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Maternal serum levels of pregnancy-associated murine-1 (PAMP-1) during pregnancy in the rabbit

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Introduction

Pregnancy associated murine protein-1 (PAMP-1) (*Hau et al.* 1978) which has a mw of 150,000 Dalton is found intracellularly in the liver (*Chemnitz et al.* 1982), in peripheral blood leucocytes (*Sarcione et al.* 1986) and in the gut-associated lymphoid tissue (*Udagawa et al.* 1986). The molecule is present in the circulation of pregnant as well as non-pregnant female mice, but is undetectable by rocket immunoelectrophoresis in healthy adult male mice. In juvenile mice of both sexes PAMP-1 is undectable in the circulation until shortly before puberty when it increases in concentration. During puberty PAMP-1 disappears from the male circulation, but rises to normal adult levels in females (*Hau et al.* 1982a). Plasma levels are significantly higher during dioestrus than during the other stages of the oestrus cycle (*Hau et al.* 1991). During pregnancy the serum concentration increases dramatically (*Hau et al.* 1978), peak levels being reached at mid-gestation in outbred females and a couple of days later in inbred females (*Hau et al.* 1985). Immunochemically identical PAMP-1 analogues have also been detected in the rat (*Hau & Porstmann* 1984), rabbit (*Hau* 1986) and several wild rodent species (*Krog & Hau* 1992). PAMP-1 exhibits partial immunological cross reaction with human pregnancy zone protein (PZP, alpha2-PAG) (*Hau et al.* 1981) indicating that PAMP-1 may be a useful model for the study of human PZP. In the mouse and rat PAMP-1 is regulated by growth hormone (*Frohlander et al.* 1987, *Eriksson et al.* 1988, *Hau et al.* 1990ab).

The aim of the present study was to develop a sensitive enzyme linked immunosorbent assay (ELISA) for quantitative measurements of PAMP-1 in compartments with low levels; and to measure levels throughout pregnancy in the rabbit.

Materials and methods

Animals

The animals used were Mol:Chinchilla rabbits (Møllegaard Breeding Ctr., Køge, Denmark). The animals were between 20 and 75 weeks old and fed a commercial diet (Altromin 3013, Brogaarden, Denmark) and tap water *ad libitum*. The light:dark cycle was 12h:12h and the rabbits were kept individually in steel-plastic rabbit cages. The rabbits were clinically healthy and screened microbiologically every 3 months (*Møllegaard* 1990).

Twelve females were mated and blood samples were obtained twice weekly from the marginal ear vein (5–10 ml) from each female following the mating. Serum was stored at –20°C until analysis.

Quantification of PAMP-1

Antibody preparations against murine PAMP-1

Precipitating antibodies against murine PAMP-1 were produced in goats as previously described (1982b). The monospecificity of the antibody preparations was determined by crossed immunoelectrophoresis[18],

which produced only one immunoprecipitate, PAMP-1, using the antibody preparation diluted 1:2 in the second dimension gel, and murine pregnancy serum as antigen. The immunoglobulin fraction of the sera was isolated by affinity chromatography on a Protein A-Sepharose CL-4B column (Pharmacia, Bromma, Sweden). Absorbed antibodies were eluted at pH 4.0 using a 0.5-M citrate phosphate buffer.

Preparation of enzyme-conjugated antibodies

Horseradish peroxidase was activated by dissolving 5 mg in 0.1 ml 0.1 M phosphate buffer (PBS) pH 6.8 containing 1.25 % (v/v) glutaraldehyde. The solution was incubated at room temperature for 18 h and excess glutaraldehyde was removed by dialysis against 0.15 M NaCl. Isolated goat antibodies against PAMP-1 were dialysed in PBS pH 7.2 and adjusted to 5 mg/ml protein. The activated peroxidase and 0.5 ml of the immunoglobulin solution was mixed with 0.05 ml 1 M carbonate/bicarbonate buffer pH 9.5 and incubated for 24 hr at 4°C. The solution was mixed with 0.05 ml 0.2 M glycine buffer pH 8.5 and incubated for 2 h at room temperature. Finally the solution was dialysed in PBS pH 7.2 and centrifuged 30 min at 50,000 g.

