Placental and gastric arpartic proteinases: new insights from bovine species

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Diversity of Aspartic Proteinases (AP)

- **Gastric origin: oldest family probably more than 600 MY (invertebrate => vertebrate)**

  - Prochymosin: for milk digestion in sucking animals
  - Pepsinogen A
  - Pepsinogen C (or progastricsin)

- **Placental origin: appeared 55 to 85 MY in ruminants**

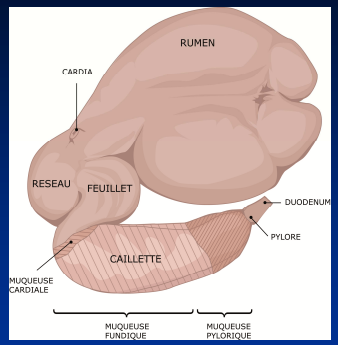
  - Pregnancy-associated glycoproteins (PAG)

- **Intermediate molecules in fetal stomach???**

Investigations on GASTRIC AP

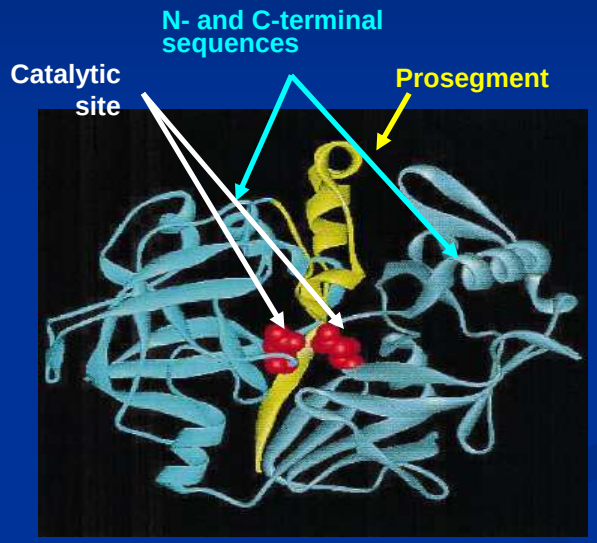
Studies on pepsinogens

- Theodore Schwann, 1810 - 1880: identification of pepsin as the substance responsible for digestion in stomach
- John H. Northrop (1930): isolation of pepsin in its crystal form (from bovine gastric mucosa) and characterization as a protein
- 1945 - … : new techniques (chromatography, SDS-PAGE, …) allowed the isolation and characterization of different gastric aspartic proteinases (AP):



Pepsinogen A (372 aa) – Pepsinogen C or progastricsin (344 to 391 aa) – Prochymosin (381 aa)

General structure of bovine zymogens



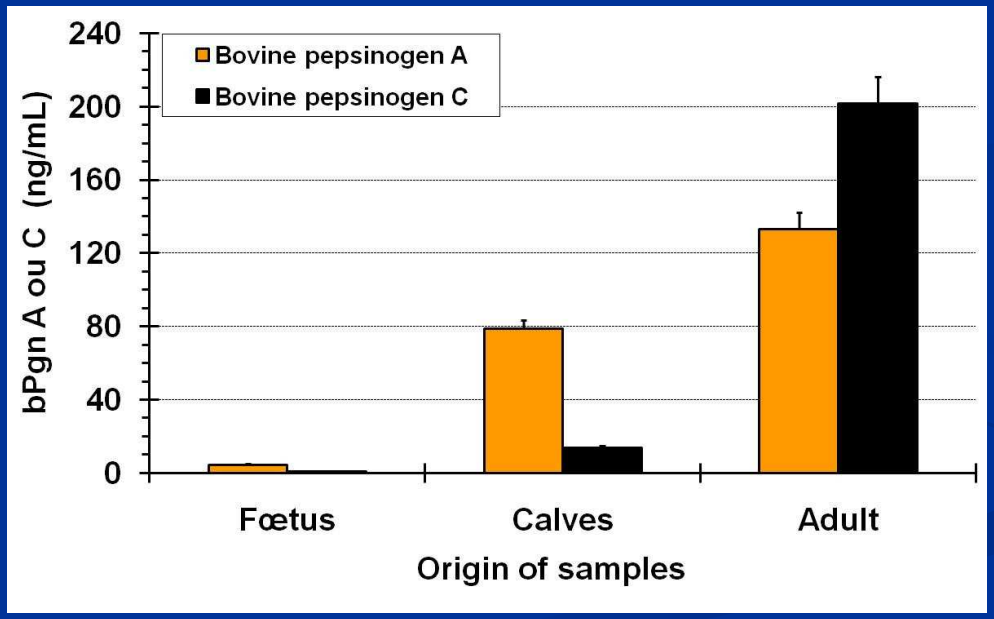
Richter et *al.*, 1998 (*Biochem J*)

N-terminal sequences of bovine gastric AP:

- **Pepsinogen A:** MSVVKIPLVKKKSLRQNLIE — Harboe et al., 1974
- **Pepsinogen C:** LVKIPLKKFKSIREIMKE — Foltmann et Pedersen, 1977
- **Prochymosin:** AEITRIPLVKKKSKRQNLIE — Pedersen et Foltmann, 1975

