Exfoliation of mammary epithelial cells in milk is linked to lactation persistency in dairy cows

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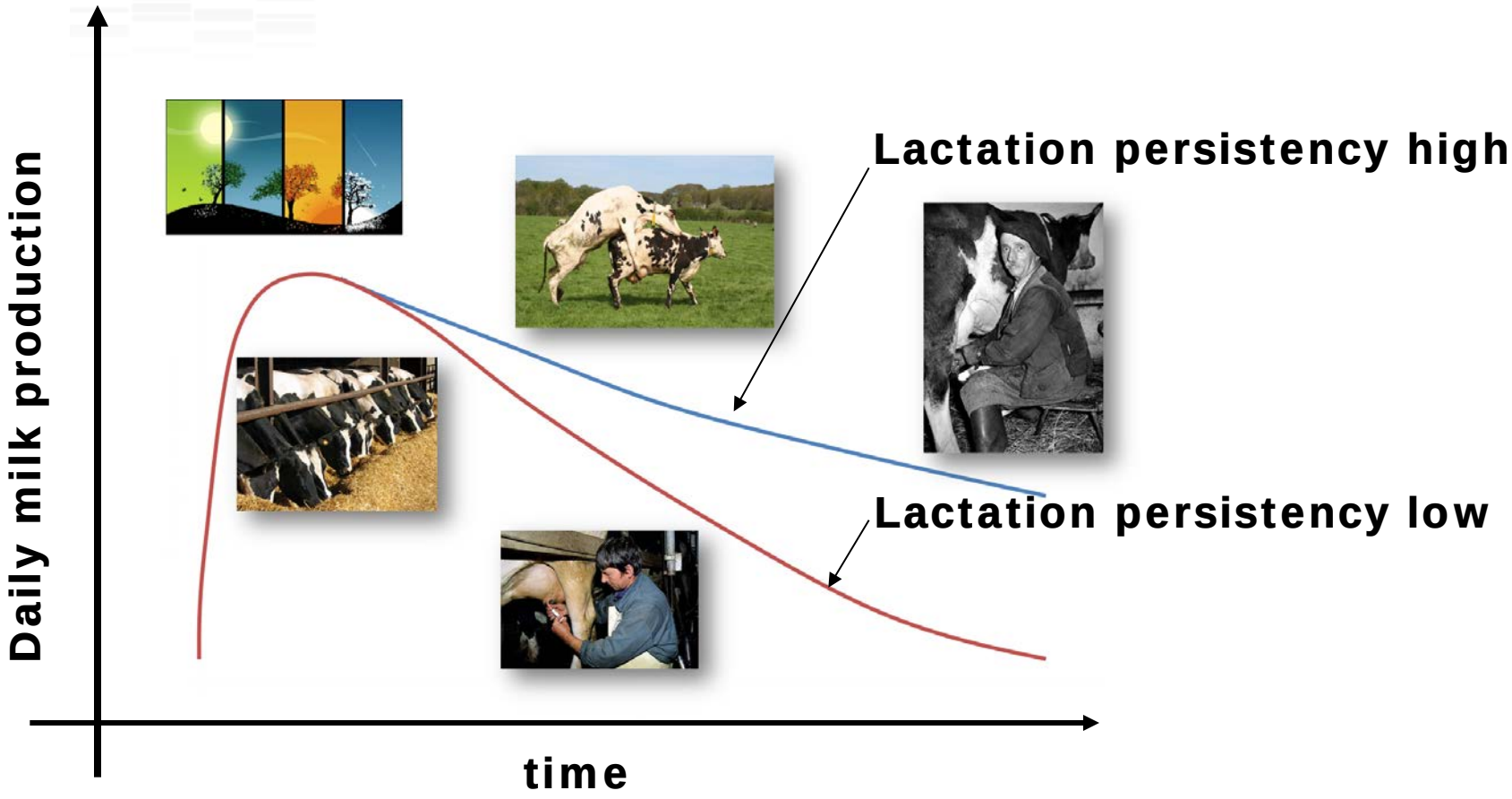
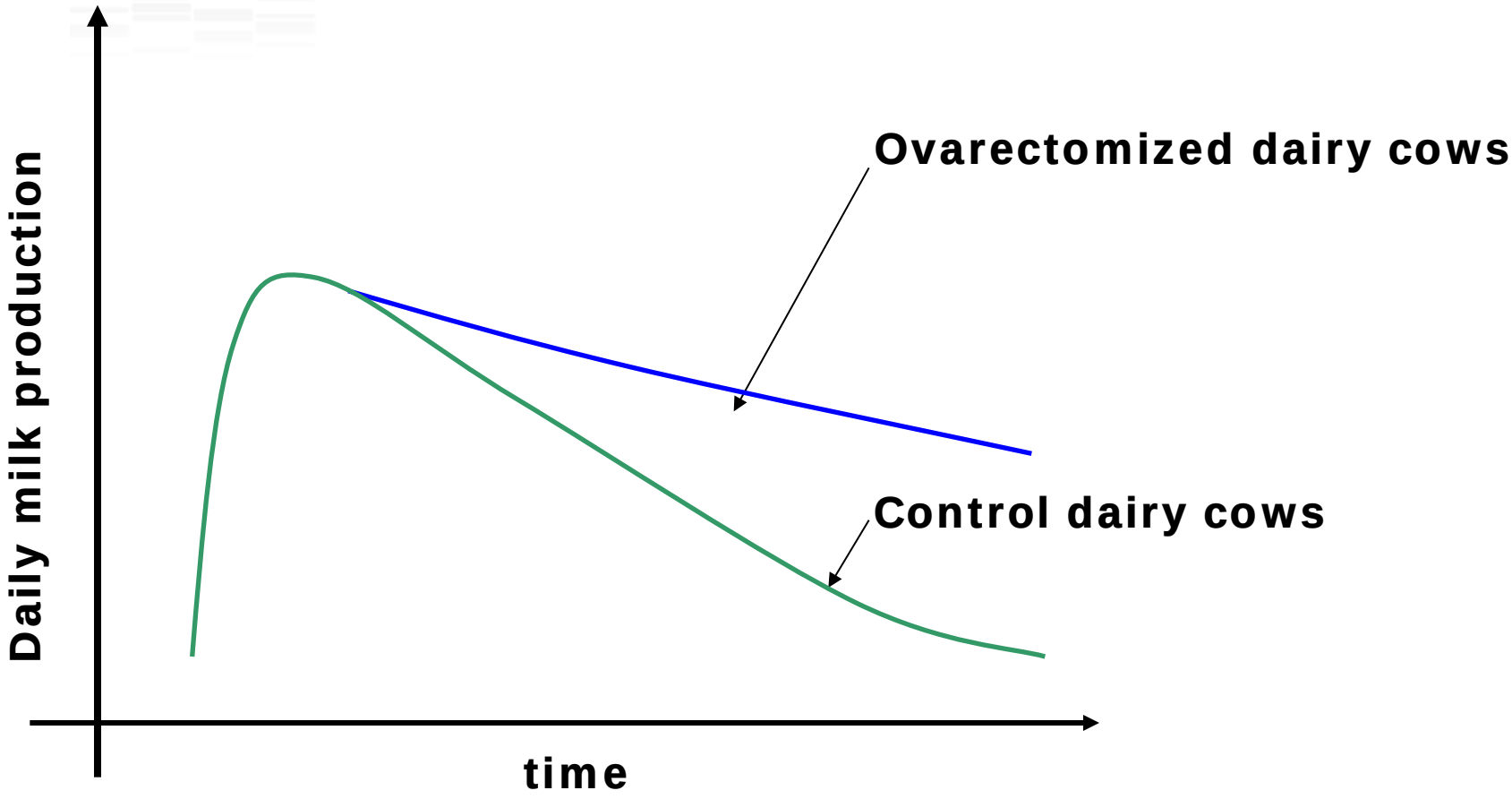
Lactation persistency



