

---

---

## Pregnancy detection from milk samples obtained for routine milk yield measurements - results and evaluation

A. Monostori, H. Rácz, J. Seenger Kóti, L. Dégen

Livestock Performance Testing Ltd. H-2100, Gödöllő, Dózsa Gy. út 58. Hungary  
(Corresponding author: Monostori.Attila@atkft.hu)

The side effect of increasing milk production compromises the reproductive result in many high producing dairy herd in Hungary. The challenge is how to decrease the calving interval which has a detrimental effect on profitability of the dairy farm. The sooner detection of pregnancy shortens the calving intervals by identifying open cows earlier. Chemical tests for early pregnancy diagnosis that use qualitative measures of pregnancy-associated glycoproteins (PAGs) originating from the placenta have been developed and commercialized. PAGs are produced specifically by the placenta, the presence of PAGs in blood can be used to accurately determine pregnancy status. Recognizing the emerging importance of early detection of pregnancy, our company has introduced a PAG based bovine pregnancy testing from milk by IDEXX Milk Pregnancy ELISA test in April of 2013. The same sample which is routinely analyzed under milk recording procedure also can be appropriate for PAG test. The test can be applied since 35 days of pregnancy (nowdays 28 days). Up to now, a large number of samples has been examined and the results are continuously evaluated on the basis of reported number of calving. Inclusive January 2015, 29 440 tests were performed. By the end of April 2015, 92 stocks numbering 27 823 cows were involved in the service. The efficiency of the method was verified by taking reported number of calving as "golden standard" assuming 285 days pregnancy time +/- 14 days tolerance threshold. On the basis of our rationale the test fulfills the specificity and sensitivity criteria expected from a bovine pregnancy test and might be a reliable new method in detecting bovine pregnancy.

---

---

### Abstract

**Key words:** Milk, pregnancy test, PAG, milk-recording

The profitability and the competitive position of a dairy farm depends on many factors. Recently the profitability of a dairy farm is fundamentally influenced by its reproductivity. As the milk production is increasing, the reproductive problems occur more frequently, the calving interval dramatically becomes longer (437 days), the average conception rate is above 3. To maintain profitability a 400-day calving interval is preferable. Regarding the conception rate 2 or less than 2 is acceptable, in any dairy herd 80% of the cows should produce calves.

The key factor to decrease calving interval is the sooner detection of pregnancy. The average economic loss due to the reproductive problems is about 40.000-80.000 HUF (155-315 Euros) per cow per year in Hungary (Ózsvári 2012). One "empty day" in a dairy herd means a loss of 500-900 HUF (an average of 700 HUF = 2.7 Euros). This means 60.000 HUF reproductive loss per cow. In a dairy herd of 1000 cows this represents 60 million HUF (233.000 Euros) loss that can be 9-11% of the revenues of a dairy herd. Probably the reproductive problem represents the largest economic damage in Hungary.

---

---

### Introduction

An appropriate pregnancy test should be sensitive and specific. Sensitivity means the safe and correct identification of the pregnant cows (avoiding iatrogenic abortion), the specificity means the accurate detection of empty cows. The consequences of false negative results (pregnant cows diagnosed empty) which come from the low sensitivity of the test is more serious. False positive results (open cows diagnosed pregnant) coming from the low specificity of the test, can cause the later detection of the empty cows. The early and the late embryonic loss may influence the accuracy of the pregnancy test, the earlier the pregnancy test is done the more significant is this distortion. However the cow is defined empty by the test it must be examined by palpation and ultrasound methods (avoiding iatrogenic abortion), but due to the infertility testing it is also proposed. To use these two additional above mentioned examinations, we can evaluate the early embryo and fetus loss. The table chart below helps us to evaluate the losses well (Szelényi, 2014):

