Early pregnancy diagnosis in cattle using blood or milk samples

Loïc Commun[1],
Kathy Velek[2], Jean-Baptiste Barbry[1], San Pun[3], Anna Rice[2], Chistroph Egli[3], Serge Leterme[2]

[1]UMR1213 Herbivores INRA (Husbandry systems Team), Vet school of Lyon (France)
[2]IDEXX Laboratories Inc., Westbrook (USA)
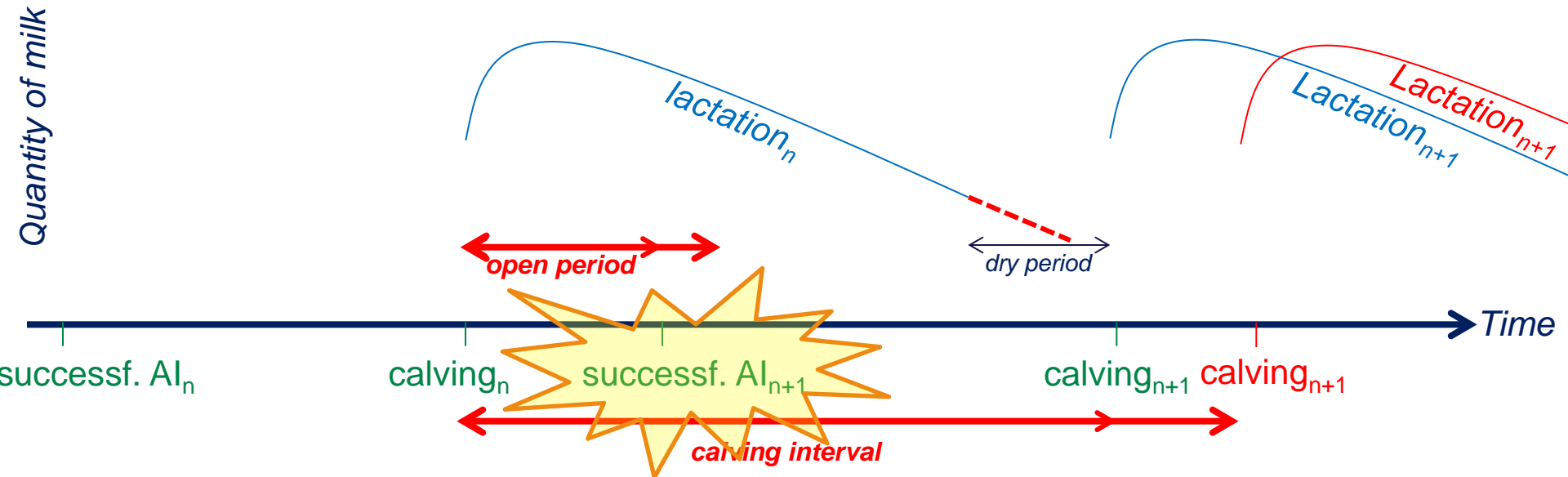*[3]IDEXX, Liebefeld-Bern (Switzerland)*

VetAgro Sup
VetSchool of Lyon

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT

INRA

Context

Production cycle of a dairy cow

- Accurate and early diagnosis of open cows :
  - → Allows farmers and vets to readapt their practices, (heat observation, hormone treatment…)
  - → Permits to control the length of the open period !
  - → Limits economical loss = € 0.57 to € 0.70 /day *(Inchaisri et al., 2010)*

 Early pregnancy diagnosis : a key point of management programs in cattle.

Context

- How to diagnose pregnancy in cow ?

  - Observation of estrus signs 3 weeks after AI, **but** heat detection rate ?

  - Transrectal examination (after 50 d after AI *(Youngquist, 2007)*), not really early ?

  - Ultrasonography

  Se = 100% from 28 d post-AI *(Filteau et al., 1998)*
  but : specific and expensive instruments
  and experienced operator

  - Biochemical methods
    - Progesterone : oldest, early, also on milk *(Pieterse et al., 1990)*. but with a poor specificity (39.3%).
    - PSPB (pregnancy-specific protein B) : early, Se=93.9% and Sp = 95.5%, but no analysis in milk *(Romano and Larson, 2010)*.
    - PAG : pregnancy-associated glycoproteins → possibility of diagnosis in blood and milk *(Friedrich, 2011)*

Context

- h

What about PAG (pregnancy-associated glycoproteins) ?

- PAG expression : 1st day of pregnancy → calving *(Gajewski et al., 2009)*.

- At least 22 transcribed genes : expression and regulation vary according to the stage of pregnancy *(Telugu et al., 2009)*.

- Some PAG expressed very early in pregnancy (by day 25) and having a short half-life (undetectable by 6 weeks post-partum) *(Green et al., 2005)*

→ making them ideal markers for detecting early pregnancy.

)) : really

990). but

= 95.5%,

010).

