



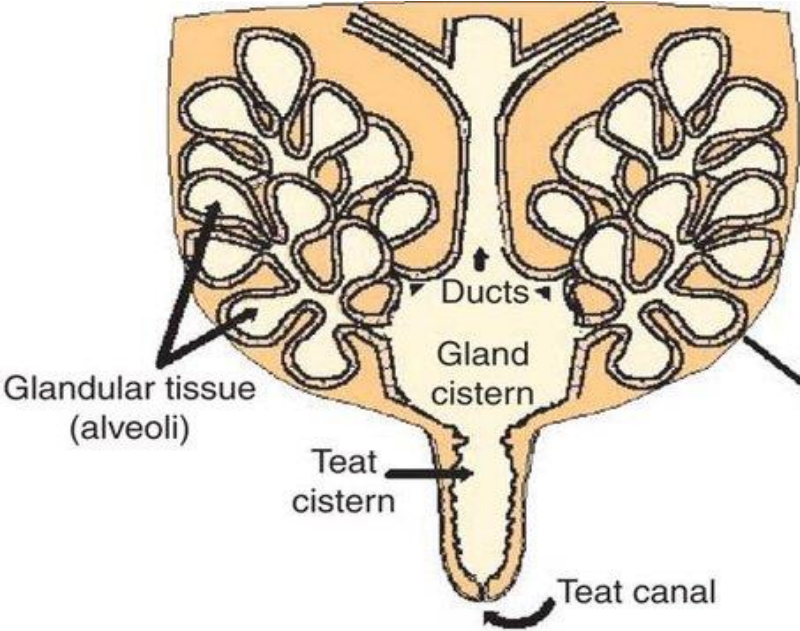
# Effects of the milk protein $\alpha$ -Lactalbumin levels and folding on milk production in dairy cows



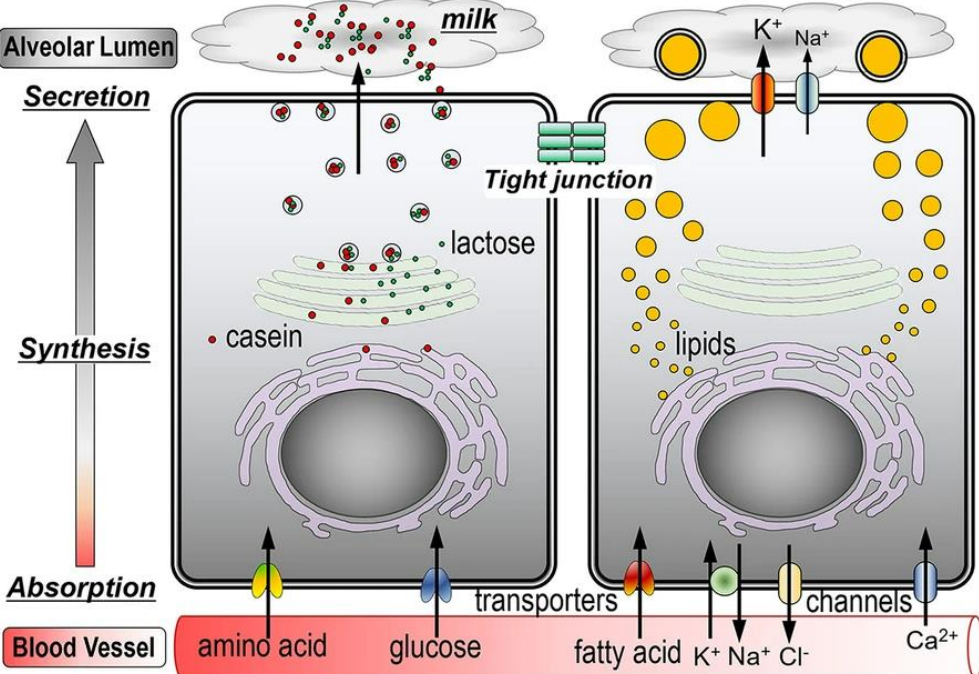
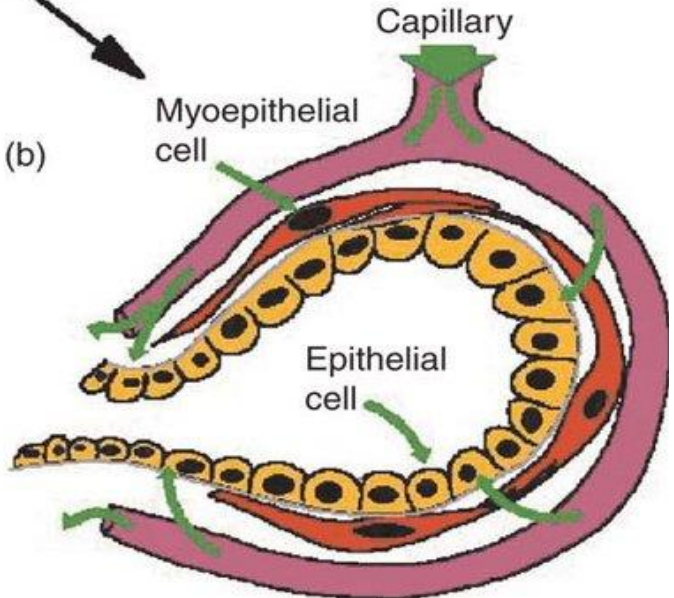
**Orit Dashevsky**

