The ability of five radioimmunoassay systems to detect pregnancy associated glycoprotein in bovine plasma

ULg UNIVERSITÉ de Liège

Ayad A.[1], Sousa N.M.[1], Sulon J.[1], Iguer-Ouada M.[2], Beckers J.F.[1]

Faculté de médecine vétérinaire

[1]Laboratory of Animal Endocrinology and Reproduction, Faculty of Veterinary Medicine, University of Liege,B-4000 Liege, Belgium
[2]Department of Organism and Populations Biology, Faculty of Life and Nature Sciences, University A. Mira, 06000 Bejaia, Algeria

Introduction

In cow, trophoblastic binucleate cells from the early conceptus synthesize substantial amounts of glycoproteins related to pregnancy.

Pregnancy associated glycoproteins (PAG) constitute a large family of molecules specifically expressed in the outer epithelial cell layer of the placenta in eutherian species (Green *et al*., 2000).

Radioimmumoassay for PAG detection in serum or plasma is currently used as a specific method for pregnancy diagnosis in cattle from day 28 (Zoli *et al*., 1992), also allows the diagosis of embryonic or fetal mortality (Szenci *et al*., 2000).

Aim

This study was conducted to compare different PAG-RIA systems: RIA-497, RIA-706, RIA-780, RIA-809 and RIA-Pool.

Materials and Methods

✓ Two groups of Holstein Friesian females: non pregnant (n=10) and pregnant (n=40) at Day 30 after AI were investigated.

✓ Plasma was obtained by centrifugation immediately after collection and was stored at -20 ℃ until assay.

✓ Polyclonal antisera were collected from rabbits immunized (R#) against different PAG preparation according to the technique of Vaitukaitis et *al*. (1971): R#497 was raised against boPAG$_{67}$, R706 against caPAG$_{55+62}$, R#780 against ovPAG$_{57+59}$, R#809 against ovPAG$_{55}$.

✓ These four antisera were mixed (R#497 one part; R#706 one part; R#780 two parts; and R#809 two parts) and used as an additional antiserum (Pool) (Ayad *et al*., 2006).

✓ The measurements were performed according to the method of Perényi *et al*. (2002).

✓ The results of the different PAG-RIA systems were arranged as follows: diagnosis pregnant correct (*a*); diagnosis pregnant incorrect (*b*); diagnosis not pregnant correct (*c*), and diagnosis not pregnant incorrect (*d*). From these values the sensitivity (100 x $a/a+d$), the specificity (100 x $c/c+b$), the positive predictive value (100 x $a/a+b$), and the negative predictive value (100 x $c/c+d$) of the pregnancy diagnosis were calculated (Szenci *et al*. 1998).

✓ A binominal exact distribution was used to calculate confidence interval for sensitivity, specificity, positive and negative predictive values (STATA/SE 8, StataCorp. 2003).

Results

Table 1. Diagnosis of early pregnancy (Day 30 after AI) in cattle by measurement of PAG using different RIA systems.

	PAG concentration (ng/mL)				
	RIA-497	RIA-706	RIA-780	RIA-809	RIA-Pool
Pregnant	1.82 ± 1.13*	5.42 ± 3.83* ;A,B	6.63 ± 2.86* ;C	5.80 ± 2.28* ;A,C	4.66 ± 2.62* ;B
(40 samples)	(0.91– 7.82)	(1.89– 17.64)	(2.59– 15.22)	(2.78– 12.45)	(1.67– 13.54)
Non-pregnant	0.21 ± 0.04* ;D	0.27 ± 0.04* * ;D	0.25 ± 0.33* * ;D	0.19 ± 0.19* * ;D	0.10 ± 0.01* * ;D
(294 samples)	(0.20– 0.57)	(0.26– 0.54)	(0.18– 2.56)	(0.16– 0.89)	(0.10– 0.26)

*, **: Significant differences between PAG concentrations from pregnant and non-pregnant females ($p < 0.001$).
A,B,C,D: Values with similar superscripts in the same row are not statistically different ($p > 0.05$).

Table 2. Mean (± SD) PAG concentration and range of PAG concentration (minmal-maximal) obtained by five PAG-RIA systems in pregnant and non-pregnant. Confidence intervals (%) are indicated in parenthesis.

RIA system	a	b	c	d	Se (%)	Sp (%)	+ PV (%)	-PV (%)
RIA-497	40	0	294	0	100 (93.0– 100)	100[a] (98.7– 100)	100[a] (93.0– 100)	100 (98.7– 100)
RIA-706	40	0	294	0	100 (93.0– 100)	100[a] (98.7– 100)	100[a] (93.0– 100)	100 (98.7– 100)
RIA-780	40	13	281	0	100 (93.0– 100)	95.6[b] (92.6– 97.6)	75[b] (62.0– 86.0)	100 (98.7– 100)
RIA-809	40	1	293	0	100 (93.0– 100)	99.7[a] (98.1– 99.9)	98[a] (87.0– 99.9)	100 (98.7– 100)
RIA-Pool	40	0	294	0	100 (93.0– 100)	100[a] (98.7– 100)	100[a] (93.0– 100)	100 (98.7– 100)

a,b: Values with different superscripts differ between rows ($p < 0.002$).

Conclusion

The present study shows clearly that the ability of PAG-RIA systems to diagnose specifically pregnancy at Day 30 after AI can be improved by selecting the antiserum and by using a combination of antisera raised against different forms of PAG.

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