

The Pregnancy-Associated Glycoproteins:

Biochemical Aspects and Clinical Application for Pregnancy Follow-up

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History of pregnancy diagnosis

- **Antiquity** – Interest for pregnancy diagnosis
- **600 years before Christ ... Egyptians**



Urine of woman supposed pregnant

⇒ **Germination of grains**

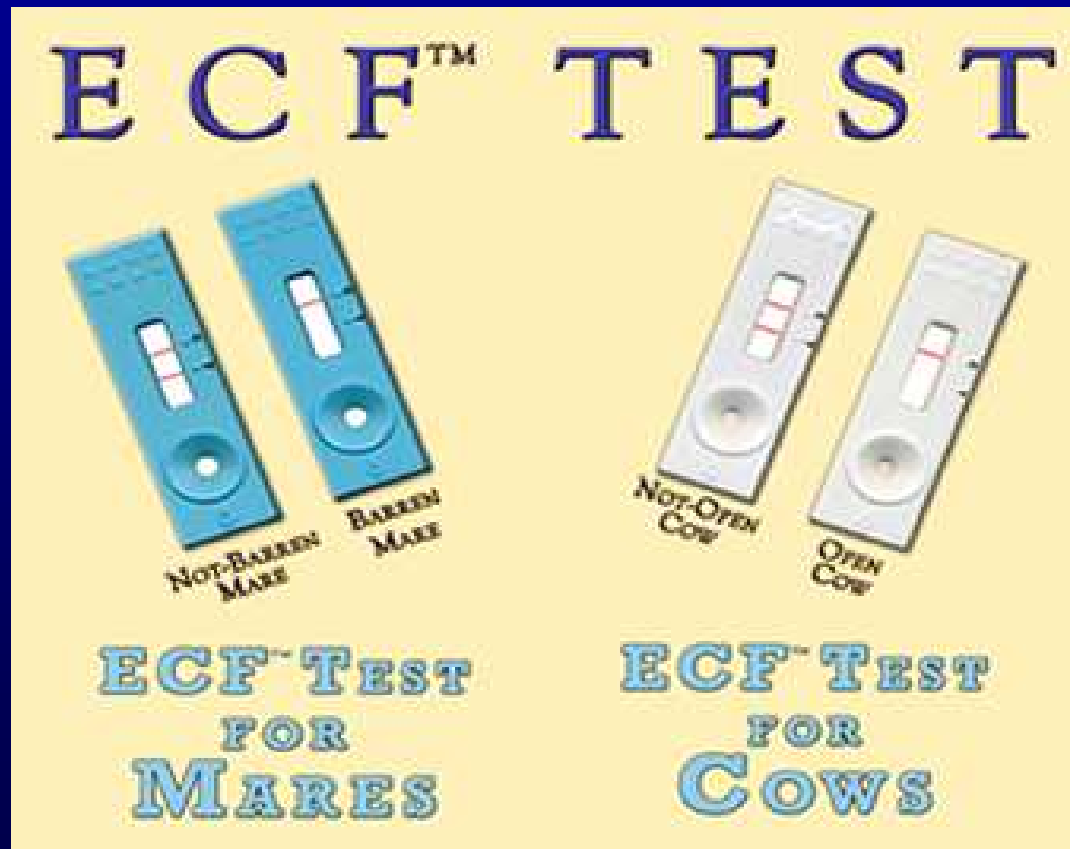
→ Fast process...

⇒ **Positive Diagnosis**

→ Slowly process...

⇒ **Negative diagnosis**

The Dream of the 21st Century



Reliability ?
Cost ?
Precocity ?

Introduction

- In human... hCG (PU)
- In horses... PMSG (eCG)
- In ruminants... no CG !

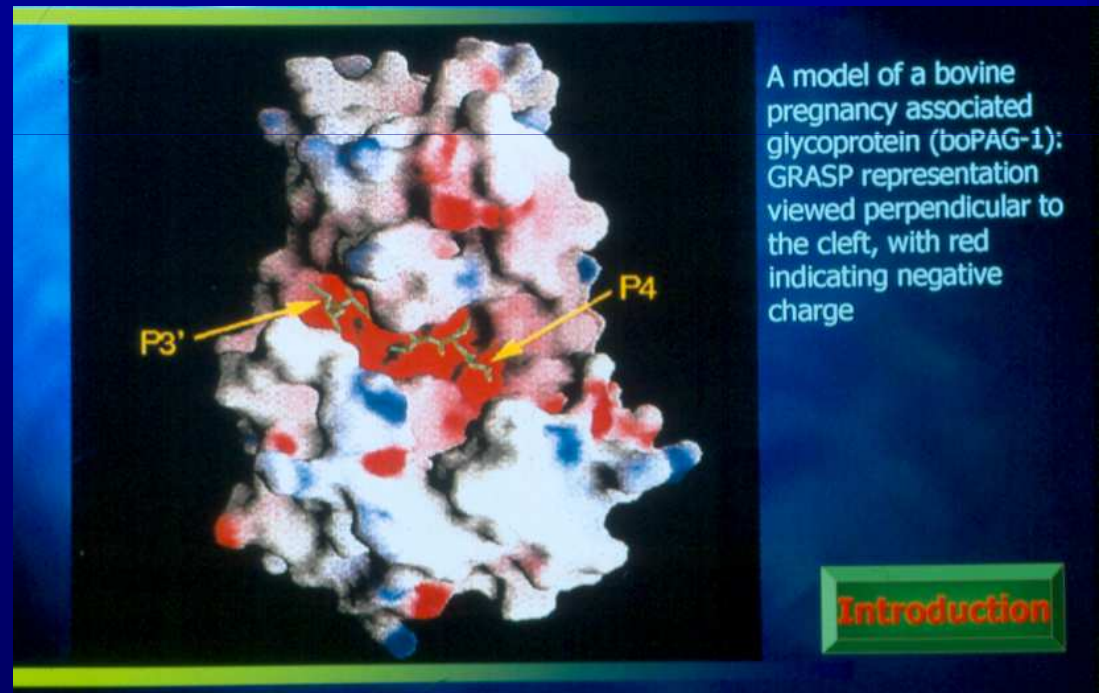
But in 1982, it became evident that some « (glyco)proteins » could be released in maternal blood and... used for pregnancy diagnosis.

PAG belong to Aspartic Proteinase gene family...

-No similarity with CG structure

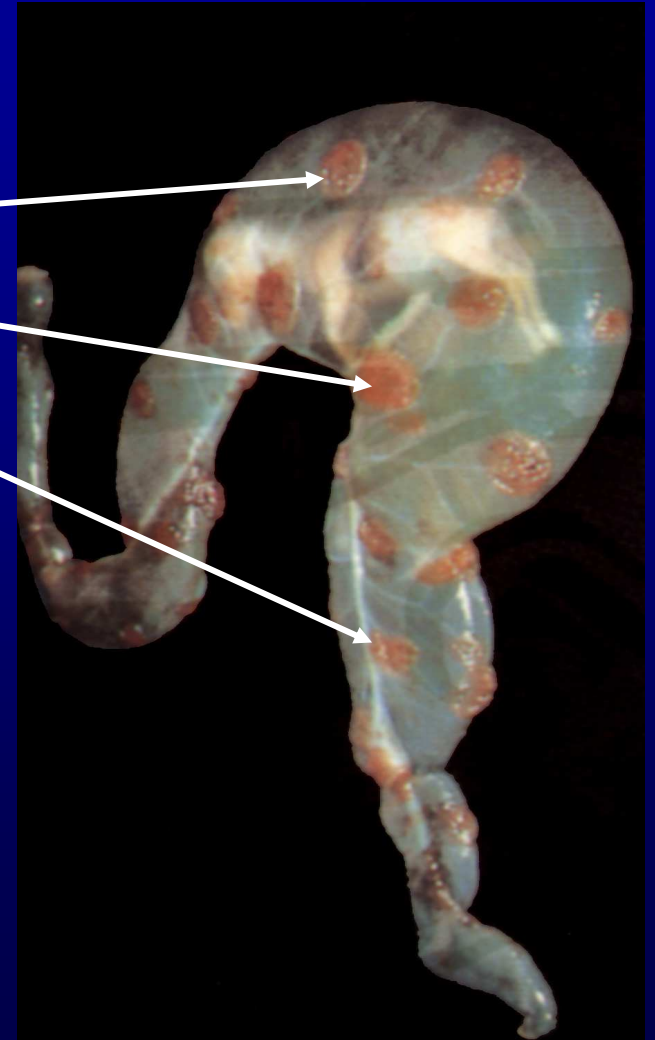
-Surprisingly ↑ 50% of similarity with pepsinogens, cathepsins D and E, ...

-No catalytic activity due to mutation in the binding cleft



Pregnancy- Associated Glycoproteins (PAGs)

**These glycoproteins
are synthesized
in the cotyledons
of ruminant
species**

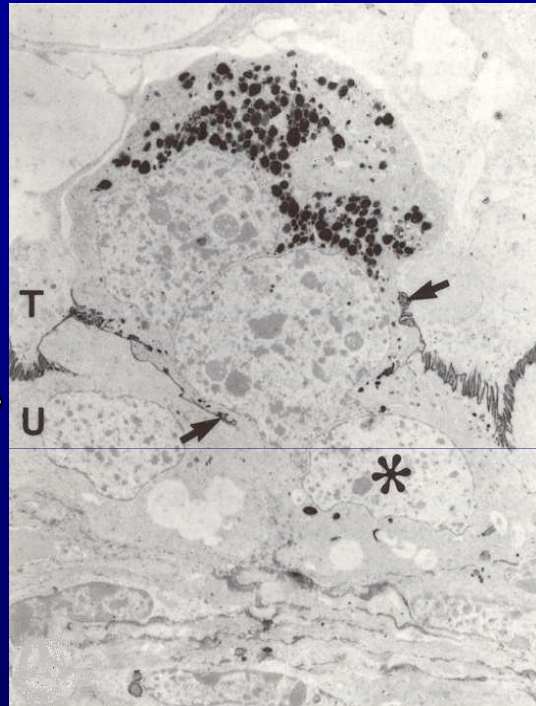


More precisely in the mono or binucleate cells of the trophoblast...

