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Serum progesterone concentration in gilts according to the phase of the reproductive cycle

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Introduction

The main reasons for swine females culling are decreased number of piglets born, anestrus condition, and return to estrus after insemination (Koketsu *et al.*, 2017). It is estimated that nearly half of the slaughtered animals due to reproductive failure are females with parity order ≤ 3 . This directly influences retention rate and production profitability (Engblon *et al.*, 2016; Gruhot *et al.*, 2017), mainly in cases where the culling decision is incorrect.

Identifying reproductive failures in pig farms is still challenging, especially when these failures relate to estrus detection management in gilts. Errors during this management can routinely occur; therefore, the decision to cull gilts due to anestrus conditions is not always accurate. Data recently published by our research group showed that approximately 30 to 40% of culling decisions attributed to cases of anestrus in gilts were incorrect. (Baldessar *et al.*, 2023).

Unnecessary culling of gilts due to failures in estrus detection could be avoided by associating regular management with serum progesterone (P4) concentration assessment (Chung *et al.*, 2002; Vela *et al.*, 2022). Nevertheless, it is still necessary to establish reference values to better interpret the P4 profile in swine females. For this reason, this study characterized the P4 concentration profile in gilts at different phases of the reproductive cycle.



Photo: Monike Willemin Quirino

Figure 1. Piglet subjected to estrus detection management through the tolerance reflex to back-pressure exerted by the male in the presence of a sexually mature male.

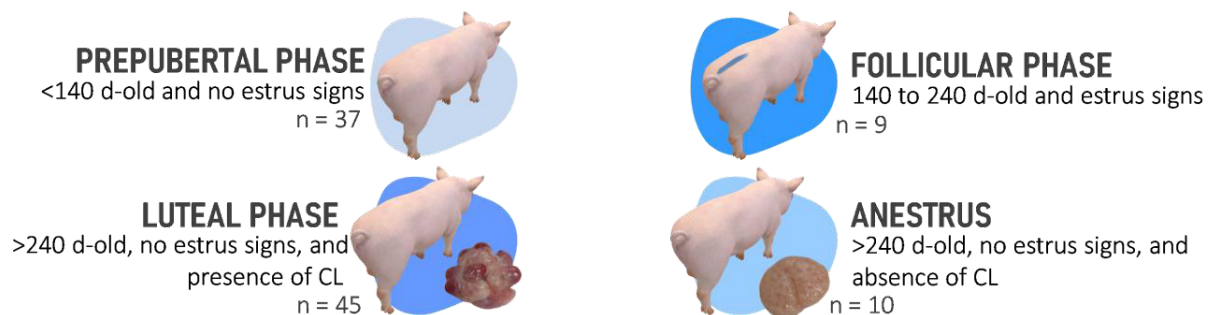
This work is linked to SDG 9 - Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation, aligned with the goal: 9.4 By 2030, upgrade infrastructure and retrofit industries to make them sustainable,

with increased resource-use efficiency and greater adoption of clean and environmentally sound technologies and industrial processes, with all countries taking action in accordance with their respective capabilities. Developing biotechnologies that promote increased productivity and prevent errors in the disposal of swine females contributes to greater profitability and efficiency in the swine production system, impacting the better use of resources and minimizing environmental impacts.

Determination of the estrous cycle phase

A total of 101 gilts (Landrace × Large White) were used in this study. Initially, females were submitted to the estrus stimulation and detection management, through the response to the back-pressure test simultaneously to the fence-line boar contact, and were classified into the following groups (Figure 2):

- **Pre-pubertal phase, n = 37:** < 140 days old, with no signs of estrus.
- **Follicular phase, n = 9:** 140 – 240 days old and with signs of estrus.
- **No estrus signs, n = 55:** > 240 days old and with no estrus signs.



The classification was based on the management of estrus stimulation and detection and the *post-mortem* evaluation of ovaries from females that did not exhibit estrus by 240 days of age. CL: corpora lutea.

Figure 2. Classification of gilts according to the phase of the reproductive cycle.



Figure 3. Schematic illustration of serum progesterone concentration analysis (ng/mL) in gilts using the chemiluminescence method.

Source: created in Biorender (Marques, 2024).

