clear that there is a large variation in the progesterone profiles of different cows and that a fixed threshold on the progesterone level would not provide good heat detection results. However, the investigated approach which uses model parameters rather than a threshold allows to accurately describe the individual profiles and provide personalized detection of heat, pregnancy and abnormalities.

In the future, this approach for data management at the cow level will be applied to the data acquired with the FO-SPR progesterone sensor and the Vis/NIR milk composition sensor to develop a valuable decision support system for dairy farmers.

Keywords. Progesterone, nutritional status, management support.

DEVELOPMENT OF A NEW IMMUNORADIOMETRIC ASSAY FOR PREGNANCY-ASSOCIATED GLYCOPROTEINS (IRMA-PAG) ALLOWING PREGNANCY FOLLOW-UP IN CATTLE BY USING MILK SAMPLES

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Pregnancy-Associated Glycoproteins (PAGs) were first described as placental antigens that were also present in the peripheral circulation of the mother soon after implantation. They are synthesized by the mono- and binucleate thophoblastic cells, some of them being secreted in maternal blood from the moment when the conceptus becomes more closely attached to the uterine wall and formation of placentomes begins. In cattle, concentrations of PAG are detectable in maternal blood from day 28 to day 30 after fertilization. Milk concentrations are 20-30 times lower than in blood samples and cannot be measured by classical radioimmunoassay systems. In the present study we report the use of a highly sensitive immunoradiometric assay for PAG (PAG-IRMA) allowing pregnancy follow-up in cattle by measuring concentrations in milk samples. In the IRMA system, an antibody was coated to StarNunc tubes and used for the immobilization of the antigen. The sample (volume until 4 ml) was incubated in the tube. After incubation, the whole sample was eliminated by several washes. Thereafter, another antibody bound to biotin (detection antibody) was added in order to quantify the antigen present in the sample. After a second wash, the reaction was revealed by adding a Streptavidin tracer (^{125}I). Purified bovine PAG 67kDa was used as standard at concentrations ranging from 100 to 50,000 pg.ml^{-1}. Highly purified immunoglobulins (hp-Ig) were obtained from two distinct rabbit polyclonal antisera by using a specific affinity chromatography (anti-PAG 4B-Sepharose gel). The hp-Ig708 (purified from polyclonal antiserum raised against caprine PAG 55kDa+59kDa) was used as capture antibody (0.01 μg/tube). The hp-Ig727 (purified from polyclonal antiserum raised against purified boPAG67kDa) was used as detection antibody (1:8,000). Radiolabeled streptavidin (125I-Strep; 50,000 cpm·100 μl^{-1}) was used to reveal the Ab-Ag-Ab-Biot complexes. Milk was collected from pregnant cows (n = 20) during the whole duration of lactation until dry-off. Samples were frozen until assay. Before analysis, milk samples were thawed at 37 °C, centrifuged (2,500 x g) and fat was removed. Samples giving high PAG concentrations were serially diluted in order to fit with standard curve range. In pregnant cows, milk PAG concentrations increased from week 10 (56.9 ± 13.1 pg·ml^{-1}) to week 11 (93.5 ± 20.4 pg·ml^{-1}) and week 12 (135.2 ± 27.7 pg·ml^{-1}). Thereafter, PAG concentrations increased regularly until week 32 (2,177.6 ± 496.2 pg·ml^{-1}) and slightly decreased until dry-off at week 35 (1,615.9 ± 663.9 pg·ml^{-1}). Immediately after parturition, PAG concentrations reached 5,615.3 ± 615.7 pg·ml^{-1} and decreased continuously until week 11 postpartum (36.6 ± 2.1 pg·ml^{-1}). In non-pregnant cows, concentrations remain lower than 40-50 pg·ml^{-1} at all time points. In conclusion, a new IRMA-PAG is available for quantitative measurement of PAG concentrations in cattle. This new test can be used for pregnancy diagnosis and follow-up in cattle.

Keywords. Pregnancy diagnosis, pregnancy-associated glycoprotein, dairy cattle.

VALIDATION OF A NEW IMMUNORADIOMETRIC ASSAY (IRMA) ALLOWING QUANTIFICATION OF PREGNANCY-ASSOCIATED GLYCOPROTEINS CONCENTRATIONS IN BOVINE MILK

Noelita Melo Sousa, Aline Flora Tchimbou, Jean-François Beckers

Development of a new immunoradiometric assay for Pregnancy-Associated Glycoproteins (IRMA-PAG) allowing pregnancy follow-up in cattle by using milk samples

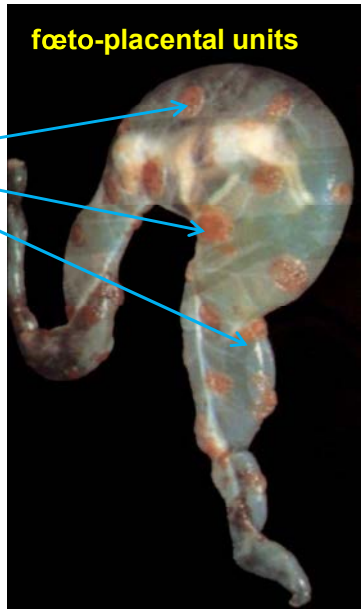
Sousa N.M., Tchimbou, A.F., Beckers J.F.