Rak Lab

# Mammary gland



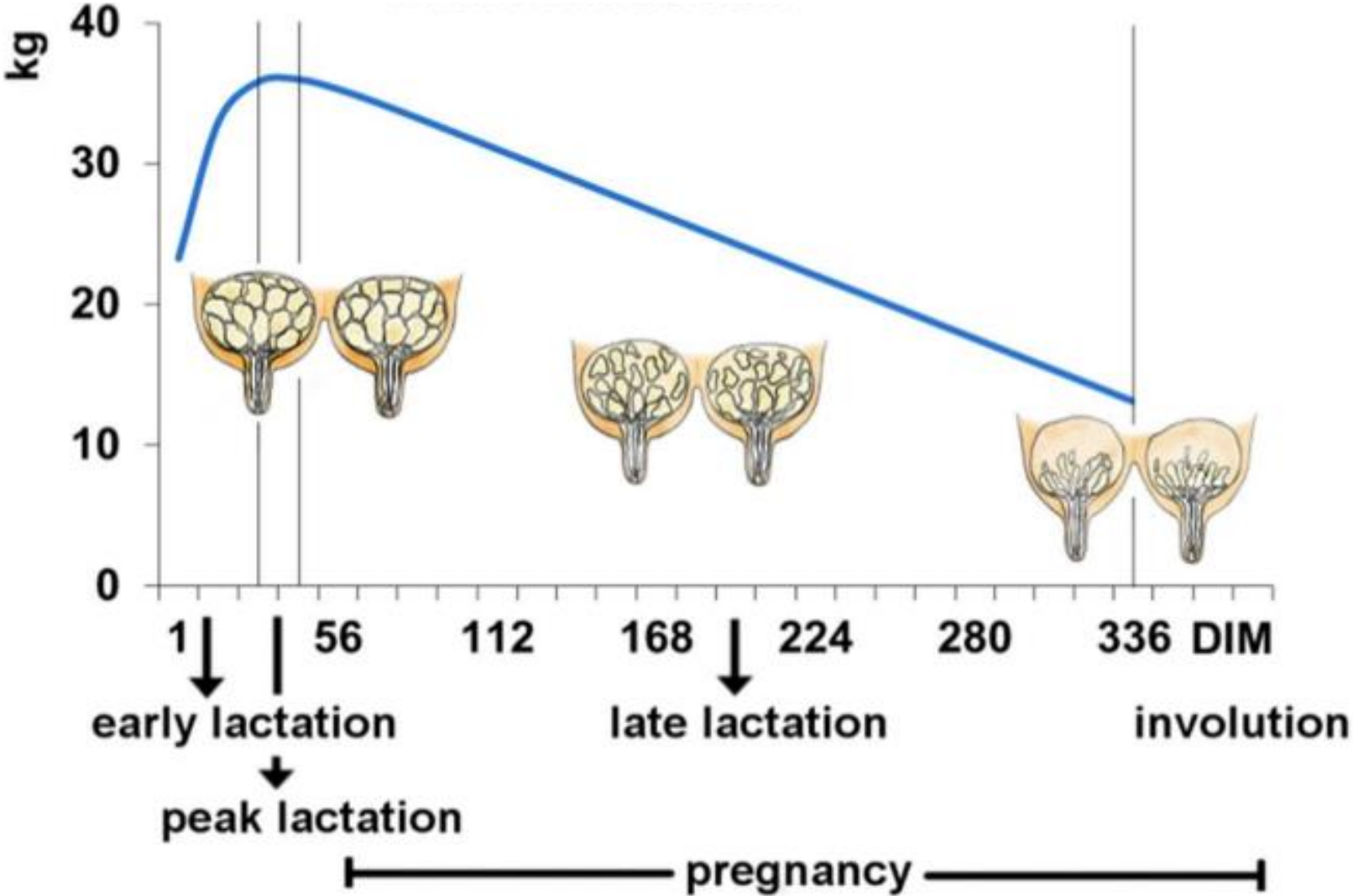
Nickerson & Akers (2011)



Kobayashi (2023)



# Milk production



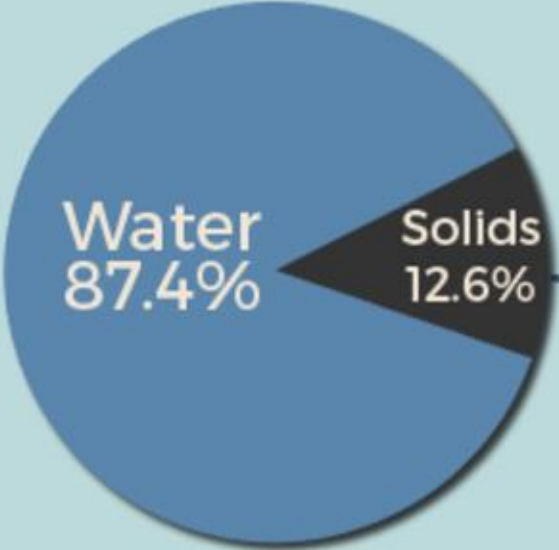
# Milk composition

## Bovine Milk

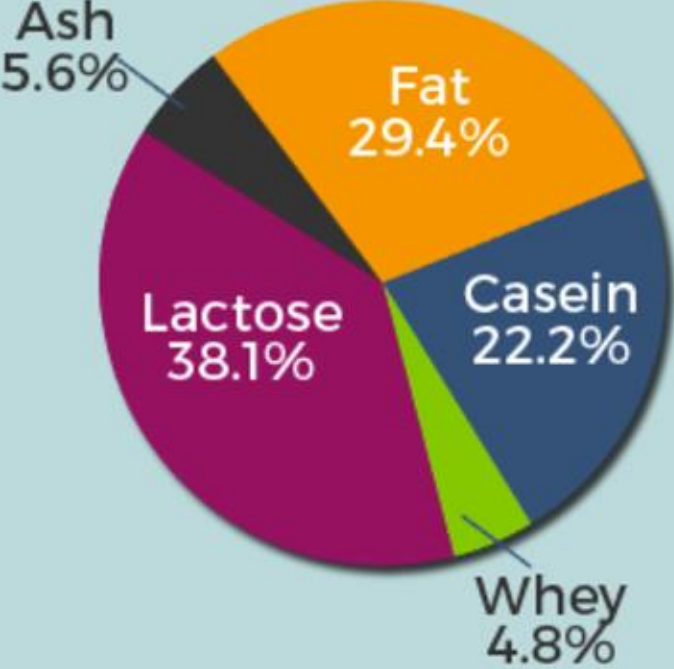


Total milk proteins:  
3.3 g/100ml

Milk Breakdown



Milk Solids Breakdown



Protein Type	Subtype	Percentage	
80% total protein	<b>CASEINS</b>	$\beta$ -casein	36%
		$\kappa$ -casein	14%
		$\alpha_{s1}$ -casein	40%
		$\alpha_{s2}$ -casein	10%
20% total protein	<b>WHEY PROTEINS</b>	$\beta$ -lactoglobulin	52%
		$\alpha$ -lactalbumin	17%
		immunoglobulins (IgG, IgA, IgM)	10%
		serum albumin	5%
		lactoferrin	1.5%
		glycomacropeptide	12%
		other	2.5%