Pepsinogen A and C concentrations in peripheral circulation of cattle

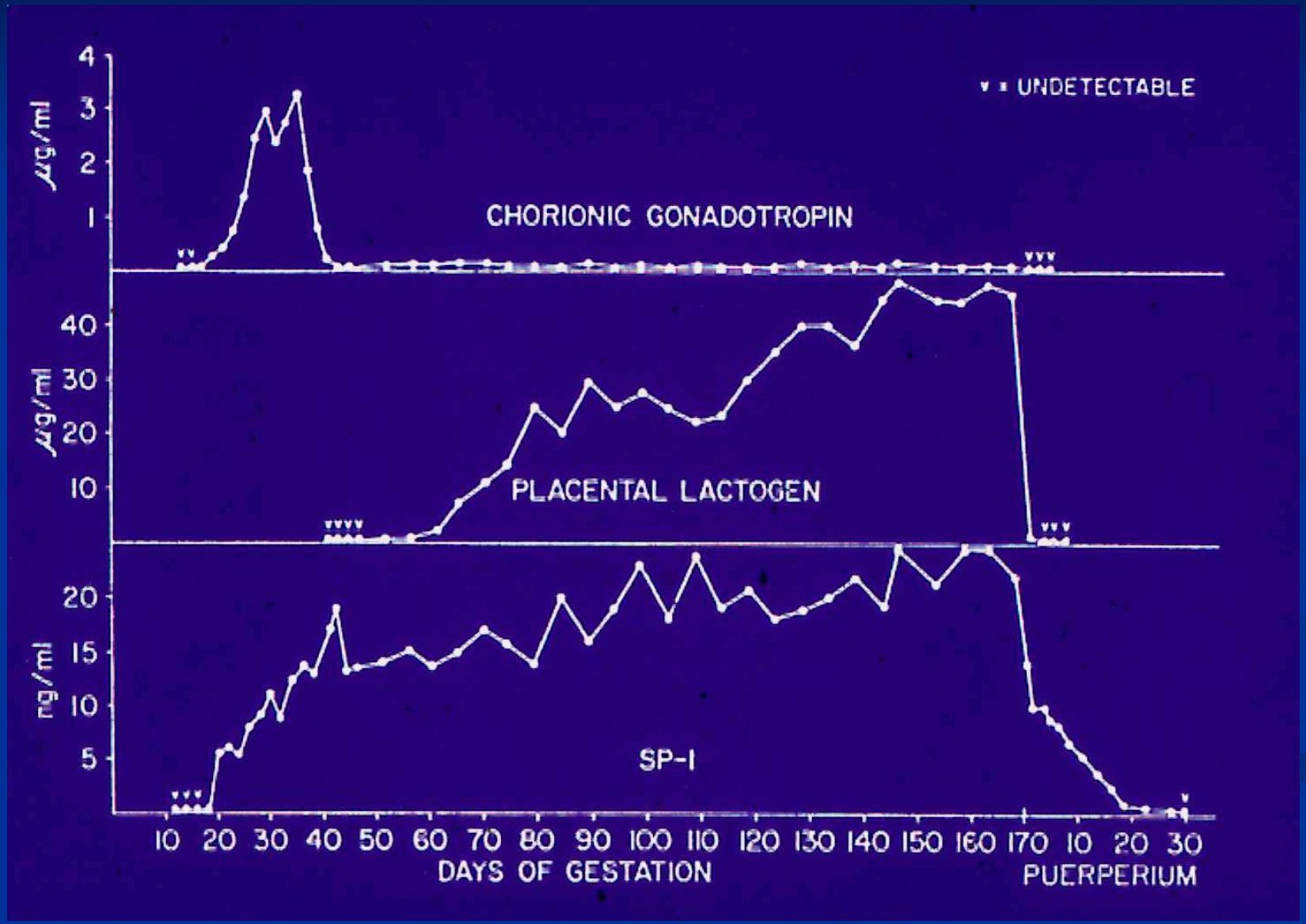
Animal	Pepsinogen A	Pepsinogen C	Ratio
Fetus	4.6 ± 0.7 ng/mL	< 0.9 ng/mL	+++
Calves	78.9 ± 6.7 ng/mL	13.5 ± 1.1 ng/mL	5.8
Adult cows	133.2 ± 17.6 ng/mL	201.5 ± 26.5 ng/mL	0.7



Mean concentration (± SD) of pepsinogen A and pepsinogen C measured in fetuses, calves and cows.

Investigations on Placental AP

Research on placental proteins in ruminant species.
Example from *Rhesus sp.* monkey



PAG 1 groupe (PSPB, PSP60…)

Discovery of boPAG-1 group…

-In 1982, Butler et al. isolated two pregnancy specific proteins:

- PSPA
- PSPB

- PSPA was identified as the alphafetoprotein
- PSPB was specific for pregnancy period… the same aminoacid microsequence than boPAG-1 isolated from bovine placenta in Liege

Lynch et al., 1992; Zoli et al., 1991

Identification of boPAG-2 group

- 1940's - Nalbandov and Casida observed a reduction in the pituitary gonadotrophic activity in the pregnant cow and suggested that the CL was replaced by additional luteotrophic substances produced elsewhere

- 1950's - Weeth and Herman (1952) and Björkman (1954) found that bovine trophoblastic cells contain glycoproteins

- 1960's - Foote and Kaushik (1963) demonstrated the presence of a LH-like activity (use of extracts of fetal/maternal cotyledons and Parlow's test)

  - Lunen and Foote (1967) suggested that a LH-like substance was present in the bovine placenta (named bovine chorionic gonadotropin or bCG). They found a significant response with the ventral prostate test and Parlow's test

- 1980's - Ailenberg and Shemesh (1983) looked for a gonadotrophic-like substance capable to stimulate the progesterone production in bovine granulosa cells culture (use of cotyledons collected during the 1st trimester of pregnancy)

Identification of boPAG-2 group

- Beckers et al. (1987) used a RRA (homogenized CL tissue used as membrane receptors) in order to identify the luteotrophic activity in fetal cotyledonary extracts.

- After several successive purification steps (90 000 times purification), they isolated a substance with high luteotrophic activity (MM 30 kDa).

- The purified hormone was distinct from hypophyseal LH as there was no line of precipitation between purified bCG and anti-bLH antiserum following radial immunodiffusion in agar gel.

