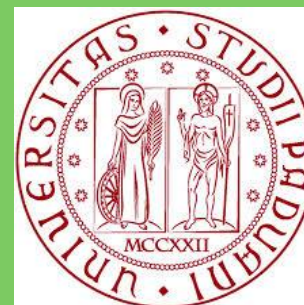


# Differential expression of mammary epithelial cell subpopulations in milk as possible biomarker of management quality and mammary gland functionality in dairy cow

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University of Torino, University of Bologna  
University of Padova, Italy



# Dairy-Agess

Home

About us

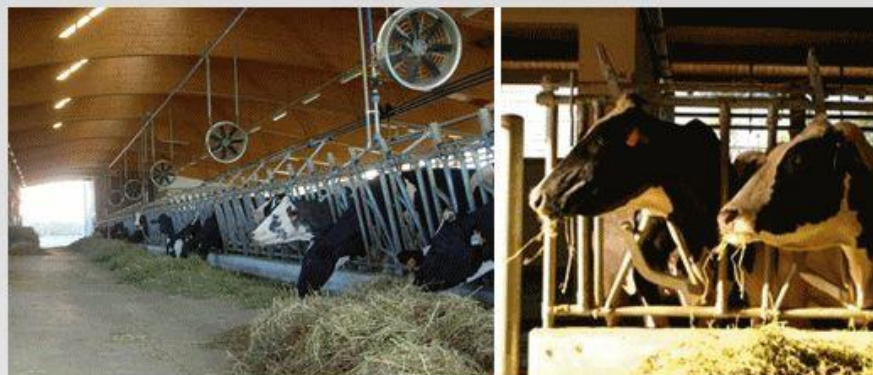
Our Project

Research Units

Partners

Contact us

*looking to the future*  
Increase dairy farming sustainability by opposing aging and stress



Welcome to our site

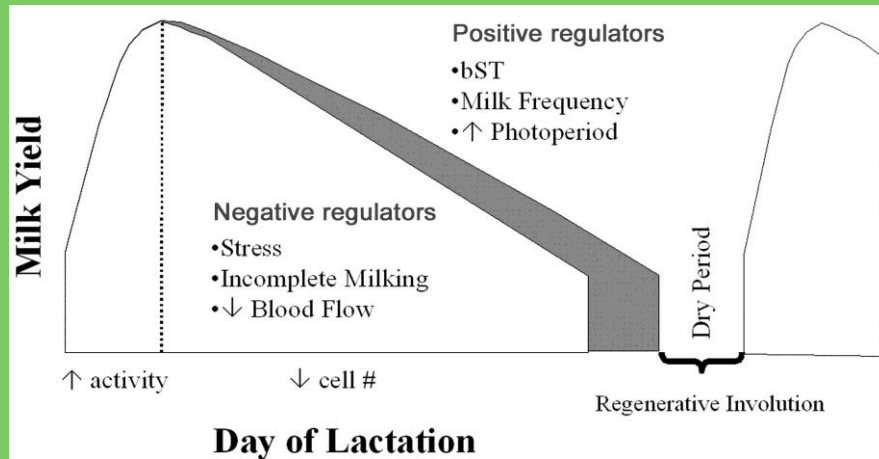
*"Sustainability between conventional dairy farm systems and longevity: models of dry period management and biomarkers of stress and aging in the mammary gland" funded by the Italian Ministry of Reserch (PRIN 2010-2011)*

[www.dairy-agess.org](http://www.dairy-agess.org)

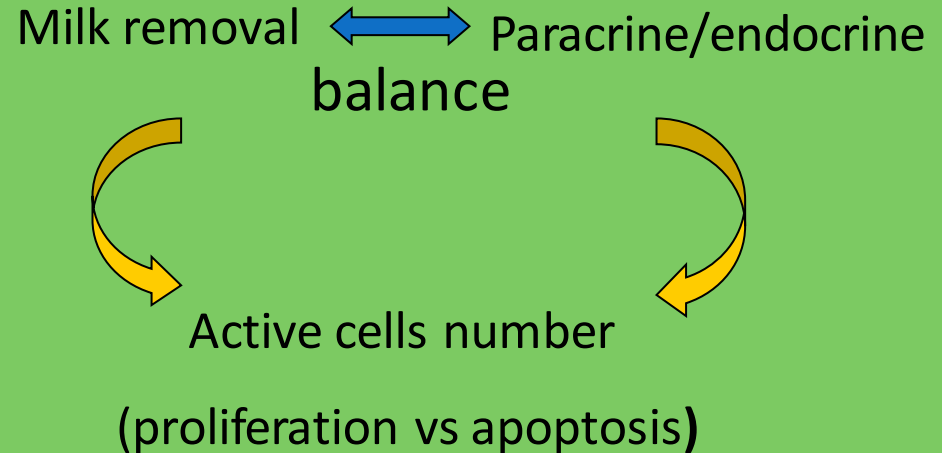
## Lines of research:

