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# Use of commercially available ELISAs to help determine estrous status in female swine

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## Summary

**Objective:** To determine the accuracy of two commercially available ELISAs in discriminating between high and low serum progesterone ($P_4$) concentrations in female swine.

**Methods:** Serum was randomly harvested from 65 crossbred females of various ages and parities. Progesterone concentrations were categorized as "high" (> 2.5 ng per mL) or "low" (≤ 2.5 ng per mL) using the Progestassay™ (Synbiotics Corp., San Diego, California) ELISA assay. Similarly, $P_4$ concentrations were categorized as "high" (> 5.0 ng per mL) or "low" (≤ 5 ng per mL) using the Target (BioMetallics, Princeton, New Jersey) ELISA assay. The specificity and sensitivity for each ELISA were determined by comparing the results of both test kits to radioimmunoassay (RIA) methods.

**Results:** Serum $P_4$ concentrations using RIA were either ≤ 0.42 ng per mL, or ranged from 3.35–17.87 ng per mL. Both ELISA test kits produce a blue color that is inversely related to serum $P_4$ concentration. Both test kits allowed a clear distinction between high and low concentrations of $P_4$ in swine serum. The Progestassay was 100% specific and 95.1% sensitive for differentiating low versus high $P_4$ concentrations. The Target test kit was 100% specific and 86.9% sensitive. For the serum samples included in the present study, the lowest concentration, as measured by RIA, that tested as "high" by the Progestassay was 3.35 ng per mL, and the lowest RIA concentration that tested as "high" by the Target assay was 5.57 ng per mL. Statistically, no differences were found between the RIA and Progestassay ($P = .25$) but differences between the RIA and Target were significant ($P = .007$). Differences between the Progestassay and Target approached significance ($P = .06$).

**Implications:** Both the Progestassay and Target ELISA test kits work well at semi-quantitative measurement of serum $P_4$ concentrations in swine. Both kits are simple to run and take only 15–25 minutes to perform on the farm. The ability to run these tests sow-side should make them a practical aid when investigating herd reproductive problems.

**Keywords:** serum, progesterone, ELISA, swine, reproduction

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Progesterone ($P_4$)-based enzyme-linked immunosorbant assays (ELISAs) have been used to assess various aspects of reproduction in cattle,[1] horses,[2] dogs,[3] and swine.[4–8] The ELISA is a semi-quantitative test which is simple to run in the field. It discriminates between high and low $P_4$ concentrations through color development. In swine, ELISAs have been investigated for their usefulness in determining pregnancy via $P_4$ concentrations in serum, plasma, blood, saliva, and feces.[4–8]

Serum $P_4$ concentrations measure luteal function and, thus, the pregnancy and estrous status of the sow. During the estrous cycle, luteal tissue forms rapidly after ovulation, causing a dramatic increase in serum $P_4$ concentration (Figure 1).[9] By the second day post-ovulation, high serum $P_4$ concentrations induce behavioral changes in the female,[9] including nonreceptivity to the boar. $P_4$ secretion by the corpora lutea (CL) continues to increase, reaching maximum serum

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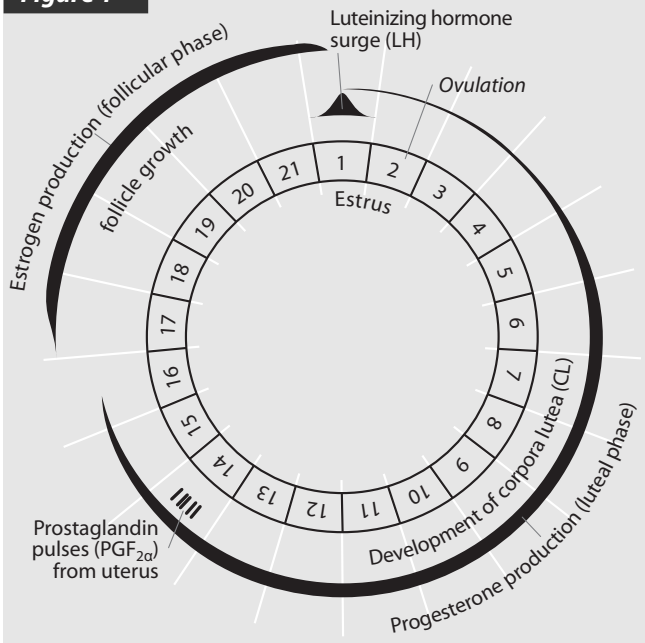
***Figure 1***




Estrous cycle of swine

concentrations as high as 33 ng per mL by day 11–12.[9] High $P_4$ secertions are maintained for an additional 2–3 days. At day 14–15 of the estrous cycle, if the uterus is nongravid, the CL rapidly regress within a 48-hour period, causing a drop in serum $P_4$ concentrations to baseline levels (< 1 ng per mL).[9] If, however, the animal is pregnant, the CLs and $P_4$ secretion are maintained and the serum $P_4$ concentration is expected to be > 5.0.