Migration of BNC till the microvillous junction



BNC fusion with a endometrial cell (*)



Trinucleate cell in the uterine epithelium

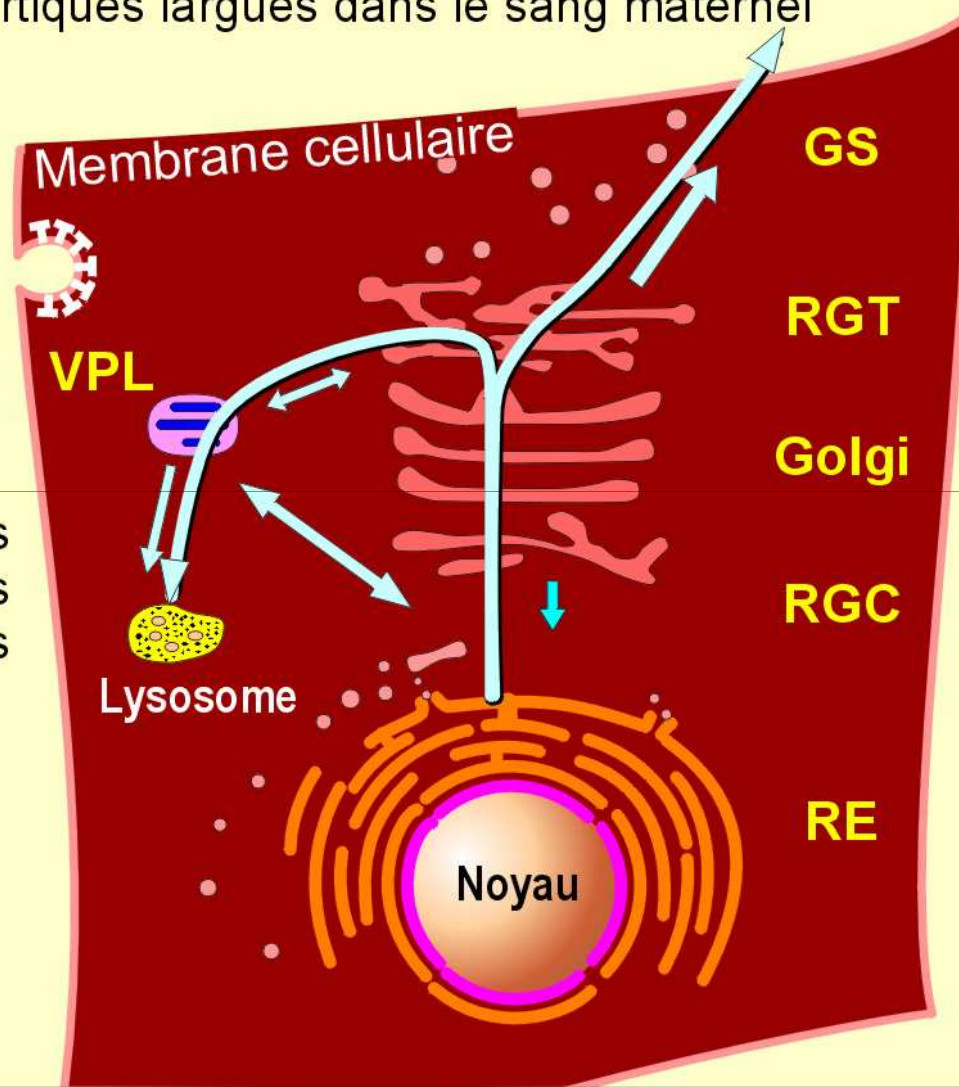


Migration of a binucleate cell in a cow placentome. **T**: trophoblast epithelium. **U**: uterine epithelium (Wooding & Beckers, *Cell Tissue Res*, 247:667).

And they are released in the maternal blood stream after migration and fusion with the maternal epithelial cells of the endometrium...

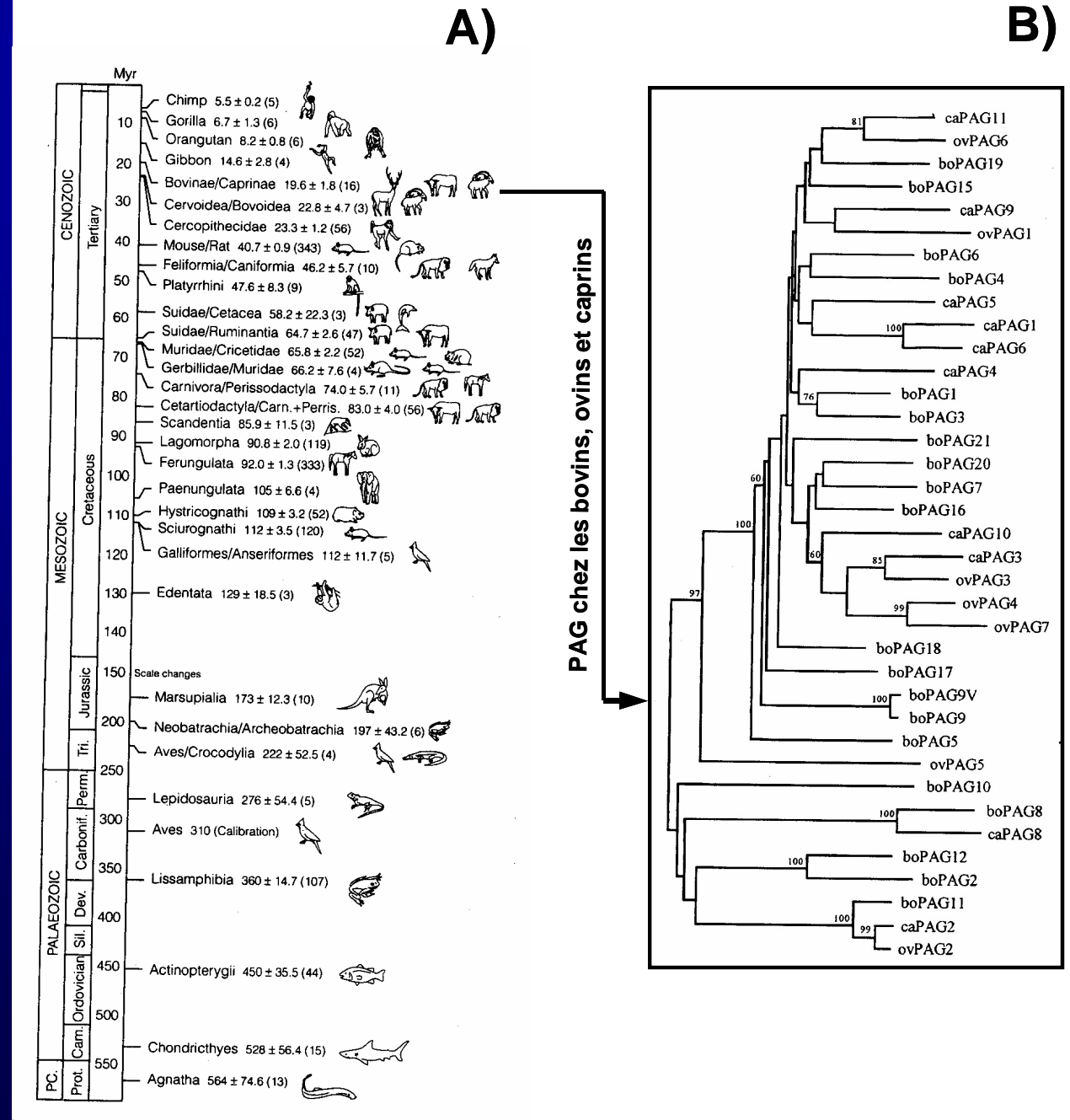
PAG, PSPB... membres de la famille des protéases aspartiques largués dans le sang maternel

