

The pregnancy-associated glycoproteins expressed in epitheliochorial placenta: new investigations by Western blot technique

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Introduction

The topography and structure of the placenta vary depending on the behavior of the egg which, at the time of its attachment, either remains in the uterine lumen or implants interstitially in the uterine mucosa.

Four types of placenta may be distinguished histologically: the epitheliochorial placenta (equidae, suidae and camelidae), the synepitheliochorial placenta (ruminants), the endotheliochorial placenta (carnivores) and the haemochorial placenta (human, primates and rodents).

Characterized in the last 25 years, the pregnancy-associated glycoproteins (PAG) constitute a large family of glycoproteins expressed in the outer epithelial cell layer (chorion/trophoblast) of the placenta (Roberts et al., 1995).

They are members of the aspartic proteinase gene family having greatest sequence identity with pepsinogens (Xie et al., 1991).

By using biochemical procedures, some molecules of the PAG family were isolated from cotyledons of bovine (bo), caprine (ca), ovine, cervid, water buffalo and European bison species. These molecules were used to immunize rabbits and antisera were produced against different PAG preparations: R#435 (anti-boPAG-II), R#497 (anti-boPAG-I) and R#706 (anti-caPAG_{55kDa+62kDa}).

Aims

The aim of this study was to describe the immunoreactivity of protein extracts from epitheliochorial placentas (equine: *Equus caballus*; porcine: *Sus scrofa domesticus* and camelidae: *Camelus dromedarius* and *Lama pacos*) against three PAG antisera by using of Western blot technique.

Materials and Methods

1) Protein Extraction and fractionation

- Uteri were collected immediately after slaughter or Caesarean section and frozen until used.
- Thirty grams of placenta from each species were extracted twice at neutral pH (10 mM potassium phosphate buffer) and in the presence of inhibitors (PMSF, EDTA and NaN₃).
- Ammonium sulfate (A.S.) precipitations were carried out at 20-50% and 50-80% saturations.
- Pellets from A.S. fractions were resuspended in 25 ml of 5 mM ammonium bicarbonate (pH 8.0) and extensively dialyzed against the same buffer (48 h) before lyophilization.

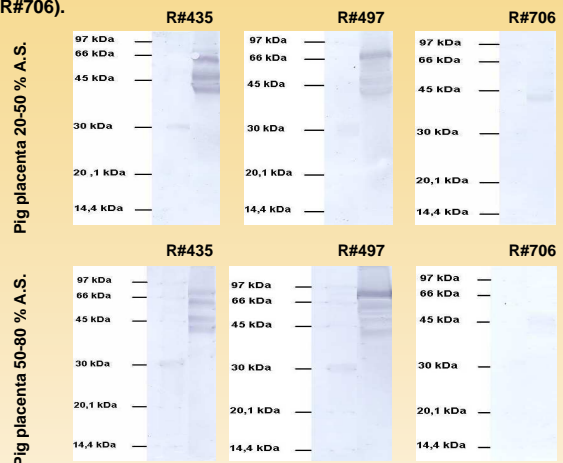
2) Electrophoresis and Western blot

- Placental proteins (100 µg in 20 µl Laemmli) in the slab gel (20 cm x 10 cm x 0.1 cm) were transferred onto a nitrocellulose membrane immediately after 1-SDS-PAGE (12% polyacrylamide gel).
- Transfer was carried out overnight at 30 V on a TransBlot Cell Apparatus. After protein visualization with Ponceau S staining, proteins were probed with the use of R#435 (anti-boPAG-II), R#497 (anti-boPAG-I) and R#706 (anti-caPAG_{55kDa+62kDa}) as previously described (El Amiri et al., 2003).

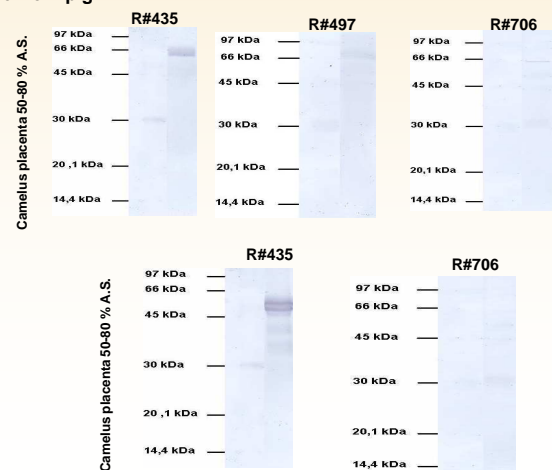
Results

No PAG immunoreactivity was found in equine placenta nor in 20-50% A.S. neither in 50-80% A.S. fraction regarding the three antisera (data not shown).

In pig placenta, both 20-50% and 50-80 % A.S. fractions gave multiple immunoreactive bands corresponding to apparent molecular masses of 45 to 66 kDa (R#435 and R#497) and 30 kDa (R#706).



Immunoreactive proteins could also be demonstrated in the 20-50% A.S. fraction from alpaca and camel. However, in these species, the number of reactive proteins were lower compared to those from pig.



Conclusion

In conclusion, placental proteins from pig, camel and alpaca were recognized by the three PAG antisera used in this study. These results open new possibilities to follow purification procedures in order to isolate and characterize PAG molecules from epitheliochorial placentas.

References

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