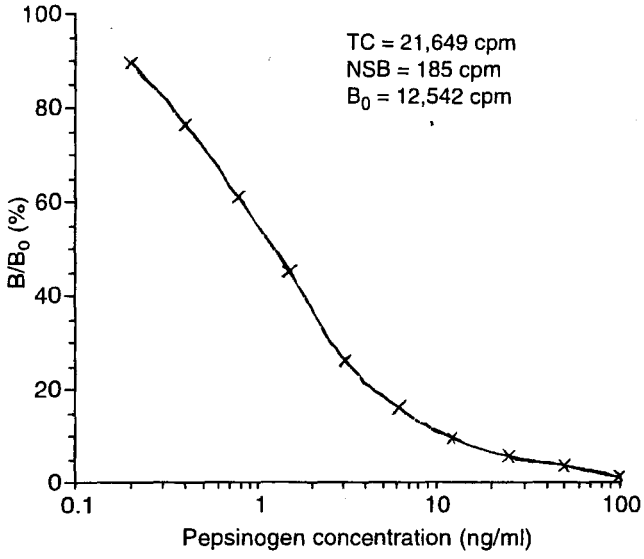


**Figure 1.** Standard curve for the radioimmunoassay of porcine pepsinogen.

**References**

Biemond I., Jansen JB., Crobach LF., Kreuning J., Lamers CB. (1989). Radioimmunoassay of human pepsinogen A and pepsinogen C. *J. Clin. Chem. Clin. Biochem.* **27**, p. 19–25.

Vaitukaitis J., Robbins JB., Nieschlag E., Ross GT. (1971). A method for producing specific antisera with small doses of immunogen. *J. Clin. Endocrinol. Metab.* **33**, p. 988–991.

Wong CR., Nakagawa Y., Perlmann GE. (1972). Studies on the nature of inhibition by gossypol of the transformation of pepsinogen to pepsin. *J. Biol. Chem.* **247**, p. 1625–1631.

## THE INACTIVE MEMBERS OF THE ASPARTIC PROTEINASE FAMILY IN THE RUMINANT PLACENTA: SPECIFICITY OF THREE DIFFERENT RADIOIMMUNOASSAY SYSTEMS

Zs. Perényi[1], J. Sulon[1], O. Szenci[2], JF. Beckers[1].

*[1]Physiology of Reproduction. Faculty of Veterinary Medicine. University of Liège. B–4000 Liège (Belgium). [2]Department of Obstetrics and Reproduction. Faculty of Veterinary Medicine. Szent István University. Budapest (Hungary).*

**Keywords.** Aspartic proteinase, radioimmunoassay, specificity.

Pregnancy-associated glycoproteins (PAGs) have been isolated from the placenta of various ruminant species in the recent decade. Molecular biology studies showed that these glycoproteins are inactive members of the aspartic proteinase family (Xie *et al.*, 1991). Radioimmunoassay developped to detect PAGs in biological fluids (Zoli *et al.*, 1992) became important tools for establishment of pregnancy diagnosis and pregnancy follow-up. As the PAGs share a high sequence homology with each other and with the other members of the aspartic proteinase family: cathepsin D, E chymosin pepsinogen and renin, in the present study the specificity of three commonly used RIA systems was tested.

In the three RIA systems 67 kDa PAG preparation was used as tracer (labelled with $^{125}$Iodine according to the lactoperoxidase method) and as standard. In RIA 1, the antiserum was raised against 67 kDa PAG purified from bovine placenta. In RIA 2 and 3, antisera contained antibodies against cPAG 55+62 and cPAG 55+59 previously isolated from caprine placenta (Garbayo *et al.*, 1998). Serial dilutions ranging from 10 ng/ml to 1 mg/ml prepared from pepsin, pepsinogen, rennin and rennet in Tween Tris buffer were tested in the three systems in comparison with the PAG standard used for assays.