Capuco et al. JAS 2003

Lactation persistency



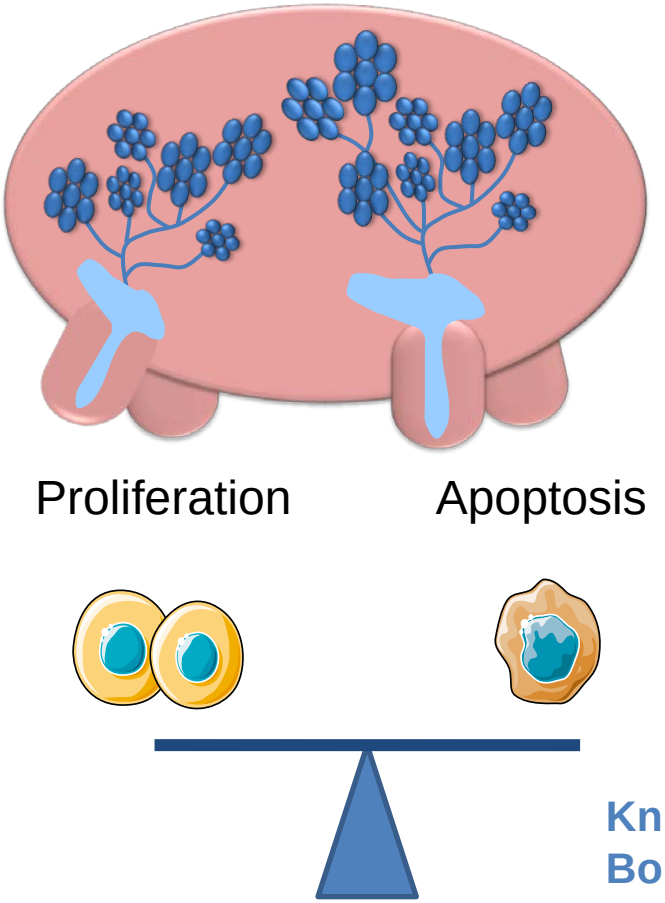
Yart et al. JDS 2012
Yart et al. Steroids 2013

Improving lactation persistency

- **Enhancing lactation persistency would allow dairy producers to extend lactation, and thus reduce the number of periods in early lactation and would reduce calving frequency and reduce the risks associated.**