Functional luteal tissue accompanied with high $P_4$ production is therefore present throughout gestation. It can also accompany certain pathologic conditions (e.g., ovarian cysts) in swine. Partial or total luteinization of the cyst wall is not uncommon with multiple, large ovarian cysts (2–6 cm diameter).[10–13] Plasma $P_4$ concentrations of 5–15 ng per mL can be observed in animals with multiple, large ovarian cysts.[11,13]

Progesterone ELISAs were devised to assess pregnancy status indirectly by determining whether high $P_4$ concentrations are present in serum at days 17–25 after service.[4–8] However, nonreturn to estrus and ultrasound diagnosis can successfully determine pregnancy, thus diminishing the diagnostic usefulness of the $P_4$-based ELISA for pregnancy checking. Progesterone ELISAs can still be of service to a clinician diagnosing management-related reproductive problems when there is a primary complaint of anestrus in the gilt pool or postweaned sows. In these situations, it is helpful to know the status of the ovaries.

The objective of this study was to evaluate two commercially available ELISAs for their accuracy in detecting high $P_4$ concentrations in swine serum.

# Materials and methods

## Animals and blood sample collection

A total of 65 randomly selected crossbred females of various ages and parities from two commercial herds were used in this study. Blood was collected in the morning from all animals via jugular venipuncture directly into silicon-coated clot tubes. Samples were allowed to clot at room temperature over a 20-minute period, and the serum decanted from the clot tubes into two separate sterile tubes. One serum tube was immediately used for $P_4$ testing using the commercial ELISA kits, and the second serum tube frozen at -70°C for later analysis with radioimmunoassay (RIA).

## Evaluation of the assays

The two commercially available $P_4$ ELISA kits tested in this study were:

- the Progestassay™ (Synbiotics Corp., San Diego, California) kit labeled for use in the cow, bitch, and mare; and
- the Target (BioMetallics, Princeton, New Jersey) kit labeled for use in mares.

Assay of $P_4$ concentrations in serum was performed with both ELISA test kits at room temperature per manufacturers' instructions. Both ELISA test kits produce a blue color, which is inversely related to $P_4$ concentrations. For the Progestassay kit, $P_4$ concentration was interpreted as "high" or "low" by comparing test well color development to color development with the $P_4$ cutoff standard (2.5 ng per mL) provided with the kit.

The Target test kit distinguishes between a "low" (< 1.0 ng per mL), "intermediate" (1.0–5.0 ng per mL), or "high" (> 5.0 ng per mL) $P_4$ concentration. To facilitate comparability between assays and with the RIA, we interpreted Target ELISA results by comparing the test well color development to a printed color guide card provided by the manufacturer, except that we combined the "intermediate" and "low" categories so that results were regarded either as "low" (blue, ≤ 5.0 ng per mL) or "high" (clear, >5.0 ng per mL) (Figure 2).

Conventional RIA procedures were used to quantitate serum $P_4$ concentrations in all samples.[10,11] The interassay coefficient of variation (CV) for the measurement of $P_4$ was 11.6%, with an intraassay CV of 7.8%. The lowest concentration of serum $P_4$ the RIA can detect is 0.25 ng per mL. For this study, those serum samples with RIA values of < 1.0 ng per mL were categorized as "low" $P_4$ concentrations, while $P_4$ concentrations > 1.0 ng per mL were categorized as "high."[5,9,14,15]

## Data analysis

The specificity of each ELISA kit was calculated as the percentage of samples identified as "low" (negative) by the ELISA test that were < 1.0 ng per mL by the RIA. Sensitivity of each ELISA kit was calculated as the percentage of samples identified as "high" (positive) by the ELISA test that were ≥ 1.0 ng per mL by the RIA. A McNemar's test was used to compare the RIA to the Progestassay and the Target, and between the Progestassay and Target.