- PAG : possibility of diagnosis in blood and milk (Friedrich, 2011)

Objectives

- To define accuracy of a PAG-based ELISA test to diagnose pregnancy, from blood and milk samples taken on cows at different days post-AI

Test With Confidence™
IDEXX

- The test exists in 2 versions:
  - one for blood (Bovine Pregnancy Test®)
  - one for milk samples (Milk Pregnancy Test®). The milk test using in our study was a prototype version, and our goal was also to define the optimal cut-off value for the milk version
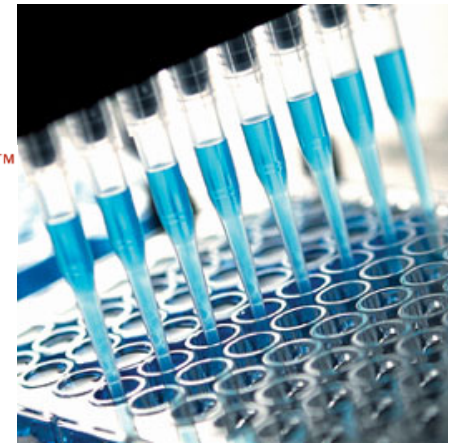
Experimental design

- **Animals and farms**
  - From 12 commercial French dairy farms (Bourgogne area)
  - 102 cows (Montbéliarde or Holstein breed) received artificial insemination (AI) were selected

Experimental design

102 cows (Montbéliarde or Holstein) from 12 French dairy farms

AI

16±1 d post-AI

Samples of :
- serum
- plasma
- milk

2 vets

30±1 d post-AI

Samples of : :
- serum
- plasma
- milk

2 vets

41±2 d post-AI

Samples of : :
- serum
- plasma
- milk

+ Pregnancy diagnosis by transrectal ultrasonography

2 vets

53±2 d post-AI

Samples of :
- milk

farmer

→ Centrifugation of blood samples + freezing of serum, plasma and milk samples within 3 hours after each sampling session
→ Analysis performed subsequently by ELISA, in lab (Bern, Switzerland), 3 months later
By a technician blinded to *the cows' pregnancy status !!!*

PAG-based ELISA test : principes
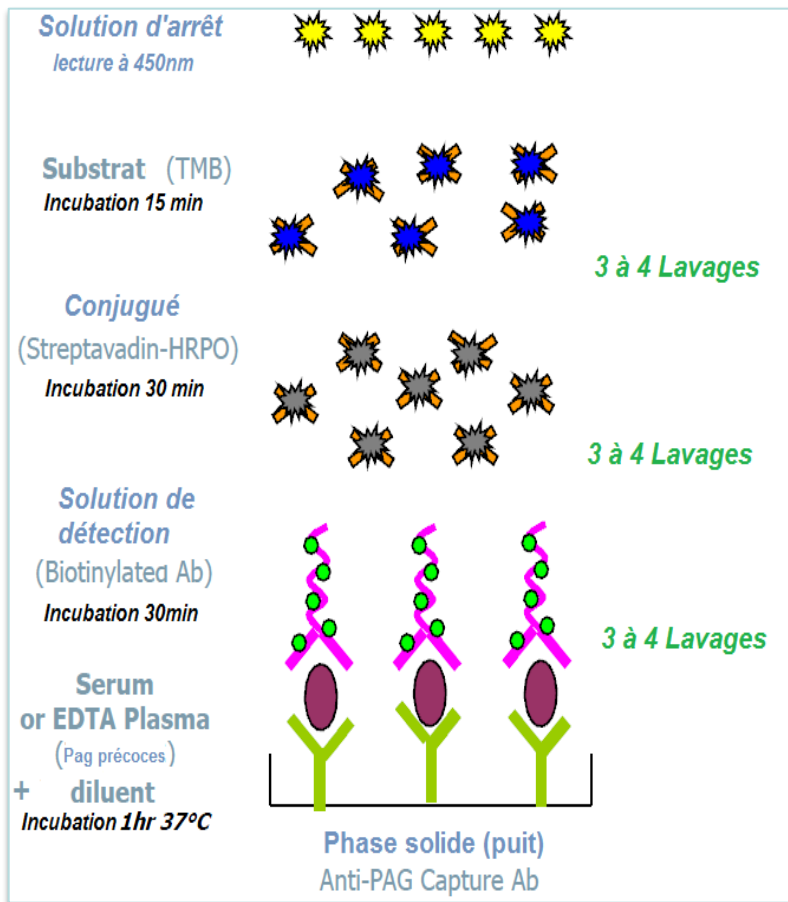
Reaction is stopped → Lecture of the plate (450 nm)

enzymatic substrate to produce a visible signal,

2nd antibody anti-PAGs, + amplification (conjugate)

PAGs in our sample

Antibody anti-PAGs in a well

ELISA analysis and interpretation of results

- Optical density (OD) of the sample → concentration of PAG
    - In **blood** : If optical density > 0.3 = cow is considered as pregnant by the test
    - In **milk** : we searched the optimum cut-off value to interpret ELISA on milk using a ROC curve method.

Pregnancy diagnosis given by the test
(in lab by a technician blinded to the cows' pregnancy status)

Versus

Pregnancy diagnosis by transrectal ultrasonography
Performed at 41 days post AI

→ Sensitivity (**Se**) and specificity (**Sp**) of the ELISA /session and /each type of sample

Results

- 63/102 cows were confirmed as pregnant by transrectal ultrasonography around 41 days post AI.