- Animal welfare in dry period vs oxidative stress during lactation
- Antioxidant (flax seed) administration in the dry period and oxidative stress in early lactation
- Ageing (mammary gland functionality) vs n# lactations

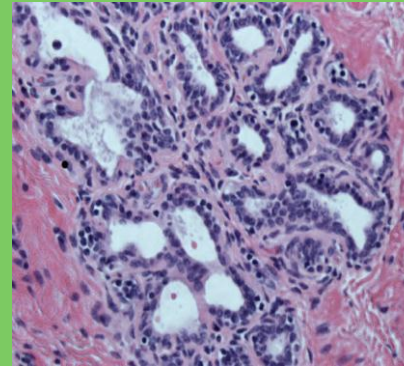
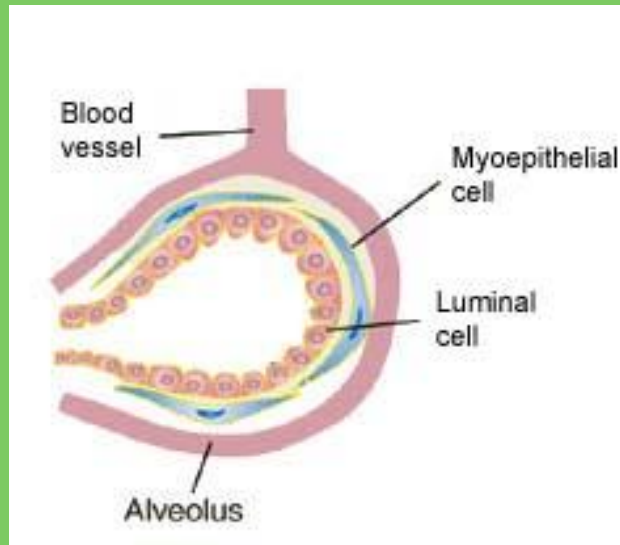
# What happen in mammary gland during lactation ?



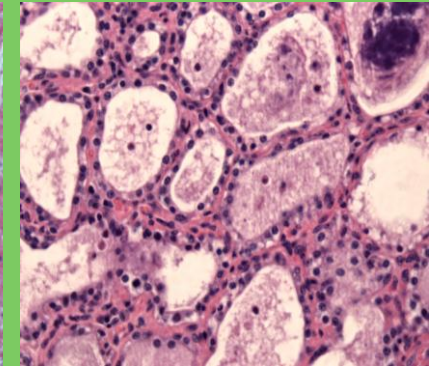
(Capuco & Ellis, 2003)