Physiology of Reproduction, Faculty of Veterinary Medicine, ULg

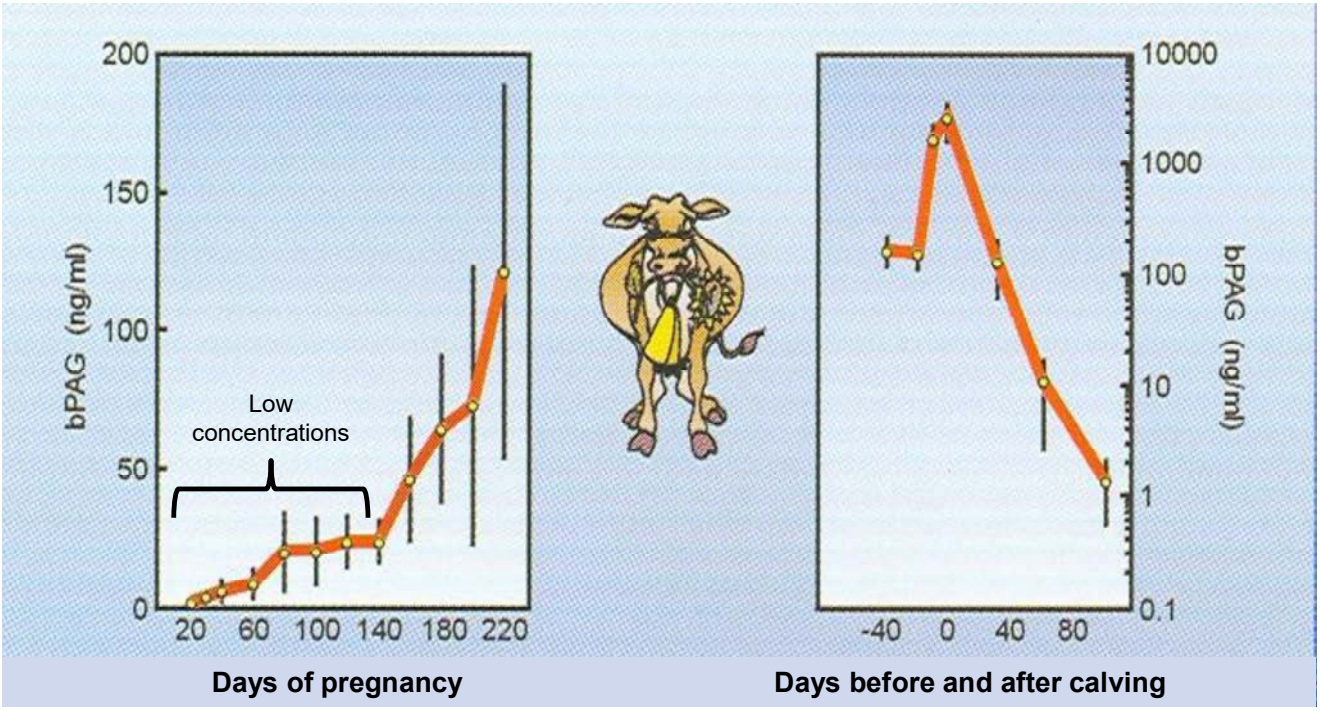
Pregnancy Associated Glycoproteins (PAGs)

First purified in the 80's in USA by Butler et al. (1982), in Belgium by Beckers et al. (1988), and in France by Mialon et al. (1993).

- **Synthesized in fetal cotyledons**
- **Glycoproteins MM between 35 and 70 kDa**
- **Belong to the family of aspartic proteases**
- **Also synthesized in intercaruncular area (Touzard et al. 2013)**



Profile of PAGs concentrations during pregnancy



Concentrations of PAG in bovine plasma or serum

Zoli et al. (1992)

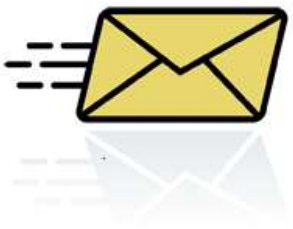
Assay of PAG in blood samples…

- PAGs molecules are stable

  Long half life in *vivo*

  Also stable in blood samples during transportation

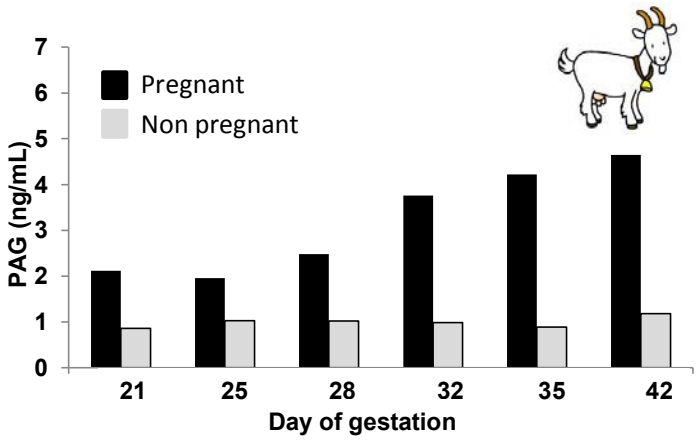
- Blood samples **can be sent by post !**



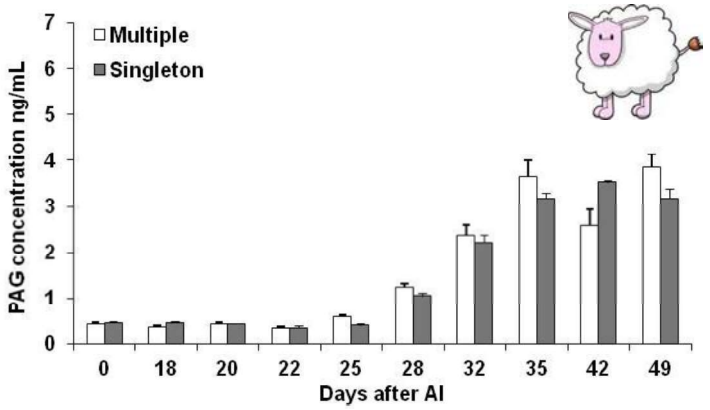
PAG in milk samples from small ruminants

The assay by the classical RIA allows the quantification of concentrations in milk of ewes and goats.

Pregnancy diagnosis is valuable from Day 32 after mating.