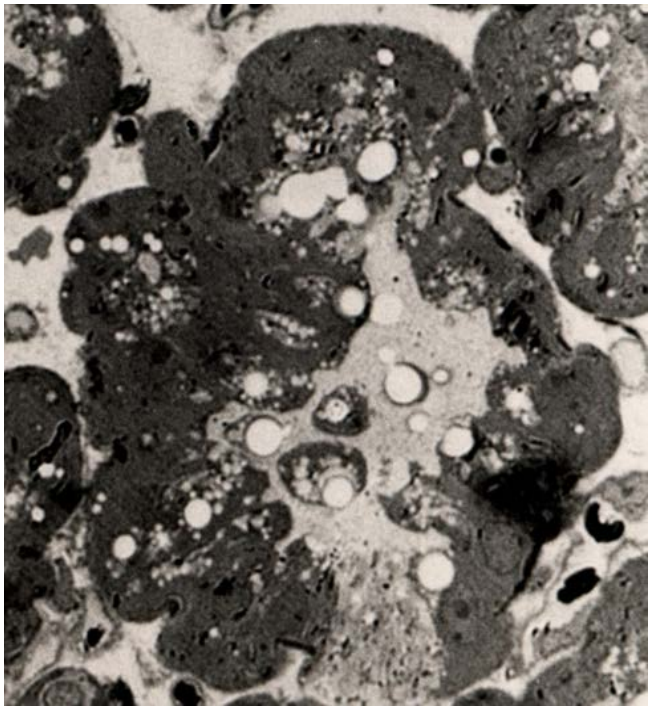
→ **Necessary to understand the mechanisms responsible for the regulation in lactation persistency**

Lactation persistency depends on the number of Mammary Epithelial Cells



Knight and Peaker Exp Physiol 1984
Boutinaud et al. RND 2004

MEC are shed into milk during the lactation process.

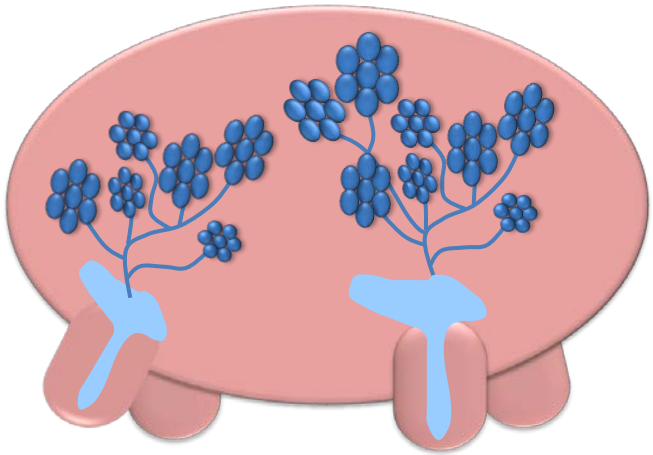


- **Milk contains MEC exfoliated during the lactation process (Boutinaud and Jammes RND 2002).**
- **MEC shedding is regulated by prolactin during lactation (Lollivier et al. ADSA 2009) and during involution (Boutinaud et al. ADSA 2013).**

→ **What are the other factors that regulate MEC shedding in milk?**

Objectives

- To characterize the exfoliation and apoptosis of MEC in milk in relation with lactation persistency



Materials and Methods

Experimental design

14 multiparous Holstein cows

Ovariectomized (n=7)

Ovariectomy
8 weeks pp.

Sham-operated (n=7)

October 2010

November 2011

Calving

Sample 1
wk 5

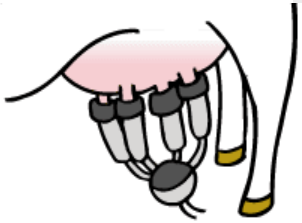
Sample 2
wk 21

Sample 3
wk 37

Sample 4
wk 47

Sample 5
wk 54

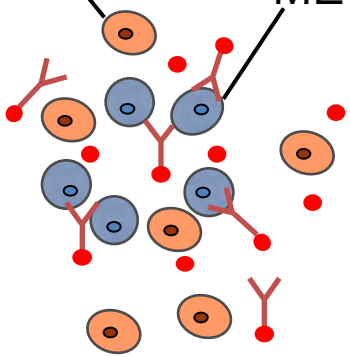
MEC purification from milk and analyses

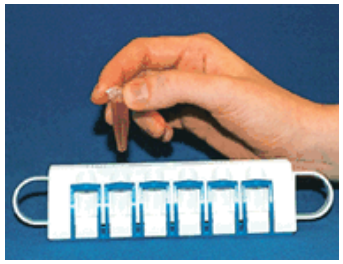


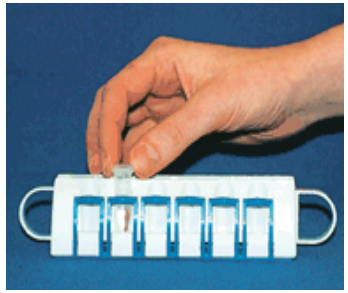
Isolation of milk cells by centrifugation

