# Isolation of the Pregnancy-Associated Glycoprotein 1 (PAG-1) from Zebu *(Bos Indicus)* Placenta

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## Introduction

**Pregnancy-Associated Glycoproteins (PAGs):**

- First described as a placental antigen of cattle *(Bos taurus)* placenta that was also present in the blood serum soon after implantation
- Expressed by the invasive mono/binucleate cells of the trophectoderm of ungulate species
- Belonging to the Aspartic Proteinase (AP) family (50% amino acid identity to pepsinogen, renin, cathepsin D and E)
- Isolated from cotyledons of cows, ewes and goats by classical biochemical procedures
- Characterized in *Bos taurus* as an acidic glycoprotein constituted by 380 aa, and showing 4 molecular isoforms with 67 000 molecular weight
- Purification of PAG molecules → development of sensitive RIA systems for pregnancy diagnosis in ruminant species

## Aim

> To investigate the presence of pregnancy-associated glycoproteins in zebu *(Bos indicus)* placenta by use of a one-step affinity chromatography

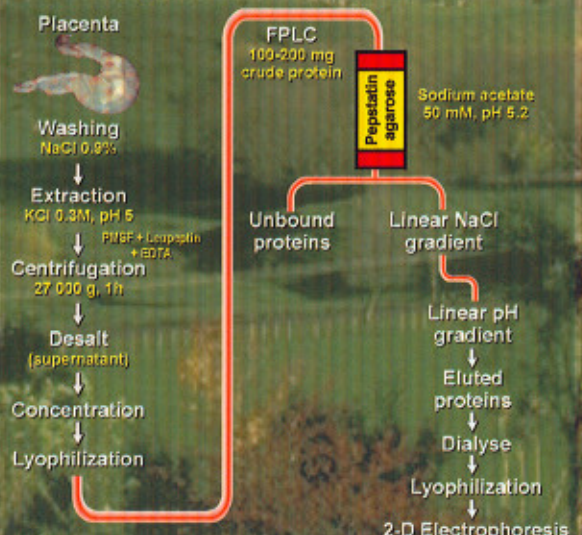
## Materials and Methods

### ① Animals

Uteri were collected from 3 pregnant zebu females immediately after slaughter.

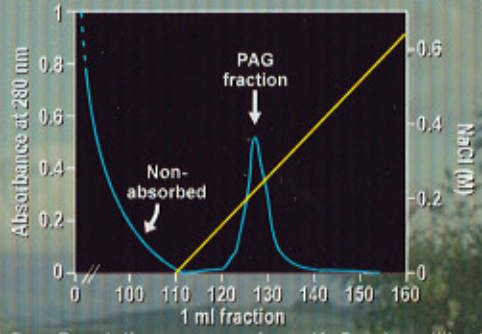
Gestational ages were between 31 and 32 weeks (≈ 7 months).


### ② PAG purification procedure




## Results

### ① Pepstatin-agarose affinity chromatography




FPLC on Pepstatin-agarose column (1x5 cm) equilibrated in 50 mM sodium acetate buffer, pH 5.2.
The yellow line indicates the slope of linear salt gradient.

### ② 2-D electrophoresis and microsequencing

Three major spots with 67 and 84 kDa molecular masses

Microsequencing of N-terminus of the 67 kDa spot :

R G S X L T T H P L R N I K

*(boPAG-1 sequence; Q29432)*

## Conclusions

- This study shows that bovine *(Bos taurus)* PAG-1 is also present in zebu *(Bos indicus)* placenta.
- PAG, like other enzymatically active aspartic proteinases, can bind to pepstatin A, a potent enzymatic inhibitor.
- Investigations are in progress in order to isolate other pregnancy-associated glycoproteins and to compare the electrophoretical patterns of Bos indicus and Bos taurus placental proteins.

References

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