- Using a receiver operating characteristic curve method, we defined

an optimal cut-off value of the

optical density of the milk test at 0.25, slightly lower than for the blood

Area Under Curve = 0.976
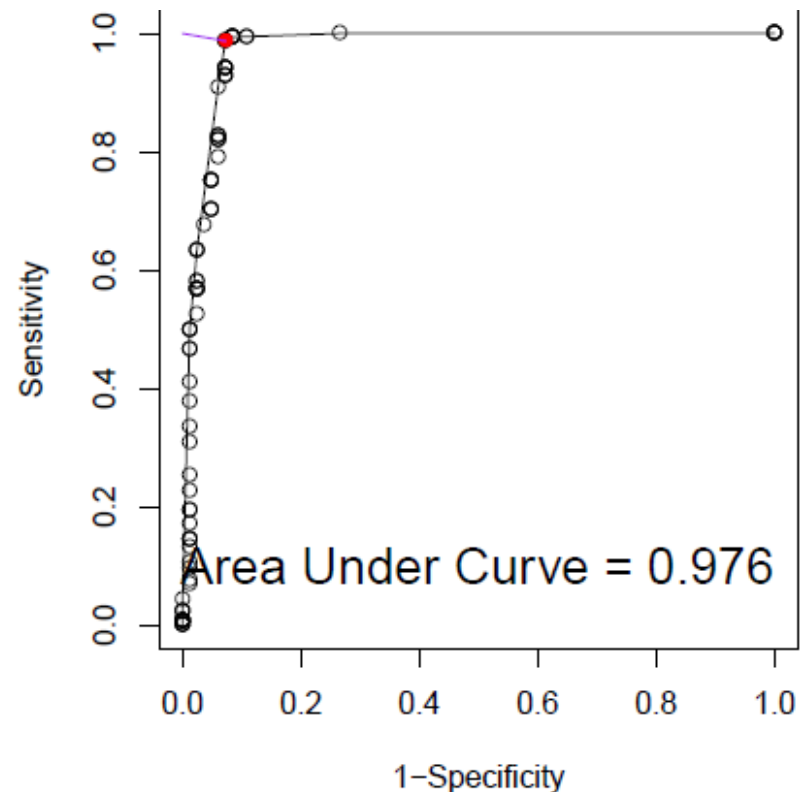
Results of the ELISA test in serum samples from the 63 pregnant cows
*(= cows diagnosed **PREGNANT** by ultrasonography at 41±2 days post-AI)*
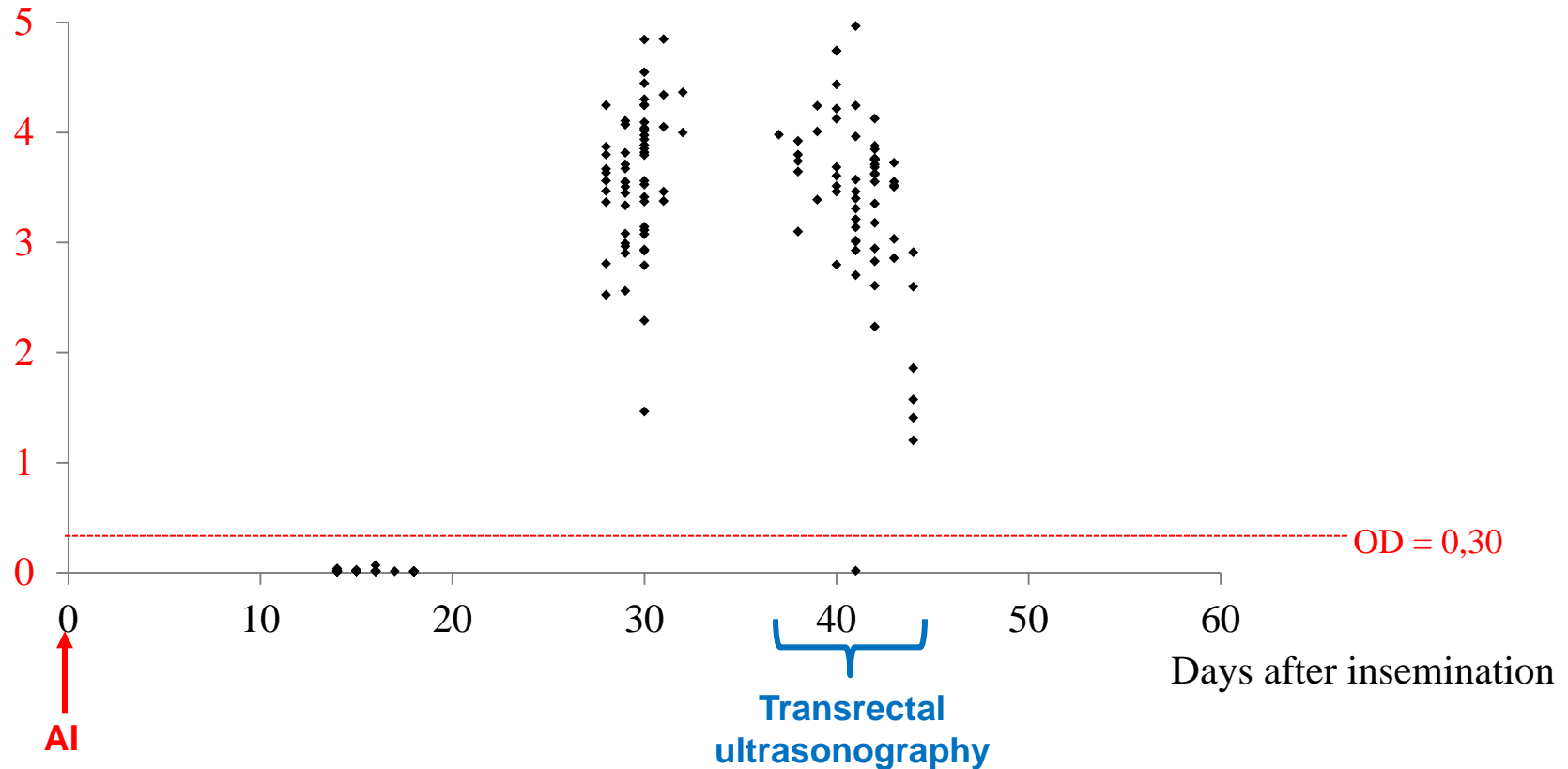
COWS diagnosed PREGNANT
by ultrasonography 41±2 d post-AI
n=63