Gonzalez et al. (2001)



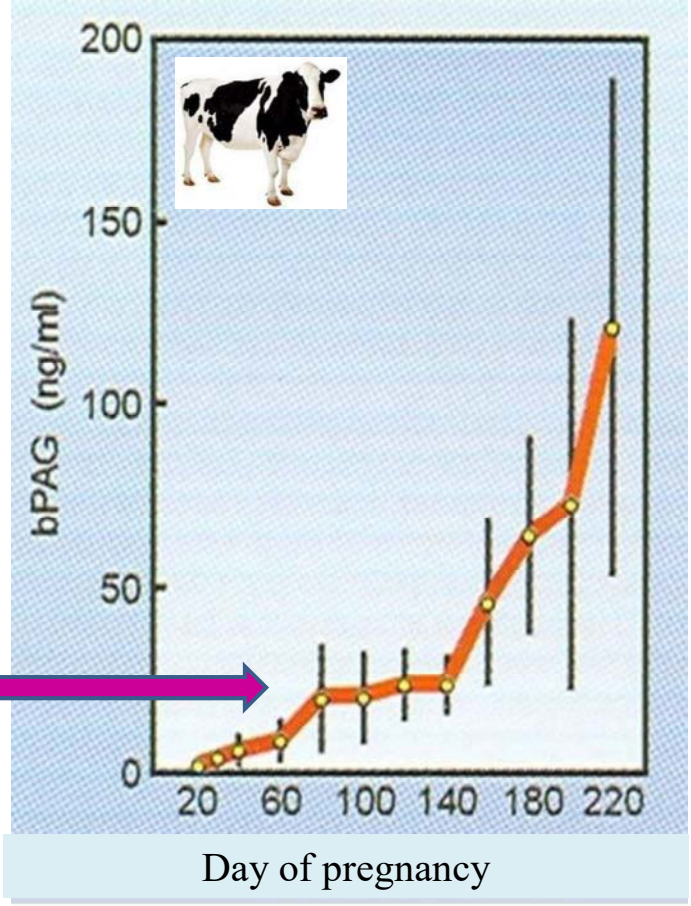
El Amiri et al. (2015)

PAG in milk samples from cows !

Unfortunately, in bovine species, blood concentrations are relatively low till the 6th month of gestation.

Moreover, in cow, concentrations of PAG in milk are much more lower (20 to 30 times) than in serum.

Plasmatic concentrations are low till the 6th month after fertilization

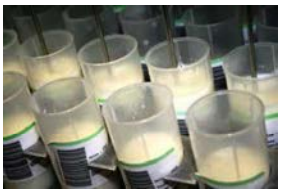


An ASSAY in milk samples

The challenge:

- **Milk contains high concentrations of lipids, casein micelles, cells, membrane fragments etc…**
  - **A competitive assay in milk can be compared to a « jogging in a marsh land ».**
  - **Non specific effects alter the speed of reaction between molecules and stability of reagents.**

The choice of IRMA technique



- **IRMA technique uses coated antibodies in excess**

  … aims to capture a maximum of the antigens present in the sample

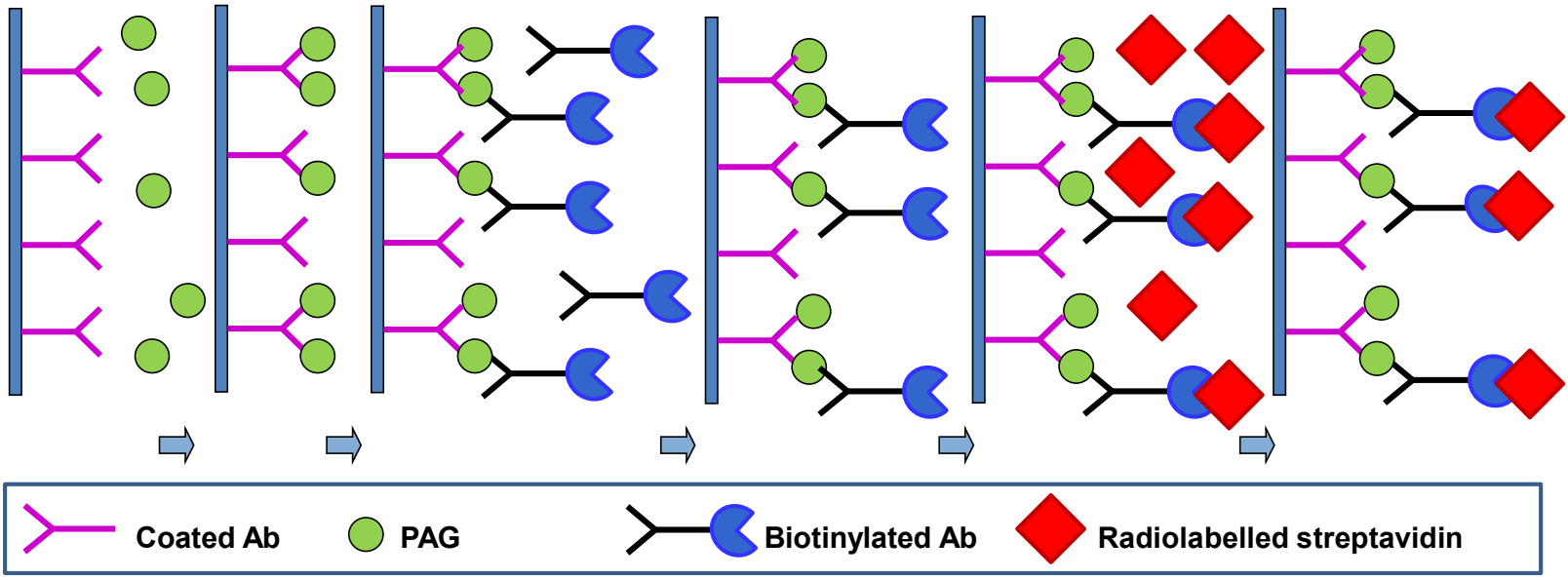
- **IRMA technique uses different antibodies for capture and revelation**