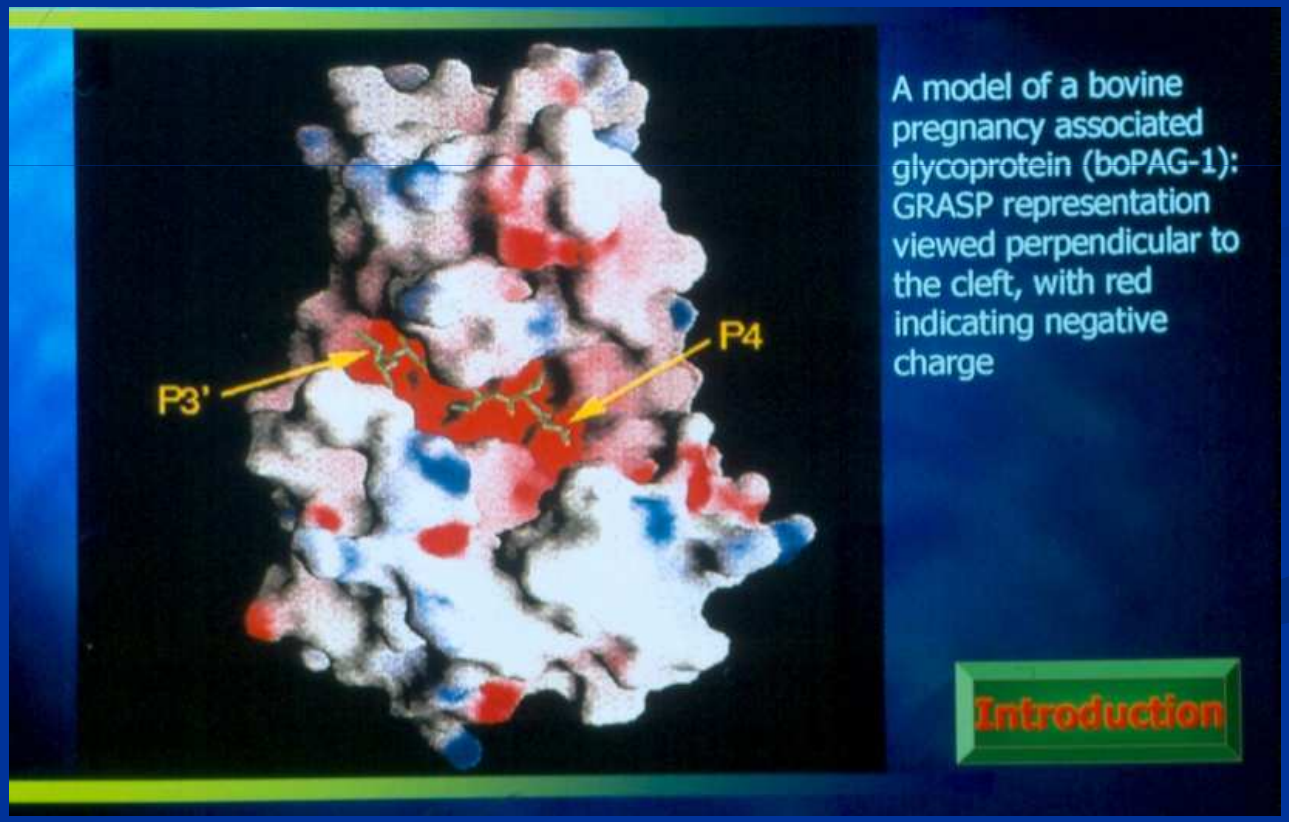
- However, this purified hormone was closely related to pituitary LH as the anti-bCG serum tested presented a cross-reaction with this substance.

- Xie et al. (1994) showed that the cDNA sequence corresponding to the bovine chorionic gonadotrophin was closely related to that of bovine pregnancy-associated glycoprotein (PAG) subfamily

boPAG-1 and -2 belong to AP gene family…

- No similarity with CG structure

- Around 50% of similarity with pepsinogens, cathepsins D and E, …



Comparison of amino acid sequence identities between PAG and gastric AP

	Pepsin	Chymosin	boPAG-1	boPAG-1v	boPAG-2	ovPAG-1	ovPAG-2	poPAG-1	poPAG-2	eqPAG
Pepsin	100									
Chymosin	59.5	100								
boPAG-1	49.5	42.5	100							
boPAG-1v	50.8	42.9	86.1	100						
boPAG-2	50.8	45.6	57.8	58.8	100					
ovPAG-1	49.4	42.3	70.6	71.6	58.5	100				
ovPAG-2	50.5	45.9	60.4	60.2	63.4	60.4	100			
poPAG-1	48.6	43.5	48.8	50.5	48.5	47.4	52.5	100		
poPAG-2	52.9	44.3	56.2	55.1	56.7	54.2	57.4	61.8	100	
eqPAG	58.6	52.3	54.9	55.2	55.4	55.5	54.6	55.3	58.5	100

Guruprasad et al. (1996)

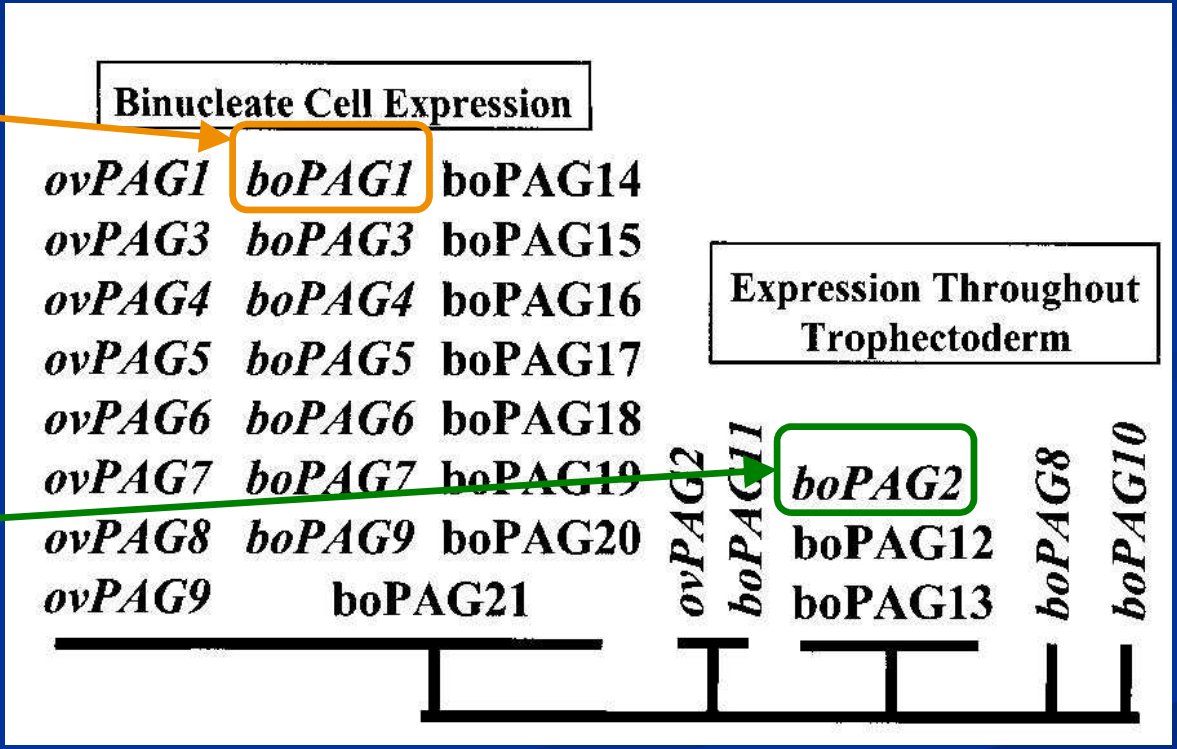
boPAG-1 versus boPAG-2 expression in bovine placenta