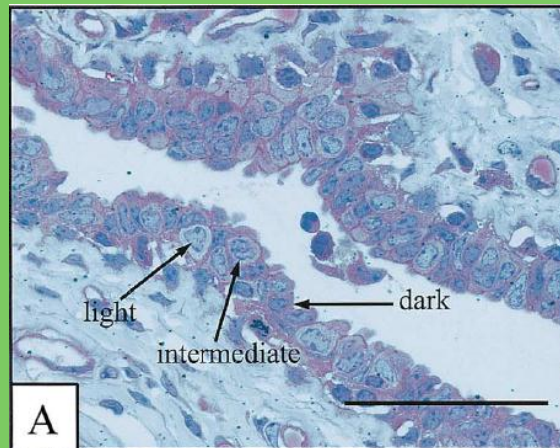
# The mammary gland unit



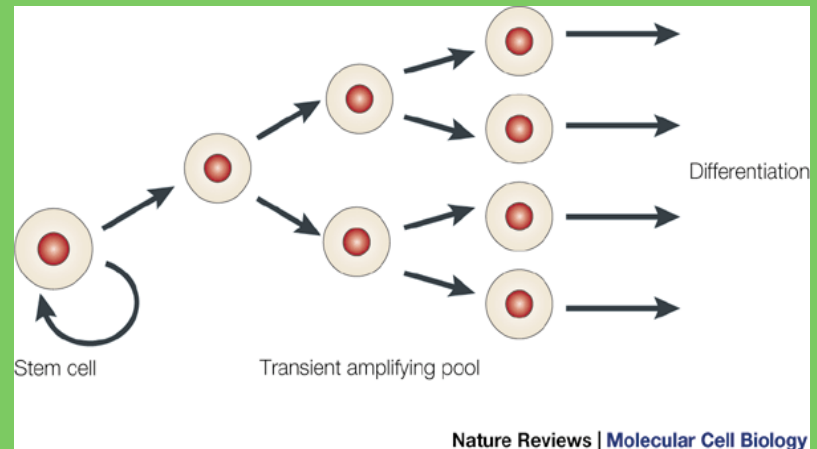
quiescent



lactating



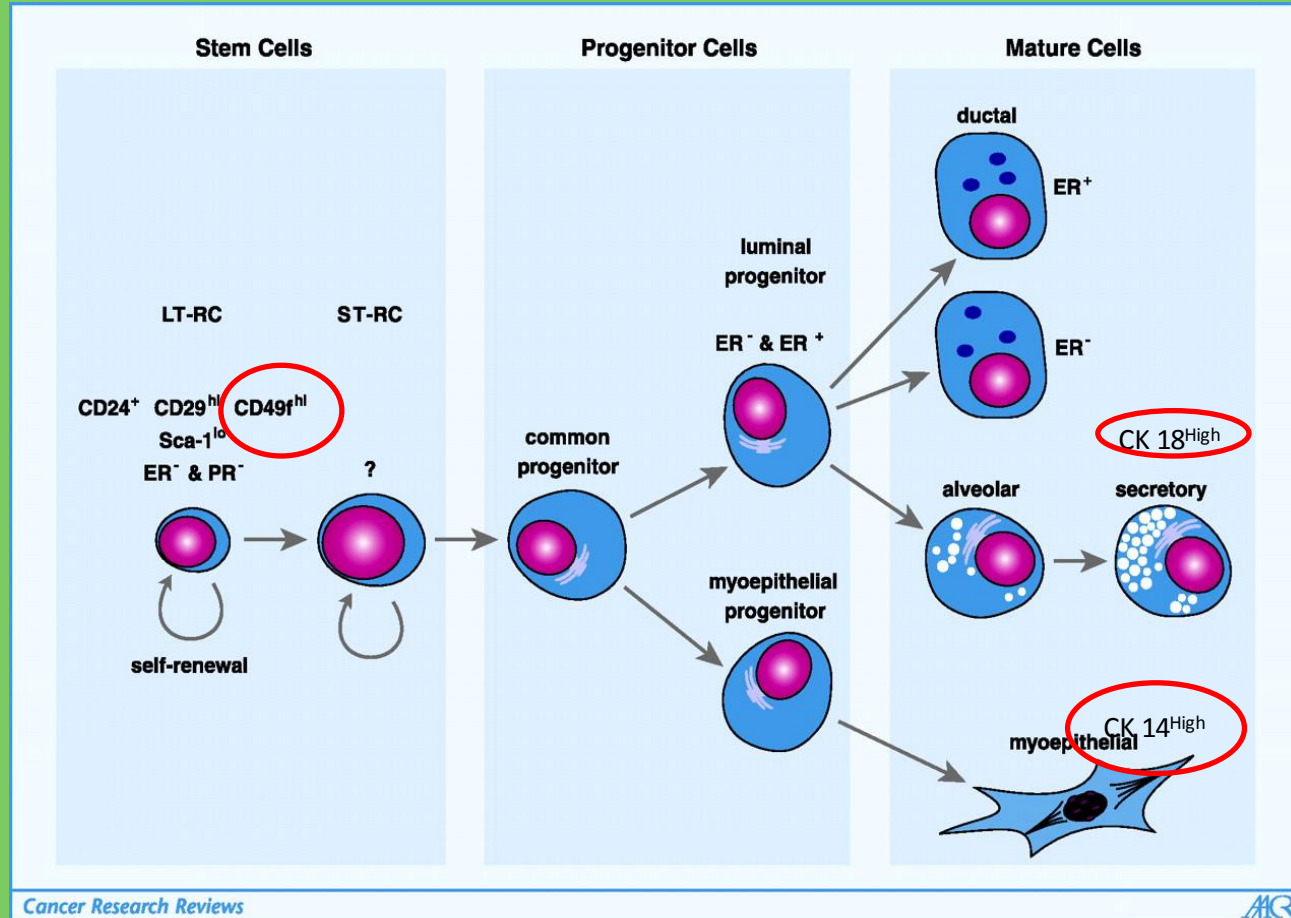
Capuco et al., 2007



Knoblich JA, 2001, Nature Reviews Molecular Cell Biology



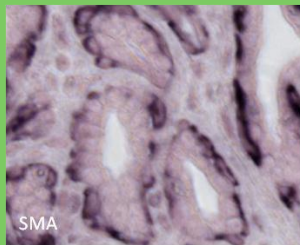
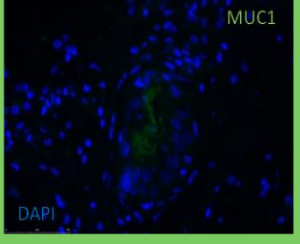
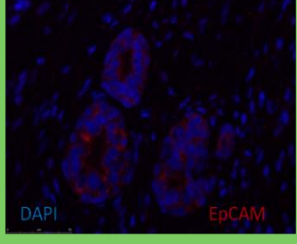
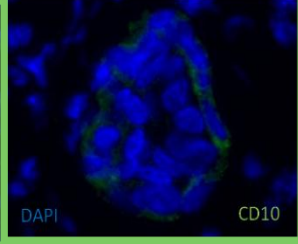
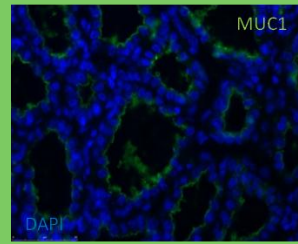
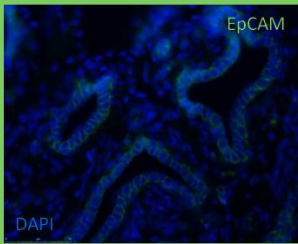
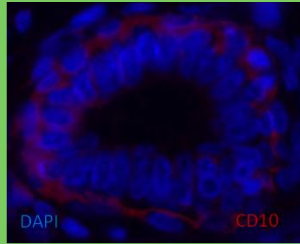
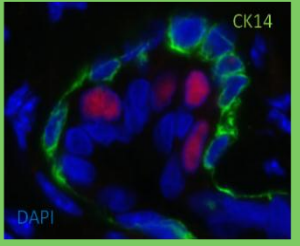
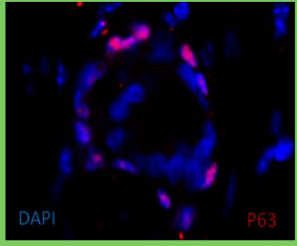
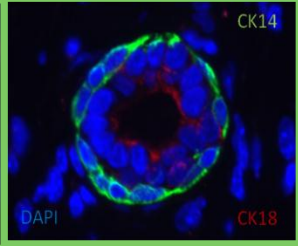
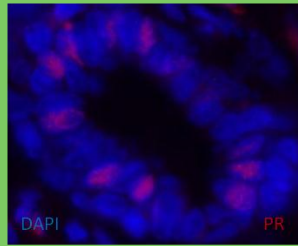
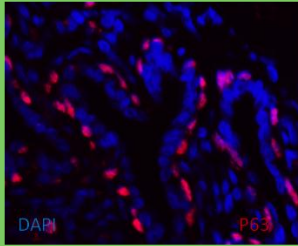
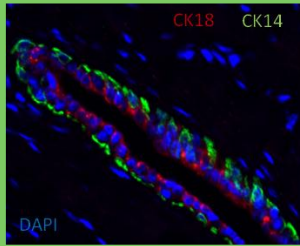
# Bovine mammary stem cells



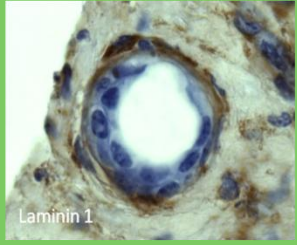
