Preliminary Study of Sex Hormone Levels Throughout the Estrous Cycle in the Saliva of 4.5-year-old Sows Born by ART

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Estradiol (17-beta-estradiol, E2) and progesterone (P4) play a key role in the regulation of the estrous cycle. Their levels fluctuate throughout the cycle, triggering processes that are essential for the expression of mating behaviour. The rising levels of E2 during the follicular phase triggers the initiation of behavioural and physiological changes associated with heat in sows, whilst P4 is essential for early embryo development and uterine preparation for implantation.

The evaluation of sex hormones is typically performed on plasma or serum. However, daily blood collection puts stress on the animals and requires specialized staff, and the extraction may alter the results of some metabolites. Since the small size of these molecules allows them to penetrate the membranes, such as those of the salivary glands, using saliva to measure sex hormones could offer a non-invasive approach to facilitate the collection of sex hormone data. Yet, studies on sex hormones in pig saliva are very scarce, limiting its application in the field.

Here, the correlation between the levels of E2 and P4 were evaluated in plasma and saliva, at three separate timepoints, in 22 Large White and Landrace crossbreed sows. These animals were born through different assisted reproductive technologies from a previous study (Paris-Oller *et al.,* 2021), with their ages around 4.5 years old. Their exclusion from production lines allows them to have natural estrous cycles, be kept in an open enclosure, and fed under identical conditions. Furthermore, 12 sows of the same herd [6 born via artificial insemination (AI group) and 6 born after surgical transfer of *in vitro*-produced embryos (IVP group)] were used to measure the fluctuation of both E2 and P4 in saliva during the estrous cycle.

Plasma was obtained through the centrifugation (1000 G, 10 min) of ophthalmic venous sinus blood collected in lithium heparin tubes. Saliva samples were collected using Salivette® tubes containing a polystyrene sponge previously chewed by the sows for 10 secs. These tubes were then centrifuged (1000 G, 5 min) and the samples stored (-80ºC) until hormone analysis. Both plasma and saliva samples were later analysed by quimioluminescence (Immulite).

The correlation between the levels of E2 in saliva and plasma, analysed using a Pearson correlation coefficient, showed a statistically significant correlation ($p < 0.05$) with a moderate negative relationship (-0.311). The average level of plasma E2 was 25.78 pg/mL whilst the saliva E2 was 49.05 pg/mL. However, the correlation between P4 in saliva and plasma was not significant, with an average concentration of 15.23 ng/mL and 0.54 ng/mL in plasma and saliva, respectively.

Analyses of the hormone levels in saliva showed no significant differences for both hormone levels between the AI group and IVP group, nor for P4 levels among the days of the estrous cycle. Nevertheless, significant differences were found in the levels of E2

per day of the estrous cycle ($p < 0.001$). Moreover, in addition to the peak expected towards the end of the follicular phase, another peak was observed around days 12-14 of the cycle. This peak is essential for the maternal recognition of pregnancy in pigs and has been identified in blood, but has never been reported in saliva of non-pregnant animals.

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