Protéases actives impliquées dans les remaniements cellulaires



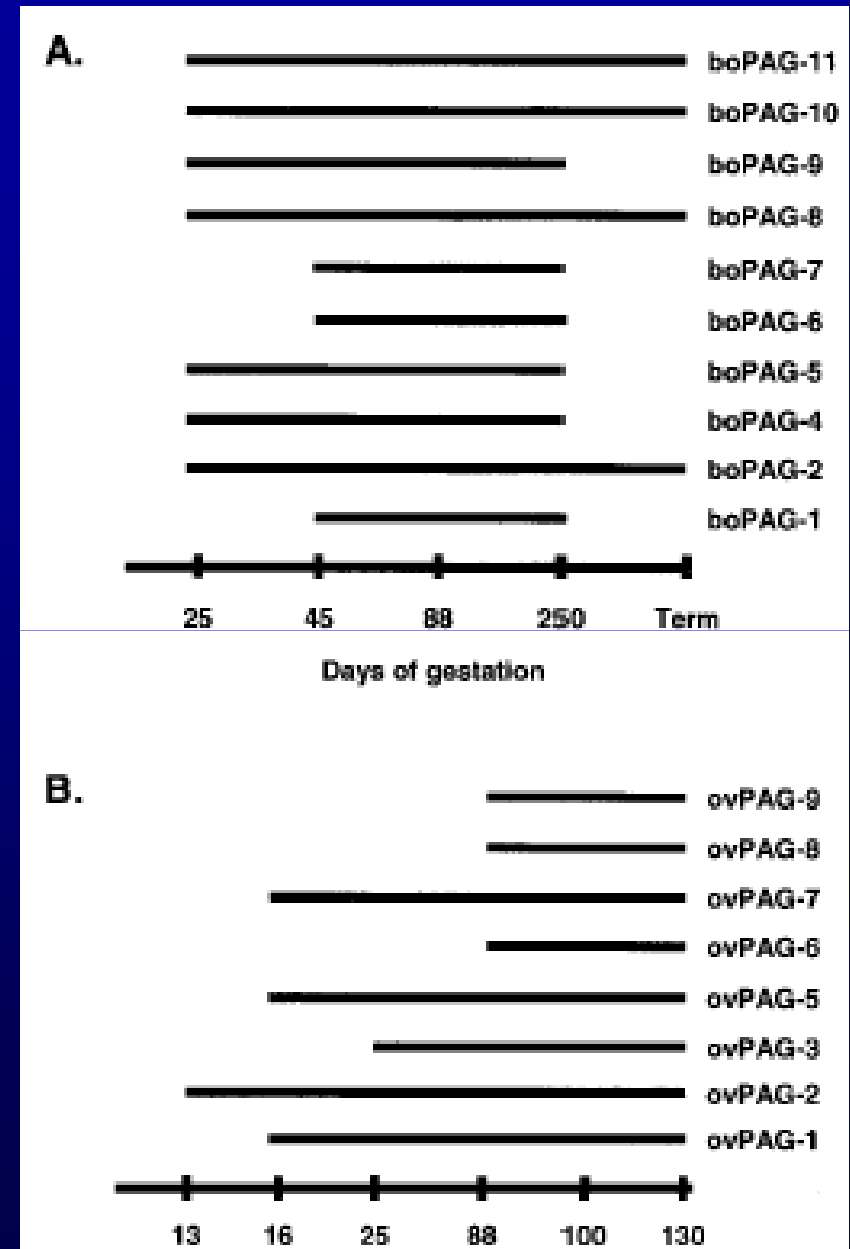
Panel of cDNA expression of PAG in ruminant species

Few of them could be purified from placenta tissue in bovine, ovine, caprine, bison and buffalo spp. (work in progress)

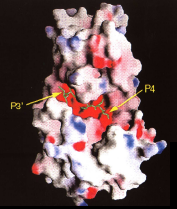


Temporal expression of PAG in bovine and ovine species

Green et al. (2000)



CHARACTERISTICS OF PAGs



boPAG-1

★ Stability of PAGs

- Long half-life *in vivo* (\approx 8.5 days)
- Stables *in vitro*

★ Not degraded by red blood cells (not necessary to centrifuge just after taking blood)

★ Blood samples can be kept at 4-25°C for 1-5 days before analysis → **Tubes can be sent by post !**

★ In milk, PAGs are only measurable in sheep and goat...
Unfortunately not in cows !!!

IMPORTANCE OF PAG RIA TESTS

Measurement of PAG concentrations in maternal blood permits:

 **Pregnancy confirmation**
(reproduction management)

 **Follow up the trophoblastic function**
(reproductive physiology, pathologic conditions affecting pregnancy)

Diagnosis of early pregnancy in cattle



Day of gestation	n	Se (%)	Sp (%)	PPV (%)	NPV (%)	Ref.
29-30	138	92.0	82.6	81.6	92.5	(1)
35	430	98.9	87.5	93.0	97.9	(2)
37-38	125	100	84.0	83.5	100	(1)

Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value

SP never reach 100% due to the embryonic mortality !

(1) Szenci et al., 1998; (2) Zoli et al., 1992

PAG RIA TESTS

★ Pregnancy diagnosis :

★ **RIA:** Belgium => 6,000 / year

France => 95,000 / year

Spain => 1,000 / year

Scotland => 1,000 / year

Perugia => ...now available

★ **ELISA:** Canada => 16,000 / year

Assay of PAG in research programs

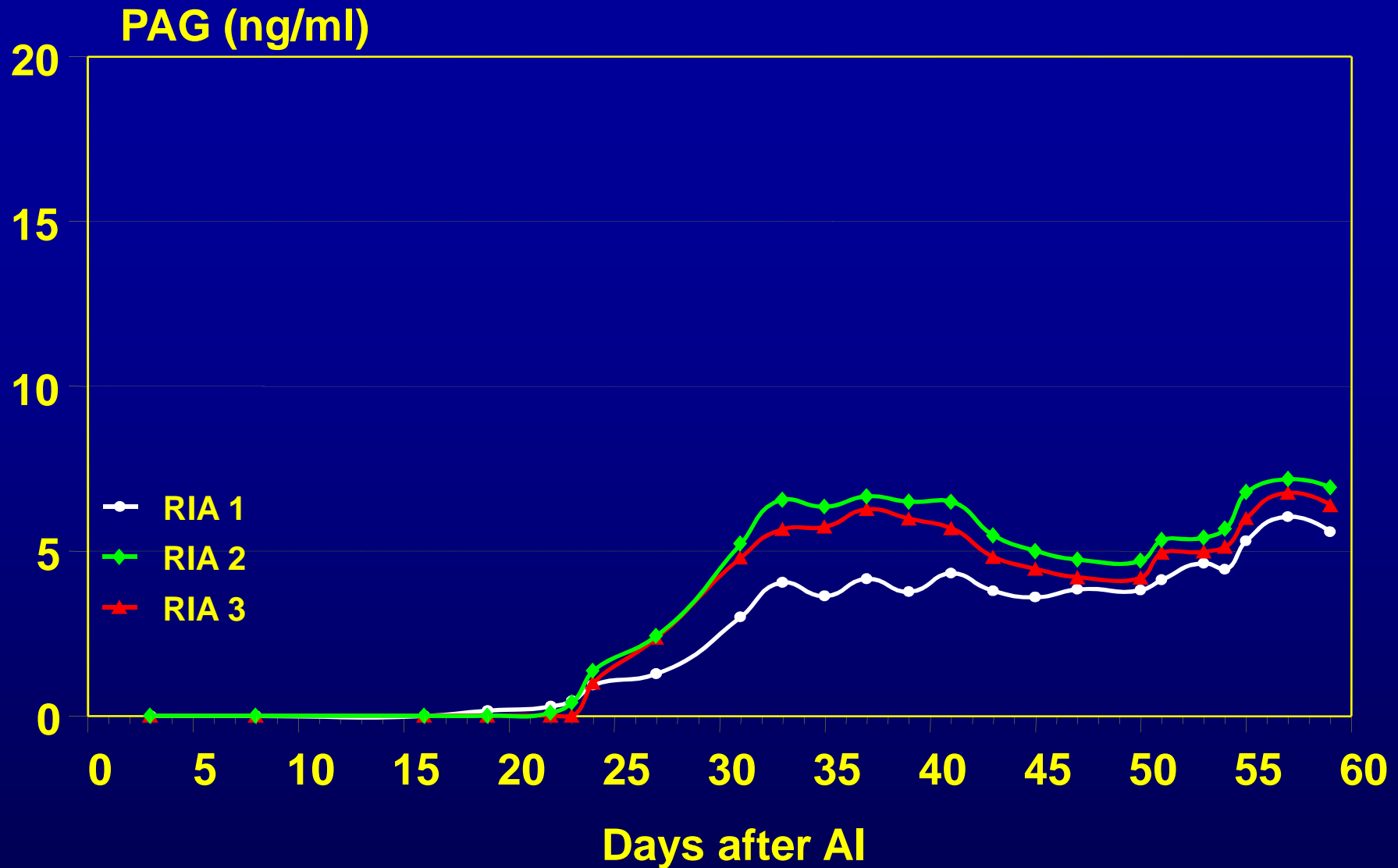


Studies on ongoing pregnancies

In the lab we try to improve the efficacy of the assay ...