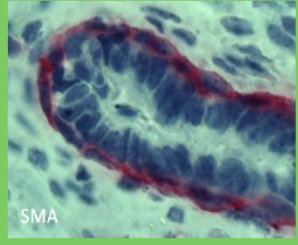
Visvader JE, 2006, Cancer Research Reviews

The regenerated structures show a proper polarization in vivo and expression of markers comparable to that of bovine tissue

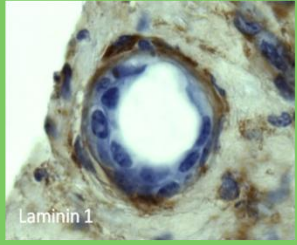
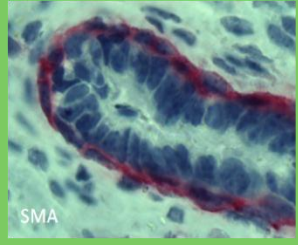
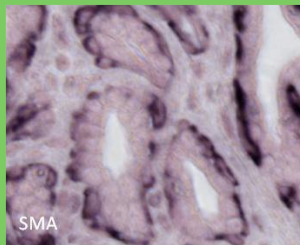
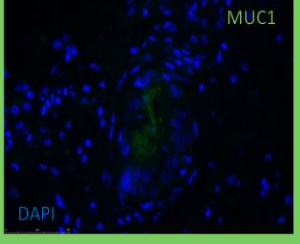
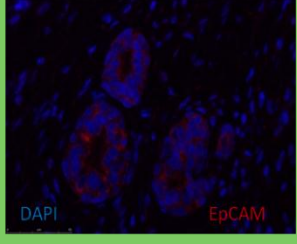
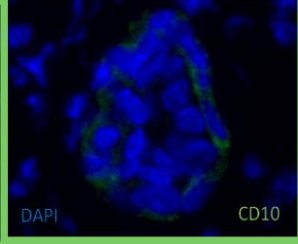
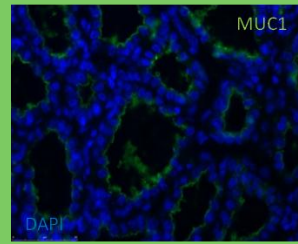
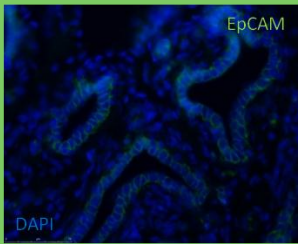
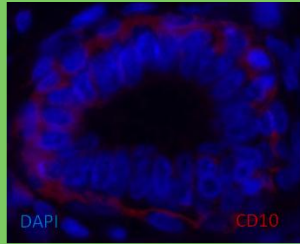
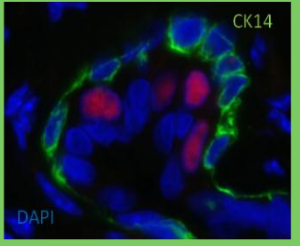
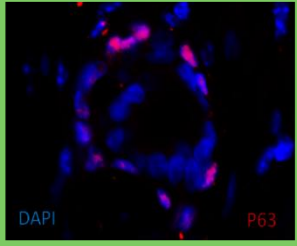
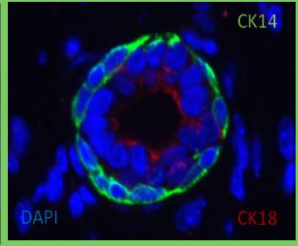
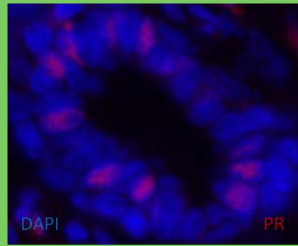
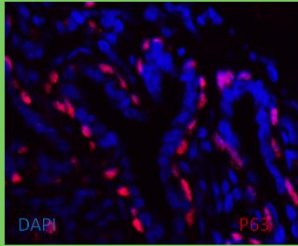
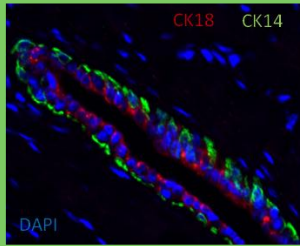
### Bovine tissue