There was weak inhibition of binding caused by the four preparations examined in the concentration range of 10 ng/ml – 100 mg/ml. Pepsinogen caused a mild inhibition of binding in RIA 2 system at 500 mg/ml (B/$B_0$=92.81%) and 1 mg/ml (B/$B_0$= 90.07%) concentrations. In the case of pepsin slightly

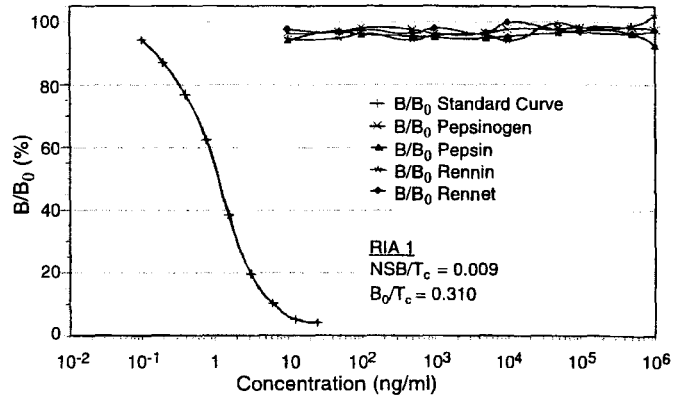


**Figure 1.** RIA 1: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

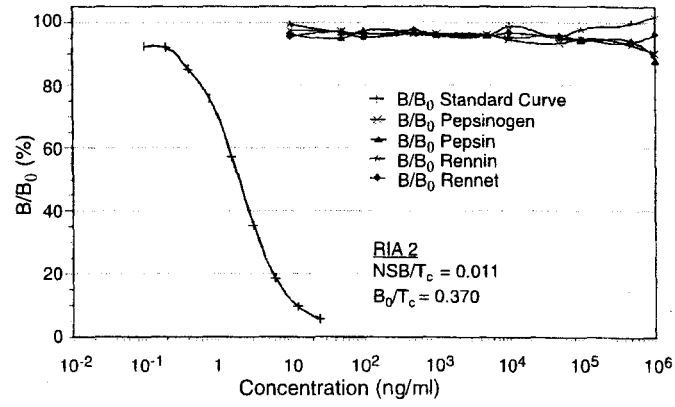


**Figure 2.** RIA 2: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

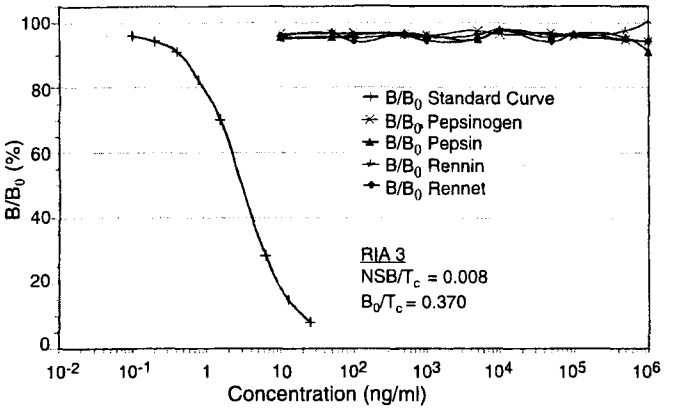


**Figure 3.** RIA 3: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

stronger inhibition of tracer binding could be observed in RIA 1, 2 and 3 at 1 mg/ml concentration ($B/B_0$= 92.55%, 87.86% and 90.86% respectively).

In our experiment only pepsin and pepsinogen could decrease the binding of the radiolabelled PAG tracer to the antibodies, and were able to slightly crossreact with the antisera used. As the pepsin, pepsinogen, PAG are belonging to the aspartic proteinase family, the binding inhibition caused by the pepsin, pepsinogen can be explained by sequence identity between these products (by the ability of the antisera to recognize epitops having the same amino acid sequence). It is also possible that the enzymatically active pepsin (or the activated pepsinogen) attacks the radiolabelled PAG molecules in the solution during the incubation phase of the assay.