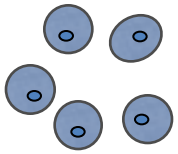
Leucocytes

MEC





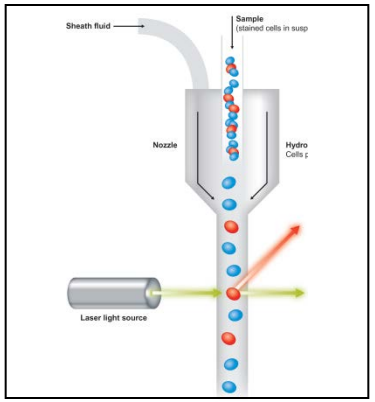




Purification of MEC using magnetic beads coated with anti-cytokeratin antibody on MPC (Dynal)

Boutinaud *et al.* JDS 2008

1. cell count determinations

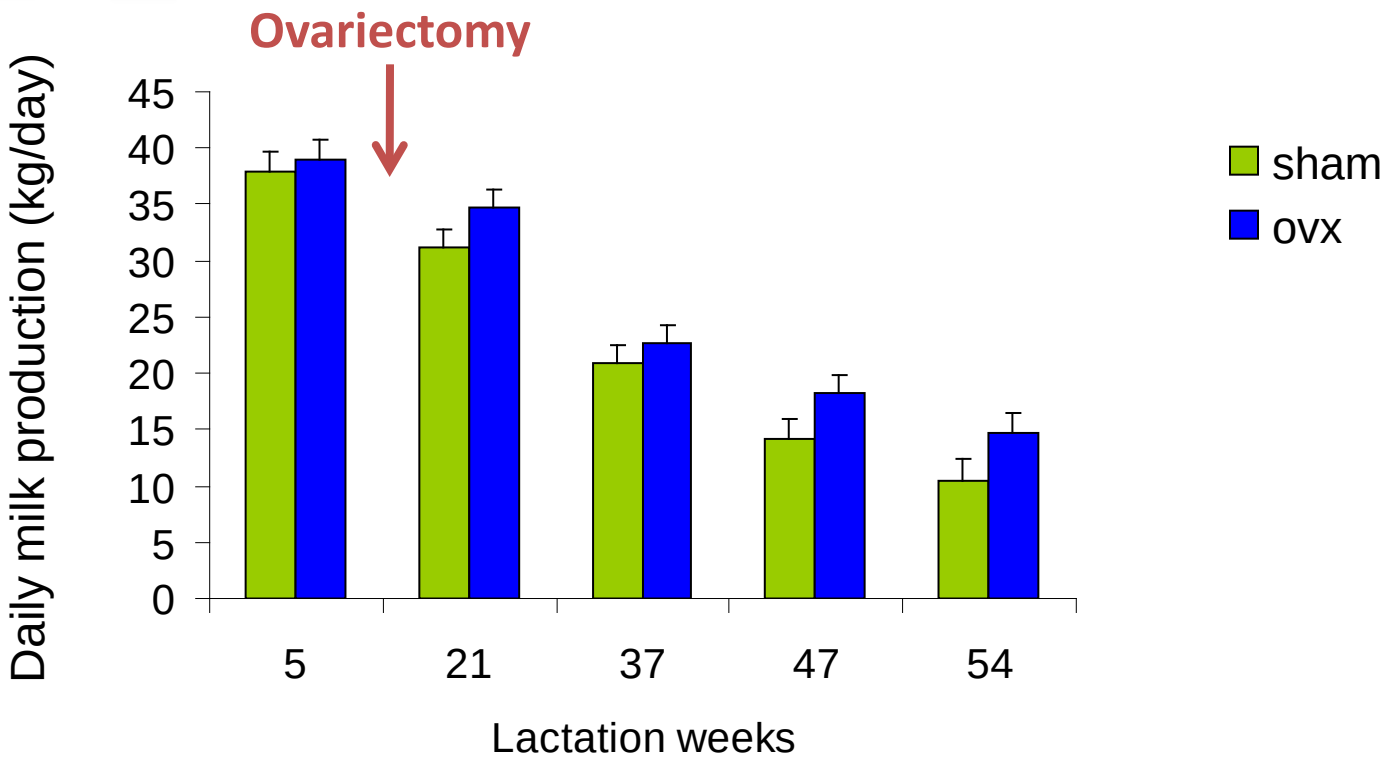