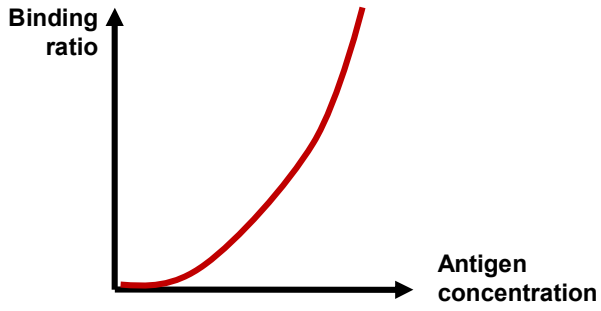
  … the best « pairs » of antibodies (capture and revelation Ab) were selected

- **In IRMA system, by using coated tubes (with immunoglobulins) for the « capture », the sample can be eliminated (washed) after the first incubation.**

  … the assay continues in a clean medium minimizing non specific interferences… and interactions with the tracer.

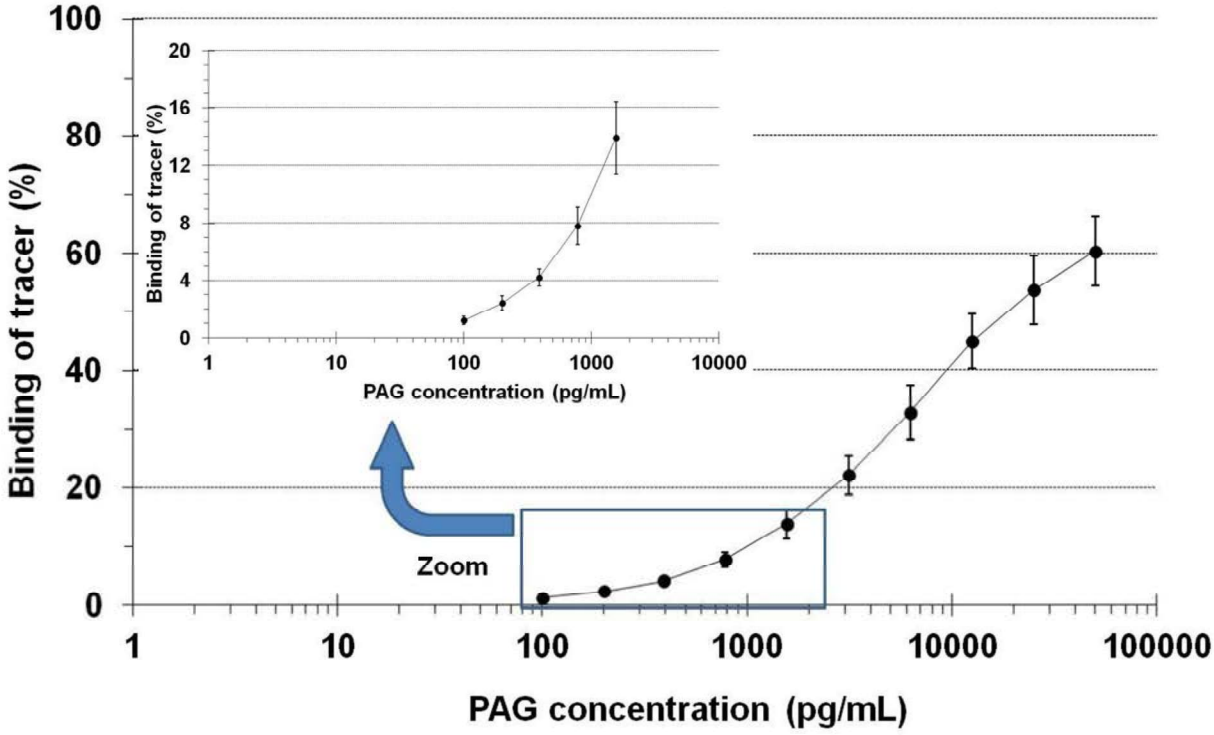
IRMA using biotiniled Ab and radiolabelled streptavidin





Standard curve - IRMA

Results



Binding ratio of eleven standard curves assayed by IRMA –PAG (mean ± SD). In the graphic inside (zoom) a focus was done on the std concentrations ranging from 0 to 1,560 pg/mL. Coated antibody was hp-Ig708 (0.32 µg/mL). Incubation with standard was performed ON-RT (under agitation). Incubation with biotinylated Ab (hp-Ig727 diluted at 1 :8,000) was performed at ON-RT (under agitation). Streptavidine tracer (50,000 cpm/100 µL) was incubated during 2 h (RT) before washing and counting.