COWS diagnosed OPEN
by ultrasonography 41±2 d post-AI
n=39

~ 16 days post AI

~ 16 days post AI

OD values of serum samples
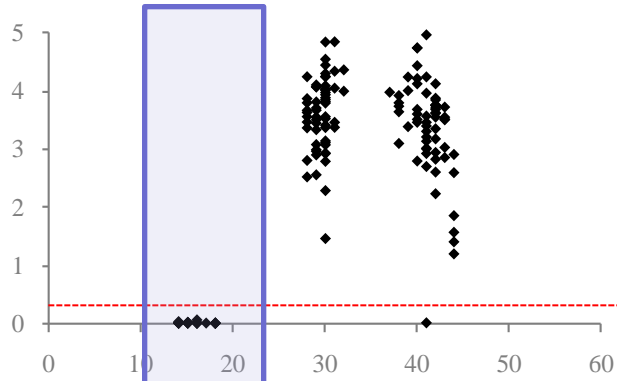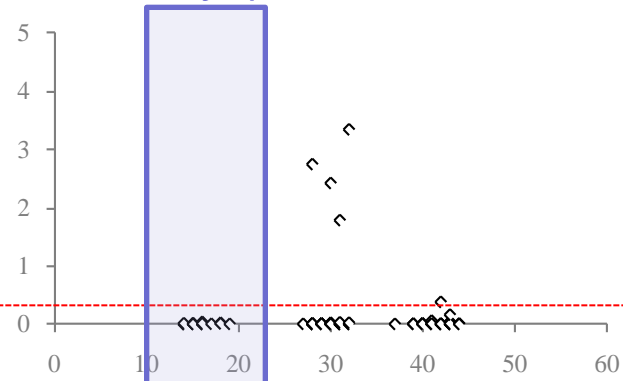
OD = 0,30

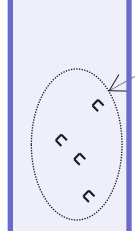OD values of plasma samples
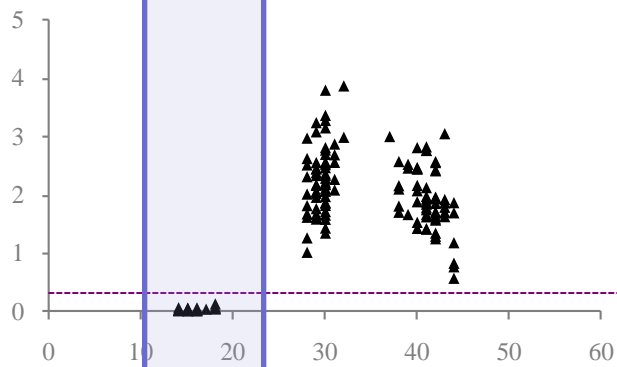
OD = 0,30
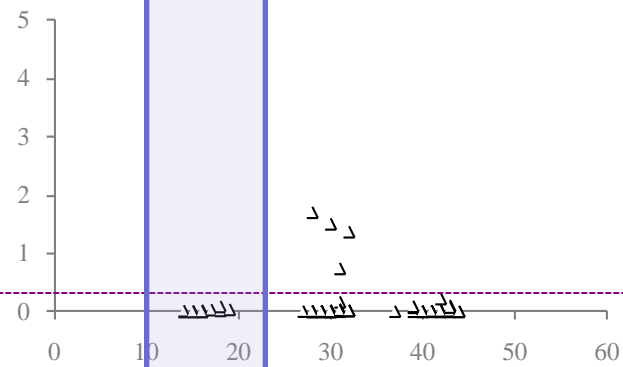
OD values of milk samples

OD = 0,25

Days post-AI

Days post-AI

COWS diagnosed **PREGNANT** by ultrasonography 41±2 d post-AI n=63

COWS diagnosed **OPEN** by ultrasonography 41±2 d post-AI n=39
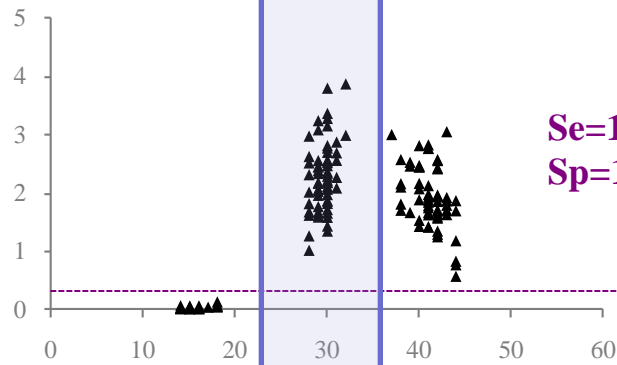
~ 30 days post AI