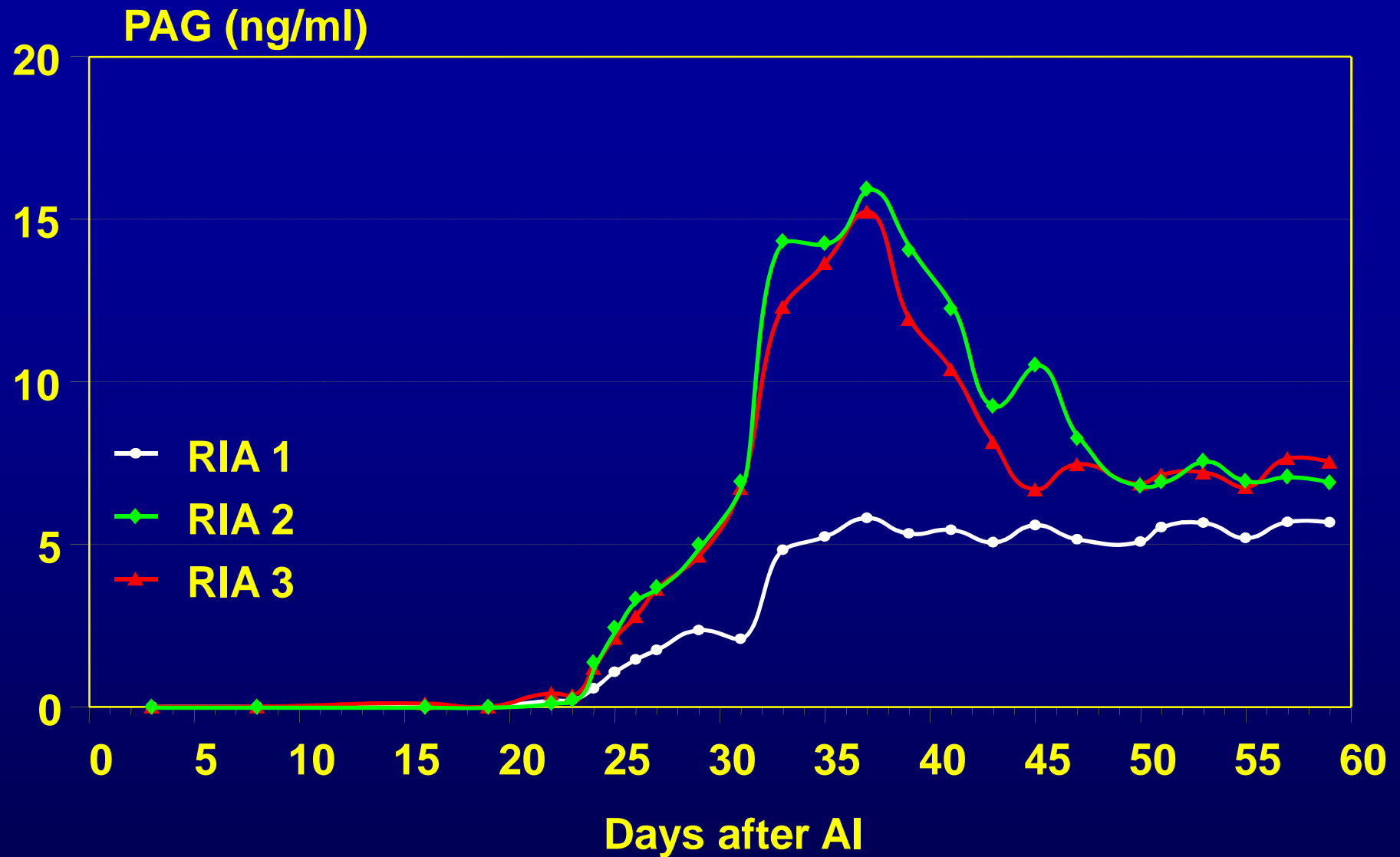
1st RIA : RIA 1 (AS 497) since 1992

2nd and 3rd RIA : RIA 2 (AS 706) and RIA 3 (AS 708) since 2000



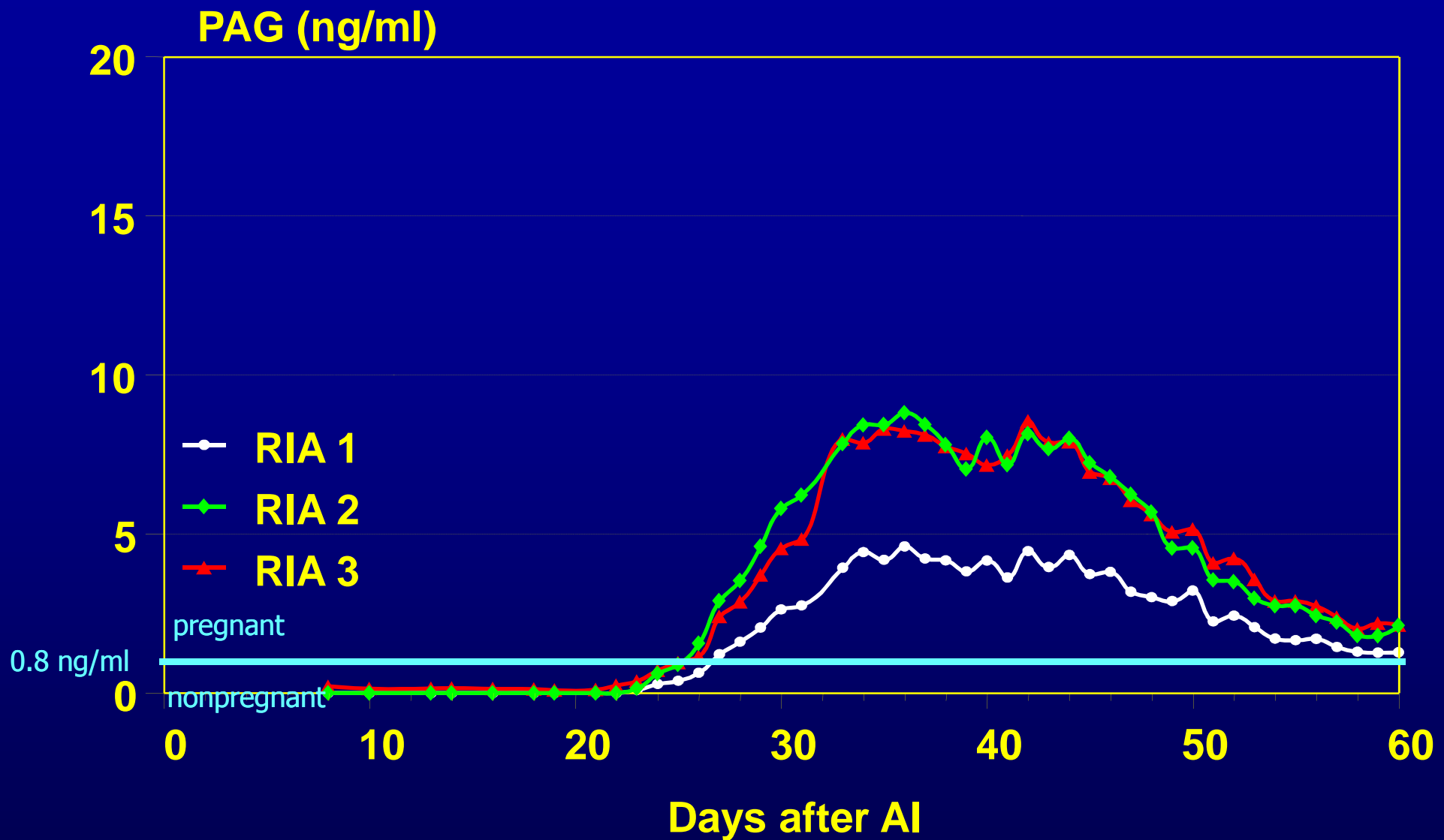
PAG profile of heifer n. 209 during the first 60 days after AI using 3 PAG-RIA systems. RIA 1 (AS 497), RIA 2 (AS 708) and RIA 3 (AS 706).

(Perenyi et al., 2002)



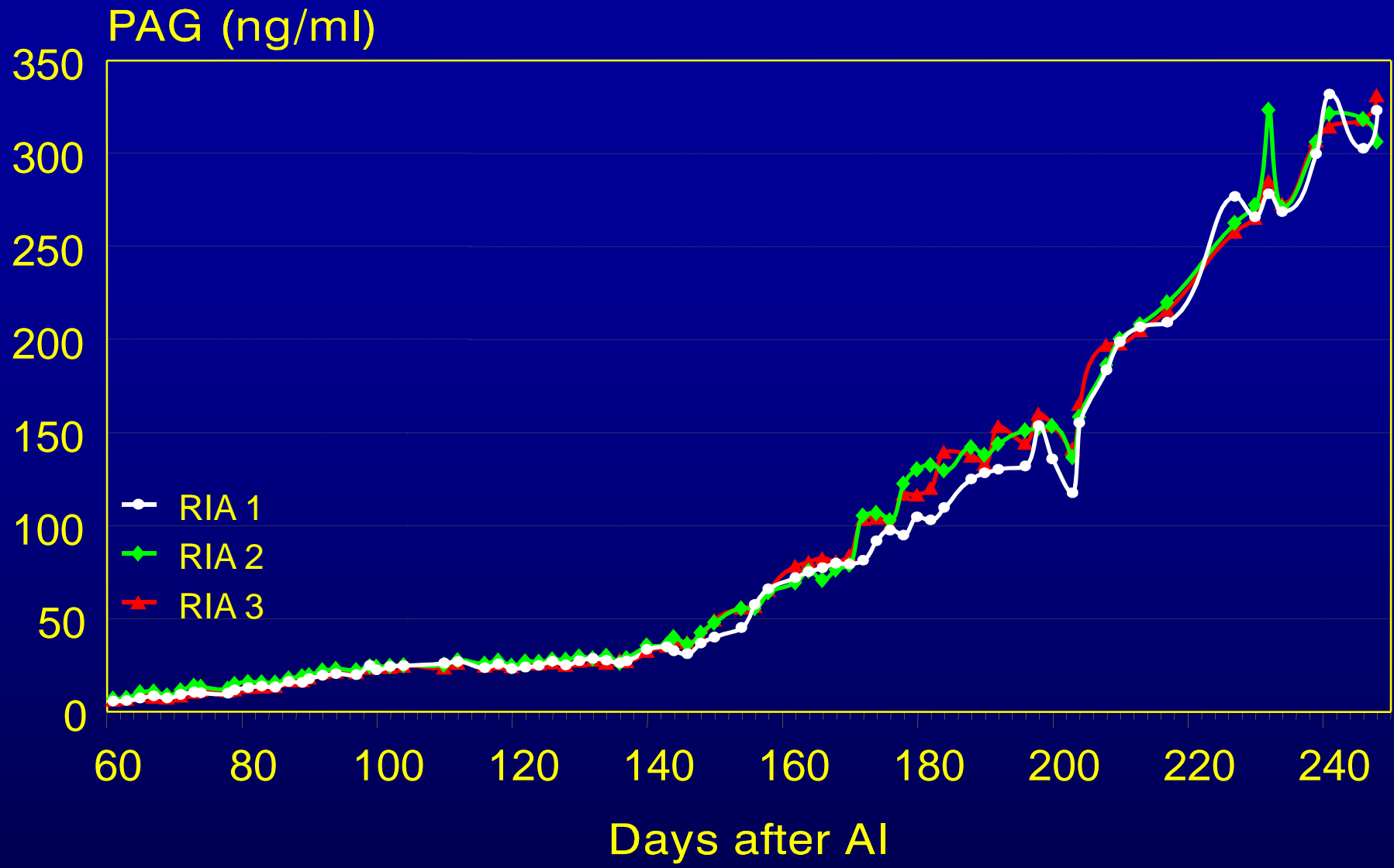
PAG profile of heifer n° 1654 during the first 60 days after AI using 3 PAG-RIA systems. RIA 1 (AS 497), RIA 2 (AS 708) and RIA 3 (AS 706).