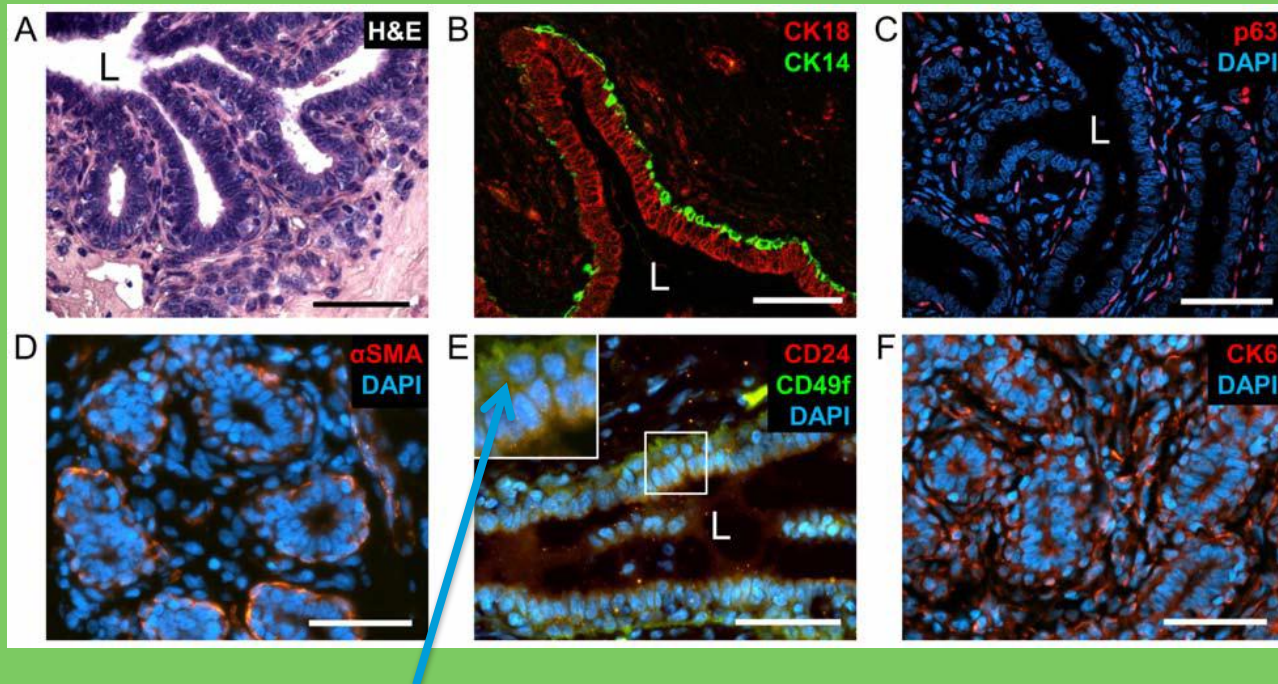


(Martignani et al, PloSOne 2010)