# Results

Of the 65 blood samples collected, eight serum samples showed various degrees of hemolysis. When comparing the ELISA test kit $P_4$ concentrations to their respective RIA values, no false readings were found, indicating that hemolysed serum does not appear to effect either test kit.

Manufacturer's directions on both ELISA kits were easy to follow. Color development could more easily be assessed for the Progestassay kit against a nonreflective white background (e.g., notebook paper) for the test wells. The Target test provided its own white background.

Serum $P_4$ concentrations measured using RIA ranged from the lowest sensitivity limit of the assay (0.25 ng per mL) to 17.87 ng per mL. Four samples (6.2%) fell into the "low" $P_4$ concentration category by RIA (≤ 0.42 ng per mL) (Figure 2). The remaining 61 serum samples (93.8%) ranged from 3.35–17.87 ng per mL ($\chi \pm sd = 10.56 \pm 3.19$ ng per mL), and therefore fell into the "high" category by RIA. None of the $P_4$ concentrations found in this study group fell between 0.42–3.35 ng per mL.

When compared to the RIA, the lowest $P_4$ concentration identified by Progestassay as "high" was 3.35 per mL, and the lowest $P_4$ concentration identified by Target as "high" was 5.57 ng per mL. Both kits, however, falsely identified as "low" serum samples (three for Progestassay, seven for Target) that RIA identified as high.

The Progestassay had a 100% specificity and 95.1% sensitivity for differentiating low versus high serum $P_4$ concentrations compared to the RIA standard (Figure 2). The Target test kit was also 100% specific, but sensitivity was slightly lower at 86.9% in comparison to the RIA standard. Statistically, no differences were found between the RIA and Progestassay ($P$ = .25) but were significant ($P$ = .007) between the RIA and Target. Differences between the Progestassay and Target approached significance ($P$ = .06).

# Discussion

Both ELISA test kits examined in this study clearly distinguished between high and low concentrations of serum $P_4$ in swine. Both the Progestassay and Target kits are acceptably sensitive and specific, as assessed by their comparability to the RIA "gold standard."

Our decision to combine the three results categories for which the Target assay was designed into two ("high" and "low") categories enlarged the "gray zone" between high for the Target ELISA (≥ 5.0 ng per mL) and high for the RIA (≥ 1.0 ng per mL, Figure 2). This decision caused the 3.35 ng per mL sample to be called "low" by the Target test but "high" by RIA, thereby affecting the test's overall sensitivity score.

Because none of the $P_4$ concentrations in the serum samples from this study (as measured by the RIA) fell between 0.42–3.35 ng per mL, it is possible that the sensitivity of these ELISA kits may be greater or less than what we have reported. The chance for false positives with either kit, however, seems unlikely, because the ELISAs set their cutoffs higher than the standard RIA cutoff.

Because the ELISA assay cutoffs were between 2.5–5.0 ng per mL higher than the RIA cutoff, there is a 9.5%–15% chance that, if choosing normally cycling sows randomly, one would take a serum sample from a sow whose $P_4$ concentration would fall within the "gray zone" between high and low, as defined by both ELISAs. For this reason, the results of these ELISAs, when used to assess $P_4$ concentration in an individual animal, should be interpreted with caution. Either of these assays, however, will provide reliable evidence to the clinician in diagnosing management-related problems when used on a group of anestrus females (10 animals or 10% of the females, whichever is greater). Alternatively, sequential samples taken from the same female at a 3-day interval can provide a reliable evaluation of her serum $P_4$ concentrations.

Although statistically the Target test was less accurate than the Progestassay, the sensivity and specificity of both ELISAs were still adequate to justify their use in swine.

## Application in practice

Veterinarians are frequently confronted with complaints about noncycling females. Knowing $P_4$ concentrations can help when addressing reproductive management problems related to anestrus in the gilt pool or postweaned sows. With the ELISA $P_4$ assays, serum can be taken from a representative portion of the gilt pool and tested on the farm.