2. flow cytometry analyses after tunel staining

3. RNA extraction and Real Time qPCR

Results

Milk production

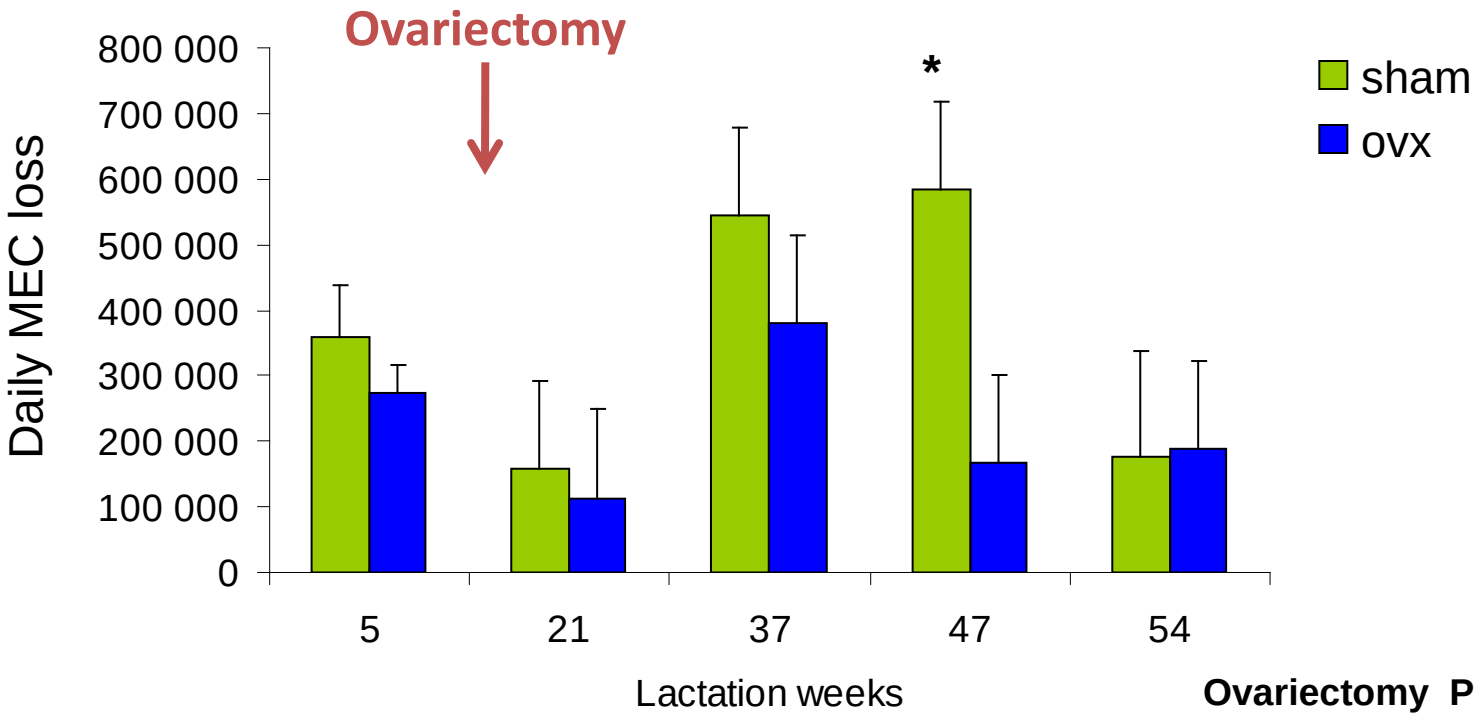


Ovariectomy P = 0.02

Lactation stage P < 0.001

As expected daily milk yield was reduced as the stage of lactation advanced whereas ovariectomy limited the decline in milk yield.

