Pregnancy testing in dairy cows using a PAG test in milk samples: Different thresholds for different stages of the pregnancy

Jean Durocher, Robert K. Moore, Simon Dufour, Sebastien Buczinski, Shereen Hassan, Stephen LeBlanc, Daniel M. Lefebvre

Lactanet
RÉSEAU CANADIEN POUR L'EXCELLENCE LAITIÈRE
<table>
<thead>
<tr>
<th>Condition</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S-N) ≥ 0.25</td>
<td>Pregnant Cow</td>
</tr>
<tr>
<td>(S-N) ≥ 0.10 to &lt; 0.25</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>(S-N) &lt; 0.10</td>
<td>Open Cow</td>
</tr>
</tbody>
</table>
PAG Secretion During Pregnancy
Early Pregnancy Diagnosis

At this stage of pregnancy...

The average PAG value of pregnant cows

... is considerably greater than the proposed threshold for identifying open cows ($S-N < 0.10$)

In theory, this should positively influence the test’s **sensitivity**

... and limit the risk of declaring that a pregnant cow is « open »
Early Pregnancy Diagnosis

However, the average PAG value of pregnant cows

... is considerably greater than the proposed threshold for identifying pregnant cows ($S-N \geq 0.25$)

Given that:

- The embryo mortality rate is high at this stage of gestation
- The half-life of PAG after embryo loss is approximately 4 days
Early Pregnancy Diagnosis

The return of PAG levels to below the proposed threshold for identifying pregnant cows (S-N ≥ 0.25) … may take several days following embryo loss

In theory, this should have a negative influence on test specificity

... and increase the risk of declaring that a cow that has recently lost an embryo is « pregnant »
PAG Decrease Following Embryonic Loss

Source: Durocher (2015)
## Early Pregnancy Diagnosis

28 - 45 days post breeding

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>315</td>
<td>32</td>
</tr>
<tr>
<td>Open</td>
<td>3</td>
<td>290</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>640</strong></td>
<td></td>
</tr>
</tbody>
</table>

Excluding Inconclusive Results (16 / 656 = 2.4%)

**Sensitivity:** 99.1%

**Specificity:** 90.1%

Pregnancy Confirmation

At this stage of pregnancy ...

The difference between the average PAG value of pregnant cows and the proposed threshold for identifying open cows (S-N < 0.10) ...

... is much smaller than in early pregnancy

In theory, this situation should negatively influence the sensitivity of the test

... and increase the risk of declaring that a pregnant cow is « open »
Pregnancy Confirmation

The rate of embryo loss is relatively low at this stage of pregnancy

And ...

The difference between the average PAG value of pregnant cows and the proposed threshold for identifying pregnant cows (S-N ≥ 0.25)

... is much smaller than in the previous period
Pregnancy Confirmation

Consequently, with embryo loss...

The return of PAG levels to below the proposed threshold for identifying pregnant cows (S-N ≥ 0.25)

... will on average take place more quickly than in the previous period

In theory, this situation should positively influence the specificity of the test

... and decrease the risk of declaring that a cow that has recently lost an embryo is « pregnant »
Pregnancy Confirmation
46 - 65 days pregnant

Sensitivity: 96.4%
Specificity: 100%

Early Pregnancy Diagnosis

**Negative Predictive Value > 99%**

The economic impact of declaring a pregnant cow « open » is highly significant

**Positive Predictive Value > 95%**

The economic impact of declaring a cow that has recently lost an embryo « pregnant » is less significant

... in a context where the strategy includes pregnancy confirmation
Bayesian estimation of sensitivity and specificity of a milk pregnancy-associated glycoprotein-based ELISA and of transrectal ultrasonographic exam for diagnosis of pregnancy at 28–45 days following breeding in dairy cows

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\textsuperscript{c} Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, QC, J2S 7C6, Canada
\textsuperscript{d} Technology Assessment Unit, Royal Victoria Hospital, 687 Pine Avenue W, QC, H2A 1A1, Canada
Durocher (2015) Clinical Trial
GESTALAB in Early Pregnancy Diagnosis
28 - 37 days after breeding

Source: Durocher (2015) Clinical Trial
Durocher (2015) Clinical Trial
GESTALAB in Early Pregnancy Diagnosis
38 - 45 days after breeding

Source: Durocher (2015) Clinical Trial
Validation of test performance according to the probability of calving

- by range of days after breeding
- by range of PAG

Analysis of the Canadian Milk Recording Data Bank

Date of Successful Breeding
Date of PAG test
Date of Subsequent Calving
Duration of Pregnancy: 275 - 285 days

Source: Valacta and CanWest DHI (2018)
GESTALAB
28 - 37 days after breeding

PAG < 0.15

<table>
<thead>
<tr>
<th></th>
<th>TOTAL</th>
<th>Pregnant</th>
<th>Open</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6,249</td>
<td>26</td>
<td>6,223</td>
</tr>
</tbody>
</table>

Negative Predictive Value: 99.6%

Source: Durocher (2018)
GESTALAB
28 - 37 days after breeding

PAG
≥ 0.15 and < 0.25

<table>
<thead>
<tr>
<th>TOTAL</th>
<th>Pregnant</th>
<th>Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>469</td>
<td>6</td>
<td>468</td>
</tr>
</tbody>
</table>