(Perenyi et al., 2002)



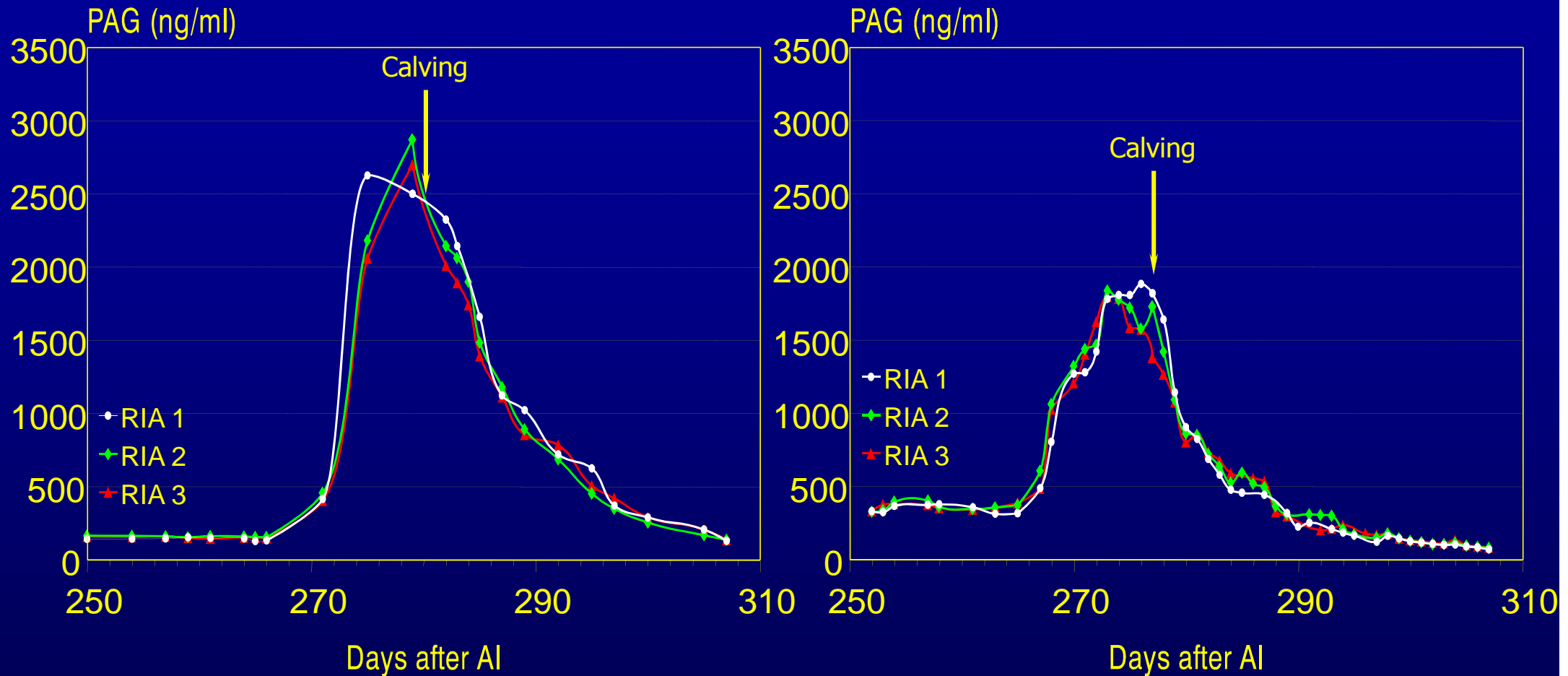
PAG profile of heifer n. 9447 during the first 60 days after AI using 3 PAG-RIA systems. RIA 1 (AS 497), RIA 2 (AS 708) and RIA 3 (AS 706).

(Perenyi et al., 2002)



PAG profiles of heifer n. 209 during 60-250 days of pregnancy using 3 PAG-RIA systems. RIA 1 (AS 497), RIA 2 (AS 708) and RIA 3 (AS 706).

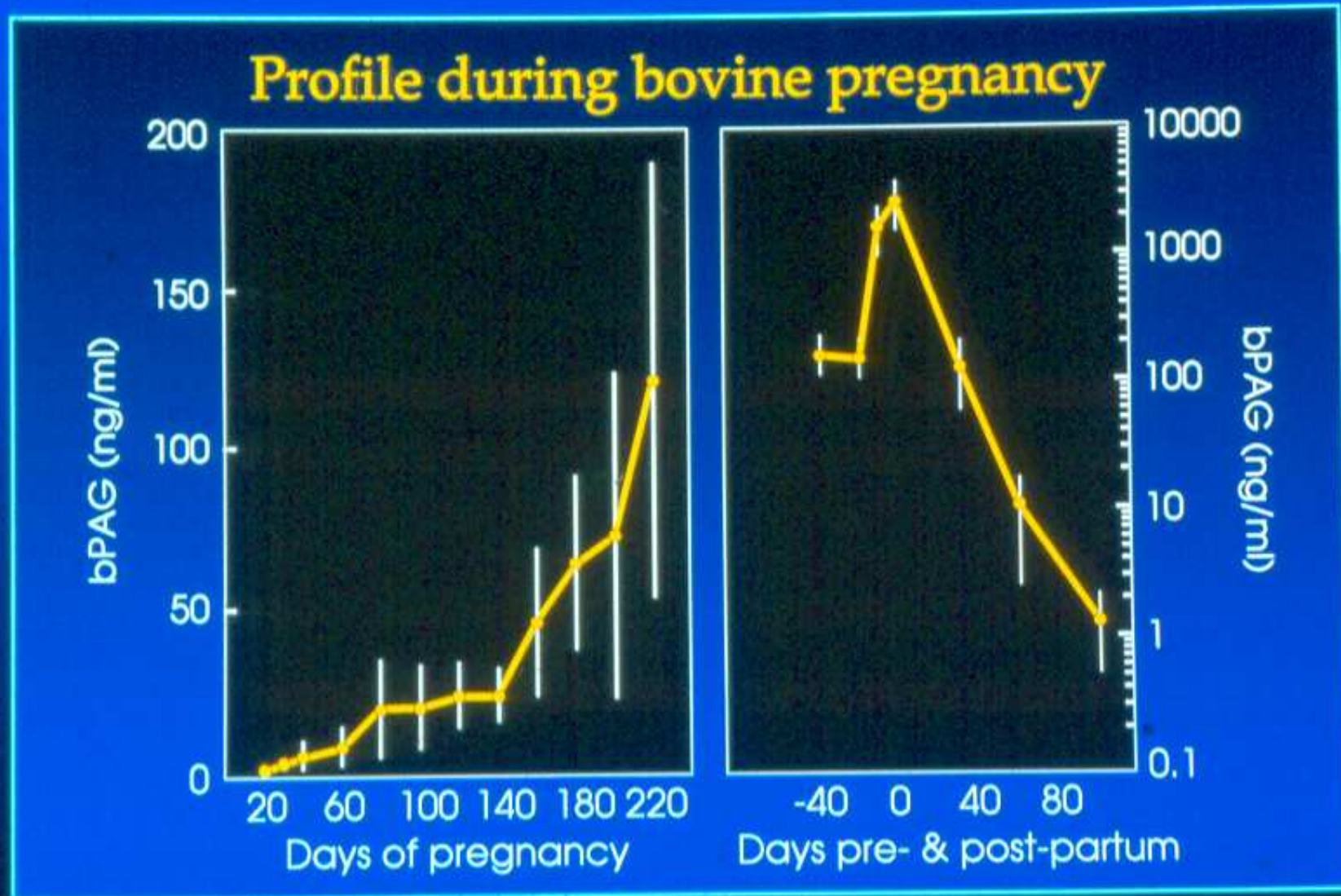
(Perenyi, 2002)



PAG profiles of heifers n. 209 and 579 after day 250 using 3 PAG-RIA systems.
 RIA 1 (AS 497), RIA 2 (AS 708) and RIA 3 (AS 706).