Daily MEC loss



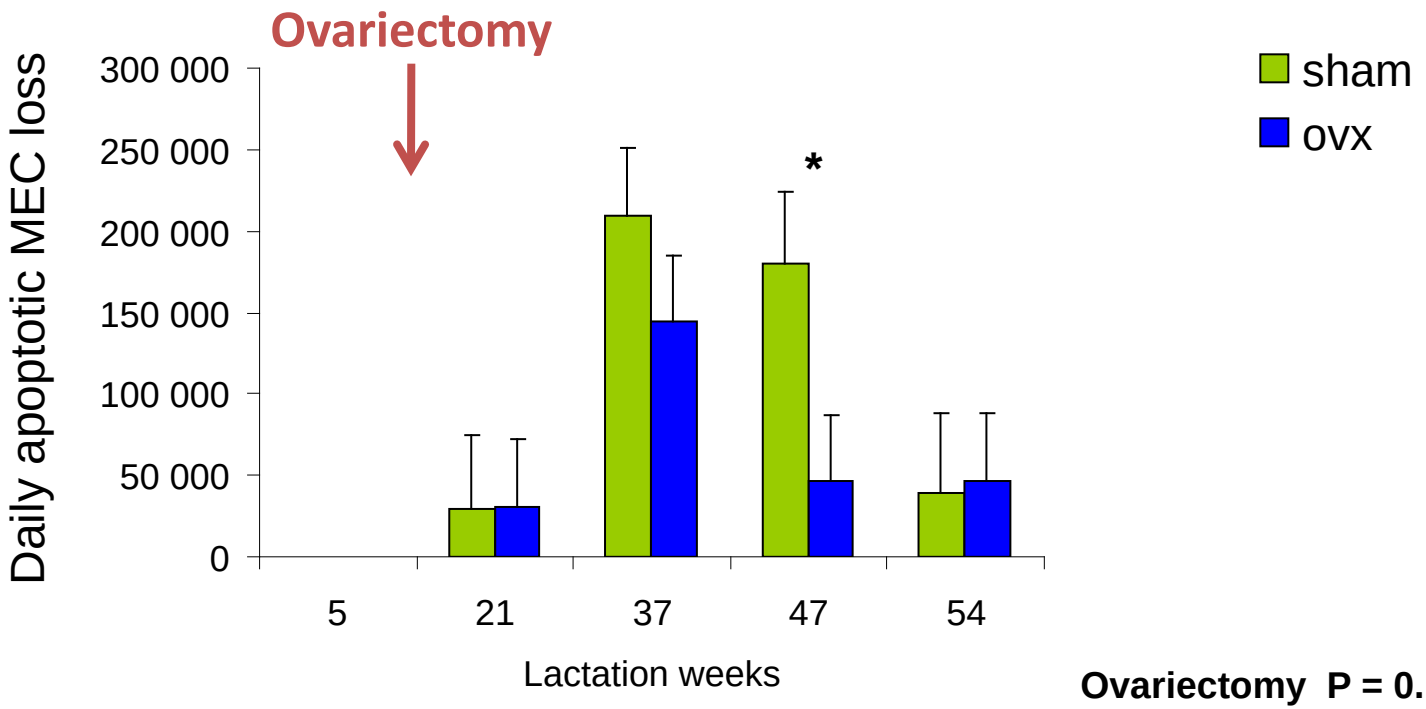
Ovariectomy P = 0.16

Lactation stage P = 0.07

A trend toward MEC being more exfoliated in milk as the lactation stages avanced. Ovariectomy decreased it at 47 weeks of lactation.

Daily apoptotic MEC loss

Both stage of lactation (P=0.03) and ovariectomy (P= 0.08) affected the percentage of apoptotic MEC in milk: 26 vs 21 % for sham and OVX, respectively.

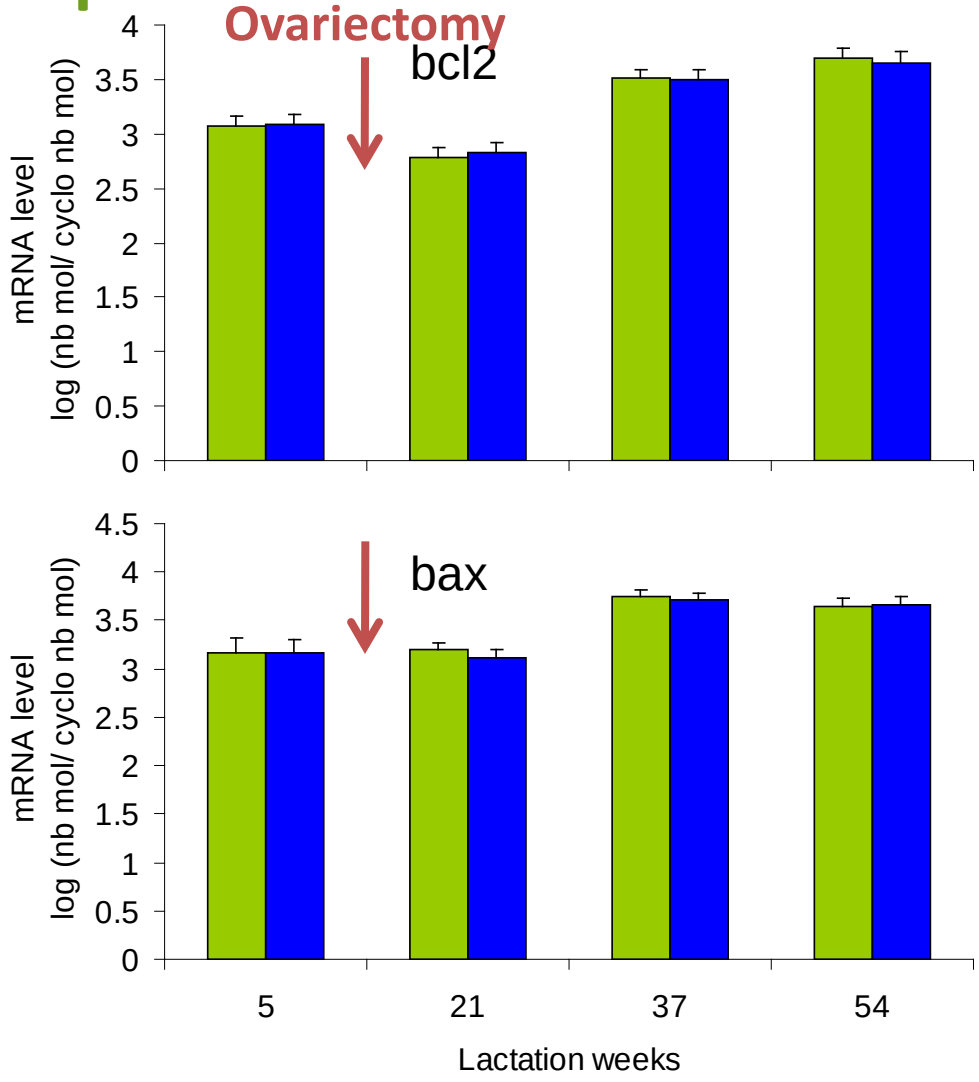


Ovariectomy P = 0.14

Lactation stage P = 0.005

As a consequence, the stage of lactation significantly affected the apoptotic MEC loss and ovariectomy decreased it at 47 weeks.