OD values of serum samples

Se=100%
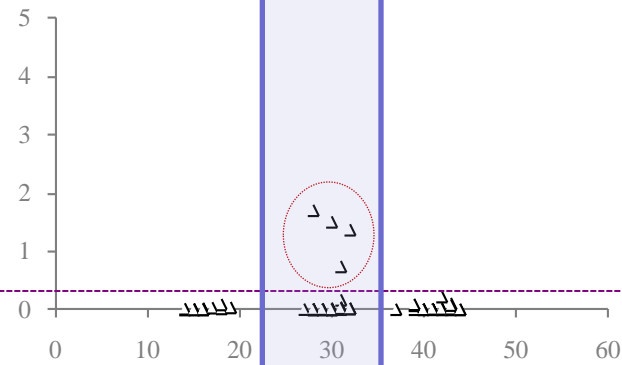Sp=88.6%

OD = 0,30
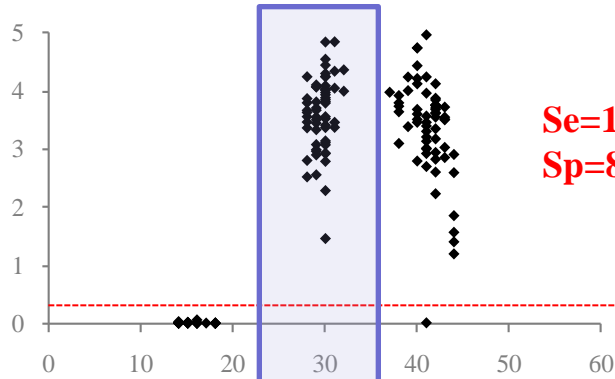
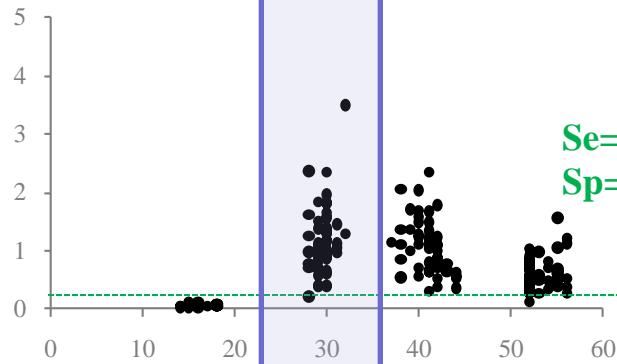**4 supposed early pregnancy losses**

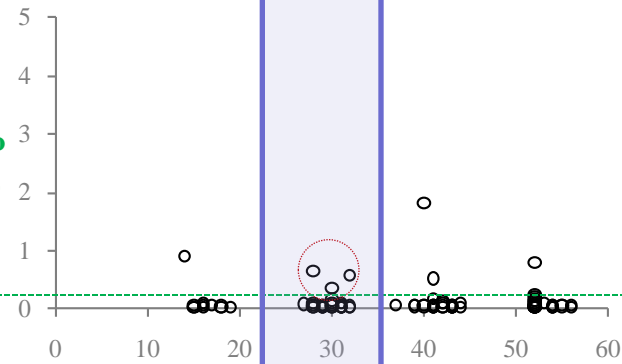OD values of plasma samples
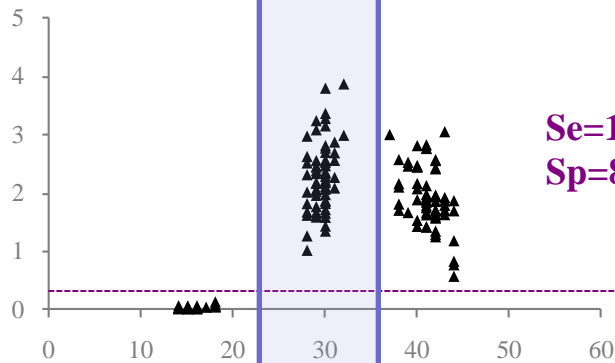
Se=100%
Sp=88.9%

OD = 0,30

OD values of milk samples
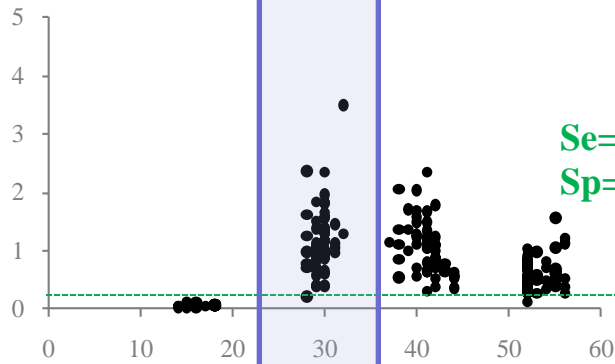
Se=98.1%
Sp=91.3%

OD = 0,25

Days post-AI

COWS diagnosed **PREGNANT**
by ultrasonography 41±2 d post-AI
n=63
~ 30 days post AI