Experimental animals and sampling

Results

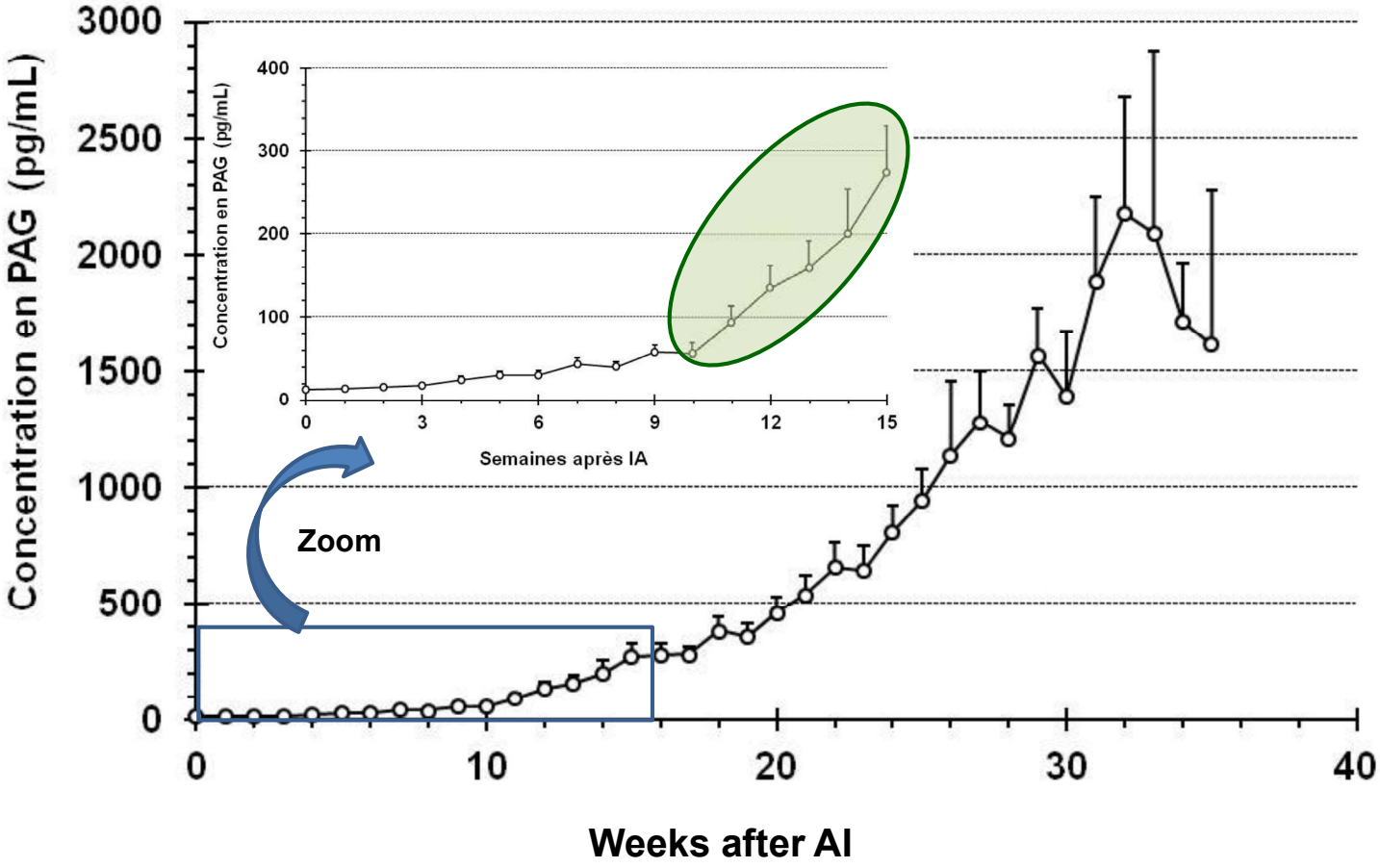
Milk farm (90 dairy cows) from plateau de Herve – Belgium

Animals used for sampling: **20 cows**

Duration of sampling: **20 months**

Frequencies: Milk sampling: weekly

Blood sampling: monthly



Mean PAG concentrations (+ SEM) measured in milk during pregnancy and before dry-off period.

Week	Mean	SEM
0	13.1	1.8
1	13.5	1.5
2	15.9	2.2
3	17.5	2.1
4	24.5	4.2
5	29.8	4.9
6	30.1	6.0
7	43.6	7.6
8	40.0	6.5
9	57.5	8.4
10	**56.9**	13.1
11	**93.5**	20.4
12	**135.2**	27.7
13	159.7	32.2
14	200.1	53.9
15	274.1	56.1
16	278.0	49.7
17	282.5	35.3
18	382.7	57.6

1st trimester

2nd trimester

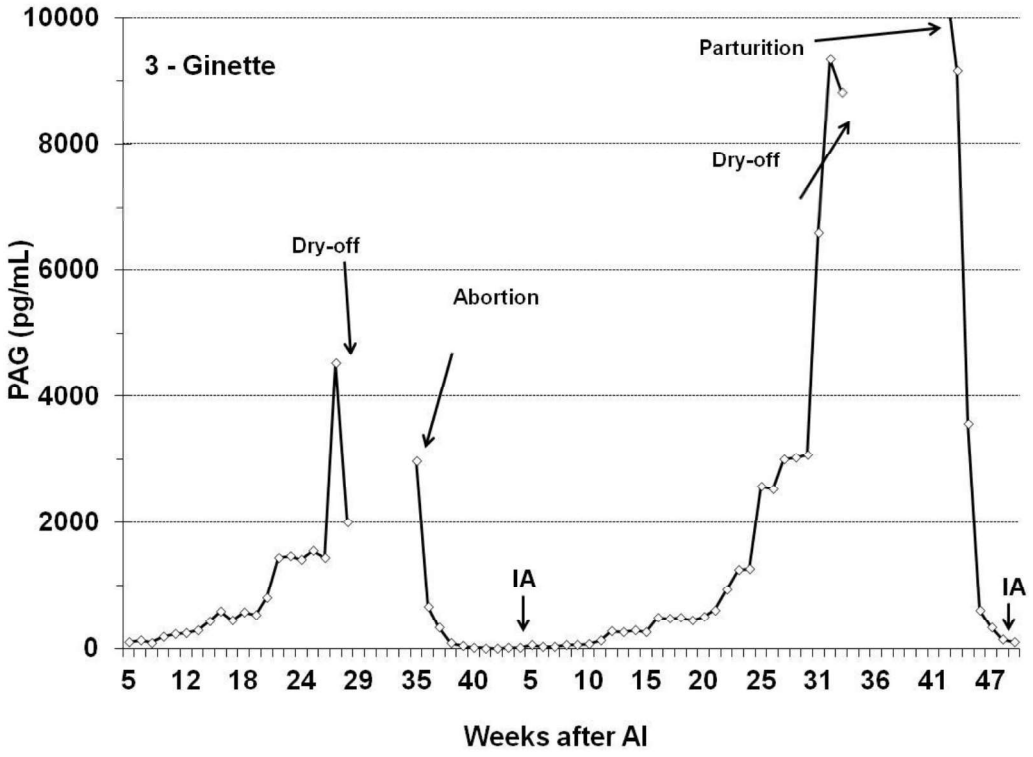
Week	Mean	SEM
19	360.2	56.7
20	460.8	64.3
21	539.5	84.4
22	660.1	106.0
23	644.5	103.1
24	805.4	112.6
25	945.5	132.3
26	1136.2	319.0
27	1282.2	215.2
28	1211.1	143.1
29	1567.7	199.5
30	1391.7	279.9
31	1888.4	358.6
32	2177.6	496.2
33	2093.1	786.1
34	1713.6	249.6
35	1615.9	663.9
36-40	Dry-off	-