*Table 1. Evaluation of losses (Szelényi, 2014)*

| Early embryonic death<br>0 – 16 day | Late embryonic death<br>16 – 42 day | Early fetal death<br>42 – 90 day | Late fetal death<br>90 – 150 day | Abortion<br>150 – day |
|-------------------------------------|-------------------------------------|----------------------------------|----------------------------------|-----------------------|
|-------------------------------------|-------------------------------------|----------------------------------|----------------------------------|-----------------------|

#### **PAG ELISA pregnancy Test**

The milk pregnancy test is an enzyme-linked immunosorbent assay kit (ELISA) that is suitable for the detection of the placenta produced pregnancy related glycoproteins (PAGs) from milk sample. It makes possible to detect pregnancy from the 35th day after the insemination so it has a very important role in early identification of empty cows. The test can be used from 60th days after the preceding calving due to the persistency of the PAGs produced during the previous pregnancy. The pregnancy proteins (Pregnancy Associated Glycoproteins - PAGs) are the family of almost 100 different identified protein molecules produced by the placenta both the fetal and mother part. PAG can be detected from milk samples from the 35-40th days after the insemination till 60 days after calving accurately. The quantity and the contents of the PAGs measured in the milk samples are variable. The variance between individual cows is bigger. The threshold of the method (cut off line) considers the quantitative variations. The level of the PAGs decreases gradually after calving. Their half-life period is long. However they completely empty from the body by the 60-90 days after calving.

#### **Integration of pregnancy testing into the milk-recording system**

The milk pregnancy test can be easily integrated in the activities made by the Livestock Performance Testing Ltd. The calving interval may be reduced by the early detection of the empty cows. The advantages of including milk pregnancy test into the routine animal recording programme as follow:

- Milk sampling connected to animal recording made by sample technicians (certainly it can be made more frequently).
- Not necessary to hold and tie the animals.
- Not invasive intervention.
- No extra delivery and sampling cost.
- No extra labour needed.
- Fast and reliable results (within 48 hours).

The summary of the test criteria:

- The test can be performed from the 35th day of the conception.
- The test can be performed from the 60th day of the preceding calving.
- The test is not reliable in mastitis infection.

The milk samples are currently being received by two ways. With samples arriving through animal recording we know all the breeding data, so we can analyse them as "gold standard" taking the date of calving declared as a basis. In case of samples sent separately we can state the result.

## **1. Data preparation for the sampling**

The PAG samples are originated from test day milk samples (it can be defined of the same sample, the result is not influenced by preservatives). The first step - as usual during the animal recording - is data capture. The first data is being uploaded according to the general system. The cows are assorted with a data handler called WinTell Prior to the milking

## **Technological description**

## **2. Sampling**

The laboratory use violet-coloured sampling vials for pregnancy test. These vials are the same as the other ones used for general milk recording, only their colours are different.

During the test day when the cow is identified in the milk stand, the program warn the sample technicians (M-first sample, E-control sample) that PAG sample must be taken as well. In case of these cows the samples are taken into violet-coloured vial instead of white one. Using TruTest the white vial must be changed to violet one in the marked milk stands. In case of installed sampling equipment the vials must be changed in the sample collecting track (stand positioned vials) and the samples must be poured into these vials. The further procedure of the milk recording does not change.

## **3. Laboratory**

Pregnancy test is based on a kind of sandwich ELISA method. The test takes 4-4 ½ hours. The milk test laboratory provides the results to further data with IDs (barcodes) of the vial.

## **4. Evaluation, data providing**

In the data processing department we assign the test results to the cows and we send the reports to the farms. We provide the test results within two days after the arrival of the samples. We can provide the test results also in e-mail in data file, on demand. The report contains the number of the tested samples and the test results in a table chart.