Apoptotic marker mRNA levels



Ovariectomy P = 0.97

Lactation stage P < 0.0001

Ovariectomy P = 0.63

Lactation stage P < 0.0001

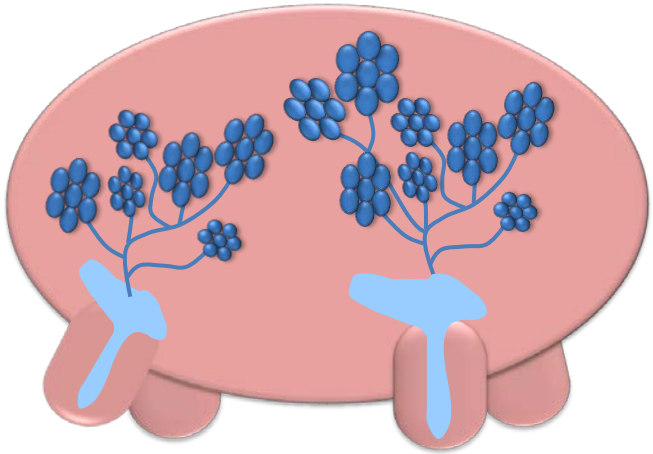
Anti and pro apoptotic mRNA rose as the lactation stage advanced. Ovariectomy did not affect these transcripts in milk purified MEC.

Conclusions

Conclusions

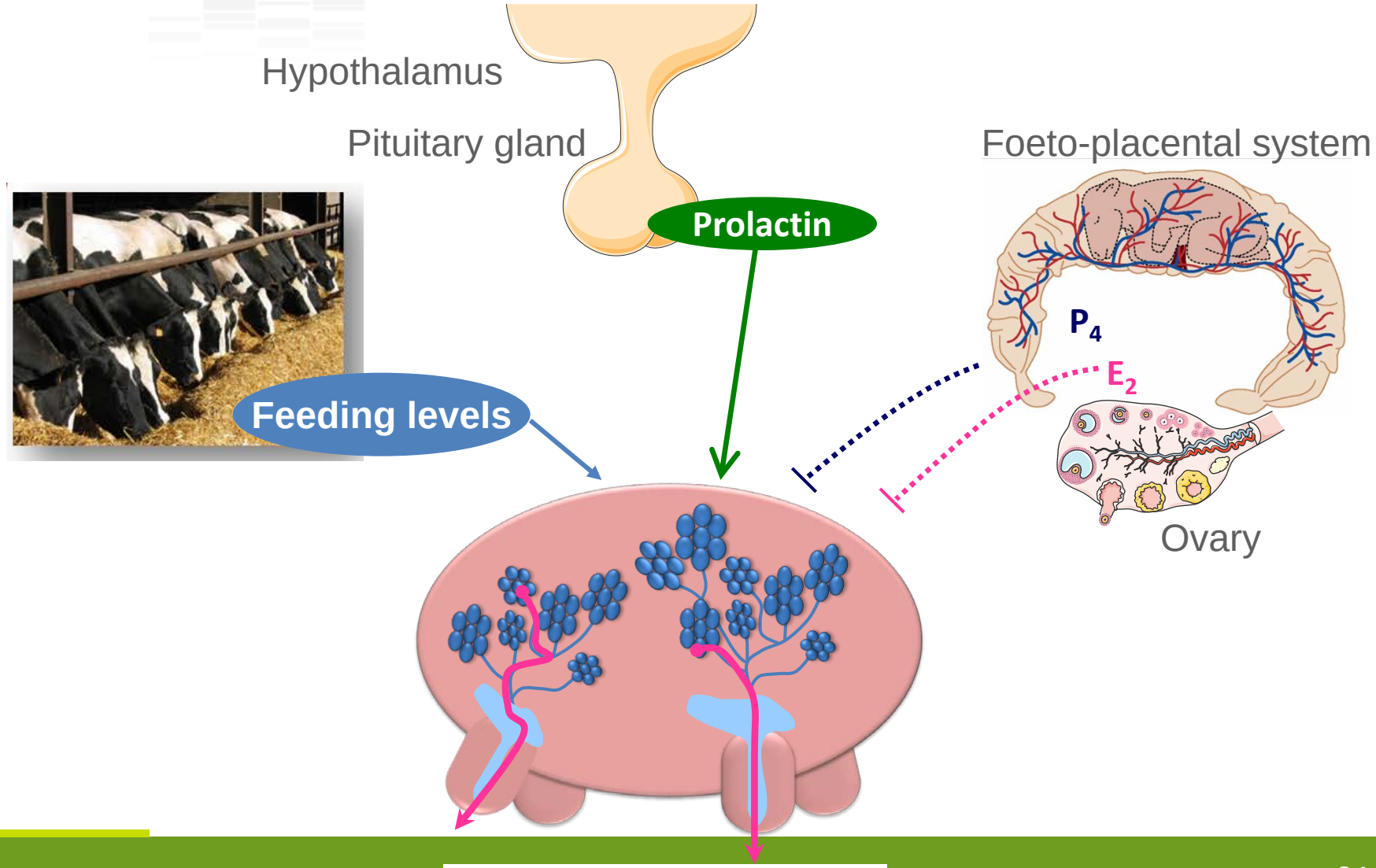
- The decrease in milk yield with the advanced stages of lactation was associated with more apoptotic MEC exfoliation in milk and ↗apoptotic marker mRNA levels.