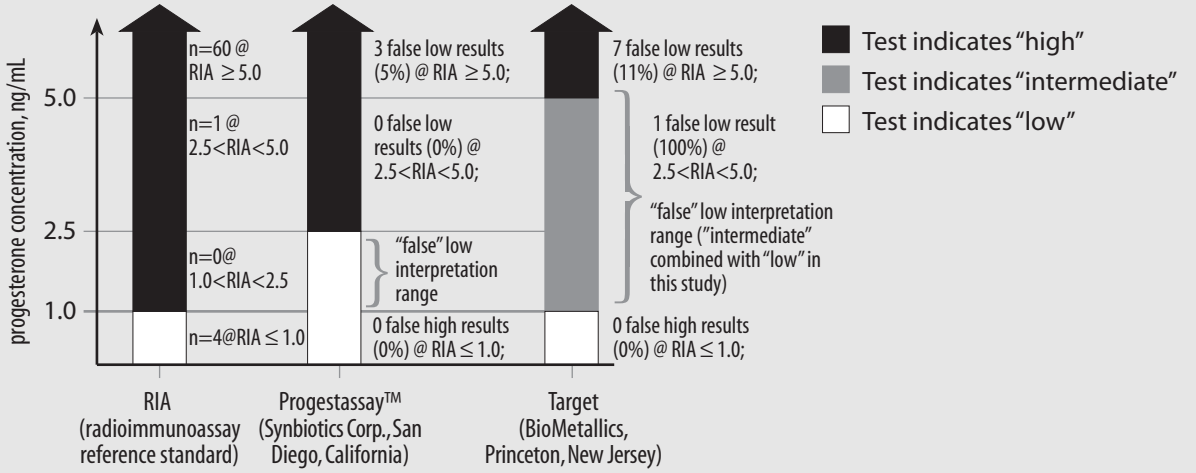
High serum $P_4$ concentrations strongly indicate that sows are cycling. If the majority of animals show high $P_4$ concentrations, changes in the heat detection program are warranted because these animals probably cycled but were missed by the heat detection regime.

Low serum $P_4$ concentrations can indicate either gilts in estrus or can help confirm the producer's observations of noncycling gilts. Estrus gilts can be easily identified by performing heat detection with a good teaser boar on these animals at the time of the visit. Once estrus is ruled out as a cause of low serum $P_4$ concentrations, suggested changes in gilt pool management can be made. In this situation, it would first be prudent to determine the onset of puberty in these gilts. Running ELISA $P_4$ assays on an adequate number of gilts across age

***Definitions***

**Specificity:** the percent of RIA <1.0 ng/mL where corresponding ELISA results are "low"
**Sensitivity:** the percent of RIA ≥1.0 ng/mL where corresponding ELISA results are "high"

***Figure 2***




Interpretation of test results

defines an age window for onset of puberty for the herd. Once age of puberty is identified for a given producer's gilts, strategies to control puberty—such as controlled stressors (e.g., movement, re-grouping, change in environment, etc.), boar exposure, and heat detection—can be strategically implemented.

Suspicion of postweaning anestrus in sows can also be confirmed or refuted using an ELISA $P_4$ assay. If $P_4$ concentrations are low, then she has problably not cycled, and therefore management suggestions can be made to enhance the probability of normal wean-to-estrus intervals (e.g., different weaning times, postweaning handling, changes in lactation or gestation management, nutrition, etc.). If $P_4$ concentrations are high, it suggests the sows are cycling, and therefore management attention should first be directed at honing the heat detection program.

Progesterone ELISAs can contribute to the diagnosis of cystic ovaries in the herd. In swine, the prevalence of multiple ovarian cysts has been reported at <10%.[10–12] In a subpopulation of animals culled specifically for reproductive disturbances, there was a 14% prevalence of multiple, large ovarian cysts.[16] Thus, females with cystic ovaries make up a small percentage of the breeding population, including those culled specifically for reproductive problems. In the past, the ability to diagnose this pathologic condition antemortem was limited, allowing these sterile animals to persist in the breeding herd.

Determining estrous cycle activity is very important when trying to differentiate a cystic sow from one in diestrous or one that is pregnant. While the ELISA $P_4$ assay alone cannot definitively diagnose animals with cystic ovaries, it can be used in conjunction with realtime ultrasound (RTU) to make a definitive diagnosis. The large, cystic structures are easily visualized transcutaneously or transrectally when viewing the ovaries.[17] Multiple, large cysts are usually found present on both ovaries. Partial luteinization of the cyst walls can frequently be seen on the monitor.[17] With anamnesis followed by RTU, these sterile animals can be culled earlier from the breeding herd, reducing nonproductive sow days.

