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Second Belgian Workshop on Animal Endocrinology
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The second Belgian Workshop on Animal Endocrinology has been held in the University of Leuven. The special topics of the workshop focused on leptin mechanism in ruminant and poultry. One of the essential roles of this hormone is to inform the organism about the level of fat reserves. The leptin gene is expressed in ruminants and poultry adipose tissues. Recent results on variations in plasma leptin and/or levels of leptin mRNA in adipose tissues show positive effects of body fatness and feeding level, and an inhibitory bêta-adrenergic effect in cattle. In other respects, *in vitro* leptin production is stimulated by glucocorticoids and insulin, whose effects are inhibited by growth hormone. Progress in knowledge about leptin will allow to better understand and control the adaptations of energy metabolism and reproductive activity of ruminants to seasonal variations in day length and food supply, as well as variations in carcass fatness of growing animal (for more information see review by Chilliard *et al.* (1999). La leptine chez le ruminant. Facteurs de variation physiologiques et nutritionnels, *INRA Prod. Anim.*, **12** p. 225–237). The third Belgian Workshop on Animal Endocrinology will be organized by the University of Namur, Facultés universitaires Notre-Dame de la Paix, in October 2001.

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Abstracts

ISOLATION AND PARTIAL PURIFICATION OF PREGNANCY-ASSOCIATED-GLYCOPROTEINS FROM SHEEP PLACENTA

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The placenta is a source of a wide range of hormones and proteins, placental lactogen, steroids and pregnancy specific or associated proteins. During the last decade, pregnancy associated glycoproteins (PAGs) were purified from bovine and caprine placenta. The PAGs belong to the aspartic protease family, in which they coexist with cathepsin D and E, chymosin, pepsinogen and renin. In 1997, Xie *et al.* identified three different PAGs molecules from the culture medium of sheep placenta removed around day 100 of pregnancy. In a recent study by means of the Ouchterlony method we have shown that the extracts of ovine placenta removed at different stage of pregnancy revealed different precipitation lines (El Amiri *et al.*, 1999). These results suggest that PAGs molecules vary during pregnancy.

The aim of this study was to isolate and further to characterize the PAGs from placental extracts by monitoring the fractions using the Ouchterlony technique, the SDS-PAGE, Western blot and heterologous RIA. Our procedure was based on that initially described by Zoli *et al.* (1991) for boPAG-1.

PAGs were isolated from cotyledons removed from pregnant ewes at the third part of gestation. The tissue was extracted in phosphate buffer containing proteases inhibitors, then the proteins were submitted to acidic and ammonium sulfate precipitations, anion and cation exchange chromatographies (DEAE Sephadex and CM Ceramic columns, respectively). Two fractions (0.04 M NaCl and 0.08 M NaCl) from the DEAE (**Table 1**) were loaded separately onto the CM Ceramic column. In the optical density profile of the CM Ceramic column, peaks were pooled, dialyzed and lyophilized. The peaks exhibiting activity in RIA were used to immunize rabbits. The antisera were tested at different dilutions.

The electrophoresis analysis of immunoreactive peaks after the CM Ceramic column showed at least three proteins between 67 kDa and 30 kDa and justified further investigations before characterizing the ovine PAGs.

Tested in buffer and without preincubation with the bovine PAG tracer, the antisera gave the following titers three months after the first immunization (**Table 2**).

Further investigations are in progress to purify till homogeneity the most abundant ovine PAGs and to characterize by amino acid sequencing after two-dimensional electrophoresis.