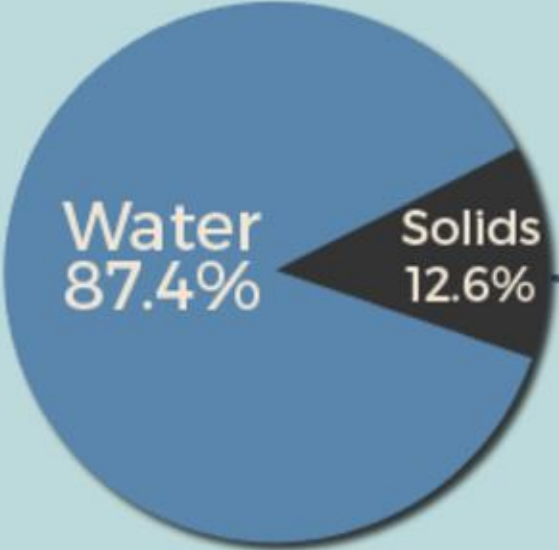
# Milk composition

## Bovine Milk

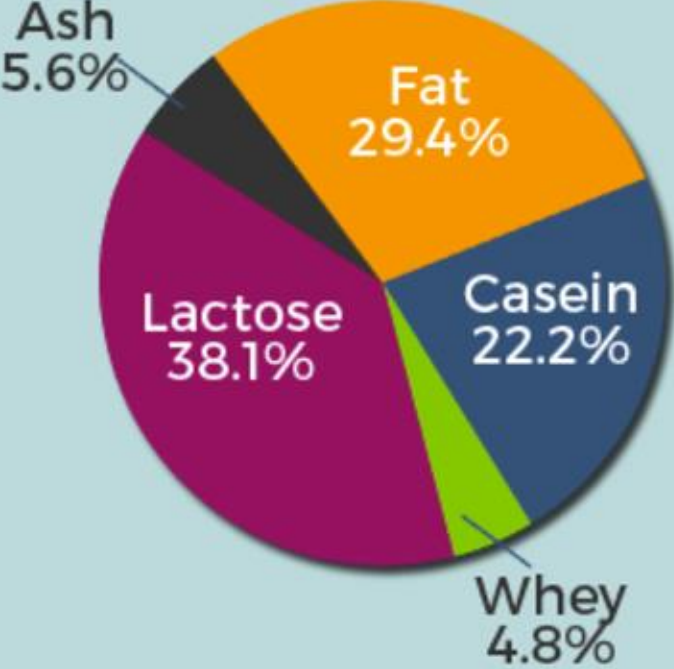


Total milk proteins:  
3.3 g/100ml

Milk Breakdown



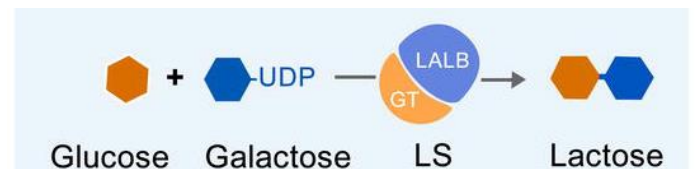
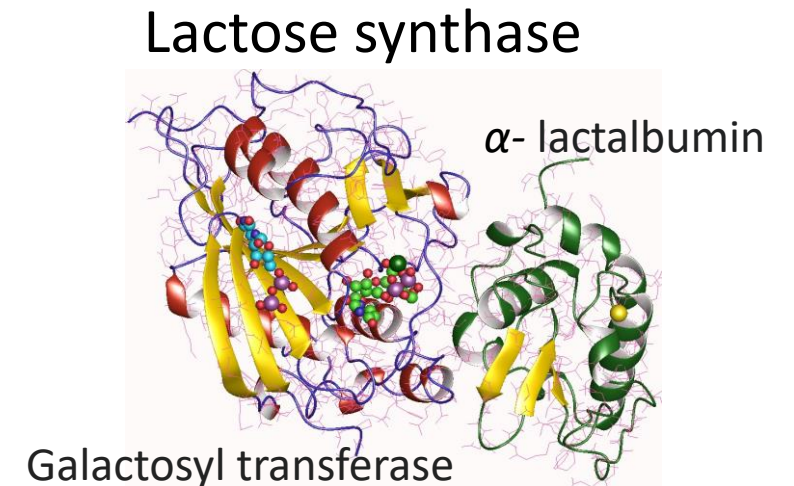
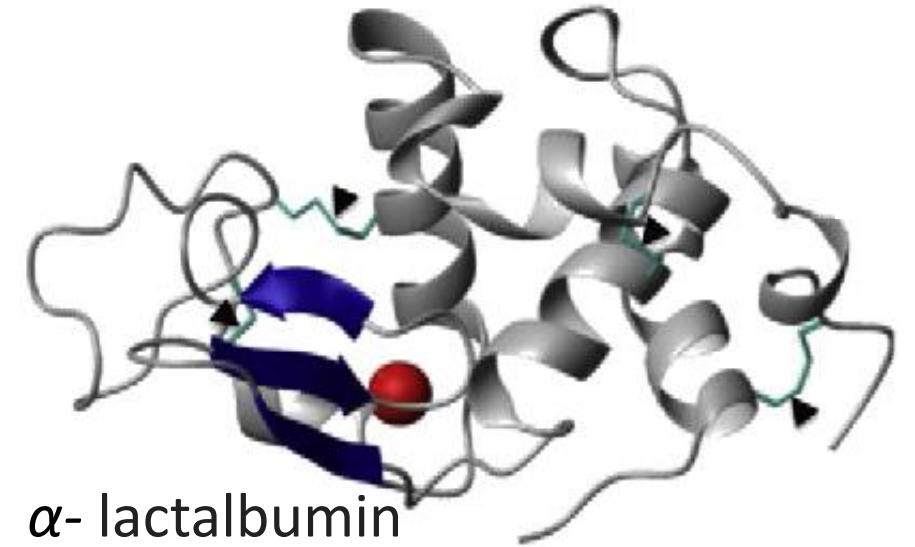
Milk Solids Breakdown



