

# PREGNANCY-ASSOCIATED GLYCOPROTEINS ISOLATED FROM THE EWE PLACENTA REMOVED AT TWO DIFFERENT STAGES OF GESTATION

B. El Amiri<sup>1,2</sup>, B. Remy<sup>1</sup>, N.M. Sousa<sup>1</sup>, H. Banga-Mboko<sup>1</sup>, Zs. Perényi<sup>1</sup>, N. Gerardin-Othiers<sup>3</sup>, and J.F. Beckers<sup>1</sup>

<sup>1</sup> Physiology of Reproduction, Faculty of Veterinary Medicine, ULg, B41, B-4000, Liege, Belgium <sup>2</sup> INRA, CRRA Saïs et Moyen Atlas, Meknès, BP 578, Morocco  
<sup>3</sup> Laboratory of Biochemistry, Institute of Chemistry, ULg, B6, B-4000, Liege, Belgium

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## 1. INTRODUCTION

The Pregnancy-Associated Glycoproteins (PAGs) are belonging to a multigene family closely related to the aspartic proteinases

Some PAGs are released in maternal circulation during the whole pregnancy period. In veterinary practice, their measurements in maternal bloodstream are useful for both pregnancy confirmation and follow-up of the trophoblastic function

In the sheep, using biochemical approach, the PAGs were first identified under three names including SBU-3, oPSPB, and ovPAG

In 1993, Atkinson *et al.* used the SBU-3 (monoclonal antibody) and identified 3 binucleate cell-specific glycoproteins having different sequences:

IXPLRNTKHLVYLXNIHXGNPYQ (Q9TRE0)  
SXLTLPLRNMKDIFYRGTIGGP (Q9TRE1)  
QGSXVTILPLRNMKDIFYVGTITDTP (Q9TRE2)

In 1995, Willard *et al.* identified the ovine PSPB and they did not report any amino acid sequences

In 1997, Xie *et al.* identified four ovPAGs from ovine placenta:

RDSXVTILPLRNMKDIFYVGT (65 kDa)  
RGSXLXIHPLRNVKDVVYLYG (61 kDa)  
RGSXXRIHPLRNHRD (60 kDa)  
SQIXLTVSXLTXNL (55 kDa)

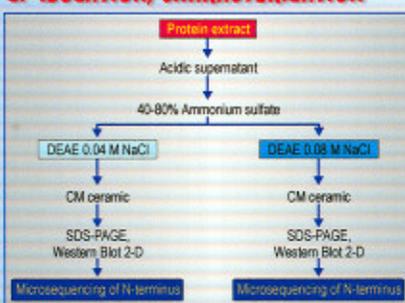
Recently, molecular biology studies identified 9 full cDNA sequences and suggested a variable spatio temporal expression of PAGs.

The biochemical approach brought interesting results confirming some data of Molecular biology and discovers for the first time novel PAG molecules

## 2. AIM

> The aim of our study was to identify and to characterize the PAGs isolated from placenta removed at two different stages of pregnancy.

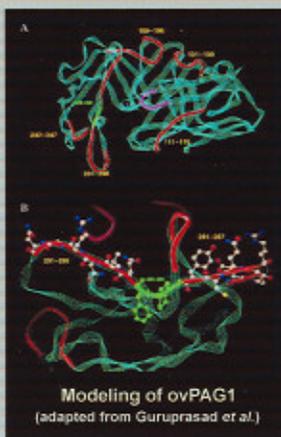
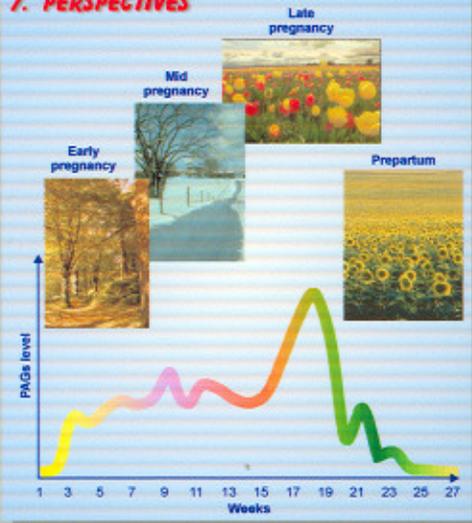
## 3. ISOLATION, CHARACTERIZATION