boPAG-I subgroup

is synthesized in binucleate cells (BNC) of trophectoderm…

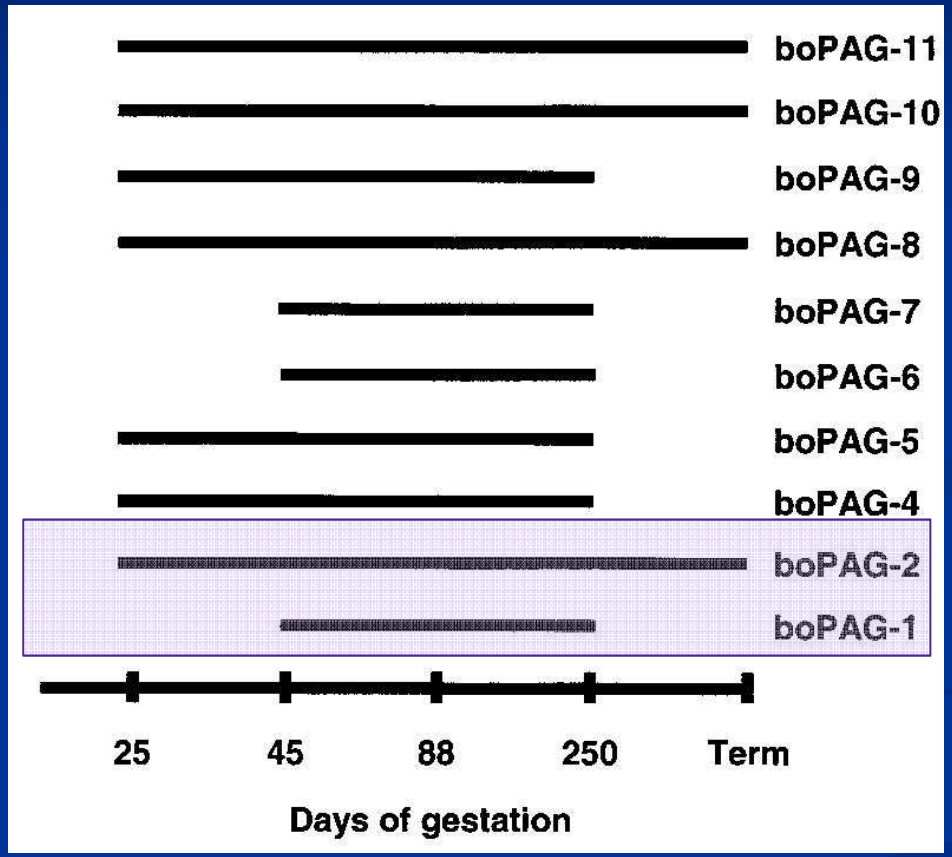
boPAG-II subgroup

is expressed in both mononucleate (MNC) and in BNC throughout the trophectoderm



Green et al. (2000)

Pregnancy- Associated Glycoproteins (PAGs)



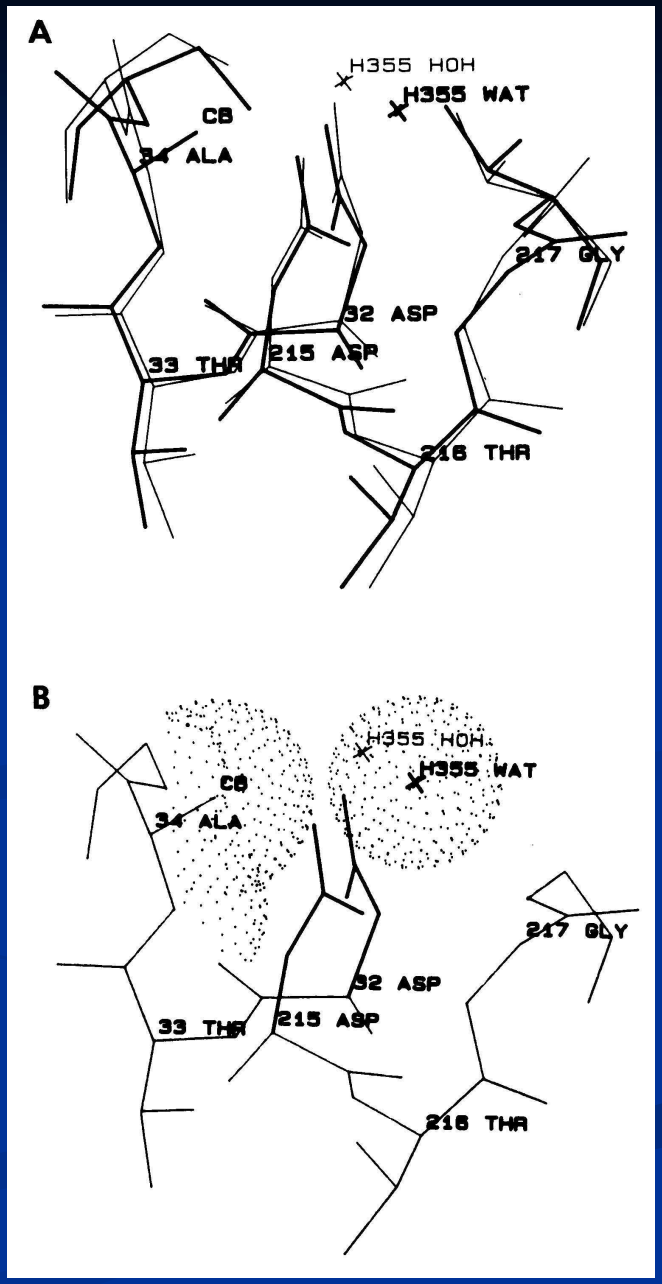
- **Temporal expression of bovine PAG-1 and PAG–2 during pregnancy in bovine species (adapted from Green et al. 2000)**

A) Computer modeling of highly conserved regions of PAG-1 (thin lines), homologous to active site of porcine pepsin (bold lines).

B) Computer modeling of active site of AP in comparison of porcine pepsin (Asp-32 et Asp-215). The van der Waals surface of water molecule H355 WAT and Ala-34 from PAG model are depicted areas.