2nd trimester

3rd trimester

Mean (+ SEM) concentrations of PAG during pregnancy

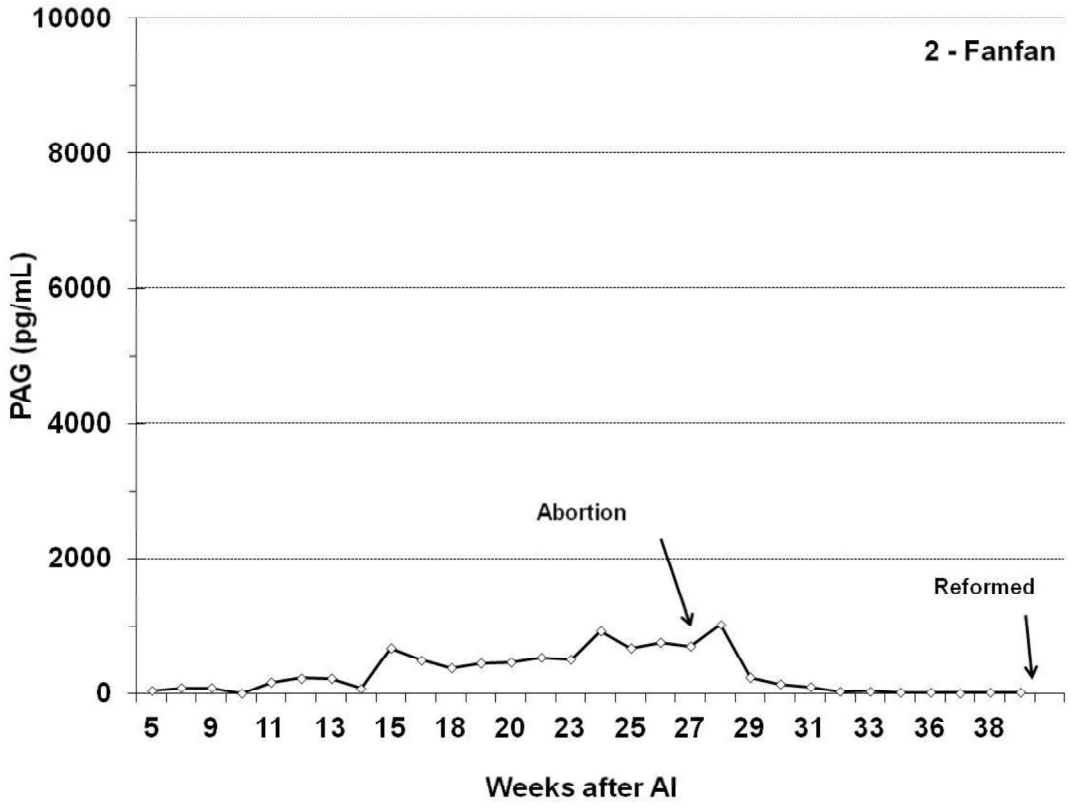
Analysis of individual profiles



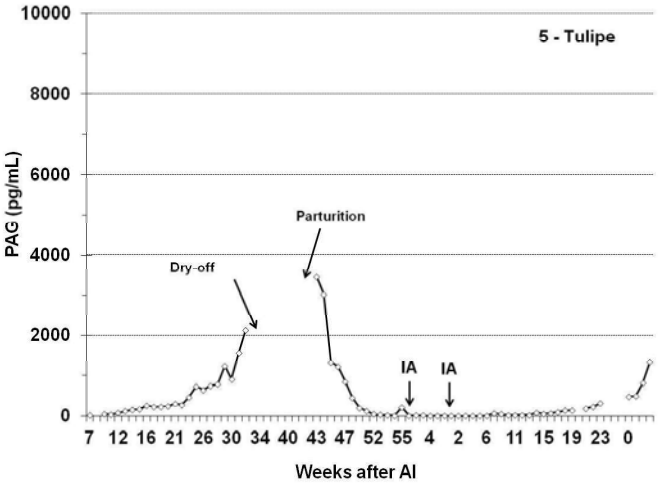
Individual profile of PAG concentrations in one cow followed during two consecutive pregnancies:

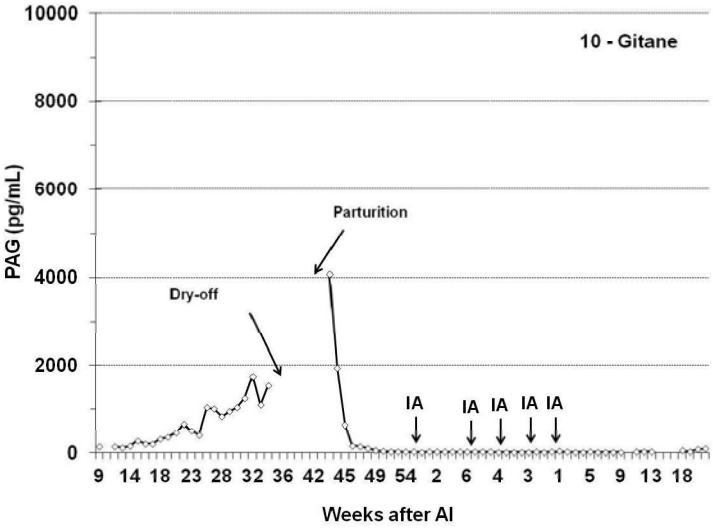
1st lactation : abortion at the 35th week of gestation

