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A non-invasive method for measuring mammary apoptosis in dairy animals

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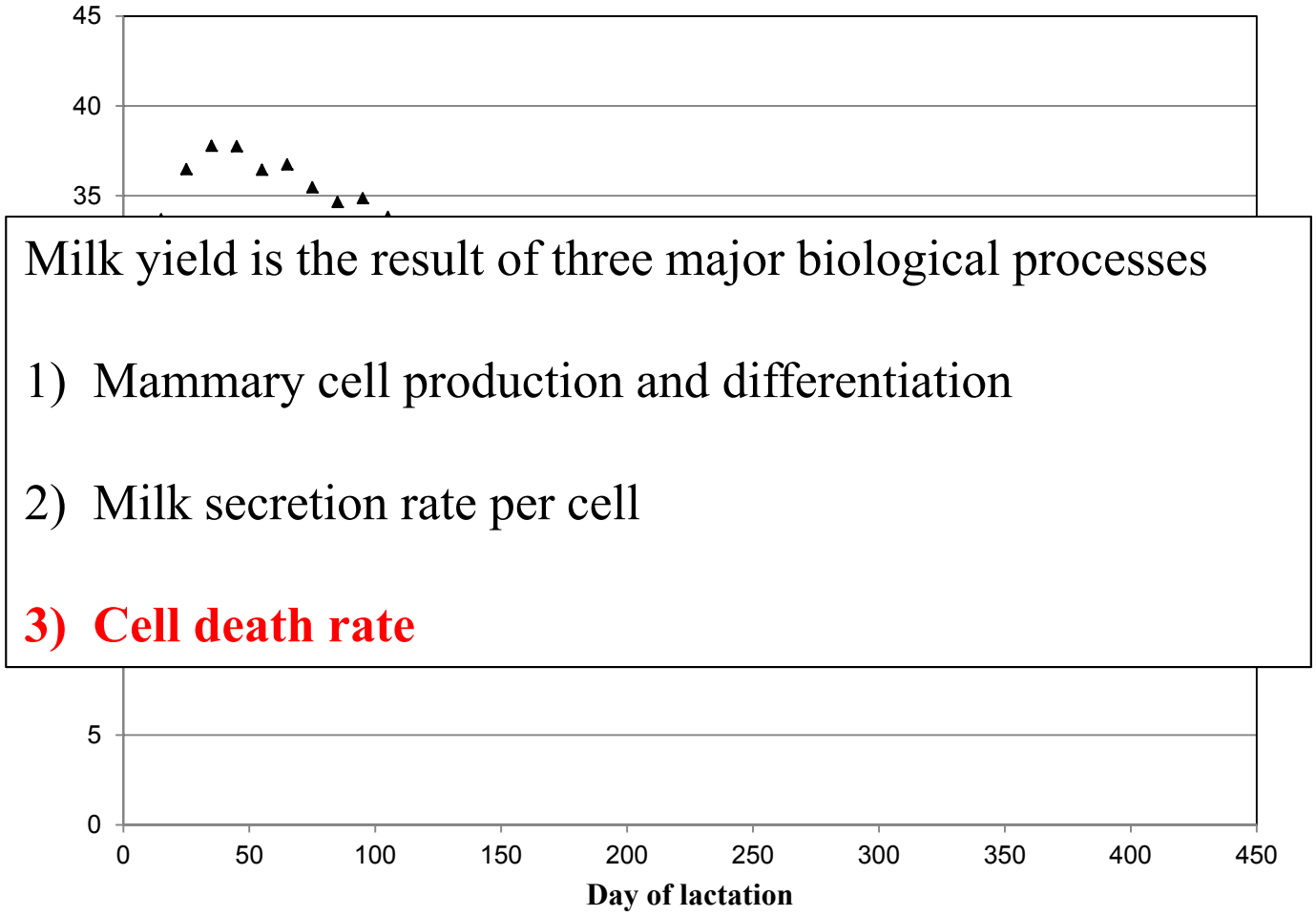
What determines milk production?