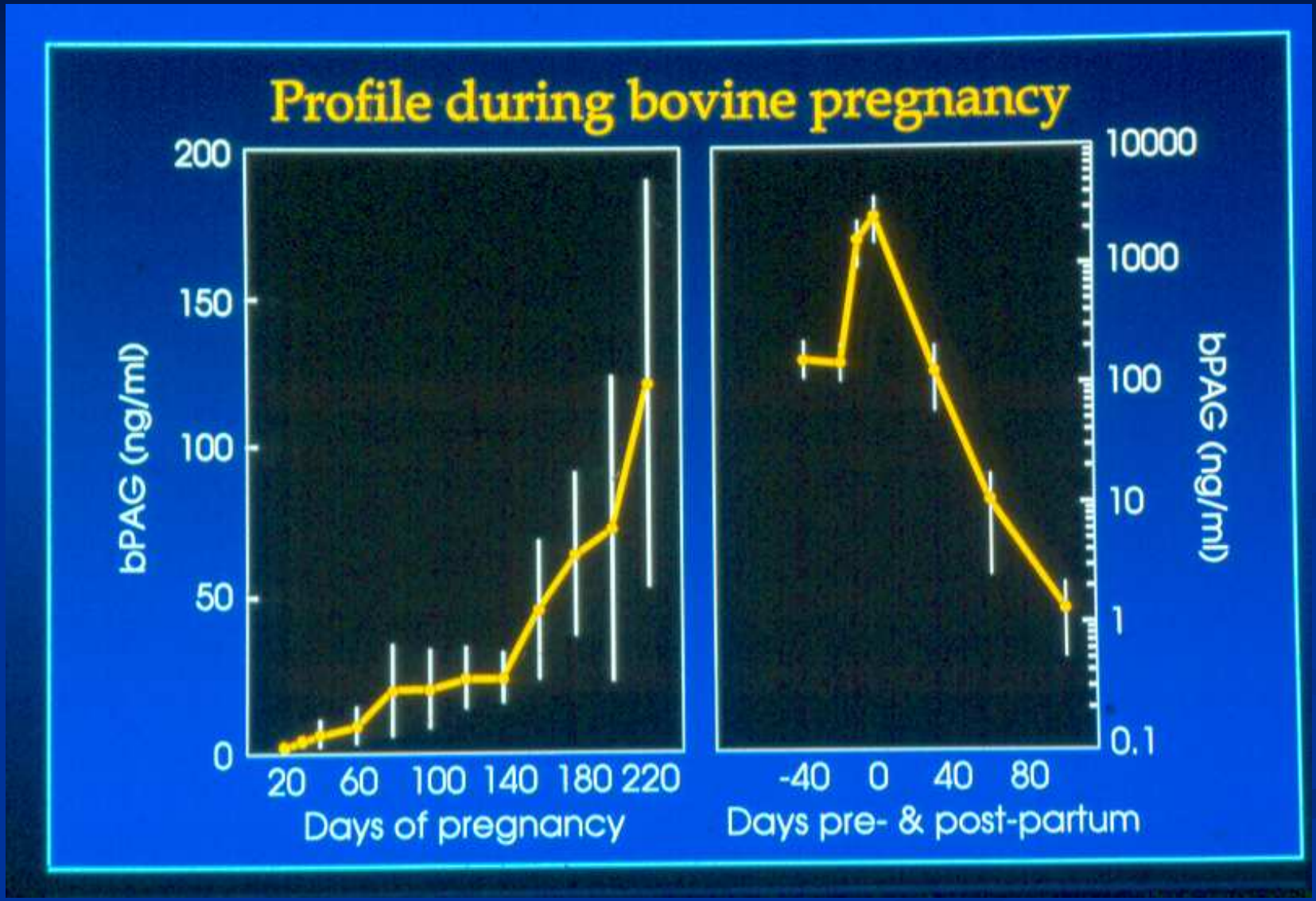
The native position of solvent is altered due to mutation around Asp

No catalytic activity



Xie et al.,1991

boPAG-1 concentrations on ongoing pregnancies



Zoli et al., 1991

Investigations on fetal pepsinogen

Pepsinogen F

- First characterized as a prenatal specific fetal pepsinogen expressed in rabbit stomach (Kageyama et al., 1990)
- A similar molecule was identified in rat stomach and in yolk sac and stomach of neonatal mouse (Chen et al., 2001)
- Closely associated molecule was identified in outer chorionic cell layer of the equine placenta (eqPAG) => its deduced aa sequence exhibited 69%, 65% and 66% identity with rabbit, rat and mouse pepsinogen F, respectively (Green et al., 1999)

Pepsinogen F

- Closely related cDNA were identified in placental tissue from cat and zebra (Gan et al., 1997)

- Recently, by using GNOMON-NCBI method, a 65% identical pepsinogen F-like molecule was predicted from genomic sequence from dog.

- In general, pepsinogens F identified in gastric mucosa more closely resemble PAG family members than other AP including pepsinogen A, progastricsin and cathepsin D

In summary…

Different families of **GASTRIC AP** were identified:

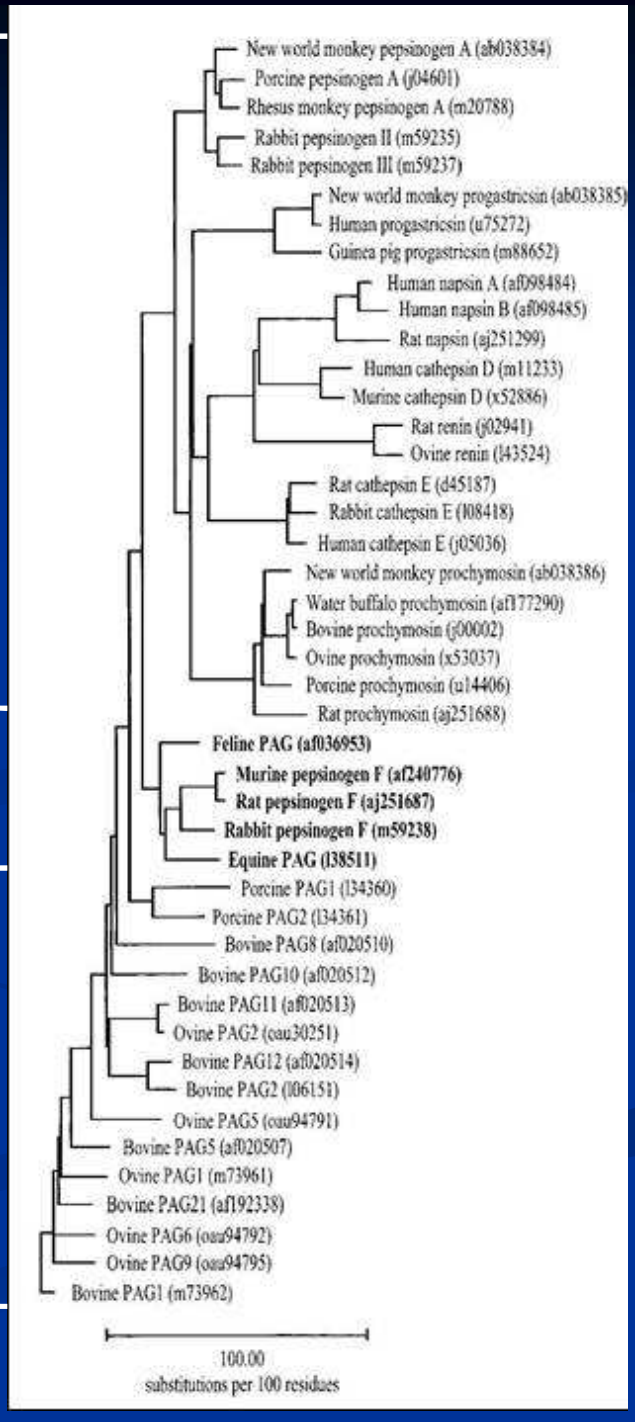
- Pepsinogens A
- Progastricsins (pepsinogens C)
- Prochymosins