(Perenyi, 2002)

PAG on ongoing pregnancies



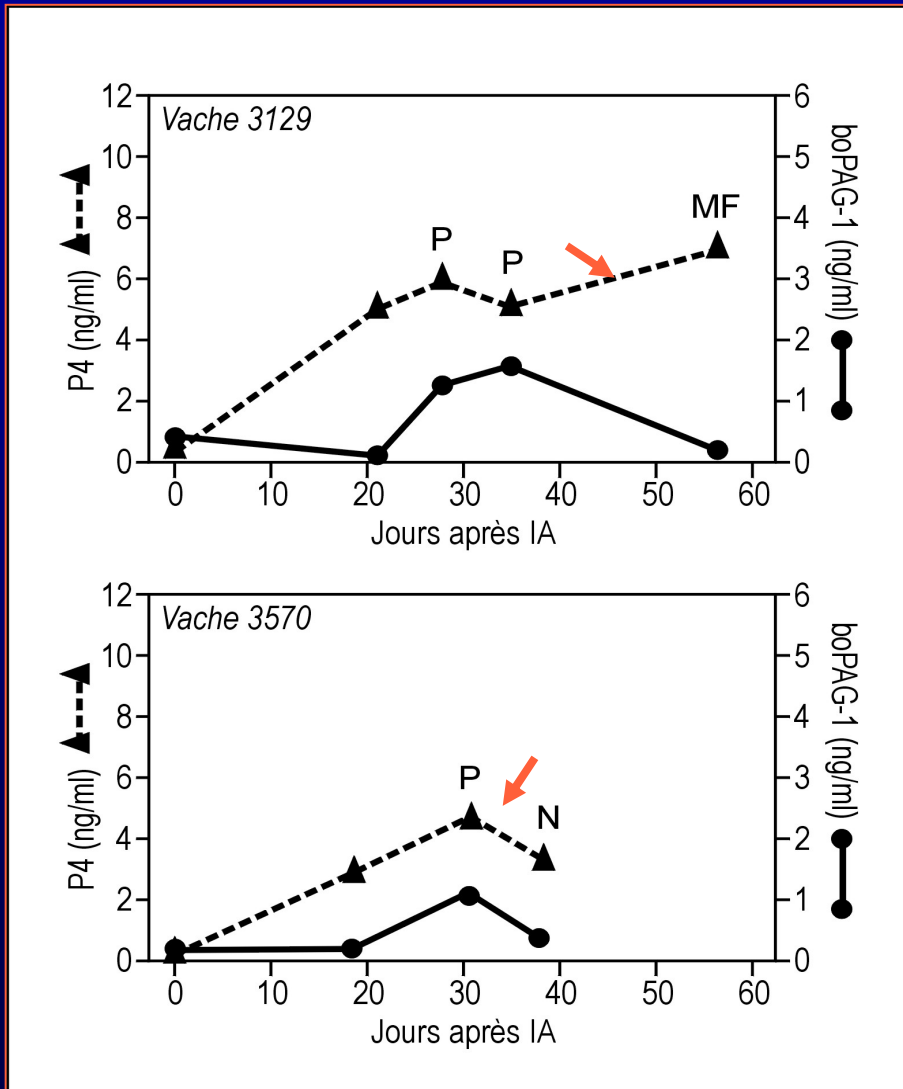
Zoli et al., 1991

Assay of PAG ...



Studies on embryonic or fetal mortalities

Embryonic or fetal mortalities

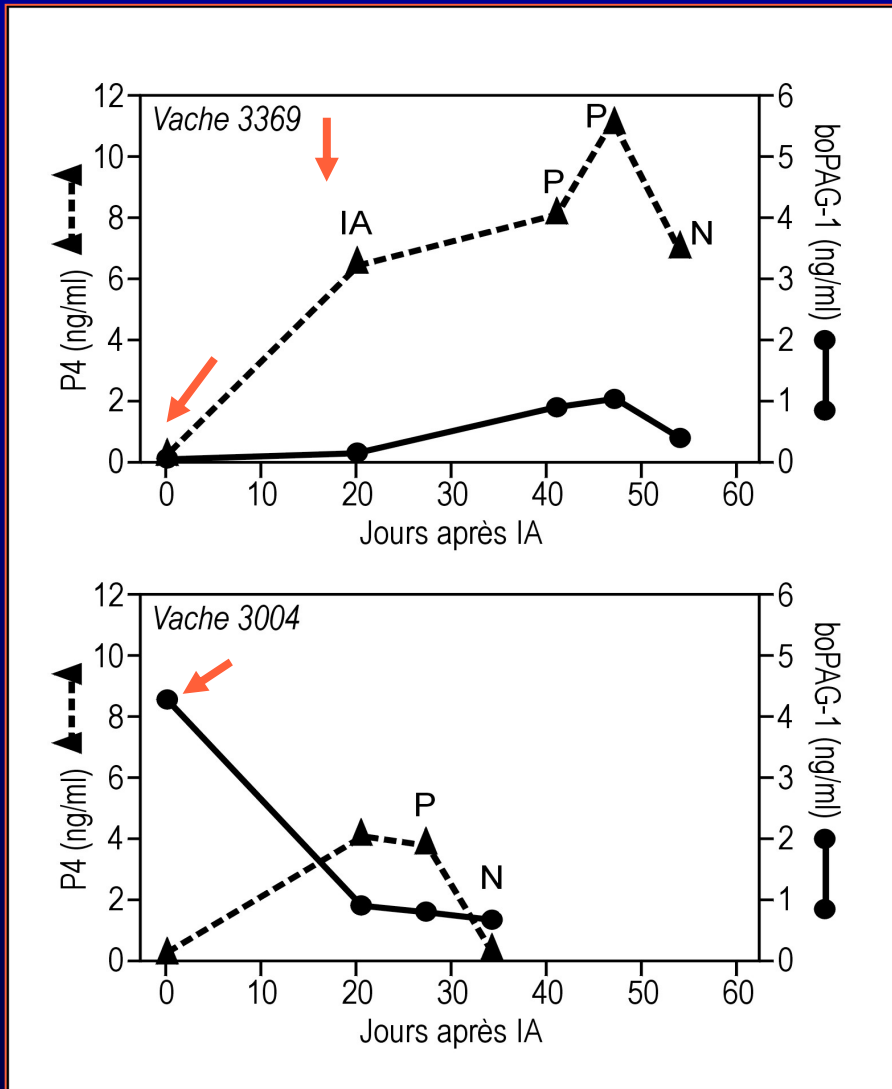


➔ **Cow 3129 – Fetal mortality with a functional corpus luteum (↑ P4 conc.)**

➔ **Cow 3570 – Early embryonic mortality with ↓ in concentrations of P4**

Plasmatic profiles of PAG and P4 associated to positive (P) or negative (N) ultrasound pregnancy diagnosis

Embryonic or fetal mortalities



➔ Cow 3369 – Embryonic mortality after a second AI

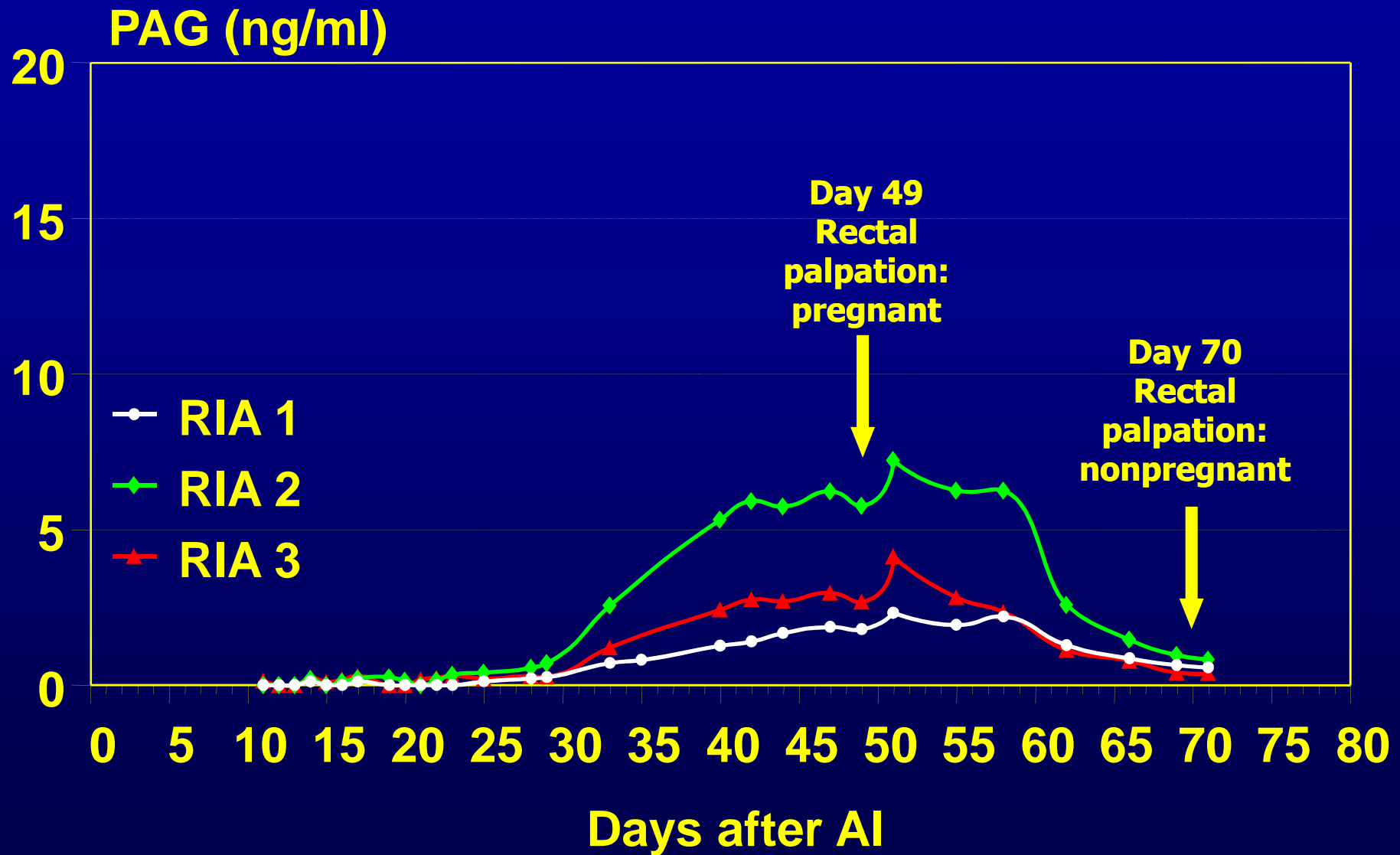
➔ Cow 3004 – Early embryonic mortality during the postpartum period

Plasmatic profiles of PAG and P4 associated to positive (P) or negative (N) ultrasound pregnancy diagnosis