Reproduction is the foundation of production. As such, clinicians are frequently confronted with the task of identifying the cause(s) of poor reproductive performance in a swine herd. A typical approach to this problem includes analyzing the herd's reproductive records, an on-site examination of the herd and its management practices, and selected diagnostic tests that provide objective data on the breeding herd. Through this approach, a clinician can usually distinguish between an infectious, chemical, or noninfectious cause for the poor reproductive performance.

When the cause is infectious or chemical, therapeutic and/or preventive herd management strategies are usually implemented and producer compliance is usually high. If, however, the problem is believed to be noninfectious (e.g., the result of poor heat detection, poor breeding technique, inappropriate timing of breeding, etc.), and changes in management are recommended to aid in diagnosing as well as resolving the problem(s), producer compliance often dwindles, decreasing the clinician's chances of diagnosing and, more importantly, resolving the problem. The $P_4$ ELISA can provide the simple on-farm evidence that will optimize producer compliance with the recommended change in management.

## Implications

- Both the Progestassay™ and Target ELISA test kits accurately discriminate between high and low serum $P_4$ concentrations in swine.
- Both kits are simple to run and take only 15–25 minutes to perform while on the farm.
- The ability to run these diagnostic tests sow-side should make them a practical and essential component when investigating herd reproductive problems.

## Acknowledgements

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## References

1. Langley OH, Power MJ, Diskin MG, et al. Measurement of progesterone in whole blood using a rapid ELISA test. *Irish Vet J.* 1987;41:311–313.

2. Elmore RG, Shull JW, Varner DD, et al. Using progesterone assay kits to determine equine luteal function. *Vet Med.* 1988;83:250–253.

3. England GCW, Allen WE, Porter DJ. A comparison of radioimmunoassay with quantitative and qualitative enzyme-linked immunoassay for plasma progestogen detection in bitches. *Vet Rec.* 1989;125:107–108.

4. Glossop CE, Foulkes JA, Whitworth A, et al. Use of an on-farm progesterone assay kit to determine pregnancy in sows. *Vet Rec.* 1989;124:115–117.

5. Saiz Cidoncha F, Cruz Lopez M, Martin Rillo S, et al. An enzymoimmunoanalysis (EIA) to evaluate progesterone in swine and cattle. *Proc 11th Intl Cong Anim Reprod AI, Dublin*. 1988;3:67–69.

6. Sanders H, Rajamahendran R, Burton B. The development of a simple fecal immunoreactive progestin assay to monitor reproductive function in swine. *Can Vet J. 1994;35:355–358.*

7. Moriyoshi M, Nozoki K, Ohtaki T et al. Measurement of gestagen concentration in feces using a bovine milk progesterone quantitative test EIA kit and its application to early pregnancy diagnosis in the sow. *J Vet Med Sci*., 1997;59:695–701.

8. Johmen M, Toshihiko N, Nobuo T et al. Enzyme immunoassay of progesterone in swine serum. Japan. *J Anim Reprod.* 1980;26:77–80.

9. Henricks DM, Guthrie HD, Handlin DL. Plasma estrogen, progesterone and luteinizing hormone levels during the estrous cycle in pigs. *Biol Reprod.* 1972;6:210–218.

10. Nalbandov AV. Anatomic and endocrine causes of sterility in female swine. *Fertil Steril.* 1952;3:100–114.

11. Miller DM. Cystic ovaries in swine. *Cont Ed Pract Vet*. 1984;6:S31–35.

12. Wrathall AE. Ovarian disorders in the sow. *Vet Bull.* 1980;50:253–272.

13. Close RW, Liptrap RM. Plasma progesterone levels in sows with induced cystic ovarian follicles. *Res Vet Sci.* 1975;19:28–34.

14.Stabenfeldt GH, Akins EL, Ewing LL, et al. Peripheral plasma progesterone levels in pigs during the oestrous cycle. *J Reprod Fert.* 1969; 20:443–449.

15. Edqvist LE, Lamm AM. Progesterone levels in plasma during the oestrous cycle of the sow measured by rapid competitive protein binding technique. *J Reprod Fertil.* 1971; 25:447–449.

16. Dalin AM, Gidlund K, Selling L. Post-mortem examination of genital organs from sows with reproductive disturbances in a sow-pool. *Acta Vet Scand.* 1997;38:253–262.

17. Dorka VA, Plonait H. Ergebnisse fortlaufender untersuchungen an sauen mit zystisch entarteten ovarien mittels sonographie und serum-hormon-bestimmung. *Dtsch Tierarztl Wschr.* 1995;102:16–21.