Intermediate family between **GASTRIC** and **PLACENTAL AP**:

- Fetal pepsinogens (pepsinogen F)

Placental AP are represented the pregnancy-associated glycoproteins (PAG) subfamilies.

- PAG group - 1
- PAG group - 2



Phylogenetic tree illustrating the evolution of AP in domestic mammals. Adapted from Chen et al (2001).

Aim

The present research aimed to investigate the expression of new molecules from AP family in gastric mucosa of bovine fetuses

Gastric fetal mucosa

M & M

Early pregnancy-Fundic mucosa (D127)

Protein extraction

↓ ← PMSF+EDTA+NaN3+pepstatin →

20-70% A.S. precipitation

↓

DEAE Sephadex D 52

↓

Fraction 27-54

↓

Sephadex G-100

↓

Fraction 38-47

(Pool A)

↓

Mono Q anion exchanger

↓

Fraction 12-30

(Pool D)

Late pregnancy-Pyloric mucosa (D242)

Protein extraction

↓

20-70% A.S. precipitation

↓

DEAE Sephadex D 52

Fraction 155-163	Fraction 164-177
↓	↓
Sephadex G-100	Sephadex G-100
↓	↓
Fraction 21-30 (Pool B)	Fraction 38-54 (Pool C)

Early pregnancy-Fundic mucosa (D127)

Results

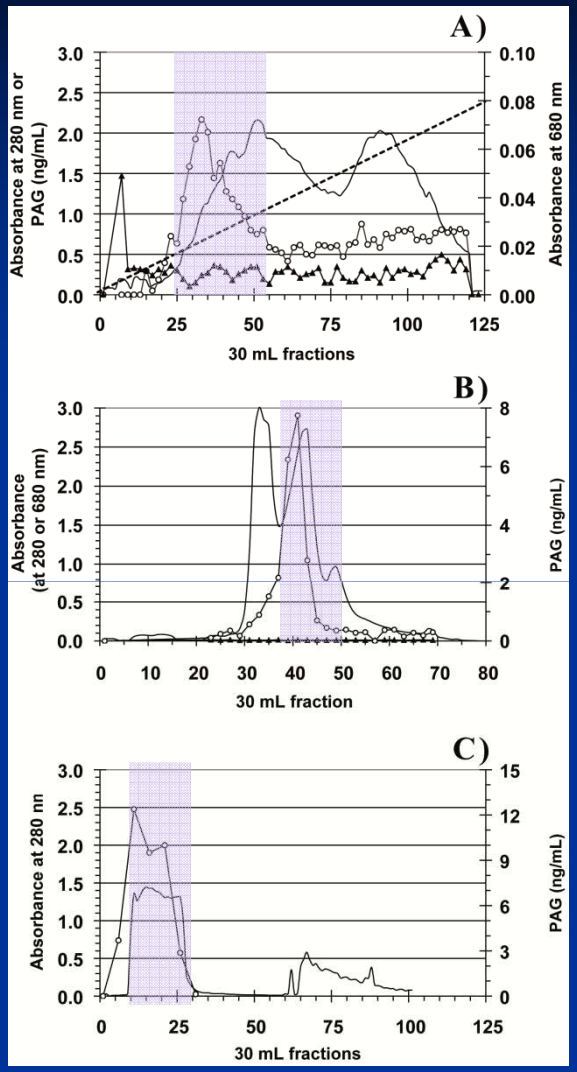


Figure 1. Elution profiles. A) DEAE anion exchanger; B) Sephadex G-100; C) Mono Q (cation exchanger)