2nd lactation : gestation until parturition

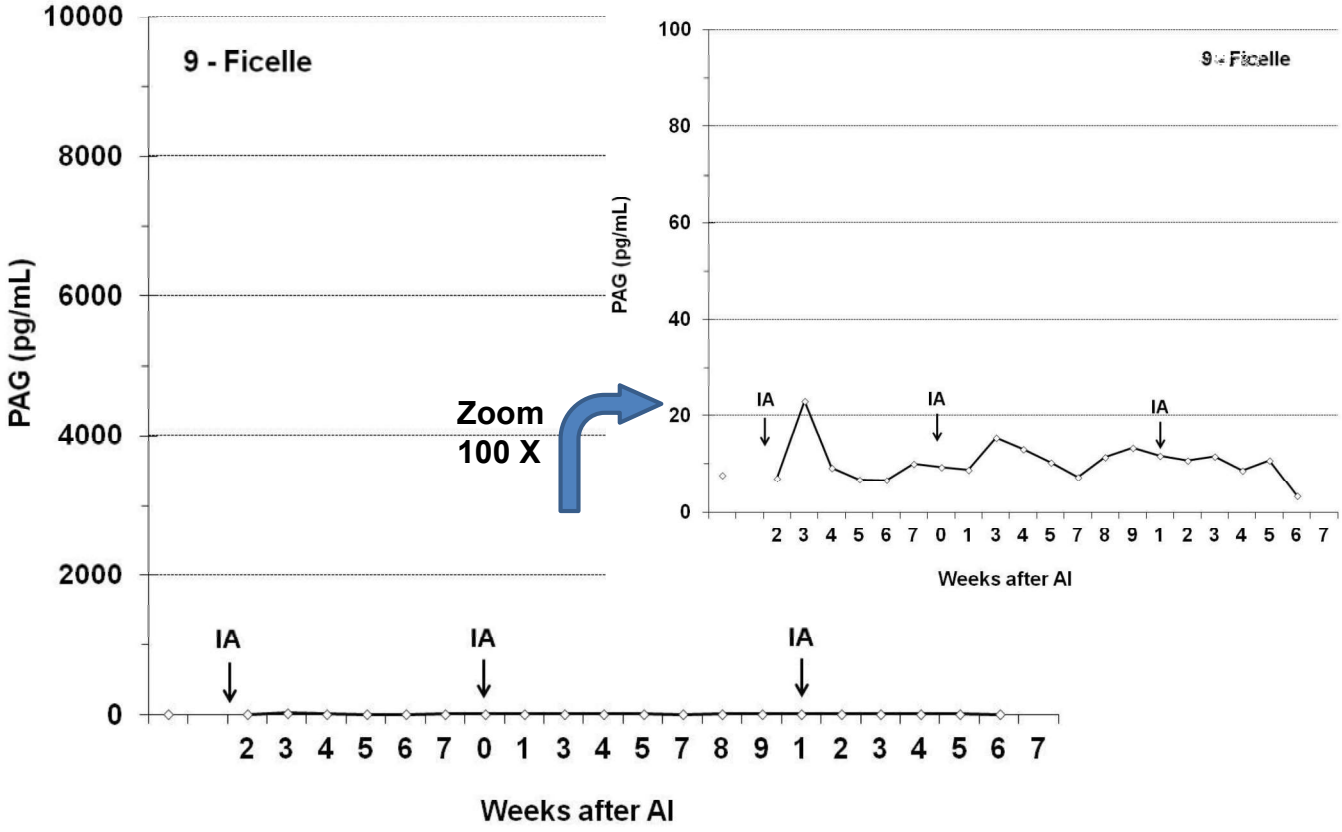


Profile of PAG concentrations in one cow suffering abortion at the 28th week of pregnancy





Indivudual profiles of PAG concentrations in two cows inseminated several imes with success (Cow Tulipe, left side) or without success (Cow Gitane, right side).

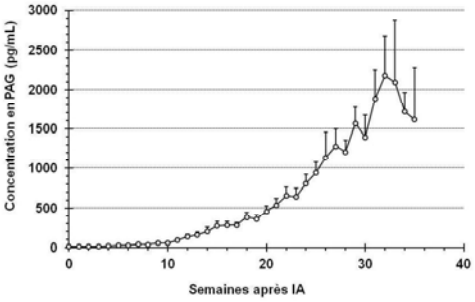


Individual profiles of PAG concentrations in one cow inseminated several times without success.