> Two different stages of pregnancy are used, placentas removed: at third trimester of gestation ( $\geq 100$  days) and from 66 to 100 days. The protein extract was first fractionated by acidic and ammonium sulfate precipitation. The 40-80% ammonium sulfate immunoreactive fraction was submitted to anion exchange chromatography (DEAE-cellulose). The fractions 0.04 M and 0.08 M NaCl were separately and directly submitted to the CM ceramic column (for the placentas removed at third trimester of gestation) or treated before by the gel filtration for the placentas removed from 66 to 100 days.

> The main spots transferred onto PVDF were submitted to the Edman degradation. The NH2 sequences obtained in this study were compared to each other and then to 7 known PAGs in sheep identified by purification or immunoprecipitation. To determine the identities two algorithms were used: the Pileup and the Olddistances.

## 7. PERSPECTIVES



## 4. RESULTS

Seven N-terminal sequences isolated from the ewe placenta are presented below, and compared to each other.

The aligned amino acid sequences of different ovPAGs identified in this study and their consensus

Gestation stages	Sequences
$\geq 100$ days	RGSNLTIHPLRNIRD ISSRVSLTIHPLRNIMDML RGSXLTIPLRNMRDIVY
From 66 to 100 days	RVSLTIHPLRNIIIDRYVG RDSNVTILPLRNMKD RGSNLTIHPLRNIRDIFYVGT RGSNLTIHPLRNITKDLVYLG
Consensus	R-S---I-PLRN-----

## 6. CONCLUSIONS

- > Our study shows that seven different molecules could be purified from sheep placenta. All were sequenced on the N-terminal part
- > Three of the isolated PAGs were never identified even by molecular biology studies
- > As placenta of two different ages were considered in our approach, our data confirm the temporal expression of the PAG and open the way for the development of new homologous RIA system in sheep

## 5. DISCUSSION

- > The sequences we obtained in this work are different from those isolated by Xie *et al.* and Atkinson *et al.*
- > Except the sequence (ISSRVSLTIHPLRNIMDML) which start by the isoleucine all sequences identified in this work started by Arg residue like in the most PAGs found in other species
- > The three PAGs isolated from the placentas ( $\geq 100$  days) were all novels
- > The PAGs isolated from the placentas from 66 to 100 days have all their mRNA defined
- > One molecule purified till homogeneity (RGSNLTIHPLRNITKDLVYLG)

Identities (%) of the sequences found in the present study to each other

Sequences	1	2	3	4	5	6	7
RGSNLTIHPLRNIRD	100	93	93	86	93	80	80
RGSNLTIHPLRNIRDIFYVGT		100	80	72	86	60	70
RGSNLTIHPLRNITKDLVYLG			100	83	87	65	70
RGSXLTIPLRNMRDIVY				100	93	72	61
RDSNVTILPLRNMKD					100	73	73
ISSRVSLTIHPLRNIMDML						100	65
RVSLTIHPLRNIIIDRYVG							100

Comparison of the sequences we found to these found by other groups (SBU-3 and ovPAGs)

Sequences	SBU-3			ovPAGs			
	57	62	69	55	60	61	65
RGSNLTIHPLRNIRD	56	69	60	33	73	87	87
ISSRVSLTIHPLRNIMDML	45	67	53	46	60	65	65
RGSXLTIPLRNMRDIVY	58	87	78	33	66	83	88
RVSLTIHPLRNIIIDRYVG	46	56	50	40	53	70	65
RDSNVTILPLRNMKD	67	77	80	33	68	80	93
RGSNLTIHPLRNIRDIFYVGT	43	61	60	26	73	80	76
RGSNLTIHPLRNITKDLVYLG	84	61	60	33	73	80	80

## REFERENCES

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- Willard *et al.*, 1995. *J Anim Sci*, 73, 960-966.
- Xie S *et al.*, 1997. *Proc. Natl. Acad. Sci.* 94:12809-12816