**Pregnancy detection from milk samples  
obtained for routine milk yield**

**Table 2. PAG measurements between 04/2013 – 03/2014 (1 year).**

| Month             | Number of samples           | Pregnant | Open           | To repeat    |
|-------------------|-----------------------------|----------|----------------|--------------|
| All measurement   |                             |          |                |              |
| 04/2013 – 03/2014 | 14315                       | 8288     | 5383           | 640 (4,47 %) |
|                   | Through the Milk laboratory |          |                |              |
|                   | 6383                        | 3174     | 2992           | 220          |
|                   | Through the Data processing |          |                |              |
|                   | 7932                        | 5114     | 2391           | 420          |
| Days of gestation |                             |          |                |              |
| 0-34 days         | 394 (NE)                    | 166 (NE) | 199 (NE)       | 29 (NE)      |
| 35-45 days        | 2661                        | 1368     | 1035 (38,89 %) | 168          |
| 46-60 days        | 1901                        | 1028     | 724            | 149          |
| Over 60th days    | 3066                        | 2555     | 435            | 76           |

NE= not evacuate.

**Table 3. Detailed review of the PAG samples (04/2013 – 03/2014)**

| Days passed since AI   | PAG           | Results of pregnancy examination |                           |      |   |
|--|---------------|----------------------------------|---------------------------|------|---|
|  |               | Cows                             | Notification              | Cows | Remark  |
| Based on gestation days (passed time since AI in days). Pregnancy: 285 +/14 days | 1268 pregnant | 916                              | In time                   |      |   |
|  |               | 143                              | False notified date of AI |      | Calving of earlier AI   |
|  |               | 304                              | No calving                | 97   | Late embryonic death ~ 16,32 %<br>Waste or out of measurement |
|  |               | 1035 open                        | 1028 Pregnant             |      | 99,32%  |
|  |               | 168 to repeat                    | 6 Pregnant                |      |   |
|  |               | 162                              | Open                      |      |   |
|  |               | 728                              | In time                   |      |   |
|  |               | 102                              | False notified date of AI |      | Calving of earlier AI   |
|  |               | 148                              | No calving                | 57   | Early fetal death ~ 8,85 %<br>Waste or out of measurement     |
|  |               | 724 open                         | 720 Pregnant              |      | 99,44%  |
| 36 - 45 days   | 1028 pregnant | 40                               | Pregnant                  |      |   |
|  |               | 109                              | Open                      |      |   |
|  |               | 2172                             | In time                   |      |   |
|  |               | 239                              | False notified date of AI |      | Calving of earlier AI   |
|  |               | 144                              | No calving                | 76   | Early fetal death ~ 2,66 %<br>Waste or out of measurement     |
|  |               | 435 open                         | 430 Pregnant              |      | 98,85%  |
| More than 60 days  | 2555 pregnant | 11                               | Pregnant                  |      |   |
|  |               | 26                               | Pregnant                  |      |   |
|  |               | 50                               | Open                      |      |   |

Until the end of April this year 92 herds with 27 823 cows have used our service. We have already made 29 440 tests until the end of April 2015.

We can evaluate only the pregnancies running through our data processing because we only have breeding data of those cows. The cows detected pregnant are also distinguished according to the days passed after the conception. The selected day for sampling meet the criteria of the early pregnancy detection. As you can see, more sample arrived in the laboratory that could not meet the requirements of the test. Of course these samples were not evaluated. The most frequent mistake: the minimum 35 days have not passed after the insemination.

The data processing of the LPT Ltd. considers an insemination successful, if the recorded calving is around  $285 \pm 14$  days after the announced insemination!!! The results below are evaluated concerning this fact.

In the second table chart we can closely define the rate of fetus and embryo loss.

---

## **Results**

---

Szelényi Z., Gyori D., Bajcsy Á., Cs., Szenci O.: A korai vemhességvizsgálatok és az embrionális / magzati veszteségek értékelése egy borjúval vemhes és ikervemhes szarvasmarhák esetében. In: Akadémiai beszámolók: Klinikumok, Gyógyszertan, Toxikológia., 2014.01.27-2014.01.30. Budapest: Paper 30.

Ózsvári L.: A togygyulladás és a szaporasági zavarok által okozott veszteségek nagysága a hazai termelés-ellenorzött tejhasznosítású tehénállományokban. Holstein Magazin 20:(2) pp. 26-32. (2012).

---

## **List of references**

---