As a test for optimal labeling, a sample of the solution (0.5 ml) was subjected to high performance liquid chromatography using TSK G3000SW column. The majority of the peroxidase activity was found to be associated with the immunoglobulin fraction and a minor peak of free peroxidase activity indicated peroxidase surplus.

Preparation of purified PAMP-1 standard

Murine female serum (n = 10) was used as starting material for purification of PAMP-1. PAMP-1 was purified by HPLC ion exchange chromatography on a TSK DEATE 5PW column followed by HPLC gel filtration on a TSK G3000SW column. When analysed in crossed immunoelectrophoresis using the unabsorbed goat anti PAMP-1 antiserum the purified preparation produced only one immunoprecipitate.

Enzyme-linked immunosorbent assay (ELISA)

A double sandwich ELISA was developed and the following optimal conditions were used. Aliquots (0.1 ml) of the non-conjugated goat anti PAMP-1 antibodies, diluted 1:10, were added to the wells of polystyrene microtitre plates (Nunc, Denmark). The antibodies were allowed to bind overnight at 5°C. The microtitre plates were then washed ten times in PBS pH 7.2 containing 2 % (*v/v*) Tween 20, and incubated for 1 h at room temperature in PBS containing 1 % (*w/v*) L-tryptophan, to block additional hydrophobic absorption sites on the plates. Preliminary experiments demonstrated that blocking with tryptophan was more efficient than blocking with bovine serum albumin or Tween 20 with respect to reduction of background staining. The murine sera to be tested were added to the wells (0.1 ml per well) and incubated for 1–1.5 h, after which the plates were washed ten times in PBS and incubated for 15 min in the blocking buffer containing 1 % (*w/v*) tryptophan. The peroxidase-conjugated antibody preparation was diluted 1:100 and added to the wells (0.1 ml per well) and incubated for 1 h. The plates were washed 20 times and blocked with tryptophan for 15 min before adding the chromogen: 0.55 mg/ml orthophenylenediamine (OPD, Dakopatts, Denmark) in a 0.1 M citrate-phosphate buffer pH containing 0.16 m peroxide. After 20 min the colour reaction was stopped by adding 0.15 ml 1 M sulphonic acid. The plates were monitored on an EAR ELISA reader (SLT, Austria) at 492 nm.

The ELISA standard curves of pregnancy serum diluted in buffer, purified PAMP-1 diluted in buffer and purified PAMP-1 diluted in male murine plasma were parallel.

A five-fold dilution of mouse late-pregnancy serum (day 17 of pregnancy, n = 20) was