COWS diagnosed **OPEN**
by ultrasonography 41±2 d post-AI
n=39
~ 30 days post AI

*Calculation with exclusion
of the supposed pregnancy losses*

OD values
of serum samples

Se=100%
Sp=100%
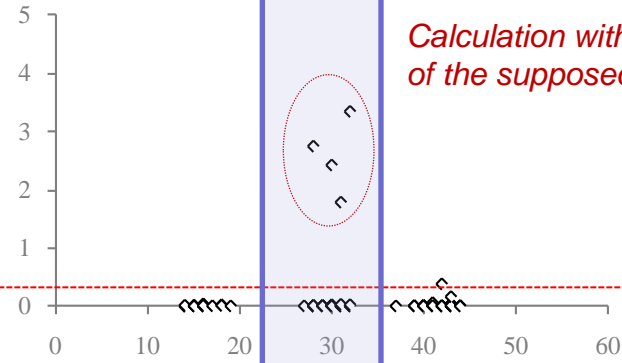
OD = 0,30

OD values
of plasma samples

Se=100%
Sp=100%

OD = 0,30

OD values
of milk samples

Se=98.1%
Sp=100%

OD = 0,25

Days post-AI

Days post-AI

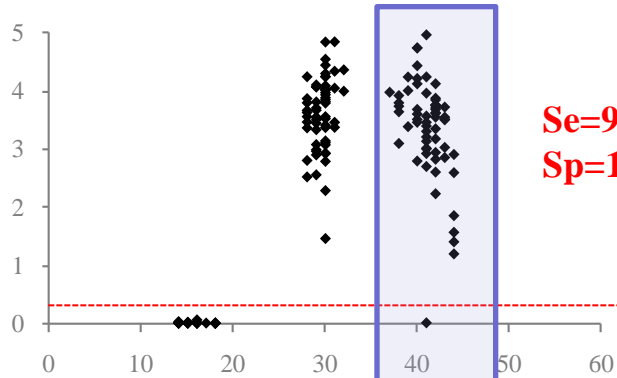**COWS** diagnosed **PREGNANT** by ultrasonography 41±2 d post-AI, n=63

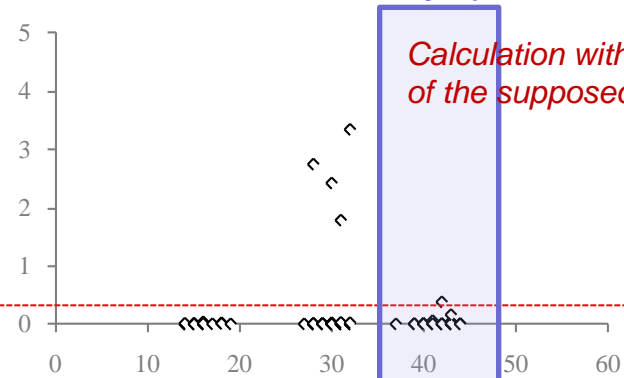**COWS** diagnosed **OPEN** by ultrasonography 41±2 d post-AI, n=39

~41 days post AI

Calculation with exclusion of the supposed pregnancy losses
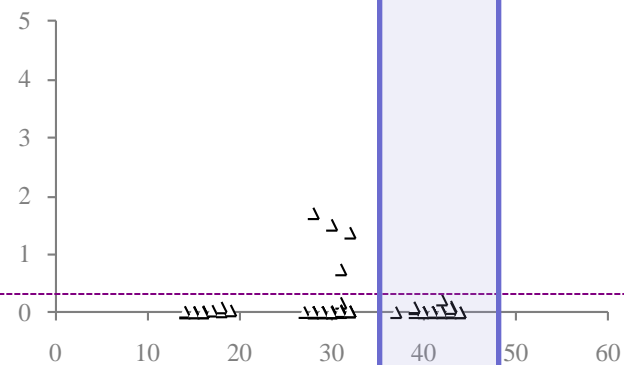
OD values of serum samples — Se=98.4%, Sp=100%, OD = 0,30
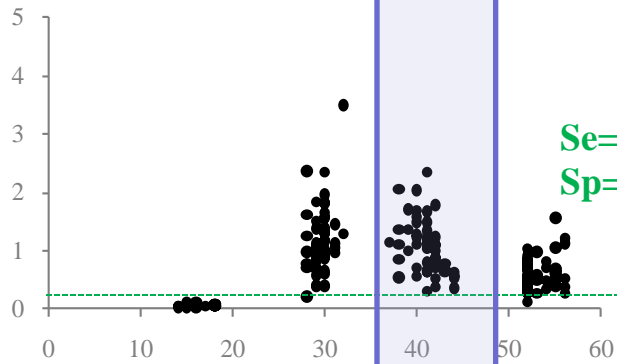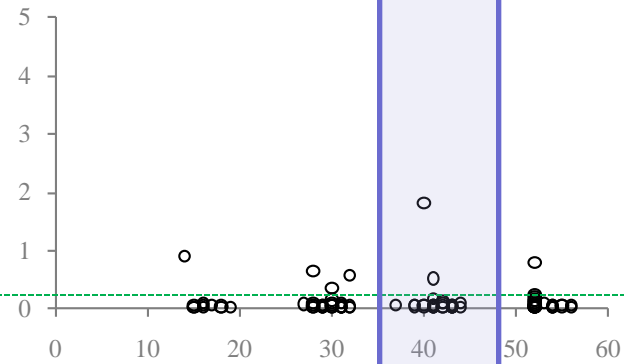
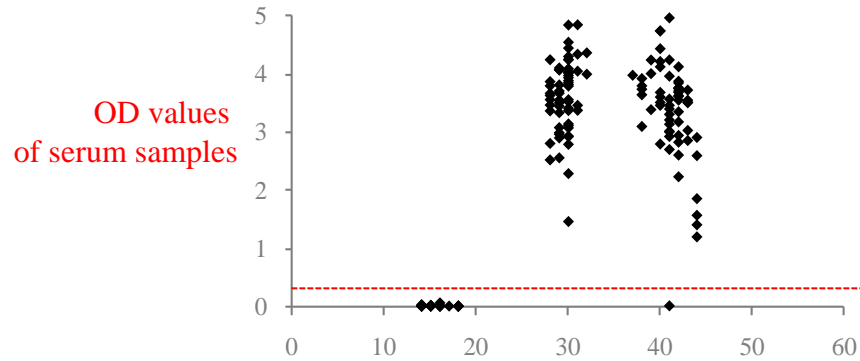OD values of plasma samples — Se=100%, Sp=100%, OD = 0,30
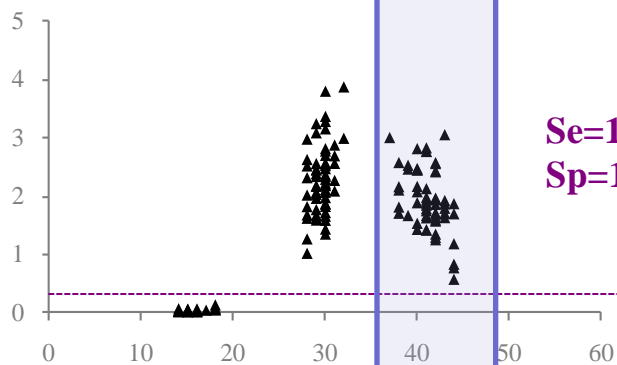
OD values of milk samples — Se=100%, Sp=92%, OD = 0,25