Milk yield is the result of three major biological processes

1) Mammary cell production and differentiation

2) Milk secretion rate per cell

3) Cell death rate

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Background

- Apoptosis of secretory cells is one of the key drivers of milk yield throughout lactation
- Past methods to measure apoptosis during lactation have been problematic
- Microparticles are released at times of cell activation and apoptosis
- Measuring microparticles in milk may help us understand one of the key determinants of milk yield – cell death rate by apoptosis

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Microparticles

- Microparticles (MP) are membrane-bound vesicles of less than 1μm diameter released from many different cell types
- Microparticles are formed by blebbing of the parent cell membrane
- During cell membrane blebbing and MP formation phospholipids become exposed on the outer leaflet of the plasma membrane and the outer surface of the microparticle
- It is the presence of these normally hidden molecules that allow for the detection of microparticles by binding to specific markers

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Milk samples

- Monthly milk samples collected from 12 cows over a 5 month period
- Farms records on milk production were taken from routine monthly recording on the farm
- 60 whole milk samples were frozen and transferred to the laboratory for MP analysis
- Microparticle density estimated using flow cytometry

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Laboratory method

- The phospholipid phosphatidylserine specifically binds to annexin V (**AV**), a calcium-dependent phospholipid-binding protein
- The negatively charged lipophilic dye merocyanine (**MC**) 540 used to detect the presence of disordered phospholipids on the membranes of MP
- MP with attached markers used to count cell numbers using flow cytometry
- More details in Journal of Dairy Science (2014) **97**:5017–5022.

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Data analysis

- Milk yield parameters plus four MP densities analysed
  - AV+ = Annexin-V positive MP density
  - MC+ = MC540 positive MP density
  - Both+ = MPs +ve for both Annexin-V and MC540 density
  - Total = all MP density
- MP = μ + Cow + b(DIM:Cow) + error
- Pearson correlation coefficients between parameters

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Results - data collected from 12 cows on 5 monthly recording days (n=57)

	Original data			Log_{10} transformed data		
	Mean	SD	Skewness	Mean	SD	Skewness
Days in milk (d)	201	68	0.11			
Milk yield (kg/d)	25.7	7.6	0.84			
Total (mp/µl)	334,705	198,669	0.91	5.44	0.29	-0.65
$Both^+$ (mp/µl)	5,207	8,753	4.26	3.35	0.59	-0.04
AV^+ (mp/µl)	120,473	104,898	1.62	4.92	0.40	-0.36
MC^+ (mp/µl)	108,686	70,374	1.04	4.94	0.31	-0.40

AV+ = Annexin-V positive microparticles; MC+ = MC540 positive microparticles;
Both+ = microparticles positive for both Annexin-V and MC540; Total = all microparticles.

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Results - ANOVA summary fitting effects of cow (n = 12) and days-in-milk (n = 5) within cow as a linear function

	DMY (kg/d)	Total (Log no/µl)	Both^{+} (Log no/µl)	AV^{+} (Log no/µl)	MC^{+} (Log no/µl)
Cow	***[1]	***	***	***	***
DIM[2] within cow	***	0.051	*	***	**
Residual	5.59	0.038	0.249	0.070	0.037
Mean cow intercept	44.9	4.9	2.7	4.2	4. 3
SE	± 2.90	± 0.080	± 0.46	± 0.027	± 0.11
Mean slope (/d)	-0.097	0.003	0.004	0.004	0.003
SE	± 0.010	± 0.0004	± 0.0024	± 0.0014	± 0.0005

[1]Probability values or *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.
[2]DIM = days in milk; DMY = daily milk yield.
AV^{+} = Annexin-V positive microparticles; MC^{+} = MC540 positive microparticles;
Both^{+} = microparticles positive for both Annexin-V and MC540; Total refers to all microparticles

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Results - Correlations between persistency and the regression slope of the 4 microparticle densities on DIM for each cow (n = 12)

	Persistency	Total	Both$^+$	AV$^+$
Total	**-0.65**			
Both$^+$	-0.32	0.37		
AV$^+$	**-0.50**	**0.69**	**0.85**	
MC$^+$	**-0.49**	**0.76**	**0.71**	**0.74**

Correlations which were > 2 SE shown in bold
SE of correlations: correlations of 0 to 0.4 - SE of ~0.27; 0.5 to 0.7 - SE of ~0.19; > 0.7 - SE of ~0.11.
AV$^+$ = Annexin-V positive microparticles; MC$^+$ = MC540 positive microparticles;
Both$^+$ = microparticles positive for both Annexin-V and MC540; Total = all microparticles.

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Implications

- Extraction of microparticles from milk is viable
- From a limited dataset we have shown that changes in microparticle density are related to the decline in milk yield in late lactation
- This is likely to be linked through apoptosis
- We have found a useful non-invasive method for monitoring apoptosis of mammary cells

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Further work

- Compare pregnant and non-pregnant cows
- Differentiate between MP from apoptosis and other cell activities
- Study MP density throughout the milking process
- Study MP production throughout more, complete lactations
- Study factors affecting MP production

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