alteration of the blood leptin concentration. It also remains speculative whether the observed alterations in plasma leptin levels are sufficient to elicit an effect on food intake or energy expenditure.

PAGs MOLECULES AS MARKERS OF PREGNANCY IN THE BOVINE: COMPARISON OF CONCENTRATIONS OBTAINED BY USING THREE DIFFERENT ANTISERA

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The mammalian placenta is a source of several proteins and hormones. In 1982, Butler *et al.* isolated two pregnancy specific proteins (PSPA and PSPB) from bovine placenta. Later PSPA was identified as α-fetoprotein, PSPB was found placenta-specific (Sasser *et al.*, 1986). In 1991, Zoli *et al.* purified and characterized a pregnancy-associated glycoprotein (PAG) from bovine fetal cotyledons later named PAG I_{67}. This PAG consists of four isoelectric variants (PI: 4.4, 4.6, 5.2 and 5.4) with a molecular mass of 67 kDa. Glycoproteins immunologically close to PAG I_{67} and PSPB were isolated from ovine placenta and later called oPAG1 (Zoli *et al.*, 1995) and oPSPB (Willard *et al.*). In 1998, Garbayo *et al.* isolated three PAGs (molecular weight: 55, 59 and 62 kDa) from mid pregnant goat placenta. Parallel to the protein purification, investigations were realized using molecular biology. After the isolation of the cDNA coding for PAG I67 and oPAG 1 it became clear that the pregnancy-associated glycoproteins are belonging to the aspartic proteinase family, sharing great sequence identity with pepsinogenes, cathepsin D, E, renin (5, 8, 13, 14). The PAG molecules purified until now are enzymatically inactive, because of mutations around the active site (Xie *et al.*, 1991). Later studies identified new DNA sequences encoding for PAG molecules in pig (Szafranska *et al.*, 1995), horse (Green *et al.*, 1994), zebra and in cat placenta (Gan *et al.*, 1997). As the PAGs are synthesized in mono, binucleate or syncitial cells of the trophectoderm (Xie *et al.*, 1991; Zoli *et al.*, 1992) and some of them are secreted on the maternal blood circulation, they can be good indicators of pregnancy and feto-placental well-being (Bohn, 1991; Sciarra *et al.*, 1963). Antisera produced in rabbit against bPSPB and PAG I_{67} allowed the development of radioimmunoassays for pregnancy detection in the bovine species from day 28 or 30 after

fertilization (Humblot *et al.*,1988; Zoli *et al.*, 1992). Also, the objective of this study was to compare the ability of three different antisera was compared to measure pregnancy-associated glycoprotein concentrations in plasma samples.

Blood samples were collected from the jugular vein into heparinized vacutainer tubes at 30–120 days after fertilization for pregnancy diagnosis (Laboratory Genes Diffusion, Douai, France). Plasma was removed after centrifugation (1000 g, 20 min) and stored at -20°C until assayed for PAGs. PAG I_{67} purified from bovine placenta was radiolabelled by the lactoperoxidase method with ^{125}I. The antisera used for the different RIA systems are shown in **table 1**. In the three RIA systems PAG I_{67} was used as standard (0.2-25 ng/ml).

The PAG measurements were realized according to the method of Zoli *et al.* (1992). Briefly, after the addition of appropriate dilutions of antisera, plasma samples and the standards were incubated overnight at room temperature. The following day, tracer (25000 cpm/tube) was added to all tubes, and they were incubated for 4 hours at room temperature. The separation of the free and bound fractions was done by centrifugation (1500 g, 15 min, 4°C) after the addition of sheep anti-rabbit-immunoglobulin, coupled to activated cellulose (DASP system). The supernatant was discarded, and the radioactivity in the pellet was determined using an LKB Wallac 1261 Multi Gamma counter. The regression and the variance analysis were realized using the PSI – Plot v. 4.0 and the GLM procedure of SAS, respectively.

Table 1. Antisera used in RIA1, RIA2 and RIA3.

Antiserum	Contains antibodies against	Dilution in RIA	System
497	PAG I_{67}	1:200000	RIA1
706	$PAG_{55,62}$	1:75000	RIA3
708	$PAG_{55,59}$	1:75000	RIA2

RIA 2 and RIA 3 systems gave significantly higher values than RIA 1 system. Moreover RIA 3 system gave higher values than RIA 2 system. Testing the same samples in RIA 1, RIA 2 and RIA 3 systems the regression parameters were calculated between RIA 2 and RIA 1, RIA 3 and RIA 1 and RIA 3 and RIA 2 systems. The correlation coefficient (r.) between the RIA 3 and RIA 1 systems (0.9403) was higher than between RIA 2 and RIA1 systems (0.9294), however the correlation between RIA 3 and RIA 2 was the highest (0.9738).

Our results suggest that antisera raised against $PAG_{55, 59, 62}$ purified from goat placenta recognize the bovine PAG molecules in maternal blood. The circulating forms of PAGs in cows are probably closer to PAG_{62} than to the other forms ($PAG_{55, 59}$). Presently we are trying to purify and characterize new forms of PAG from bovine placenta in order to improve the accuracy of early pregnancy diagnoses in cow.

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