### Xenotransplant

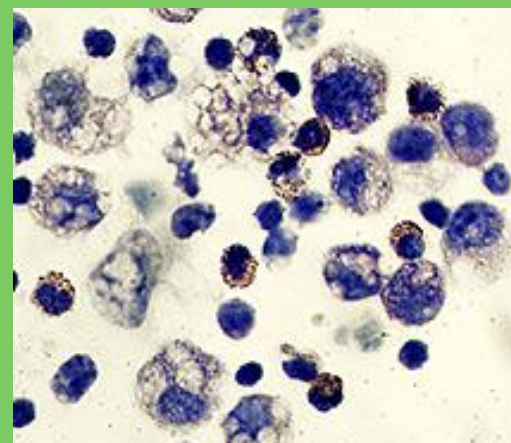
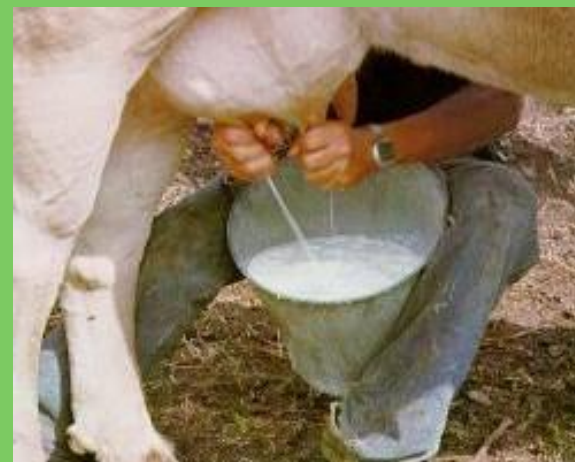
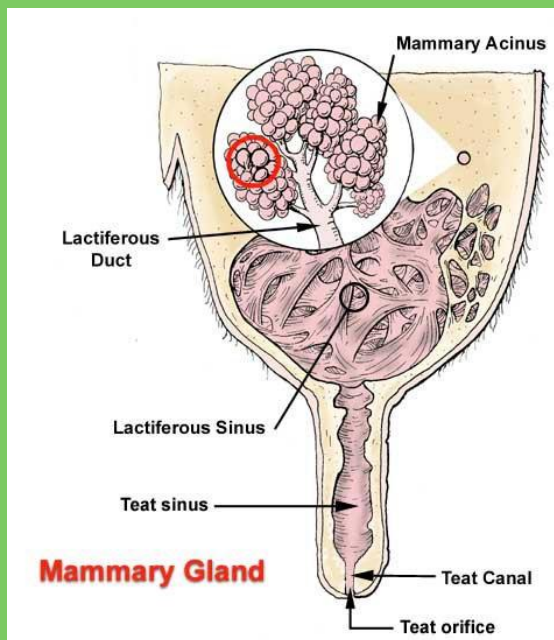




CD49f

(Rauner & Barash, Plos One 2012)





# Our questions

Which populations of epithelial cells are in milk?

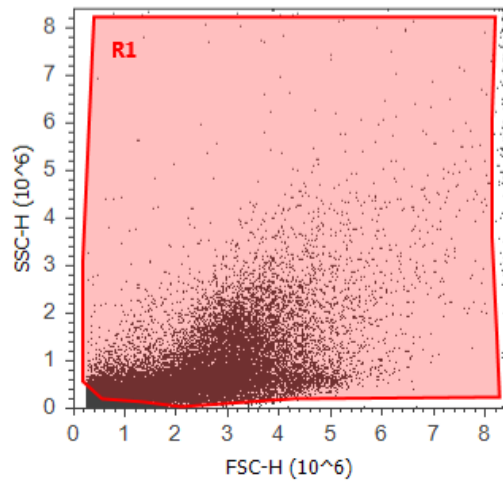
May they be considered potential biomarkers of mammary gland functionality?

Is there any relationship between these cells with cow welfare or stress during lactation?

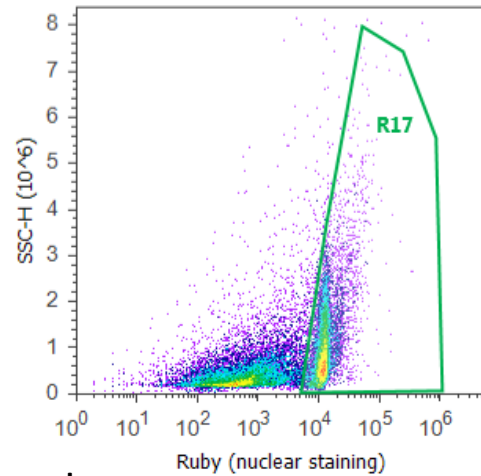
# A Six-Color Flow Cytometric Assay for the analysis of mammary epithelial cell subpopulations in milk:

- ✓ Living cells
- ✓ CD45 (positive cells for leucocytes)
- ✓ CK14
- ✓ CK18
- ✓ CD49f