Negative Predictive Value: 98.7%

Source: Durocher (2018)
GESTALAB
28 - 37 days after breeding

PAG
≥ 0.25 and < 0.35

<table>
<thead>
<tr>
<th>TOTAL</th>
<th>Pregnant</th>
<th>Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>243</td>
<td>16</td>
<td>227</td>
</tr>
</tbody>
</table>

Negative Predictive Value: 93.4%

Source: Durocher (2018)
GESTALAB

28 - 37 days after breeding

PAG
≥ 0.35 and < 0.50

<table>
<thead>
<tr>
<th>TOTAL</th>
<th>Pregnant</th>
<th>Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>88</td>
<td>216</td>
</tr>
</tbody>
</table>

Negative Predictive Value: 71.1%

Source: Durocher (2018)
GESTALAB
28 - 37 days after breeding

PAG ≥ 0.50

<table>
<thead>
<tr>
<th></th>
<th>TOTAL</th>
<th>Pregnant</th>
<th>Open</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15,302</td>
<td>13,595</td>
<td>1,707</td>
</tr>
</tbody>
</table>

Positive Predictive Value: 88.8%

Source: Durocher (2018)
Gestalab Volume by DSB

% of Samples

Days Since Last Breeding

- 0 - 23
- 24 - 25
- 26 - 27
- 28 - 37
- 38 - 45
- 46 - 49
- 50 - 90
- 91 - 150
- 151 - 207
- 208 - 279
- >= 280
## Gestalab Interpretation Grid

<table>
<thead>
<tr>
<th>Days Since Breeding</th>
<th>Thresholds</th>
</tr>
</thead>
<tbody>
<tr>
<td>26-27</td>
<td>0.10, 0.40</td>
</tr>
<tr>
<td>28-37</td>
<td>0.15, 0.25, 0.35, 0.50</td>
</tr>
<tr>
<td>38-49</td>
<td>0.10, 0.15, 0.25, 0.45</td>
</tr>
<tr>
<td>50-90</td>
<td>0.00, 0.10, 0.15, 0.25</td>
</tr>
<tr>
<td>91-150</td>
<td>0.10, 0.25, 0.35</td>
</tr>
<tr>
<td>151-207</td>
<td>0.25, 0.35</td>
</tr>
<tr>
<td>208-279</td>
<td>0.25, 0.50</td>
</tr>
</tbody>
</table>
# GESTALAB

## Producer Report

### Pregnancy Milk Test

<table>
<thead>
<tr>
<th>NAME</th>
<th>HERD NUMBER</th>
<th>PAGE</th>
<th>LAST TEST DATE</th>
<th>SAMPLING DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crackholm Farms</td>
<td>QC 7695</td>
<td>1</td>
<td>03 May 2019</td>
<td>30 May 2019</td>
</tr>
<tr>
<td>David Crack</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VETERINARIAN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Consult www.valacta.com/GESTALAB/cautionary guide to using the Gestalab report. Valacta is not responsible for losses or damages related to the improper use or interpretation of these results.

### Test Results

<table>
<thead>
<tr>
<th>Chain #</th>
<th>Cow Name</th>
<th>Last #</th>
<th>Days in Milk</th>
<th>Days Last Bred or Certified Calv</th>
<th>Sample type</th>
<th>ELISA Value</th>
<th>Test Results Interpretation</th>
<th>Recommendation</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td></td>
<td>2</td>
<td>166</td>
<td>26</td>
<td>M</td>
<td>0.06</td>
<td>Open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>255</td>
<td>2</td>
<td>313</td>
<td>29</td>
<td>M</td>
<td>0.06</td>
<td>Open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Barbade</td>
<td>3</td>
<td>92</td>
<td>29</td>
<td>M</td>
<td>0.35</td>
<td>Inconclusive</td>
<td>See explanatory note</td>
<td>7</td>
</tr>
<tr>
<td>24</td>
<td>4768</td>
<td>1</td>
<td>266</td>
<td>30</td>
<td>M</td>
<td>0.00</td>
<td>Open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Dasha</td>
<td>3</td>
<td>84</td>
<td>31</td>
<td>M</td>
<td>0.61</td>
<td>Pregnant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>5650</td>
<td>1</td>
<td>87</td>
<td>35</td>
<td>M</td>
<td>0.00</td>
<td>Open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>5843</td>
<td>1</td>
<td>121</td>
<td>66</td>
<td>M</td>
<td>0.63</td>
<td>Pregnant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>2</td>
<td>201</td>
<td>91</td>
<td>M</td>
<td>0.07</td>
<td>Probably open (98%)</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

7 An inconclusive result indicates that the ELISA value cannot confirm whether the cow is pregnant or open. This result may predict a normal pregnancy with a delayed increase in PAG (Pregnancy Associated Glycoprotein) or an embryo loss accompanied by a gradual decline in PAG. To confirm your cow’s reproductive status, please consult your veterinarian. Please note that this GESTALAB test will not be charged.

30 The majority of the cows (98%) with a similar ELISA value at this stage of the pregnancy are open.
Take Home

• PAG physiology – single threshold not optimal
• Using DSB greatly increases the value of the interpretation
• Power of DHI database allows to optimize the value of PAG test
• Vet involvement in using PAG testing in a reproduction strategy
Thank you!

Dr Jean Durocher - jdurocher@lactanet.ca