Protein Category	Sub-Category	Percentage	
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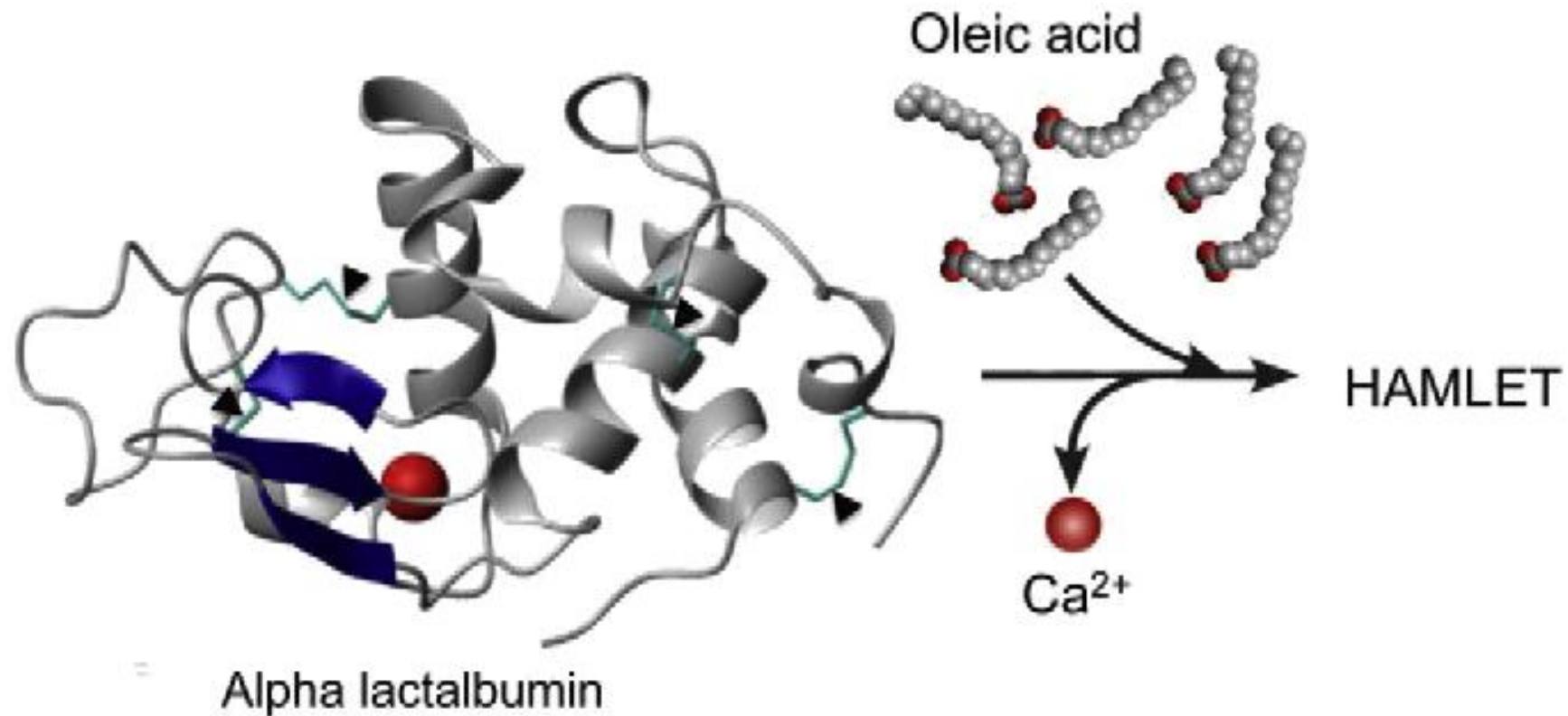
# $\alpha$ - Lactalbumin ( $\alpha$ - La)

- Increases milk production
- Part of lactose synthase
- High nutritional value
- Gut health
- Improves metabolic diseases
- Boosts immune system
- Anti-carcinogenic



# HAMLET

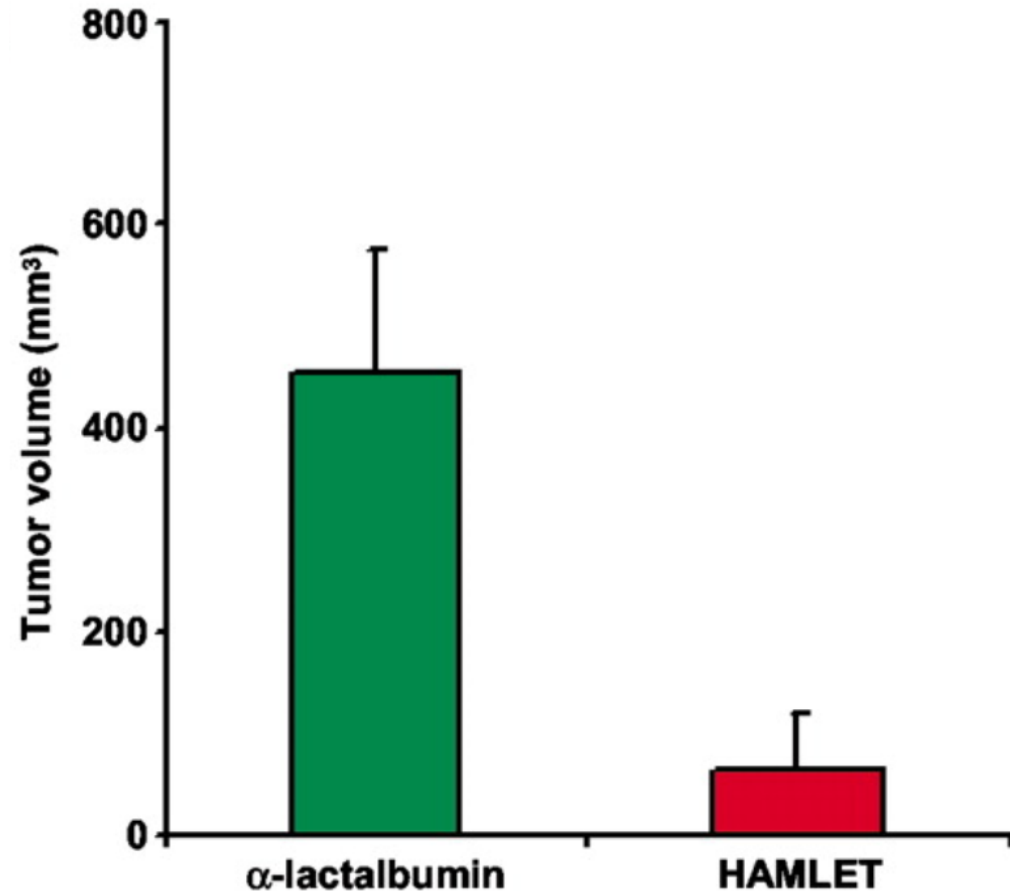
Human  $\alpha$ -Lactalbumin Made Lethal to Tumor Cells



