Milking-induced prolactin release has proliferative and survival roles on mammary epithelial cells in dairy cows.

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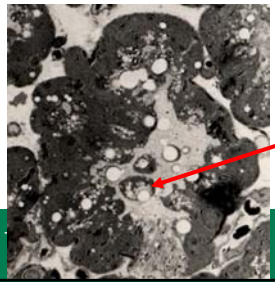
The 61st Annual EAAP Meeting
Crete, 23 - 27 August 2010

Introduction

- The ability of ruminant mammary glands to produce milk is determined by the number of cells secreting milk (**mammary epithelial cells, MEC**).
- As lactation advances, despite a permanent mammary epithelial **cell proliferation**, there is a gradual reduction in the number of milk-secreting cells, either through cell death or by the **abrasion of epithelial cells during milk ejection**.



Mammary epithelial cells released in the milk

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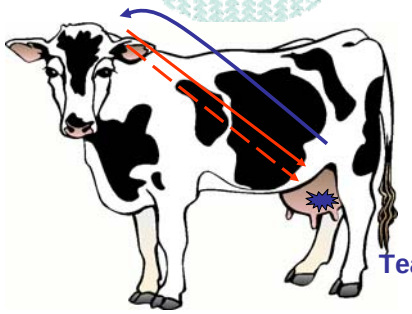
What is the control ?

Mammary epithelial cells released in the milk

Introduction

Prolactin release in blood

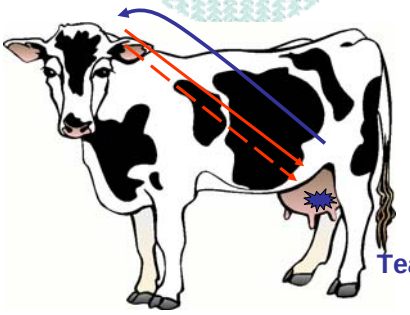


Teat stimulation

- ❑ Milking and nursing induce the release of prolactin (PRL)
- ❑ Milking-induced PRL release decreases as lactation advances (Fuchs et al., 1984)
- ❑ PRL is a survival factor for mammary epithelial cell cultivated in vitro in cows (Accorsi et al. 2002).

Introduction

Prolactin release in blood



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- Milk[illegible]ases as lactation adva[illegible]
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Is Prolactin the control ?

Hypothesis

The milking-induced prolactin release has proliferative and survival roles on mammary epithelial cells in dairy cows

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Objectives

To inhibit the release of PRL at milking, we used a dopaminergic agonist (Quinagolide) that prevents PRL secretion by the lactotroph cells in the adeno-hypophysis and to restore it by PRL injections

Methodology

- Animals:
  - **9 multiparous Holstein cows**
    (134 d of lactation)

  - Latin square design:
  - 3 5-d periods
  - 3 treatments:
    - **Quin**: daily i.m. injections of Quinagolide
    - **Quin+PRL**: daily i.m. injections of Quinagolide and twice a day i.v injections of bovine PRL
    - **Control**: daily injections of water

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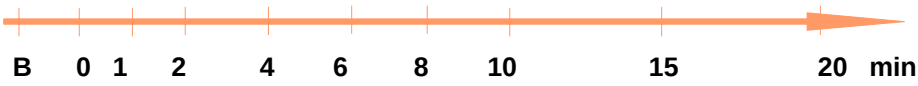
Methodology

❑ **Blood samples** were collected before and during the morning milking (4th day) for prolactin analysis by ELISA



B : Basal / 0 : installation of milking unit

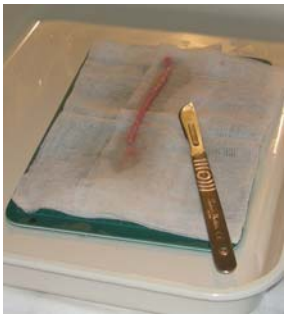
Methodology

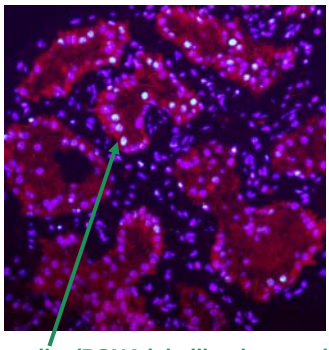
❑ **Individual daily milk yields** were measured.

❑ **Milk samples** were collected for composition (fat, protein and lactose)

Methodology

- Mammary biopsies were collected using the method of Farr et al., (1996).
- Mammary cell proliferation were analysed by immuno-histochemistry using Proliferative Cell Nuclear Antigen labelling.

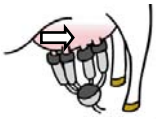




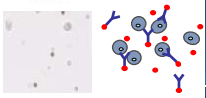
Proliferative cells (PCNA labelling in green)

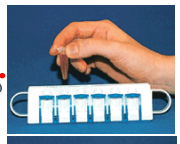
Methodology

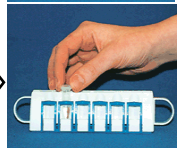
- **Mammary epithelial cells** were purified from milk



Isolation of milk cells by centrifugation







Purification of milk MEC using magnetic beads coated with anti-cytokeratin antibody on MPC (Dynal)