Days post-AI

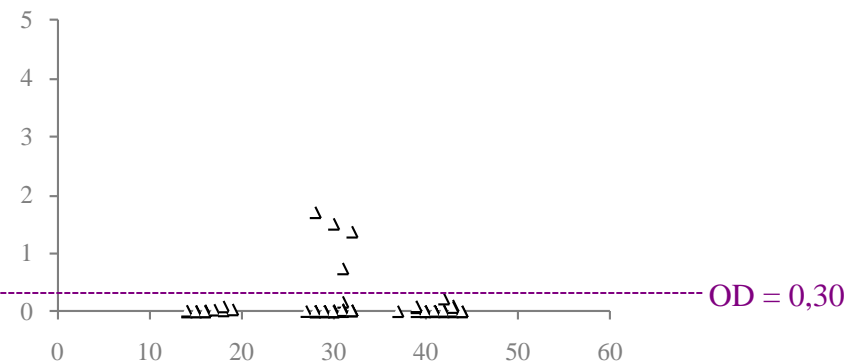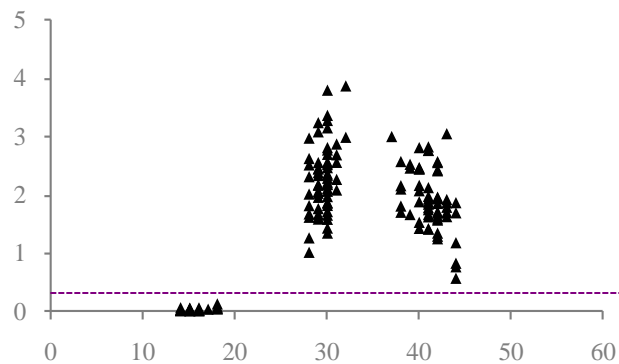**COWS diagnosed PREGNANT** by ultrasonography 41±2 d post-AI n=63