Subsequently, the gilts classified in the 'no estrus signs' group (n = 55) were slaughtered at a federally inspected slaughterhouse. After evaluation of the reproductive tract, gilts were reclassified based on the presence or absence of corpora lutea (CL; Figure 2). A total of 45 gilts had CL; therefore, these gilts were reclassified into the 'luteal phase' group, while the remaining gilts (n = 10) had no CL and were reclassified into the 'anestrus' group.

Serum progesterone assessment

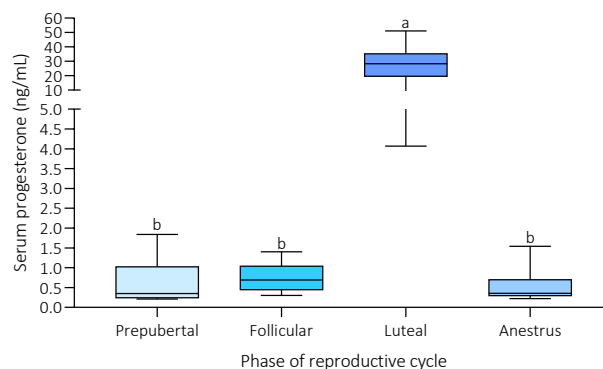
Blood from all females was collected by puncturing the jugular vein using a 40 × 12 mm needle and a tube containing clot activator. The samples were centrifuged (1500 × g for 10 minutes), and the serum was stored in microtubes at -20 °C. Then, samples were analyzed for P4 levels through the chemiluminescence method (previously validated for pig serum samples; Figure 3).

Results and discussion

Serum P4 concentrations (mean ± standard error of the mean) in gilts during the luteal phase (27.5 ± 1.7 ng/mL) were higher than levels observed in all other phases of the reproductive cycle (P < 0.01;

Figure 4). P4 concentrations were similar among gilts in the pre-pubertal phase (0.62 ± 0.08 ng/mL), follicular phase (0.76 ± 0.12 ng/mL), or the anestrus condition (0.54 ± 0.13 ng/mL; $P > 0.05$, Table 1). The minimum P4 concentration observed in the luteal phase was six, five, and seven times greater than the average levels in the pre-pubertal phase, follicular phase, and anestrus condition, respectively.

The presence of functional CL is what defines a female as being cyclic. The results confirm that evaluating P4 levels - the hormone produced by these structures - is a potential strategy to distinguish gilts truly in anestrus from those in the luteal phase, avoiding unnecessary culling of animals. It is important to note that although the data provided in this study serve as reference values, P4 results should always be associated with the reproductive cycle records in the farm routine, since low progesterone



^{a,b}Indicate significant differences between groups ($P < 0.05$). The values were compared using analysis of variance (after data transformation through the ARSINH function), and means were compared using the Tukey-Kramer test.

Figure 4. Serum progesterone levels in gilts according to the reproductive cycle phase.

Table 1. Serum progesterone levels (ng/mL) in gilts at different reproductive cycle phases.

| | Anestrus | Pre-pubertal | Follicular | Luteal |
|----------------------------|-------------------|-------------------|-------------------|-------------------|
| n | 10 | 37 | 9 | 45 |
| Minimum | 0.22 | 0.21 | 0.30 | 4.1 |
| Maximum | 1.54 | 1.84 | 1.40 | 51.2 |
| Mean | 0.54 ^A | 0.62 ^A | 0.76 ^A | 27.5 ^B |
| Median | 0.35 | 0.35 | 0.69 | 28.4 |
| Standard error of the mean | 0.13 | 0.08 | 0.12 | 1.7 |

^{A,B}Indicate significant differences among groups ($P < 0.05$). The values were compared using analysis of variance (after data transformation through the ARSINH function), and means were compared using the Tukey-Kramer test.

levels can be observed not only in anestrus but also during the follicular phase.

The P4 values in this study can also serve as reference values for identifying cyclic gilts not detected in estrus through routine estrus detection management procedures (back-pressure test simultaneously to the fence-line boar contact). For these cases, performing at least two blood collections per animal, with an interval of approximately seven days, is recommended.

Final considerations

The results confirm that the assessment of P4 concentrations can be used to (i) avoid the unnecessary culling of gilts that were mistakenly identified as being in anestrus and (ii) contribute to the identification of cyclic gilts that were not detected in estrus

through the routine estrus detection management (back-pressure test simultaneously to the fence-line boar contact).

It is essential to highlight that P4 results should always be correlated with the reproductive cycle records in the farm routine, especially when observing low progesterone levels, characteristic of both anestrus and the follicular phase.

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