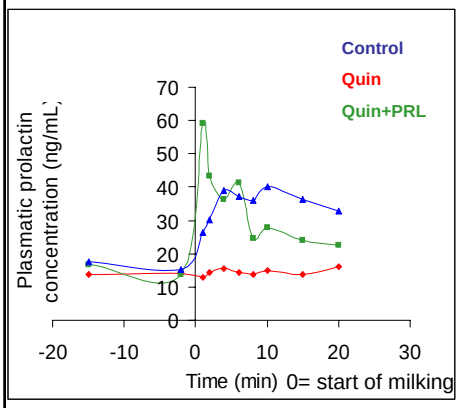
Cell count and viability determinations

Vi-CELL™, Beckman Coulter

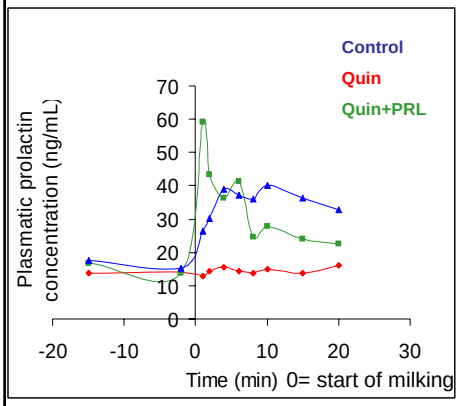
Boutinaud *et al.* JDS 2008

Results

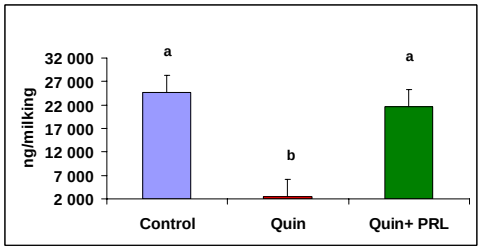
Injections of Quin reduced milking-induced PRL release…



Injections of Quin reduced milking-induced PRL release…



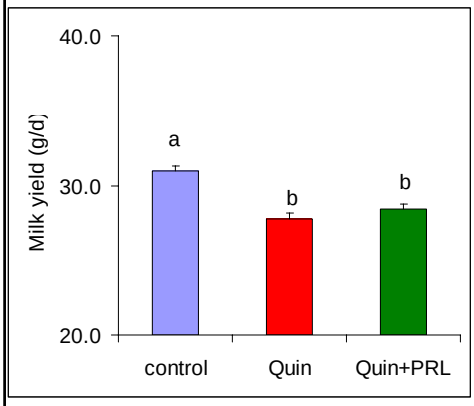
Area under the curve = Amount of PRL release



a, b: P< 0.05

… PRL injections mimicked the endogenous PRL release at milking

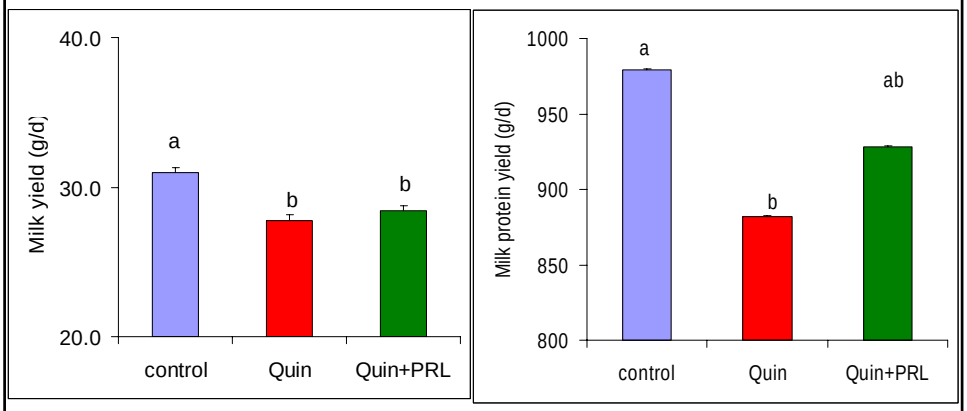
Injections of Quin decreased milk production…



a, b: P< 0.05

… PRL injections had no effect on milk yield

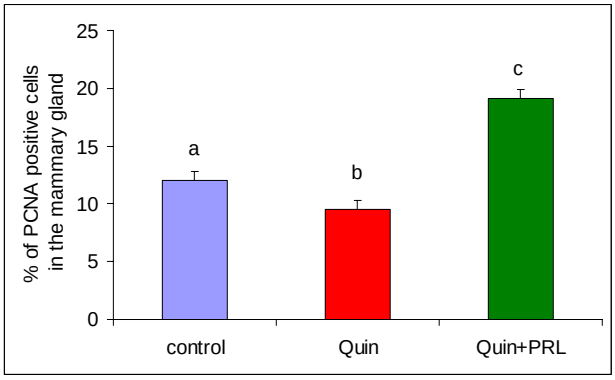
Injections of Quin decreased milk production…



a, b: P< 0.05

… PRL injections had no effect on milk yield but had a tendency to increase milk protein yield (P=0.09)

Injections of Quin decreased cell proliferation in the mammary gland



a, b, c: P<0.05

…PRL injections clearly increased mammary cell proliferation

Injections of Quinagolide increased the number of Mammary Epithelial Cells released in the milk per day

	Treatments				Contrast	
	Control	Quin	Quin+PRL	SEM	Quin effect	PRL effect
MEC/d	204,000	326,000	256,000	29,400	0.02	0.099
MEC viability (%)	62	53	69	3.9	0.85	0.008

…PRL injections tended to decrease it and increased milk Mammary Epithelial Cell viability.

Conclusions

- Chronic administrations of Quinagolide ↘ milk production in dairy cows. Quin ↘ mammary cell proliferation and ↗ MEC release in the milk.
- PRL injections at milking time were not sufficient to restore milk yield (≠for milk protein yield). PRL injections ↗ mammary cell proliferation and the viability of MEC purified from milk.
- Thus, the milking-induced PRL release is a survival factor for bovine mammary epithelial cells. Therefore, it plays a major role in the control of lactation persistency.

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