The pregnancy Associated Glycoproteins in Sheep : New Investigations by using the Ouchterlony Technique

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Introduction

- During pregnancy in several animal species, various hormones and proteins appear or increase in the maternal circulation. Many of these proteins are of feto-placental origin, and have been detected in ruminants species like bovine, ovine and caprine.
- Pregnancy proteins were first purified from bovine placental membranes by Butler et al. (1982). They isolated two pregnancy-specific proteins: PSPA and PSPB. PSPA was identified as a α-fetoprotein, which is not strictly limited to pregnancy, while PSPB was confirmed as placenta-specific (Sasser et al., 1986).
- In 1991, Zoli et al. purified from bovine fetal cotyledons a pregnancy-associated glycoprotein (PAG: later designated bPAG1). In the same year molecular biology studies allowed to classify this PAG in the aspartic proteinase family (Xie et al., 1991). The bPAG1 showed a molecular weight of 67 kDa and four isoforms with different pIs (5.4, 5.2, 4.8 and 4.4). These glycoproteins (either PSPB or bPAG1) can be detected in the maternal circulation by week 3 after breeding and have been currently used as a gestation marker in cows (Humblot et al., 1988; Zoli et al., 1992).
- The bPAG2, initially isolated by Beckers et al. (1988a) as a bovine chorionic gonadotropin, was more recently characterized by Xie et al. (1994) as an other bovine pregnancy-associated glycoprotein presenting a binding site to the LH receptors.
- PAG molecules were also isolated in sheep (Zoli et al., 1995) and goat placenta (Garbayo et al., 1998). The characterization of the caprine PAG revealed 3 different forms having molecular weights of 55, 59 and 62, and different amino acid sequences. Moreover, each form gave different isoforms varying in the isoelectric points (Garbayo et al., 1998).
- Recently, new members of the PAG family were identified by molecular cloning of DNA in horses (Green et al., 1999a), pigs (Szafranska et al., 1995), zebra (Gan et al., 1997) and cats (Gan et al., 1997).
- With regard to PAG expression during the pregnancy period, molecular biology studies developed on bovine, ovine and caprine PAGs showed that the expression pattern during gestation varies spatially and temporally (Green et al., 1999b; Garbayo et al., 1999).

AIM

The aim of this study was to investigate the presence of PAG in ewes placenta extracts collected in early and in mid pregnancy by means of Ouchterlony method.

Material & Method

❶ Preparation of antigens and antisera

Antigens

Approximately 500g of placenta were used. The tissue was minced and homogenized with a hand mixer in 50 mM phosphate buffer containing PMSF (0.2 mM) and EDTA (0.2% w:v), with ratio of buffer to tissue of 5:1 (v:w). The homogenate was stirred overnight. It was then centrifuged at 27000 x g for 1 h, and the pellet was discarded. The supernatant was dialyzed against 5 mM ammonium bicarbonate (pH 7.8), lyophilized and stored until use. The antigens preparation steps are summarized in figure 1.

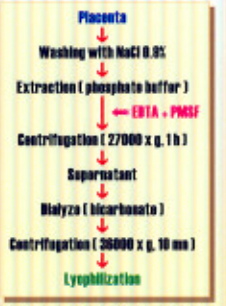

Figure 1: Steps of the antigens preparation

M & M cont'd

Antisera

Twelve antisera were used in this study (table 1).

Table 1: Groups of antisera tested and their origin.

b = bovine
o = ovine
c = caprine

Groups & antisera	N° antiserum tested	Source & reference
① Anti-bPAGII	436 437 438	Beckers et al, 1988a, 1988b
② Anti-bPAGI	497 726 727	Zoli et al, 1992
③ Anti-oPAG	494 495	Zoli et al, 1995
④ Anti-cPAG (55-59)	706 707	Garbayo et al, 1998
④ Anti-cPAG (55-62)	708 709	Garbayo et al, 1998

❷ Ouchterlony technique

The Ouchterlony technique (Ouchterlony, 1949) was used to characterize *in vitro* antigen-antibody reaction by precipitation in gelose (Hartmann & Toilliez, 1957). The antisera with their dilutions (1/1; 1/2; 1/4; 1/8; 1/16; 1/32) were placed in the peripheral wells and the central well was loaded by the placenta extracts (Fig. 2) at the concentration of 20 mg crude protein/ml of phosphate buffer. Ovine placenta from two stages of pregnancy were used in the present study (early pregnancy ≈ 5 to 6 weeks and mid pregnancy ≈ 10 weeks). After an overnight incubation, the precipitation lines corresponding to the antigen-antibody reaction could be seen by using a Coomassie bleue (R250) staining.

Figure 2: Extracts from placenta (Ag) collected in early and mid gestations were placed in the central well. Antisera (As) with different dilutions (1/2, 1/4, 1/8, 1/16, 1/32) were loaded in the peripheral wells.

Results

❶ Mid pregnancy placenta gave two precipitation lines with at least one antiserum from the different groups tested (Fig. 3 A, B, C and D). Two precipitation lines were also observed when extracts of early pregnancy placenta were tested (data not shown).

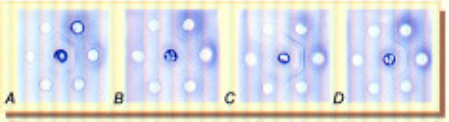

Figure 3: In all strips, the central well was loaded by extract of placenta removed in mid pregnancy. The peripheral wells were loaded respectively by antisera 437, 497, 495 and 706 (strips A, B,C and D, respectively).

❷ Mid pregnancy placenta extracts showed three precipitation lines with some antisera from oPAG group (Fig. 4).

Results (cont'd)

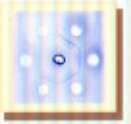

Figure 4: The placenta extract was in the center. The crude anti-oPAG (495) and its dilutions were loaded in the peripheral wells.

❸ The thickness and the position of the precipitation lines varied if we compare early and mid pregnancy placenta (Figure 5 A and B). Concerning the position, in the early placenta extract, the precipitation lines were closer to antiserum wells than in mid pregnancy placenta. This suggest a higher migration speed of the antigen in young placentas.

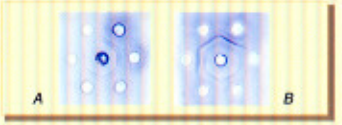

Figure 5 : Central wells were loaded by the mid pregnancy placenta extract (fig. 5A) or by the early placenta extract (fig. 5B). The peripheral wells were loaded by the antiserum anti-bPAG2 (437).

Conclusion

- This study shows that in sheep placenta extracts there are many molecules crossreacting with antisera raised against different classes of PAG.
- The differences between early and mid pregnancy placenta extracts concerning the number, the thickeness and the position of precipitation lines suggest that different forms of PAG can be expressed in early and mid pregnancy.

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