Novel milk phenotypes based on a biological model of lactation – a synthesis

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Introduction

> Lactation models have tried to use the underlying biology of the animal to describe the lactation curve (Dijkstra et al, 1997; Pollott, 2000)

> The use of such models may provide more information on which to base selection or management decisions than the methods currently used

> Can we measure components of the lactation curve from milk samples?

> Synthesis of work from 3 JDS papers and 2 EAAP presentations plus one ‘in press’ publication
Biology of lactation

Milk yield is the result of three major biological processes

- Mammary cell production and differentiation
- Milk secretion rate per cell
- Cell death rate
Biology of lactation 1

Cells produced

Week in relation to parturition

Relative scale
Biology of lactation 2
Cells dying off by apoptosis

Biology of lactation 3
Biology of lactation 5

Secretion rate per cell

Relative scale

Week in relation to parturition
Biology of lactation 6

![Milk yield](image)

Week in relation to parturition

Relative scale
Lactation curve

Milk production at 3 different lactation lengths

- 305d
- 370d
- 440d
Daily milk yield

Milk yield ($M_t$) on day $t$ of lactation is:

Number of differentiated parenchyma cells (NDPC)

minus

Number of differentiated cells dying ($NDCD_t$)

multiplied by

Cell secretion/offtake rate ($S_t$)

$$M_t = (NDPC - NDCD_t) S_{Mt}$$

If we could measure one or more of the these processes then we may be able to gain more control over milk production.
Daily milk yield

Milk yield ($M$) on day $t$ of lactation is:
Number of differentiated parenchyma cells ($NDPC$) minus
Number of differentiated cells dying ($NDCD_t$) multiplied by
Cell secretion/offtake rate ($S_t$)

$$M_t = (NDPC - NDCD_t) \cdot S_{Mt}$$

$$NDPC = \left(\frac{M_t}{S_{Mt}}\right) + NDCD_t$$
Estimating NDCD
Number of cells dying

- Apoptosis likely to be cause of decline in milk production in late lactation
- Commonly measured as persistency
- Extracellular vesicles (EV) formed by apoptosis
- Can we measure EV in milk to estimate apoptosis rate?
Extracellular vesicles

- EV - membrane-bound vesicles of less than 1µm diameter released from many different cell types
- Formed by blebbing of the parent cell membrane
- During cell membrane blebbing and EV formation phospholipids become exposed on the outer leaflet of the plasma membrane and the outer surface of the microparticle
- Presence of these normally hidden molecules allow the detection of microparticles by binding to specific markers (annexin V (AV) and merocyanin 540 (MC))
Correlations between persistency and the regression slope of the 4 microparticle densities on DIM for each cow (n = 12)

<table>
<thead>
<tr>
<th></th>
<th>Persistency</th>
<th>Total</th>
<th>Both+</th>
<th>AV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>-0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both+</td>
<td>-0.32</td>
<td>0.37</td>
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<td></td>
</tr>
<tr>
<td>AV+</td>
<td>-0.50</td>
<td>0.69</td>
<td>0.85</td>
<td></td>
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<td>MC+</td>
<td>-0.49</td>
<td>0.76</td>
<td>0.71</td>
<td>0.74</td>
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</tbody>
</table>

Correlations which were > 2 SE shown in bold
AV+ = Annexin-V positive microparticles; MC+ = MC540 positive microparticles; Both+ = microparticles positive for both Annexin-V and MC540; Total = all microparticles.
Cow EV densities by days in milk
EV density and persistency

![Graph showing the relationship between change in EV density (log cell/g/d) and persistency (g/d). The equation is given as y = -0.0467x + 0.0046 with R² = 0.2839.](image-url)
Number of differentiated cells dying

- Persistency related to EV in milk
- Relationship between EV and ‘apoptosis’ parameter needs defining
- Also the factors which affect it or cause variation need investigating
Estimating milk secretion rate per cell
Proposed secretion rates throughout lactation (Pollott, 2004)

Figure 4. Secretion rate curves for the 5 components from overall least squares means, assuming a constant milk secretion rate throughout lactation and maximal number of secretory cells = 2.8 x 10^{10}.
Gene expression study throughout lactation

**New question:** Can we use gene expression levels as an indicator of secretion rate throughout lactation?

**Supplementary question:** Can we do this with samples from a commercial herd?
14 genes studied

- Lactose – B4GALT1, LALBA, UGDH, UGP2
- Prolactin – JAK2
- Protein – CSN1S1, LALBA
- Cell cycle etc. – ARNTL2, CYSLTR2, FOXH1, UTRN
- Fat – ACACA, DGAT1, FASN, SCD

Differences found in gene expression levels between cows for all genes except DGAT1
Lactose genes
Prolactin genes
Correlations between gene expression and lactation curve parameters across cows

<table>
<thead>
<tr>
<th>Gene</th>
<th>Level of milk production (cow effect)</th>
<th>Rate of increase in milk production in early lactation</th>
<th>Rate of decrease in milk production (apoptosis?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACACA</td>
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<td>ARNTL2</td>
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<tr>
<td>CSN1S1</td>
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<td>CYSLTR2</td>
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<td>FASN</td>
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<tr>
<td>FOXH1</td>
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<tr>
<td>LALBA</td>
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<td>SCD</td>
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<tr>
<td>UTRN</td>
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<td>0.24</td>
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</table>
Implications

- RNA can be extracted from milk with biologically credible results
- Gene expression of several key genes varies across lactation
- Further analyses required to determine if GER can be used to estimate secretion rate in lactation
- GER in some genes related to lactation curve parameters
Daily milk yield- conclusions

Milk yield \((M)\) on day \(t\) of lactation is:

Number of differentiated parenchyma cells – only estimable

minus

Number of differentiated cells dying – measureable with EV?

multiplied by

Cell secretion/offtake rate – constant but varies between animals
Conclusions

- Encouraging start to investigating phenotypes in milk that may relate to lactation curve characteristics
- More work required on more cows in different environments to see how relationships vary
- Probably number of mammary cells produced has biggest effect on milk production