- The lower decline in milk yield after ovariectomy was associated with lower MEC exfoliation and apoptosis at 47 weeks of lactation.



MEC exfoliation in milk and apoptosis are negatively linked to lactation persistency

Perspectives: physiological factors that regulates lactation persistency



I would like to thank the team of the experimental farm (J. Lassalas, A. Mottin, IEPL) and S. Wiart, L. Finot, E. Leguennec and P. Debournoux for their technical assistance. Thank you for your attention.

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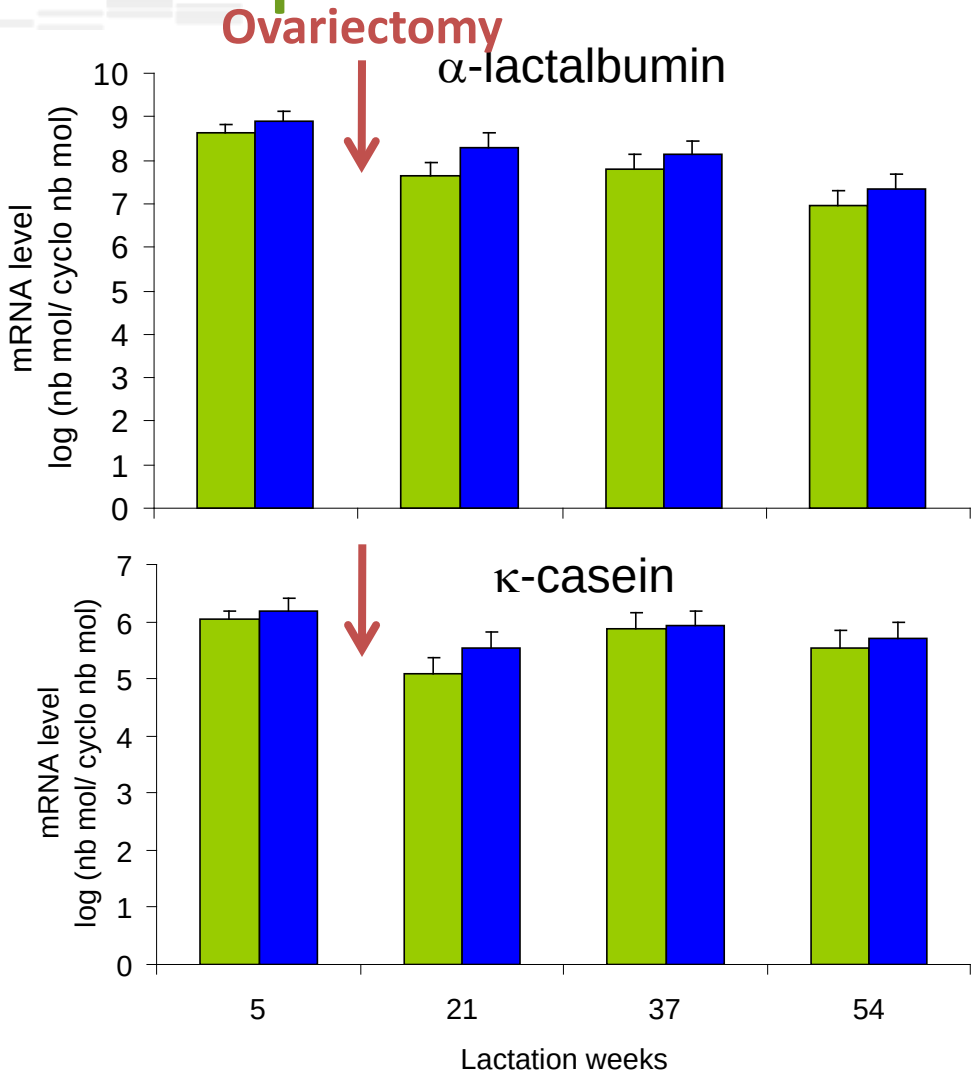
Milk protein mRNA levels



Ovariectomy P = 0.12

Lactation stage P = 0.03

Ovariectomy P = 0.38

Lactation stage P = 0.14

The mRNA level of α-lactalbumin was reduced as the lactation stage advanced.

Stastistical analyses

Data analyzed by ANOVA using the MIXED procedure of the SAS software program with REPEATED statement. Time was used as a repeated effect, and cow (treatment) was used as the subject

$$Yijk = \mu + \alpha i + \beta j + \alpha i\beta j + \gamma k + \varepsilon ijk$$

ovariectomy

lactation stage

ovariectomy x lactation stage

cow within the treatment

Data before ovariectomy used a co variable