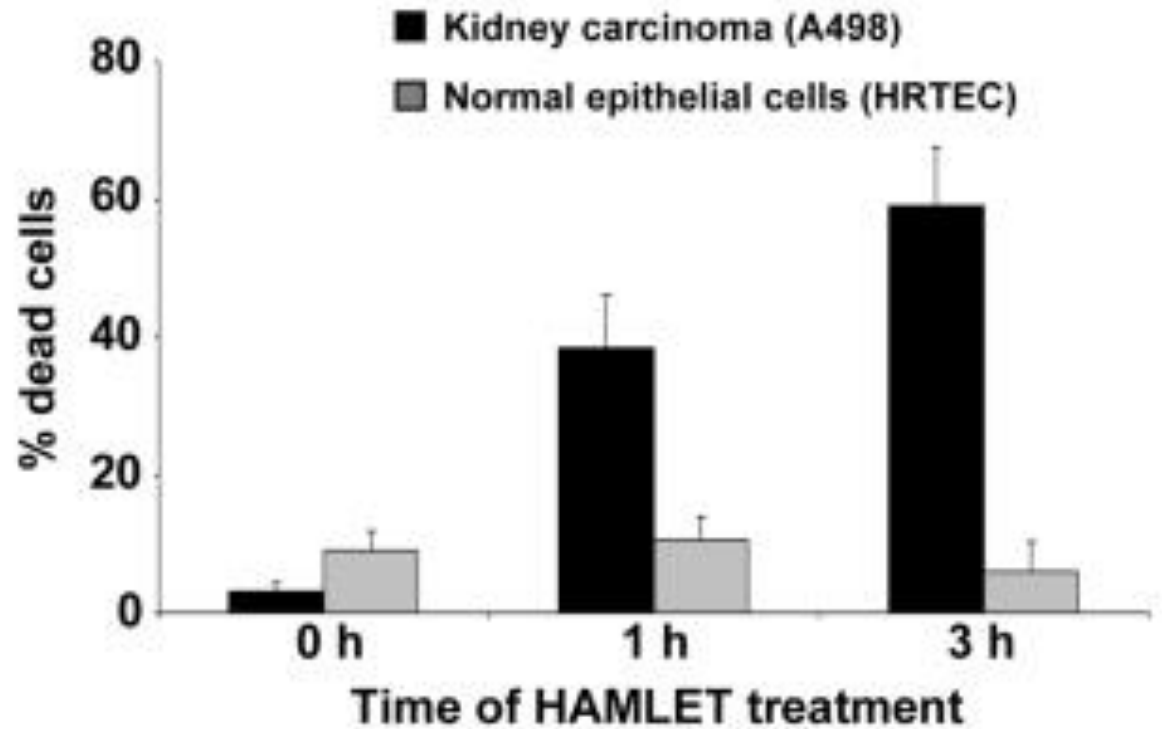
**HAMLET\BAMLET**

# HAMLET

## Human $\alpha$ -Lactalbumin Made Lethal to Tumor Cells



Fischer et al. (2004)



Gustafsson et al. (2009)