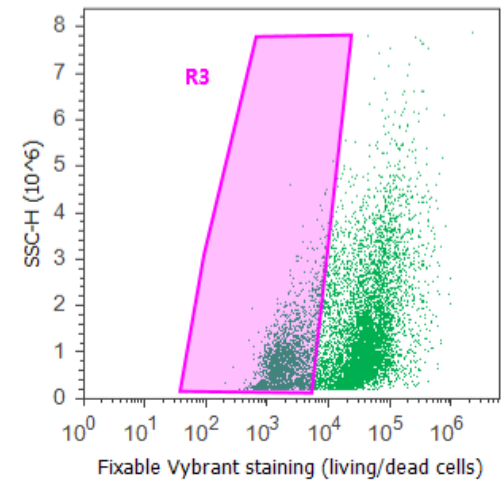
# Gate strategy



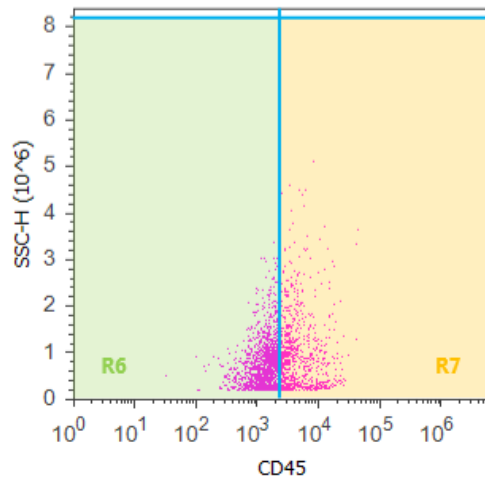
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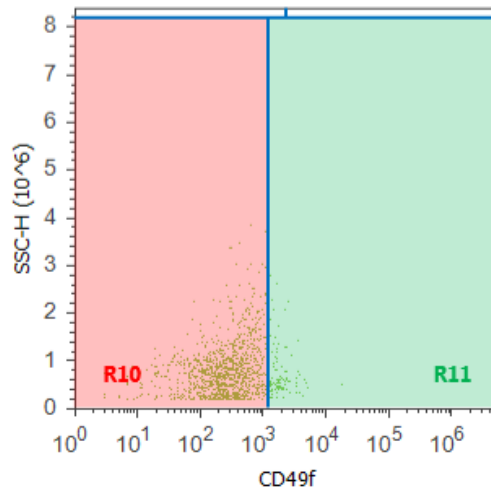
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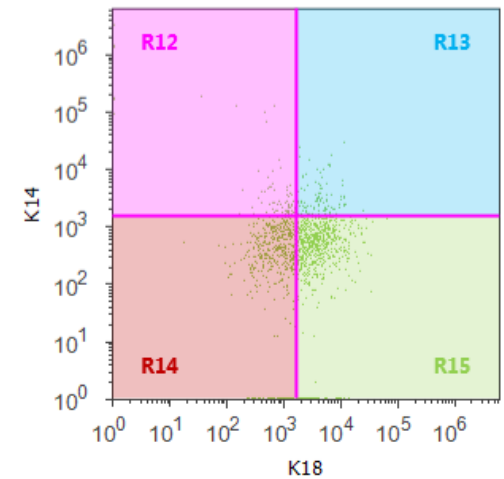
c



d



e



f



# Work in progress

## 1. Animal welfare in dry period



5 cows (each animal/9m<sup>2</sup> bedding, 21.6 m<sup>2</sup> paddock)

- Animal behaviour (video recording rumination and resting time , pedometer analysis)



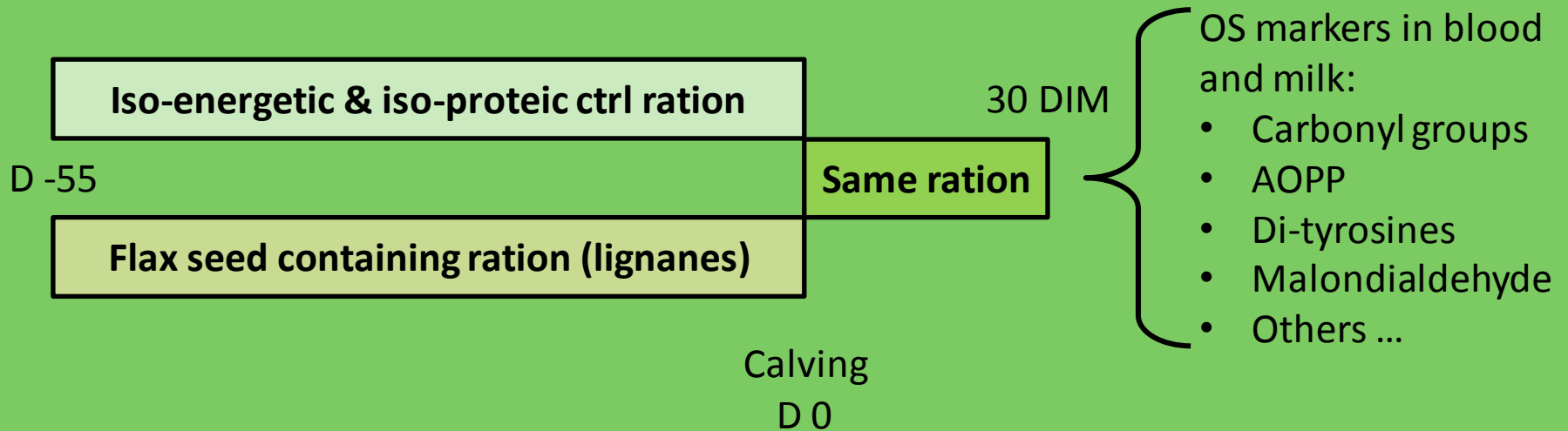
19 cows (each animal/2.4 m<sup>2</sup>, 5.6 m<sup>2</sup> paddock)