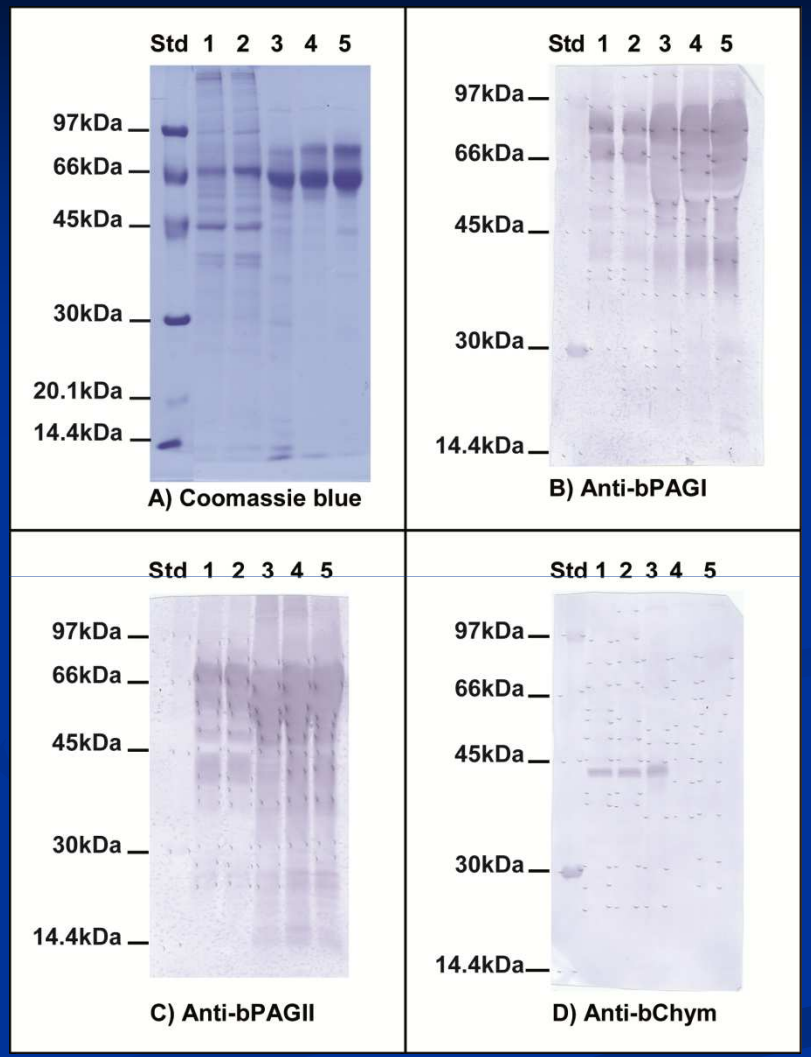


Figure 2. Lines: (1) Crude extract; (2) 20-70%AS; (3) DEAE 27-57; (4) Sephadex G100 (27-70); (5) Mono Q 38-47.

Late pregnancy-Pyloric mucosa (D242)