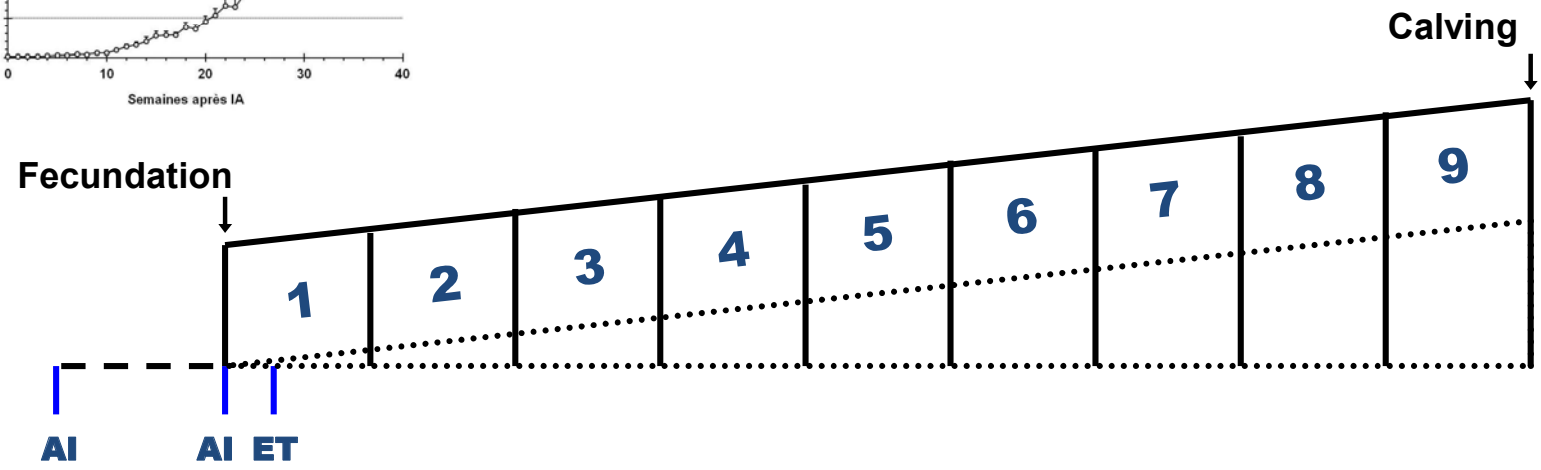
In summary

In the present study, we developed an immunoassay allowing the precise quantification of PAG concentrations in the milk of bovine species

Thus helping dairy cow breeders for follow-up of pregnancy



Perspectives offered by the project



Pregnancy follow-up

- Assay of PAG in milk
- Clinical/US diagnosis or assay of PAG in blood

Thank you for your attention!

Acknowledgements…

Participants:	Contribution:
Ms Bériot et Claessens (Trainee)	Purification PAG
Mr. C. Delhalle (ouvrier)	Slaughterhouse collection

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Team:

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Mme Ch. Poullet
Mr. B. Glaude (Ing.)
Mr. M. Machado

Collaborators concerning research in milk:

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DGA – Ministère de la Région Wallonne

Laboratoire d'Endocrinologie et de Reproduction Animale

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Final OptiMIR Scientific and Expert Meeting: From milk analysis to advisory tools (Palais des Congrès, Namur, Belgium, 16-17 April 2015)

OPTIMIR - A PROJECT AIMING THE DEVELOPMENT OF NOVEL MID-INFRARED BASED MANAGEMENT TOOLS FOR DAIRY HERDS

Paula Friedrichs [(1)], Catherine Bastin [(2)], Frédéric Dehareng [(3)], Brian Wickham [(4)], Xavier Massart [(5)], editors

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[(4)] ConsultWickham. Old Chapel Lane, 3. Clonakilty, Co. IRL-Cork.
[(5)] Association Wallonne de l'Élevage ASBL. Rue des Champs Élysées, 4. B-5590 Ciney.

The dairy industry represents 13% of the turnover of the European food industry and north-west Europe produces 60% of the European milk. However, in 2014 the milk market has experienced a substantial fall in milk prices of some 50%. At these lower prices for milk the business of many milk producers is not sustainable. Due to recent advances, the mid-infrared (MIR) analysis of milk performed within milk recording (MR) promises more information than used traditionally. Beside the established MR parameters (protein, fat, lactose, and urea), the spectra could provide additional information on cows' status for a range of characteristics (*e.g.* fertility, health, energy balance, feeding and methane emission). Thus, the spectra routinely obtained from the MIR analysis of milk offer a possibility to develop novel, cost-effective tools which enable milk producers to improve the management of their dairy herds and in turn reduce the costs for milk production. The OptiMIR project aims to improve the sustainability of the dairy sector by developing and providing innovative, economical and standardized MIR-based tools for the management of dairy herds. Additionally, the project aims to create and promote a framework for the cross-border exchange of information and practices to enhance the service of milk recording organizations (MROs) in north-west Europe and to strengthen their competitiveness. In order to achieve these objectives, in 2011 the European OptiMIR project was officially launched for a 5-year-period. The INTERREG IV B funded OptiMIR project is a cooperation between three research centers, three universities, 11 MROs, and one laboratory from six north-western European countries. To accomplish the goals, the different processes from the identification of the priority areas to the development, validation, and implementation of the novel tools were outlined in three working packages comprising 10 actions. A common transnational database combines the phenotypic data of the cows and the MIR spectra from the European MR, which enables the detection of relevant phenotypic traits and their reflection in the MIR spectra. Additionally, a standardization has been installed among all instruments of the milk analyzing laboratories involved in the OptiMIR project to ensure a stable prediction over time and a correction of deviations. Due to the monthly standardization process the developed prediction equations can be used in routine on all instruments taking part in the standardization. Different models for the prediction of the pregnancy status, energy balance, the methane emission as well as the detection of ketosis and acidosis have been developed within OptiMIR. It is worth mentioning, that those models predict the status of the animal with different accuracies and that progress on development and implementation differs between the models. Some of the tools developed within OptiMIR are already used in the field and provide dairy farmers with useful information *e.g.* about the health status or methane emission of a certain cow. The OptiMIR project also resulted in a collaborative planning on an economic interest group formation of the participating MROs. The final OptiMIR scientific and expert meeting disseminates the results obtained through OptiMIR more detailed and also provides an overview of recent advances in the development of management tools for the dairy sector. Initial application of the MIR-based tools has shown their potential for providing dairy farmers with information to improve their herd management in a cost-effective way. However, further research and development is required to finish a greater extend of MIR-based tools so that north-western European stakeholders can acquire competitive advantage in the dairy sector.

Keywords. Dairy industry, livestock management, infrared spectrometry, research, Europe.