Early pregnancy diagnosis in cattle using blood or milk samples

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• 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.
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)
• How to diagnose pregnancy in cow?
  – Observation of estrus signs 3 weeks after AI: heat detection rate?
  – Transrectal examination (generally after 50 d after AI (Youngquist, 2007)): really early?
  – Ultrasonography: Se = 100% from 28 d post-AI (Filteau et al., 1998) but: specific and expensive instruments + experienced operator
  – Biochemical methods:
    • Progesterone: oldest, ideally at 24 d post-AI, also on milk (Pieterse et al., 1990). but with a poor specificity (39.3%).
    • PSPB: pregnancy-specific protein B. At 28 d post-AI, Se = 93.9% and Sp = 95.5%, this new method is interesting, but no analysis in milk (Romano and Larson, 2010).
    • PAG: possibility of diagnosis in blood and milk (Friedrich, 2011)

What about PAG (pregnancy-associated glycoproteins)?

- PAG expression: 1\textsuperscript{st} day of pregnancy \rightarrow 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)

\rightarrow making them ideal markers for detecting early pregnancy.
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

• 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

16±1 d post-Al
Samples of:
- serum
- plasma
- milk

2 vets

30±1 d post-Al
Samples of:
- serum
- plasma
- milk

2 vets

41±2 d post-Al
Samples of:
- serum
- plasma
- milk

+ Pregnancy diagnosis by transrectal ultrasonography

2 vets

53±2 d post-Al
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)

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

**Pregnancy diagnosis by transrectal ultrasonography**
Performed at 41 days post AI
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.
Results of the ELISA test in serum samples from the 63 pregnant cows (= cows diagnosed PREGNANT by ultrasonography at 41±2 days post-AI)

Optical Density values of serum samples (~ targeted PAGs concentration)
COWS diagnosed PREGNANT by ultrasonography 41 ± 2 d post-AI
n = 63

~ 16 days post AI

OD values of serum samples

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

~ 16 days post AI

OD values of plasma samples

OD values of milk samples

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

~ 30 days post AI

OD values of serum samples

Se=100%
Sp=88.6%

OD = 0,30

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

~ 30 days post AI

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

4 supposed early pregnancy losses
COWS diagnosed PREGNANT by ultrasonography 41±2 d post-AI
n=63
～30 days post AI

Se=100%
Sp=100%

OD = 0.30

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

Se=100%
Sp=100%

OD = 0.30

Calculation with exclusion of the supposed pregnancy losses

Se=98.1%
Sp=100%

OD = 0.25

OD values of serum samples

OD values of plasma samples

OD values of milk samples

Days post-AI

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

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

OD values of serum samples

Se=98.4%
Sp=100%

OD values of plasma samples

Se=100%
Sp=100%

OD values of milk samples

Se=100%
Sp=92%

Calculation with exclusion of the supposed pregnancy losses

OD = 0.30

OD = 0.25
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 values of plasma samples

OD values of milk samples

~53 days post AI

Se=98.1%
Sp=96%

OD = 0.30

OD = 0.25
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