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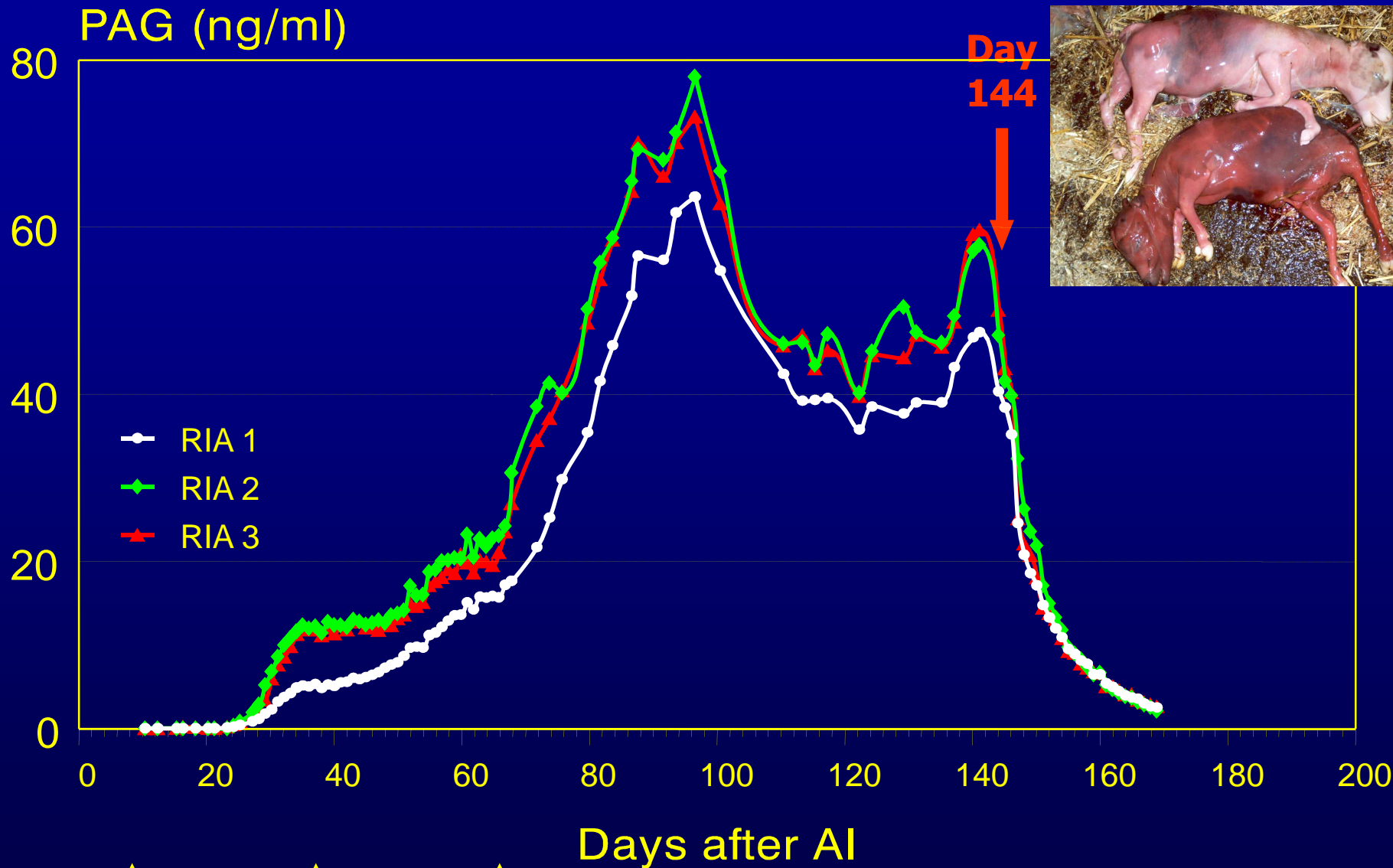
Szenci et al. 1998

PAG concentrations in animals with spontaneous pregnancy failure



(Perenyi, 2002)

PAG concentrations after fetal mortality due to a *Neospora caninum* infection (Heifer 8368)



Neospora
diagnosis

↑
-

↑
-

↑
-

↑
-

↑
+

↑
+

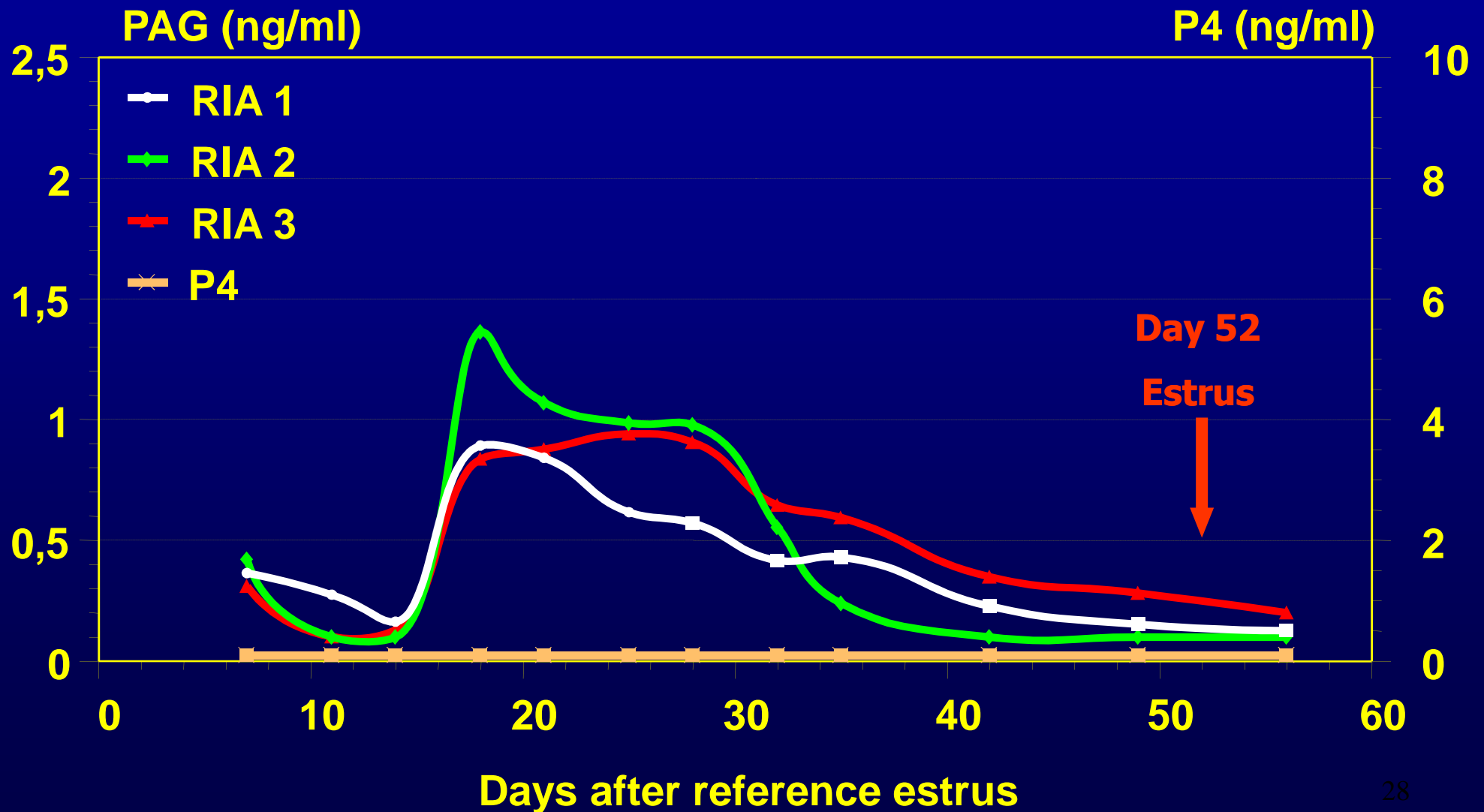
(Zs. Perenyi in cooperation
with Ch. Rettigner; 2002)

Assay of PAG ...



Embryo transfer during anoestrus...

