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ACCURACY OF A RAPID ELISA TEST KIT TO MEASURE PROGESTERONE IN SOWS

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Introduction

Despite new applications of ultrasonography, blood progesterone is still a reference diagnostic tool for the assessment of reproductive status in many species, including the sow (2, 5). Techniques based on saliva or fecal samples (3), have been investigated, but only serum semi-quantitative ELISA (1) can be implemented at low costs. This study was designed to validate a new ELISA kit, for rapid field determination of sow progesterone.

Materials and methods

Blood samples were collected in the IFIP experimental herd, from 60 females of various physiological status (table 1). Jugular blood was allowed to clot in dry tubes for 12hours at 4°C before centrifugation and serum was frozen.

Table 1 Characteristics of samples.

	Progesterone (ng/ml)			
	n	Mean *(sd)*	Min	Max
Non pubertal gilts	18	0 *(0)*	0	0
Oestrus *(2-3 days post AI)*	23	2.7 *(2.8)*	0.6	12.7
Pregnancy *(4 - 8 weeks)*	19	12.0 *(3.3)*	7.6	19.2

Reference progesterone values were determined by quantitative radioimmunoassay (RIA) in specialized laboratories *(INRA, Tours or ENV, Nantes)*. Semi-quantitative determinations were performed on the same samples, using the Pig-Reprokit® *(CEVA Santé Animale, Libourne)*. This competitive ELISA includes 2 standards : low (2.5 ng/ml) and medium (5 ng/ml). Standards and thawed samples (1 drop) are placed in antibody-coated microwells for incubation with a progesterone conjugate (15 min in dark, at room temperature). Wells are washed before another 15 min incubation with a substrate-chromogen. Subsequent coloration is visually assessed by comparison with standards. Coloration intensity and progesterone concentration are inversely related. Specificity, sensitivity and accuracy were calculated using RIA as reference and a bimodal categorical scale (≤ 2.5 ng/ml ; > 2.5 ng/ml). ELISA were performed simultaneously on the same samples by two independent observers. Agreement was evaluated using Kappa statistic.

Results

ELISA kit was tested on a large range of progesterone concentrations (0 to 19.2 ng/ml) with a balanced repartition of samples : 30% had no progesterone while low]0;2.5ng/ml], medium]2.5;5ng/ml] and high >5 ng/ml values, accounted respectively for 23, 10 and 37% of the data. At the threshold value (2.5 ng/ml), global accuracy was high (92%), with 100% sensitivity (no false negatives), Table 2. Specificity was lower (84%) due to 5 false positives. They were misdiagnosed by ELISA as]2.5;5ng/ml], but RIA values (1.5 to 2 ng/ml) were close to standards. Agreement between observers was high (Kappa=0.95, p<0.001).

Table 2 Accuracy of semi-quantitative ELISA kit

Threshold 2.5 ng/ml		RIA		
		Negatives	Positives	Total
ELISA	Negatives	27	0	27
	Positives	5	28	33
	Total	32	28	60
Specificity		84 %		
Sensibility		100 %		
Global accuracy		92 %		

Discussion and Conclusion

The good accuracy of Pig-Reprokit® confirms recent findings on mares with a similar semi-quantitative ELISA (4) or previous work on sows with different kits (1). Low sensibility is also reported with these rapid tests for intermediate values (4). However, global accuracy is suitable for rapid, on farm, real-time determination of sow cyclicity. This tool may contribute to better management of hormonal treatments. Further investigation is needed to test various possible applications in sow herds.

References

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