Validation of two homologous radioimmunoassays for measuring pregnancy-associated glycoproteins (PAGs) in ewe

B El Amiri[1,2], A Karen[3], J Sulon[1], A Alvarez[1], M Alomar[1], O Szenzi[3], NM Sousa[1] and JF Beckers[1]

❶ Faculty of Veterinary Medicine, Liège, Belgium; ❷ INRA, Meknès, Morocco; ❸ Faculty of Veterinary Science, Budapest, Hungary

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Abstract

We have recently produced different antisera against PAGs isolated from placentas collected at days 60 to 100 of gestation (R805 to R809) and at later (> day 100) stages of pregnancy (R778 and R780). The aim of this study was to validate two homologous RIA systems. Antisera R780 (against ovPAG57+59) and R805 (against ovPAG58+61) were used in RIA1 and RIA2, respectively. The specific activity of the tracer was 14,900 Ci/mmol. The minimal detection limits of RIA-1 and RIA-2 were 0.2 ng/ml and 0.3 ng/ml, respectively. The inter-assay CV of samples with low (1.0 ng/ml), medium (2.5 ng/ml) and high (4.0 ng/ml) PAG concentrations were 13%, 12% and 7% for RIA 1 and 13%, 11% and 5% for RIA 2, respectively. The intra-assay CV were 3%, 6% and 9% for RIA1 and 8%, 9% and 5% for RIA2, in samples with 1, 2.5 and 4 ng/ml of PAGs, respectively. The recovery ranged from 95% to 112%. No cross-reaction was observed with albumin and other members of aspartic proteinase family: renin, cathepsin D, pepsinogen and pepsin tested till 10,000 ng/ml. In conclusion, the two homologous RIA systems developed in this study were sensitive and specific for the detection of ovine PAGs.

Introduction

- Pregnancy-associated glycoproteins (PAGs) are synthesized by mono and/or binucleate trophoblastic cells. They constitute a large family of aspartic proteinases, showing a greatest sequence identity with pepsinogens.
- PAGs are interesting for both scientific and economic reasons in the framework of livestock breeding programs. The determination of PAG concentrations in the maternal bloodstream is the basis of tests for pregnancy diagnosis or follow-up.
- Production of new antigens isolated from sheep placentae at two different stage of gestation can be useful for the development of homologous radioimmunoassay (RIA) systems.

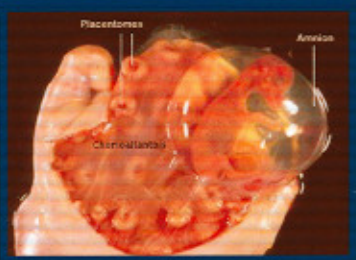
Aim

The present study describes the development of two sensitive and specific double-antibody RIAs for ovine PAGs previously isolated from foetal cotyledons.

Materials and Methods

Antigen production

Purification scheme involved sequential DEAE-cellulose chromatography, gel filtration and CM ceramic [1,2]. Two fractions were proven to be interesting by means of Western blot. They were used to produce two different antisera.



Antisera

The origin and the characteristics of the antigens used to produce the antisera are summarized in Table 1. Three adult rabbits (3-4 kg) were immunized according to the method of Vaitukaitis et al. [3]. The proteins were reconstituted in distilled water, emulsified in an equal volume of Freund adjuvant, and then injected into a rabbit. Injections were given intradermally at multiple sites along the back, which had previously been shaved. The rabbit received boosts of fresh antigen at one week intervals. Three weeks after the first injection, blood was collected from the marginal ear vein and allowed to clot for 12-24 h at room temperature, the sera obtained were removed and stored at -20°C until assayed.

Tracer

A purified preparation of ovPAG61+58 was used as tracer after iodination [4].

Table 1: Antisera used in RIA-1 and RIA-2

System	Antiserum	Antigens	Characteristics		
			N-terminal sequence	Accession number	pI
RIA-1	R780	$ovPAG_{57+59}$	ISSRVSXLTIHPLRNIMDML	P83203	4.1 ; 4.5 ; 5.5
			RGSNLTIHPLRNIRD	P83444	4.5 ; 4.8 ; 5.0
RIA-2	R805	$ovPAG_{61+58}$	RGSNLTIHPLRNTNDIDY	P83495	4.0 ; 4.1 ; 4.7 ; 4.8 ; 5.1
			RGSNLIIHPLRNIRDTFYVGNIT	P83494	4.1 ; 4.7 ; 4.8 ; 5.1 ; 5.4 ; 6.2

RIA procedure

The assay's incubation was performed under equilibrium conditions during 18 hours at room temperature. Free and bound fractions were separated using Tris-BSA (0.4%) buffer containing PEG (4%) and an anti-rabbit IgG serum. The reaction was allowed for 30 minutes. After centrifugation the supernatant was discarded and the radioactivity was determined in all the tubes with gamma counter.

RIA validation

The sensitivity of each RIA was determined as the mean of 20 Bo values minus 2 standard deviations. The precision and reproducibility were determined by evaluating the intra and inter-assay coefficient of variation (CV).

RIA validation (cont'd)

Inter-assay precision was assessed by determining the coefficient of variation of repeated measurements in seven different assays of three serum pools with low, medium and high concentrations of ovPAGs. Intra-assay precision was determined by repeated measurements (n = 10) of these same three ovine serum pools within one assay.

Accuracy of measurement of ovPAGs in serum was determined by assaying known amounts of ovPAGs (0.5; 1; 1.5; 2; 2.5; 3 and 3.5 ng/ml) that had been added to ovine serum containing no ovPAGs.

Specificity of RIA was evaluated by assaying for ovPAGs in solution of various proteins of protease family and pools of sera from pregnant and non-pregnant ewes. The following aspartic proteinases were tested: pepsinogen, pepsin, rennet, rennin and cathepsin D.

Results

- The tracer specific activity was 14,900 Ci/mmol, allowing a highly sensitive RIA with a non specific binding lower than 3%.
- The final antiserum dilution were 1/60,000 and 1/50,000 respectively for R780 (RIA-1) and R805 (RIA-2).
- The minimal detection limits of RIA-1 and RIA-2 were 0.2 ng/ml and 0.3 ng/ml, respectively.
- The inter-assay CV of samples with low (1.0 ng/ml), medium (2.5 ng/ml) and high (4.0 ng/ml) PAG concentrations were 13%, 12% and 7% for RIA-1 and 13%, 11% and 5% for RIA-2, respectively.
- The intra-assay CV were 3%, 6% and 9% for RIA1 and 8%, 9% and 5% for RIA2, in samples with 1.0, 2.5 and 4.0 ng/ml of PAGs, respectively. The recovery ranged from 95% to 112%.
- Concerning the specificity, no cross-reaction was observed with albumin and other members of aspartic proteinase family (renin, cathepsin D, pepsinogen and pepsin) tested till 10,000 ng/ml. In non-pregnant animals, PAG concentrations were lower than 0.2 and 0.3 ng/ml, respectively for RIA-1 and RIA-2.

Conclusion

In conclusion, the two homologous RIA systems developed in this study were sensitive and specific for the detection of ovine PAGs.

Perspectives

Further investigations are in progress to evaluate these two RIA systems for early pregnancy diagnosis in blood and milk samples and for prediction of single or multiple gestations.

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