- Hormonal levels: from plasma (cortisol, DHEA), from hair (cortisol),
- Stress Biomarkers in blood (AOPP),

Experimental period from 1° to 3° lactation

## 2. Nutrition in dry period

### 2. Antioxidant (flax seed) administration in the dry period and oxidative stress (OS) in early lactation



**Indicator of Lignane absorption:**

Total Enterolactone (Free + Conjugate) in Blood and Milk

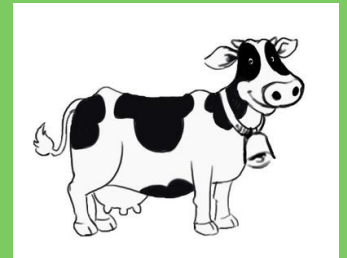
# Preliminary results for differential expression of epithelial cells in milk during lactation:

EL = 0-30 Days

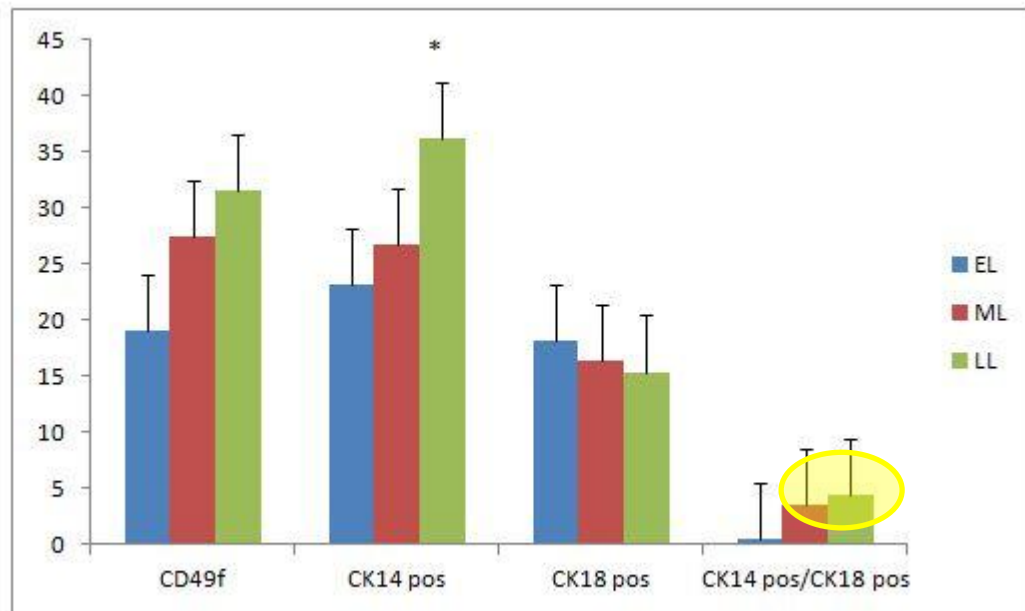
MD = 90-120 Days

LL = 210-250 Days

10 healthy with < 100.000  
SC/ml during the  
experimental period



	EL (early lactation)	ML (mid-lactation)	LL (late lactation)
cell viability %	28.8 ± 12.5	30.0 ± 15.1	48.4±13.0
CD45 <sup>+</sup> cells	75.1 ± 15.6	54.5± 27.9	28.1±11.5





- ✓ an increase CD49f<sup>+</sup> epithelial cells vs a decrease in cell viability (Spearman correlation ( $\rho = -0.611$ ,  $p = 0.027$  )
- ✓ K14<sup>+</sup> cells vs milk production in EL: first results indicate a positive correlation that has not been observed in later stage
- ✓ So far no consistent number of samples are available among groups of animals in different condition (management or nutrition) for statistic evaluation

# Conclusions

- ✓ Differential expression of epithelial cell is detected during lactation
- ✓ Many factors may influence the difference (management, nutrition, milk production, ageing, animal welfare)
- ✓ It is possible to propose that specific subpopulations may be related to mammary gland functionality
- ✓ Studies that are carrying out in our research network may give a contribution to clear some clues in this field
- ✓ If we will confirm some of preliminary data, the differential composition of primitive and mature differentiated epithelial cells in milk could be considered as biomarkers of dairy cow in production