COWS diagnosed OPEN by ultrasonography 41±2 d post-AI n=39

Calculation with exclusion of the supposed pregnancy losses

OD values of serum samples

OD = 0,30

OD values of plasma samples

OD = 0,30

OD values of milk samples

~53 days post AI

~53 days post AI

Se=98.1%
Sp=96%

OD = 0,25
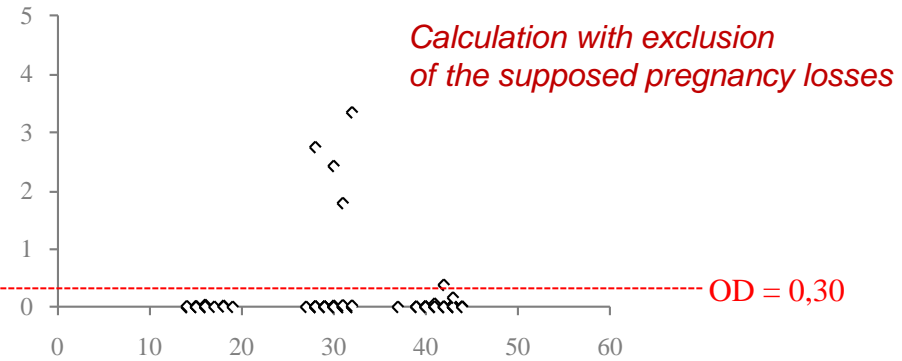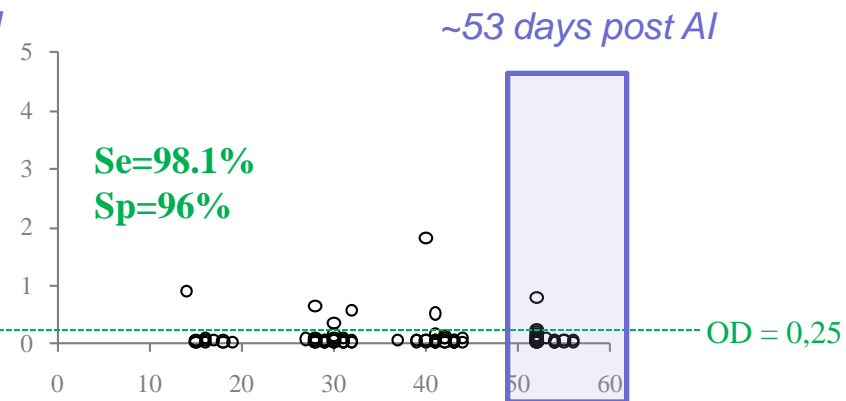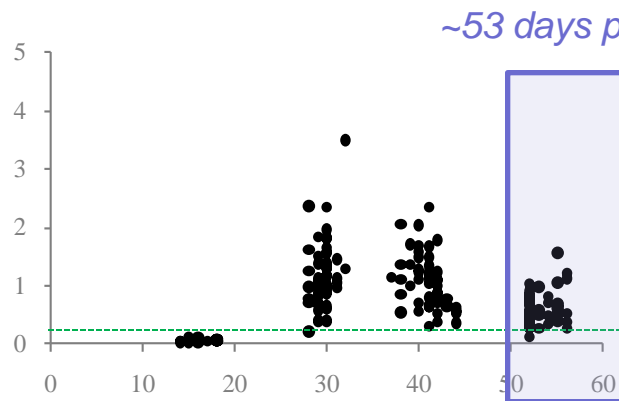
Days post-AI

Days post-AI

Discussion

Early pregnancy losses

- seems to be equal to 6% in our study
  - a pregnancy loss rate of around 3.7% in the 33 to 40 d post-AI stage *(Sterry et al., 2006)*
  - 6% between 25 and 60 days in a poster in this session *(Beharry et al., 2013)*

- As early pregnancy loss is always possible,
  → it is recommend to retest the cows 10 days later in case of doubt

Discussion

Comparison of results with other pregnancy test on milk samples

- Before this study, no reliable test on milk samples before 150 days post insemination *(Friedrich, 2011)*
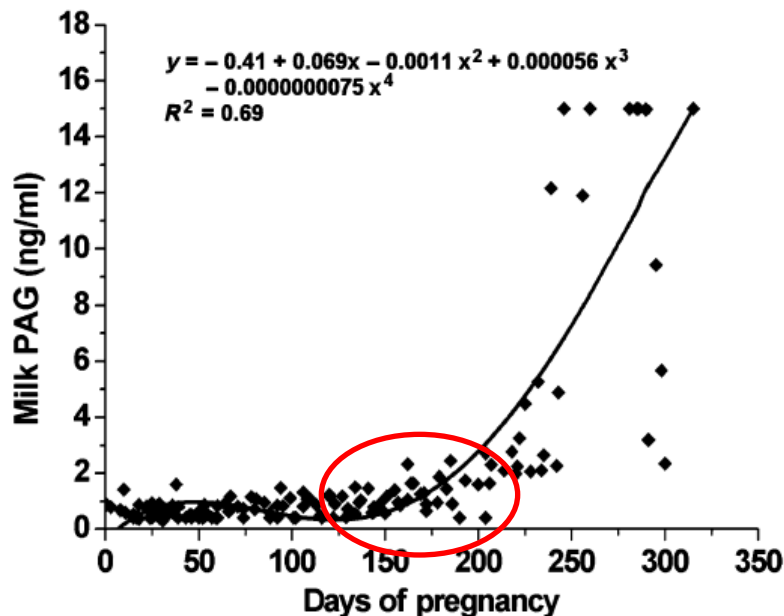
Discussion

And after 53 days post insemination ?

- Lower PAG concentrations in milk sampled at 53 d post-AI

→ decrease with the stage of pregnancy ? or because collected by dairy farmers ?

- Leblanc *(2013)* with the same test : all milk samples after 60 d post-AI

→ the tests showed very good performances (Se = 99.2% and Sp= 95.5%)

- Thus :

  – The test runs after 53 days post-AI

  – In our study, the way of collection and of storage of the milk samples at 53 days post AI (by farmers), explains probably our results concerning lower concentration of PAG in milk at this stage

Conclusion

- **Bovine Pregnancy Test**® (blood) and **Milk Pregnancy Test**® (milk) prove remarkably **reliable** to diagnose pregnancy in cows from as **early** as 27 days post-AI.

- On milk samples :
  A **new** pregnancy diagnosis test
  = a major improvement that Dairy Herd Improvement Laboratories could propose
  to their customers.

- A usefull test to diagnose pregnancy in beef cattle, by blood samples

→ Finally, this new ELISA PAG-based test is a breakthrough for pregnancy diagnosis in cattle.

Thank you for your attention