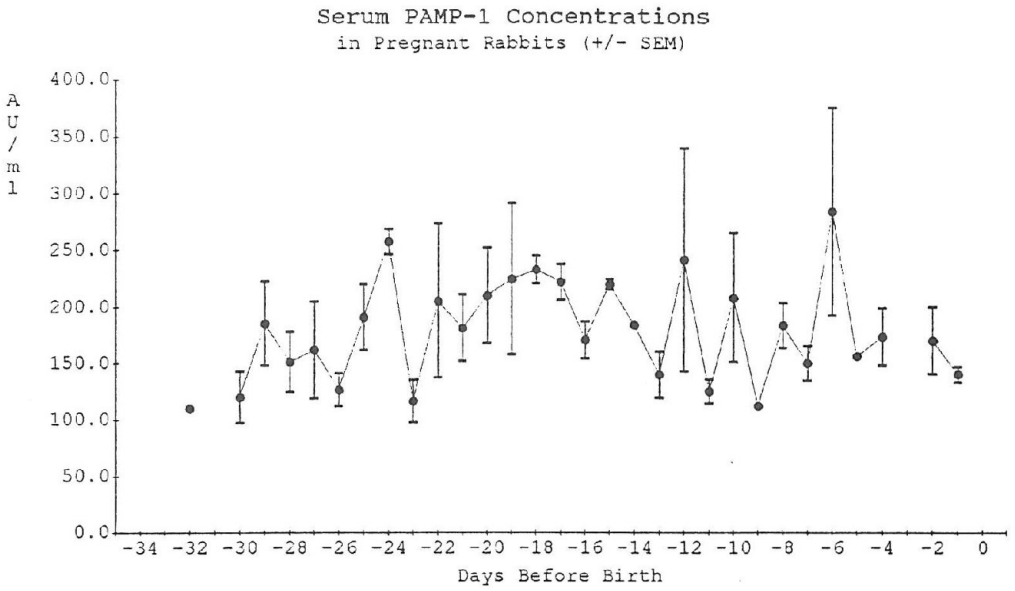

Figure 1. Levels of PAMP-1 measured by ELISA in 12 rabbits. Values were recorded twice a week for each rabbit. Each point represents the mean value ± SEM.

used as standard. The undiluted standard was arbitrarily assigned a value of 100 AU/ml.

Results

Figure 1 shows the maternal serum levels of PAMP-1 in 12 primigravid rabbits throughout pregnancy. No significant fluctuations were recorded.

Discussion

This is the first report on serum levels of PAMP-1 during pregnancy in the rabbit. PAMP-1 does not appear to be a pregnancy-associated protein in the rabbit since no significant changes were observed during gestation. This is an interesting findings which agrees well with the species differences in levels of the human PZP protein demonstrated by several authors. During human pregnancy PZP increases dramatically to reach very high levels in serum (*Westergaard et al.* 1982). By contrast, in sub-human primates such as macaques and squirrel monkeys the concentration was higher in non-pregnant females than in pregnant females (*Bohn & Ronneberger* 1973, *Lin et al.* 1976). These studies were performed using antibodies against human PZP. Using antisera raised against dog PZP it was found that in this species peak concentrations are recorded at mid-pregnancy like in the mouse (*Bohn* 1986, *Szabo et al.* 1986). Using the same antibody preparation (*Hau et al.* 1993), analysed the levels during the breeding season in the mink. In the mink PZP increases to reach a local maximum at mid-pregnancy, but the molecule does not behave as a traditional *pregnancy*-associated protein in this species either. Thus PAMP-1 and PZP do not seem to be obligatory pregnancy-associated in all species.

PAMP-1 is regulated by pituitary growth hormone in the mouse and rat, and further studies are needed in order to shed light on the hormonal regulation of PAMP-1 in the rabbit.

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Summary
An ELISA was developed to measure for the first time serum levels of Pregnancy-Associated Murine Protein-1 (PAMP-1) throughout pregnancy in the rabbit. In rodents serum levels of PAMP-1 are regulated by growth hormone. Unlike the pregnancy-associated rise in serum levels in pregnant mice and rats, PAMP-1 did not increase significantly during pregnancy in the rabbit.

Sammendrag
En ELISA-metode blev udviklet til, for første gang, at kvantitere Pregnancy-Associated Murine Protein-1 (PAMP-1) i serum gennem drægtigheden hos kaninen. PAMP-1 reguleres af væksthormon hos mus og rotter. I modsætning til den drægtighedsassocierede stigning i serum hos gnavere, øgedes serumniveauet af PAMP-1 ikke gennem drægtigheden i kaninen.

Yhteenveto / K. Pelkonen
Työssä kehitettiin ELISA, jolla määritettiin ensimmäistä kertaa koko tiineyden ajan kanista seerumin Pregnancy-Associated Murine Protein-1 (PAMP-1). Jyrsijöissä PAMP-1 tasoa säätelee kasvuhormoni. Hiirellä ja rotalla seerumin PAMP-1 taso nousee tiineyteen liittyen, mutta kanissa ei merkittävää nousua tiineyden aikana voitu todeta.

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