sampling is difficult in group-housed females. Thus, practical non-invasive tools for identification of the pre-puberty period in farms are lacking. We searched for salivary biomarkers of the pre-puberty period to develop such tools and improve the detection of the gilts to stimulate.

Material and methods
Two experiments were performed, in order to 1) identify potential salivary biomarkers of the pre-puberty period, 2) check their relevance for the detection of the period of receptivity to the boar effect.
In the first experiment, six 144 to 147-day-old Large White gilts were subjected to trans-abdominal ultrasound puberty diagnosis 3 times a week until first ovulation. At the same time, urine samples were collected for estrone assay to detect the pre-puberty period and saliva samples were collected for metabolome analysis using ^{1}H-Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) and steroidome analysis using gas chromatography coupled to tandem mass spectrometry (GC/MS/MS) to search for potential biomarkers of the pre-puberty period. Data were analyzed by multivariate and univariate statistical analyses and repeated measures one-way ANOVA.
In the second experiment, saliva samples were collected from 30 Large White x Landrace crossbred gilts that were exposed to a boar twice a day and subjected to estrus detection from 150 to 175 days of age. Among the 30 gilts, 10 were detected in estrus 4 to 7 days after introduction of the boar and were considered receptive to the boar effect, 14 were detected in estrus more than 8 days after boar introduction, 6 did not show estrus and were considered non-receptive. Saliva samples from 6 receptive and 6 non-receptive gilts were analysed for steroidome and metabolome. Four saliva samples per gilt were analysed: 26 days and 11 days before boar introduction, the day of boar introduction, 3 days later for receptive gilts or 7 days later for non-receptive gilts. Similar statistical analyses were performed.

Results

In the first experiment, puberty of the gilts occurred between 182 and 192 days. Urinary estrone concentration significantly increased 2 weeks before puberty. This period with higher estrone levels was considered as the pre-puberty period. In order to identify potential salivary biomarkers of this period, metabolome and steroidome analysis were performed on gilts saliva. Metabolome analysis allowed the identification and quantification of 23 low-molecular-weight metabolites in porcine saliva. Steroidome analysis allowed the identification and quantification of 28 steroids in porcine saliva. The concentration of some metabolites (butyrate and hydroxyvalerate, formate, malonate, propionate, dehydroepiandrosterone) and steroids (17β-estradiol and 5α-dihydroprogesterone) showed significant variations at the beginning of the pre-puberty period. Thus, these metabolites and steroids were considered as candidate salivary biomarkers of the pre-puberty period. These results show that non-invasive saliva sampling could be a welfare-friendly tool for the identification of the physiological status of the gilts.
In the second experiment, metabolome and steroidome analyses allowed the identification of 33 metabolites and 29 steroids in gilt saliva. When we looked for biomarkers candidates that could differentiate gilts that will be receptive to boar effect from non-receptive gilts, the concentrations of several metabolites and steroids tended to be different in receptive gilts compared to non-receptive gilts in saliva collected 25 and 11 days before boar exposure. However, no significant differences could be highlighted, including for candidate biomarkers, due to the large variability between gilts. Thus, in our conditions, we could not identify salivary biomarkers that could differentiate gilts that will be receptive to the boar effect from non-receptive gilts.

Discussion

These results show that non-invasive painless sampling of saliva could allow the identification of the physiological hormonal status of the gilts from immature to pubertal stage. However, we could not identify relevant salivary biomarkers to the effectiveness of the boar to stimulate puberty in our experimental conditions. Our study was an exploratory experiment with a small number of experimental gilts, making the assumption that at least one biomarker will show a marked difference between receptive and non-receptive gilts. This was not the case. Therefore, research for salivary biomarkers should continue using a higher number of animals and time points provided the existence of rapid and cheap analytical methods. Other omics approaches, such as proteomics or lipidomics, could also be of interest to search for salivary biomarkers of the period of receptivity to boar effect.

References

Camous S., Prunier A., Pelletier J., 1985. Plasma prolactin, LH, FSH and estrogen excretion patterns in gilts. J. Anim. Sci., 60, 1308-1317.