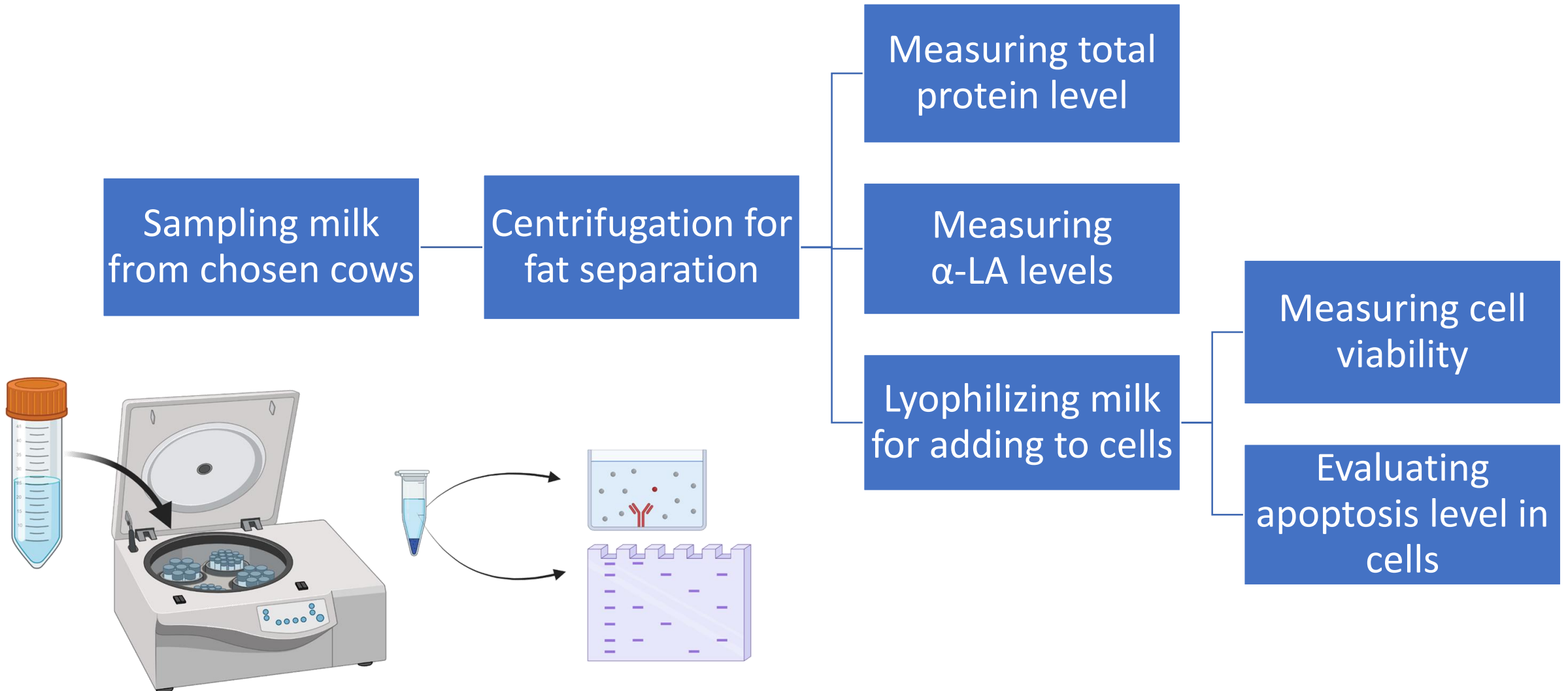


We hypothesize that

**BAMLET** that induces apoptosis  
in cancer cells **might induce apoptosis in  
mammary epithelial cells.**

Thus, **is responsible  
for the drop in milk production.**

# Developing a reliable method of measuring $\alpha$ -LA levels.



# Obtaining milk samples



## Start Milk

12 milk samples

from cows  
at the  
**start** of  
lactation



## Volcani's dairy



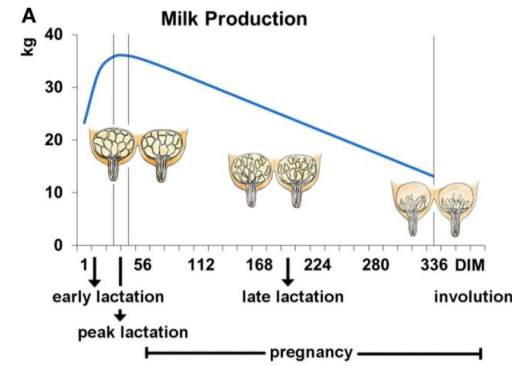
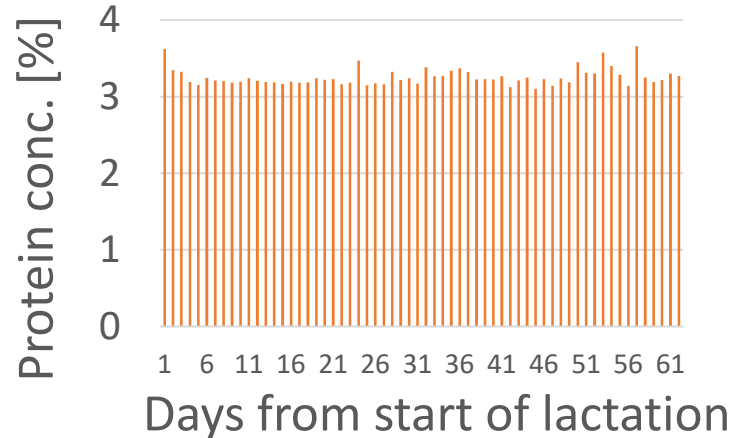
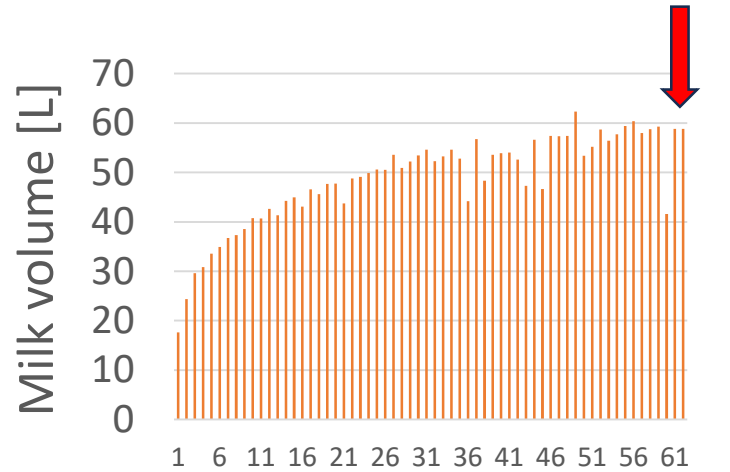
## End Milk

12 milk samples

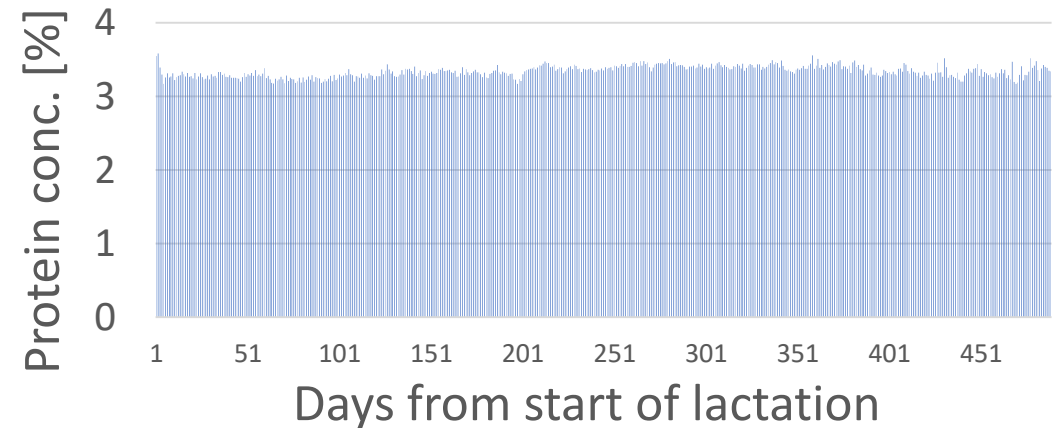
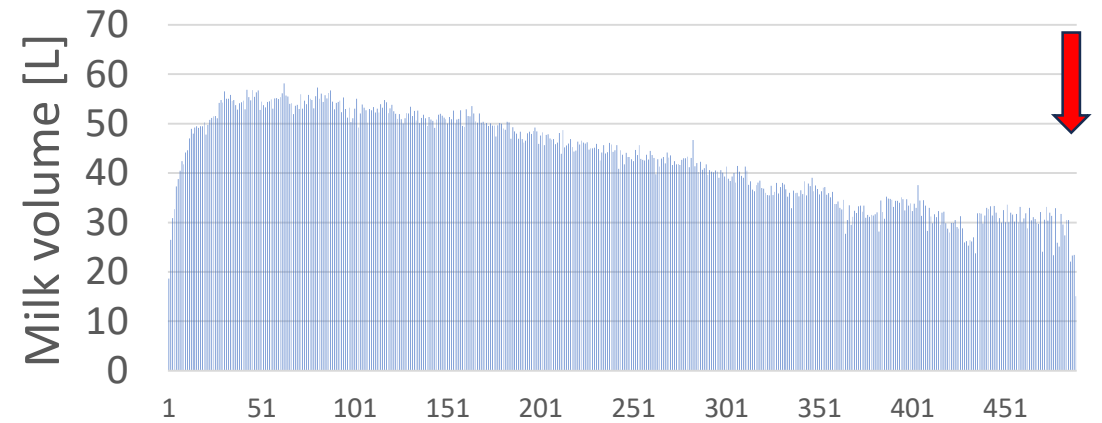
from cows  
at the  
**end** of  
lactation