In physiological conditions the levels of pepsin and pepsinogen in biological fluids never reach the concentration range where these enzymes were able to crossreact with the antisera, so that RIA 1, 2 and 3 systems can be considered as specific for the detection of PAGs in the range of concentration in plasma or serum as reported in the literature.

**References**

Garbayo JM., Remy B., Alabart JL., Folch J., Wattiez R., Falmagne P., Beckers JF. (1998). Isolation and partial characterization of a pregnancy-associated glycoprotein family from the goat placenta. *Biol. Reprod.* **58**, p. 109–115.

Xie SC., Low BG., Nagel RJ., Kramer KK., Anthony RV., Zoli AP., Beckers JF., Roberts RM. (1991). Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. *Proc. Natl. Acad. Sci. USA.* **88**, p. 10247–10251.

Zoli AP., Guilbault LA., Delahaut P., Ortiz WB., Beckers JF. (1992). Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnacy diagnosis. *Biol. Reprod.* **46**, p. 83–92.

## RADIOIMMUNOASSAY MEASUREMENT OF INSULIN-LIKE GROWTH FACTOR-I IN CAMELS: EFFECTS OF ANTICOAGULANTS AND BODY CONDITION SCORE IN SUCKLING FEMALE

M. Hammadi[1], T. Khorchani[1], M. Moslah[1], H. El-Hatmi[1], M. Chammen[1], A. Ben Arfa[1], D. Portetelle[2], R. Renaville[2].
*[1]Département des Sciences animales. IRA Médenine. 4119 Médenine (Tunisia). [2]Unité de Biologie animale et microbienne. Faculté universitaire des Sciences agronomiques de Gembloux. B–5030 Gembloux (Belgium).*

**Keywords.** IGF-I, radioimmunoassay, dromedaries.

This communication reports a study of the effects of two anticoagulants and body condition score (BCS) on the blood concentration of insulin-like growth factor-I (IGF-I) in dromedary (*Camelus dromedarius*). Three blood tubes (serum, heparin, and EDTA anticoagulant) were taken from the jugular vein of twenty suckling dromedary females (Maghrebi breed) which were divided into two BCS groups (BCS was 5.2 ± 0.9 and 3.8 ± 0.6 in group 1 and group 2, respectively; P < .0001). Females were at 3 to 5 months postpartum. Bloods with anticoagulants were immediately centrifuged while serum samples were centrifuged after an incubation of 3 h at room temperature. Serum and plasma were conserved at – 20°C until assays. The serum and plasma IGF-I concentrations were measured by a double-antibody RIA procedure. IGF-binding proteins were removed by acid-ethanol extraction. Concentration of IGF-I in samples with heparin was significantly higher (P < .0001) than IGF-I in serum and in EDTA samples (68.7 ± 42.6 ng/ml; 34.11 ± 12.5 ng/ml; 35.1 ± 6.9 ng/ml, respectively). IGF-I concentrations in serum and heparin plasma were significantly correlated (r = 0.84, P < .0001). Interaction between BCS level and anticoagulant was very significant (P < .0001). Heparin increased the detection of IGF-I in group 1 but did not affect the concentration of IGF-I in group 2 in which the concentration of IGF-I in serum was significantly lower (41.4 ± 14.2 ng/ml *vs.* 26.8 ± 2.9 ng/ml in group 1 and group 2 respectively). With EDTA, any difference between groups was noted (36.6 ± 7.5 ng/ml *vs.* 33.5 ± 6.0 ng/ml in group 1 and group 2, respectively). It was concluded that heparin increased the immunoreactivity of IGF-I probably by reducing its affinity to the binding proteins. This action was not observed with EDTA. Finally, the concentration of IGF-I is higher in animal with good body conditions score.

(This research was supported by the IRA (Tunisia) and the DGCI (Belgium) cooperation grant).