PAG concentrations after embryo transfer during anoestrus

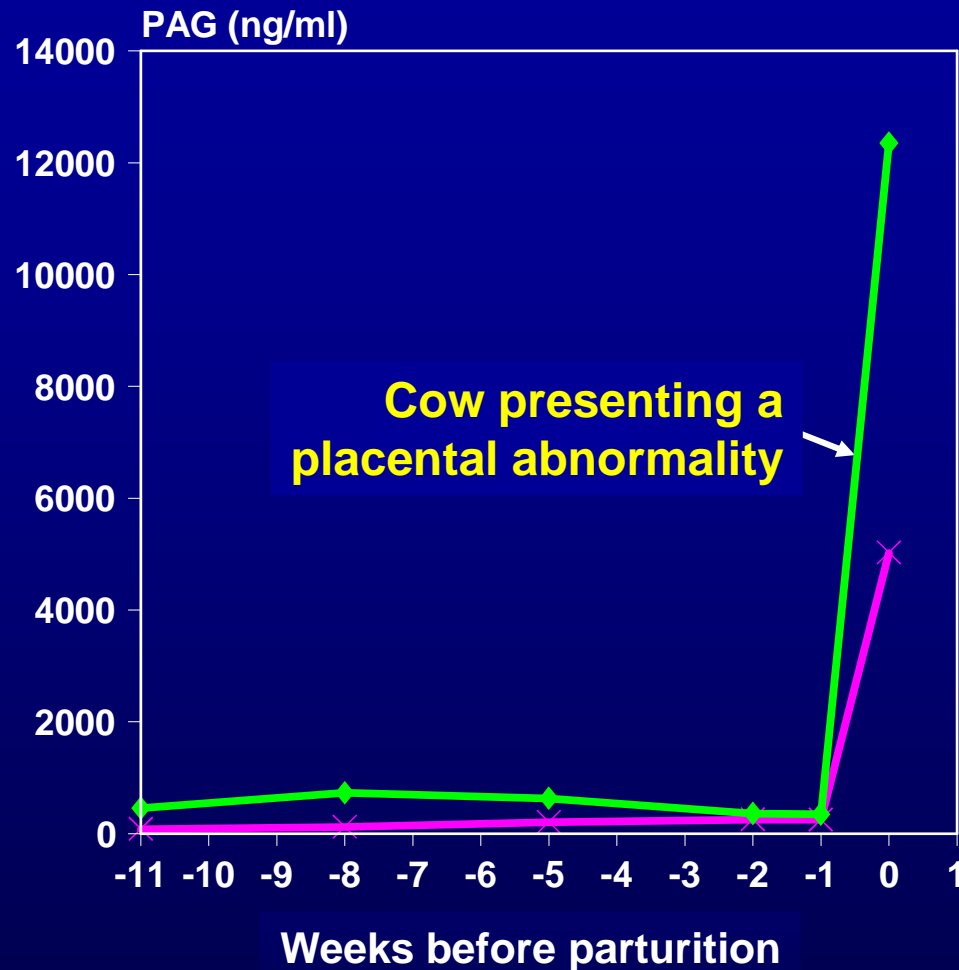


Assay of PAG ...



After clonage ...

PAG concentrations after clonage



Comparison of PAG concentrations in cows presenting a normal gestation and in one cow presenting a placental abnormality in addition to a normal fetus.

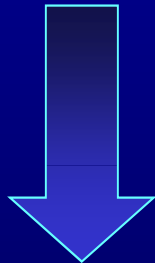
Assay of PAG ...



Possible effect of under-nutrition ...

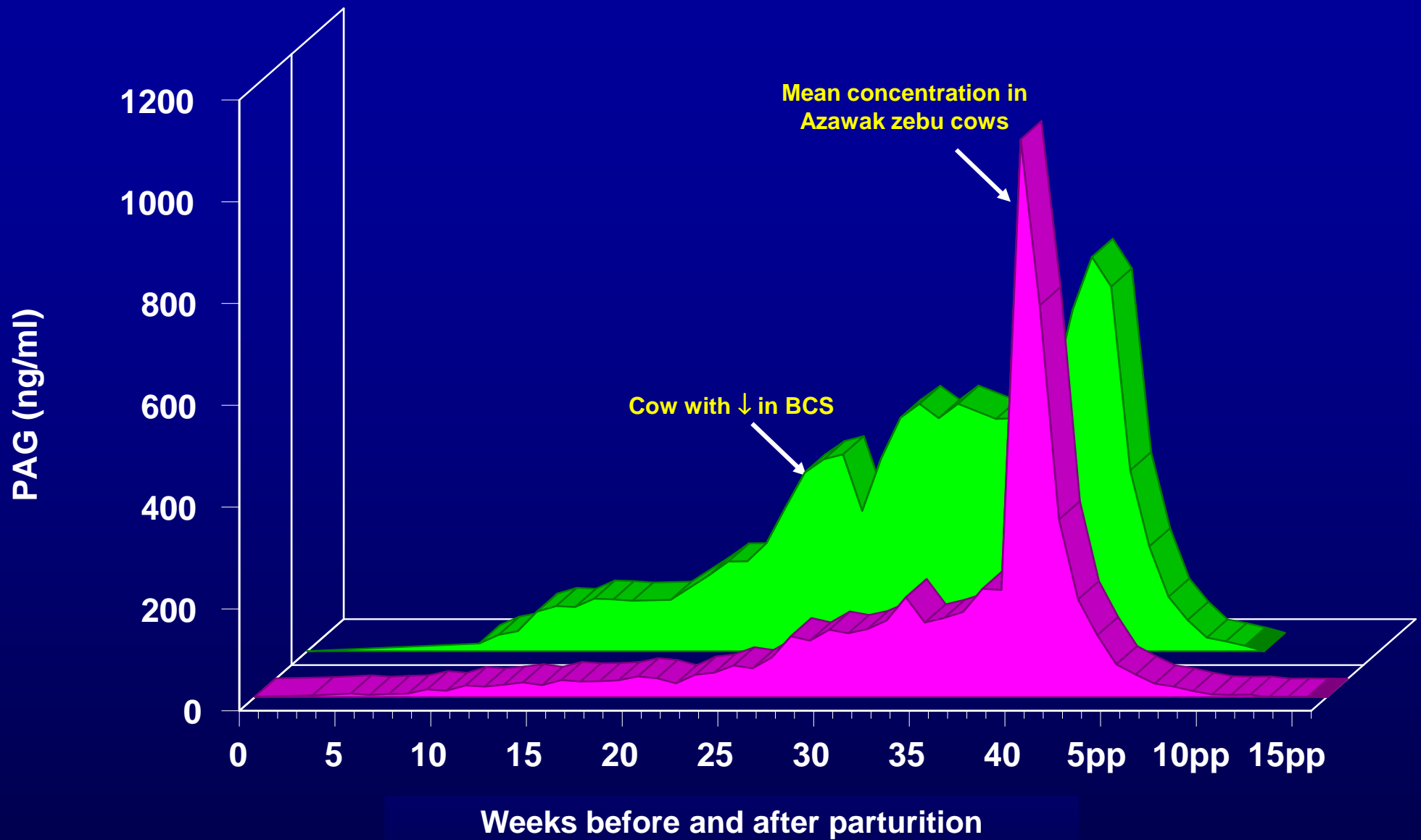
Animaux ...

- 12 Azawak zebu cows
- BCS 4-5



**Blood sampling from the 8th week
of pregnancy till the 10th week
postpartum**





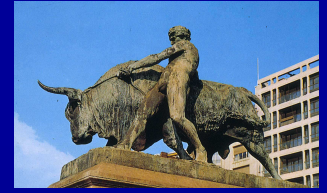
PAG concentrations in a Azawak zebu cow with a degradation of BCS.

New investigations in Buffalo...

Collaboration with Perugia (Prof. A. Debenedetti and Dr. O. Barbato)...

Preliminary studies:

- PAG concentrations higher than in cattle (9.0 ± 4.0 ng/ml) at Day 30 of pregnancy**
- PAG RIA: suitable for pregnancy diagnosis in this species and other ruminant species**



In summary ...

With PAG RIA ...

The concentrations of Pregnancy-Associated Glycoproteins can help...

Farmers... - For pregnancy diagnosis in cow, sheep, goat and buffalo ...
- Isolated or in conjunction with other sero-diagnosis:
brucellosis, leucosis, IBR, BVD, Visna Maedi, CAEV.

Veterinarians... - When developing rectal palpation or ultrasound investigations:
- in order to confirm a diagnosis...
- in pregnancy with mummified fetus...

Researchers... - When studying the embryonic or fetal mortality, the consequence of diseases, of nutrition, ...

Pregnancy diagnosis

Clinical or RIA

Clinical or RIA

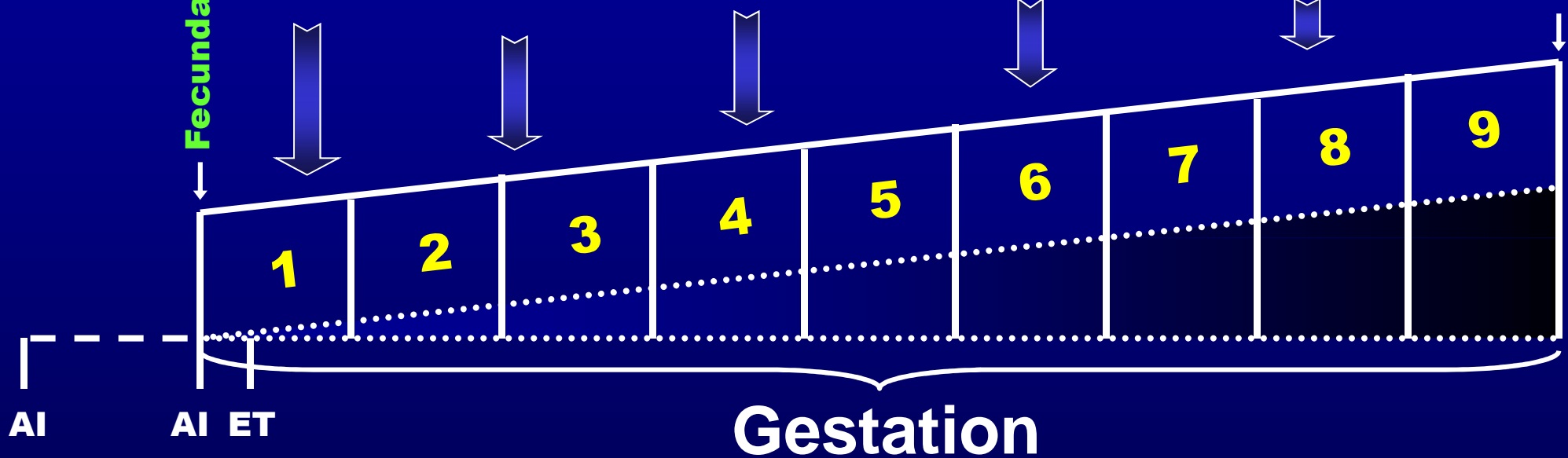
Clinical or RIA

Clinical or RIA

Clinical or RIA

Parturition

Fecundation



EEM

LEM

Abortion - Mummification - Maceration

Newborns with excessive or insufficient weights

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More information about PAG-RIA:

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