# General milk assessment

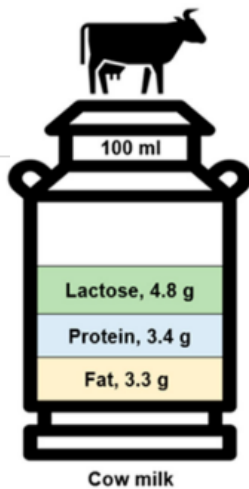
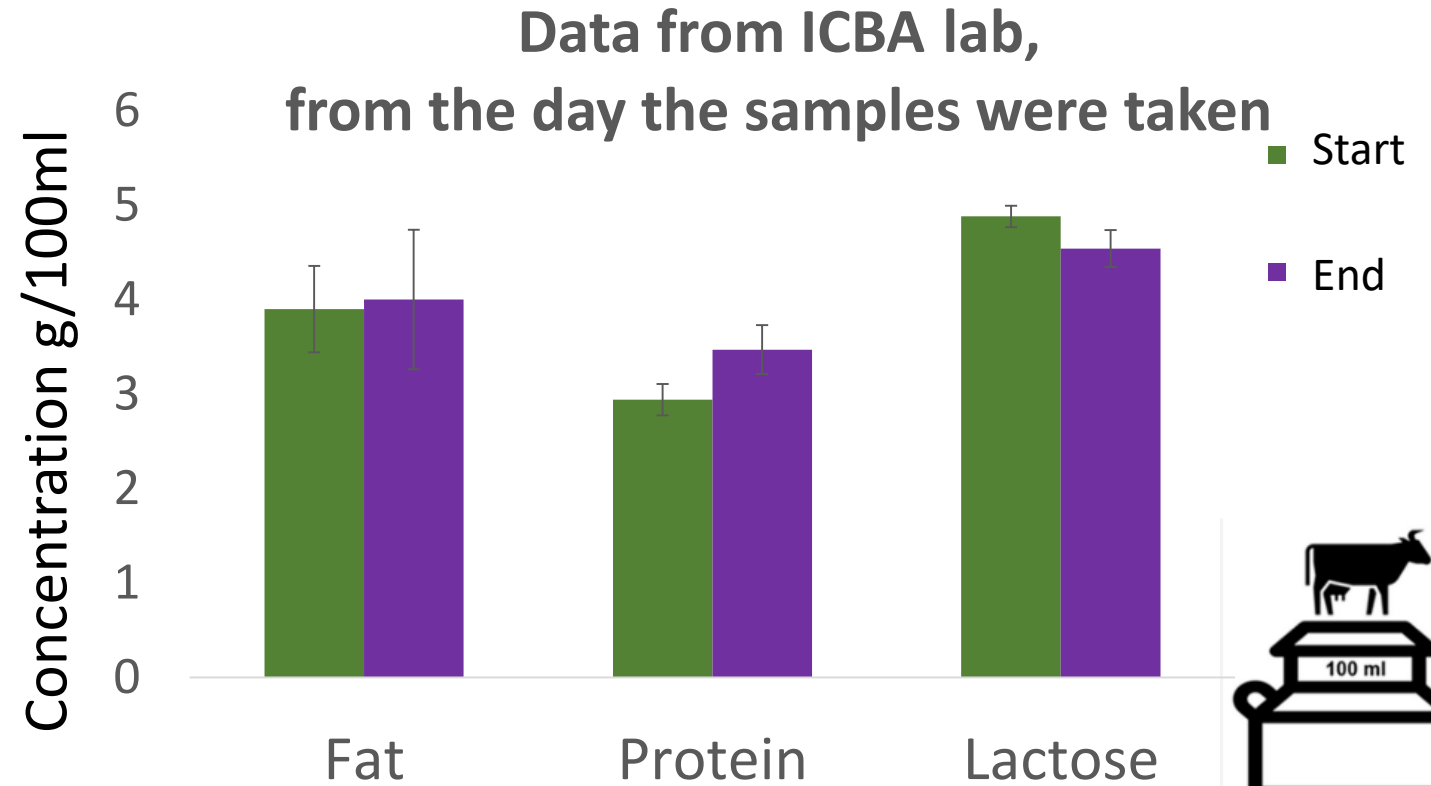
## Start Milk