Results

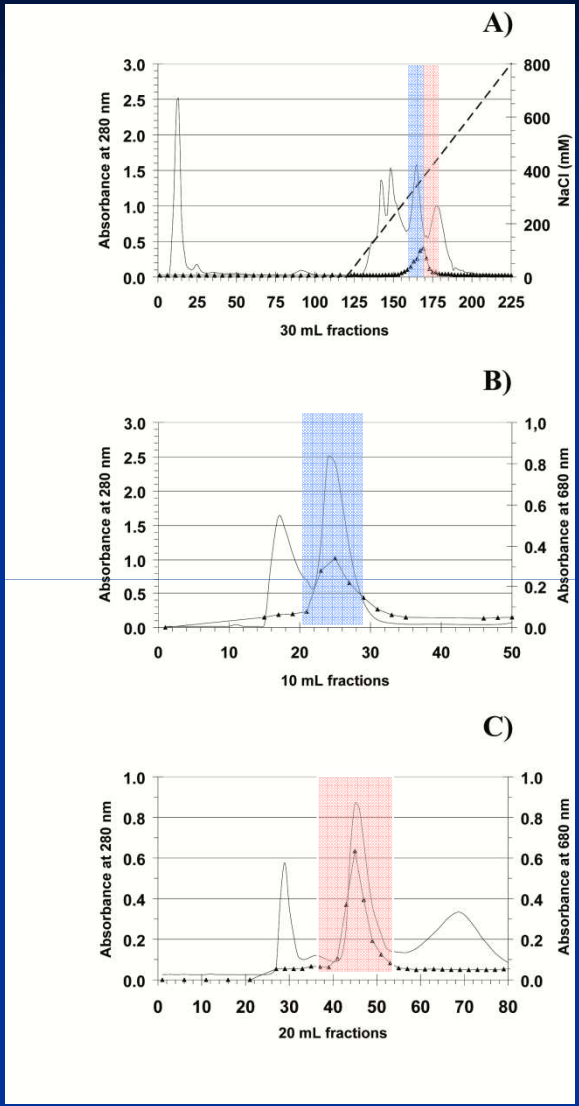


Figure 3. Elution profiles. A) DEAE anion exchanger; B) Sephadex G-100

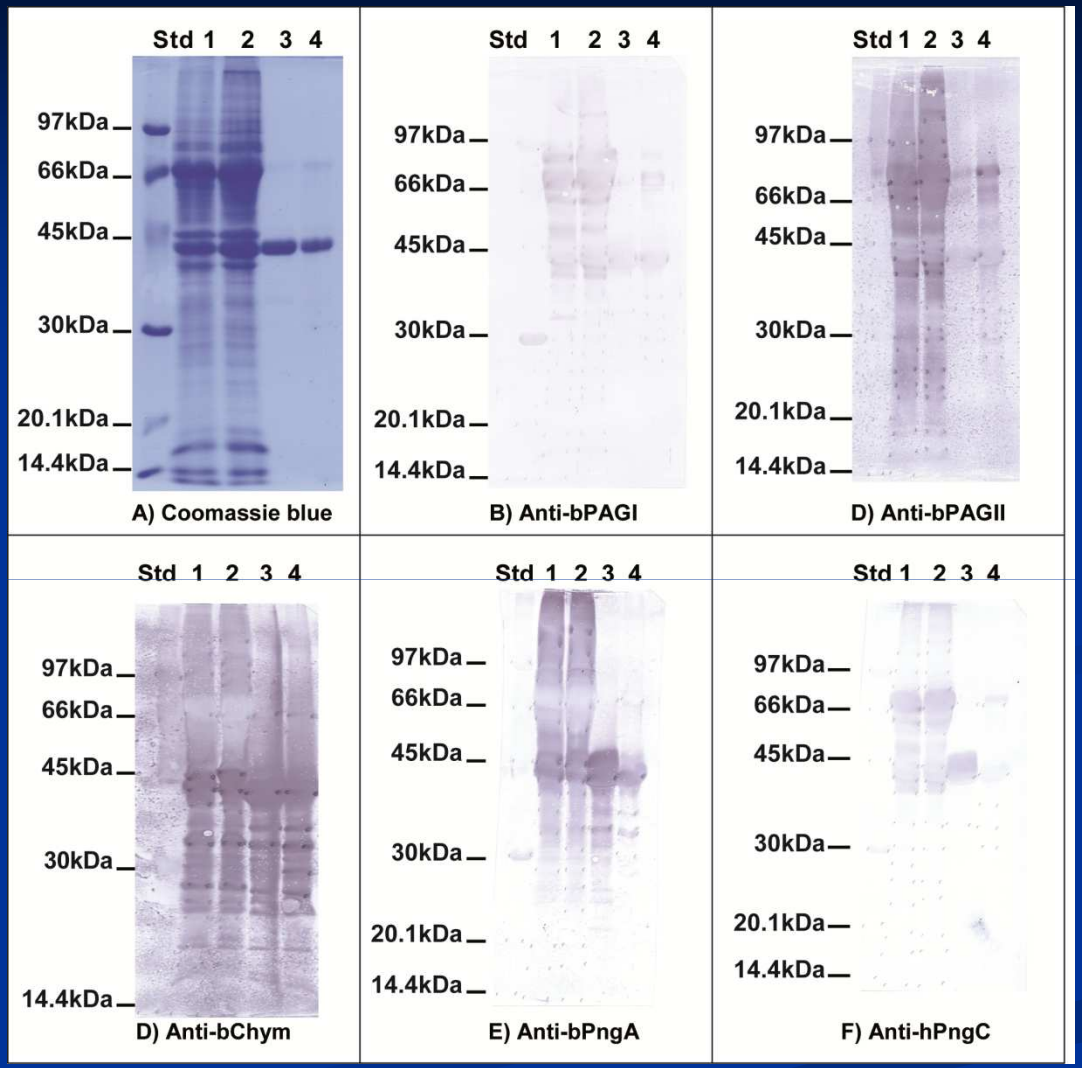


Figure 4. Lines: (1) Crude extract; (2) 20-70%AS; (3) Sephadex G100 (21-30); (4)Sephadex G100 (38-54)

Results

Table 1. Origin, molecular mass and comparison of major proteins isolated from bovine fetal mucosa with those described in databank.

Tissue	Origin	MM (kDa)	Sequence	Identity
Fundic mucosa	Pool D	63.7	IPLDQVAGYKIEALXDDPDT	Bovine Alpha-2-HS-glycoprotein or Asialofetuin (75% Id)
		65.4	GVLQGDAAQEME	Unknown protein
		72.5	DTHKSEIAHRFKDLGEEIFK	Bovine serum albumin (95% Id)
		83.4	DPEINVRSIT	Unknown protein
Pyloric mucosa	Pool B	41.5	AEITRIPLYKGKSLRKA	Bovine Prochymosin and chymosin (100% Id)
		65.4	ITDQSEIA	Unknown protein
			SPNKYWA	Unknown protein
	Pool C	41.5	AEITR	Bovine Prochymosin and chymosin (100% Id)

Conclusions…

- Our investigations allowed identifying a PAG-like immunoreactivity in extracts of stomach mucosa (fundic area) removed from fetuses in early pregnancy
- Prochymosin immunoreactivity was more abundant in pyloric area removed late in pregnancy
- Our data support the theory of switching of gene expression for aspartic proteinases during fetal, neonatal and adult phases

Acknowledgements

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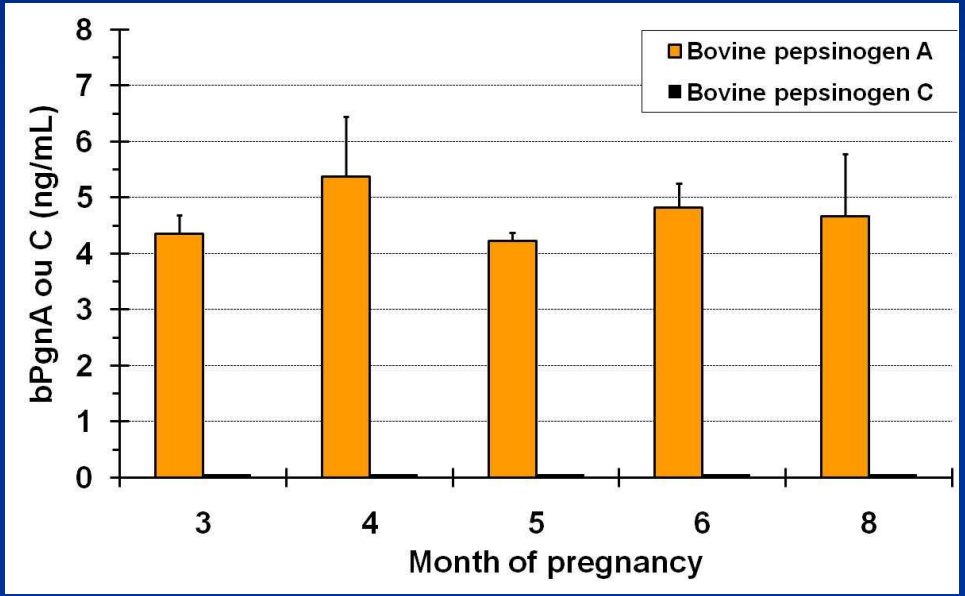
- **Mr Yves Colemonts (CER, Marloie Belgium)**
- **Mrs Nicole Otthiers (CIP, Ulg)**

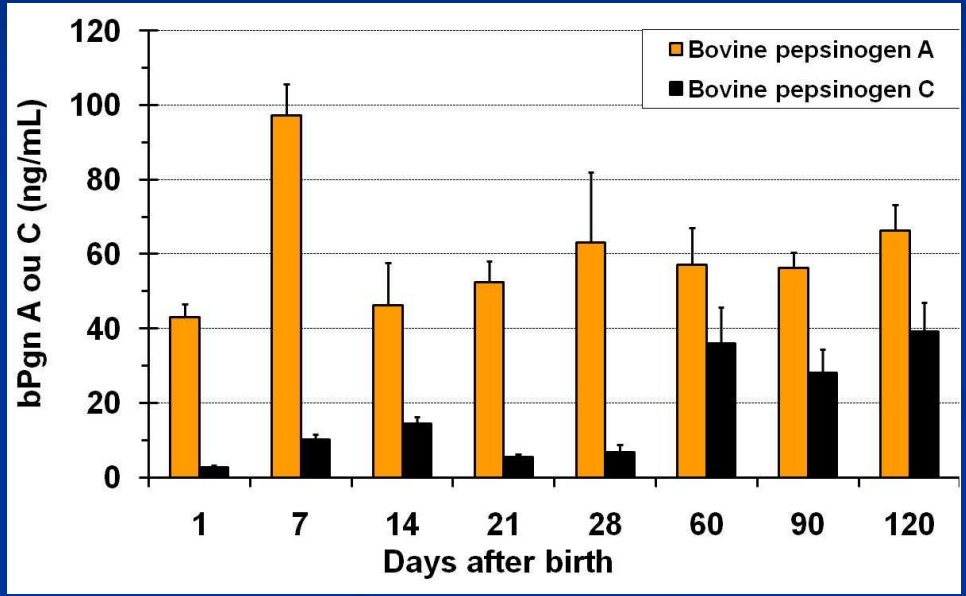
Thank you for your attention…

Concentrations of pepsinogens A and C in cattle

Foetuses



Calves



Mean concentration (± SD) of pepsinogen A and pepsinogen C measured in fetuses and calves