## End Milk

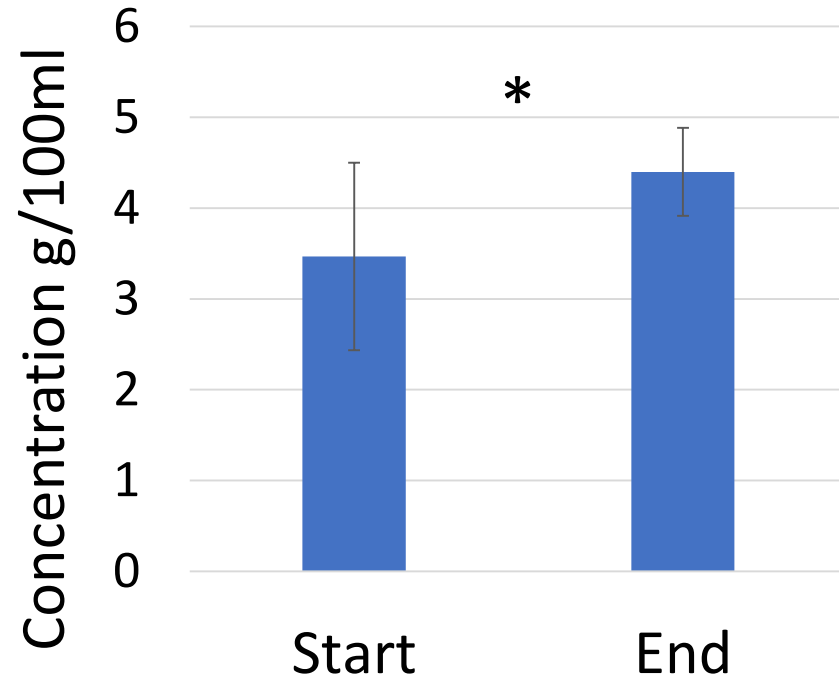


# Measuring total protein level

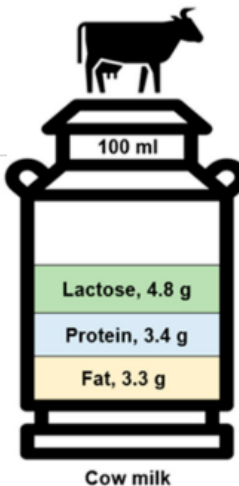
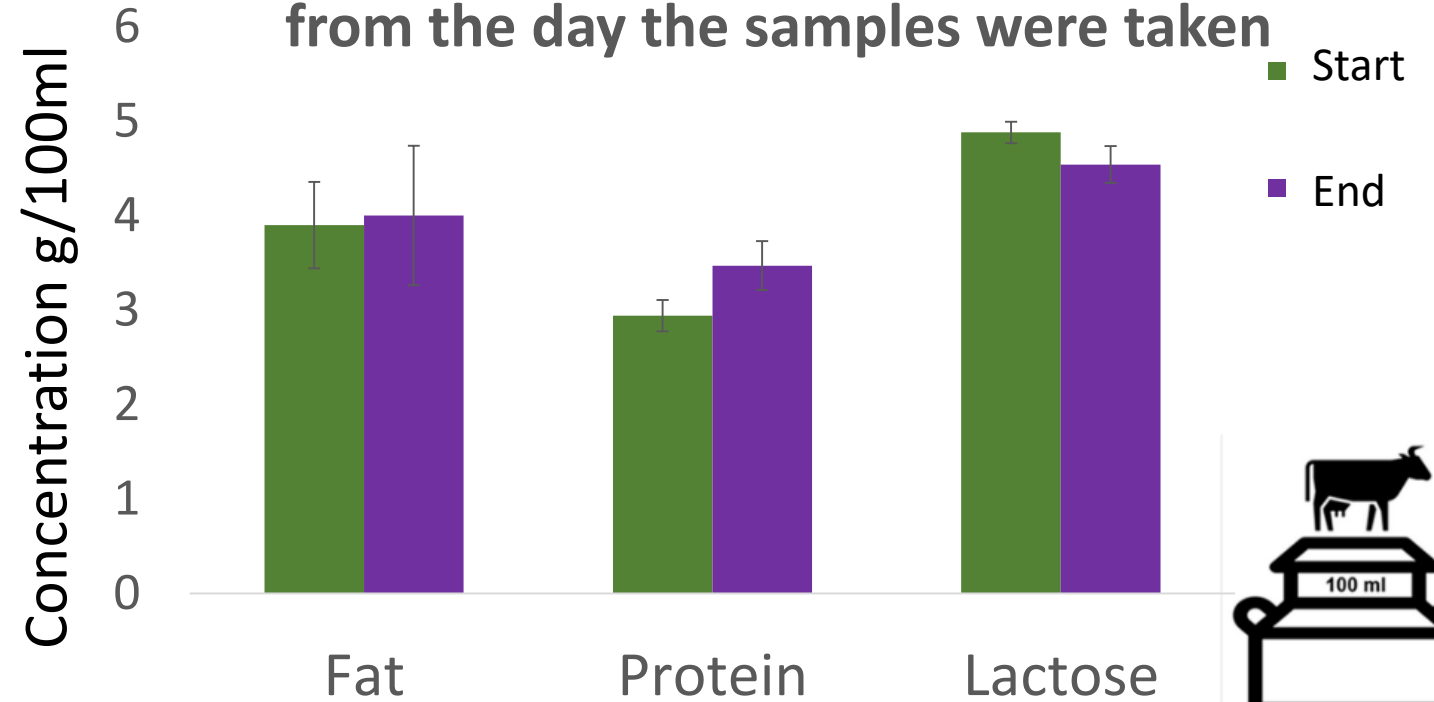


# Measuring total protein level

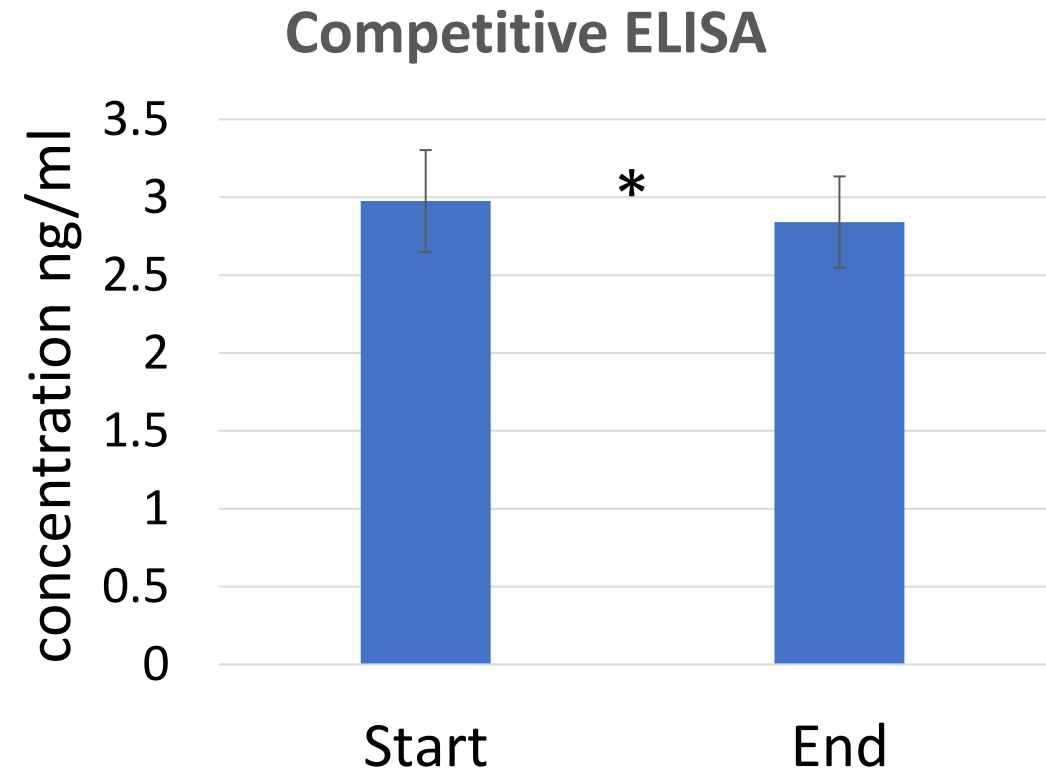
## Bradford test on samples



## Data from ICBA lab, from the day the samples were taken

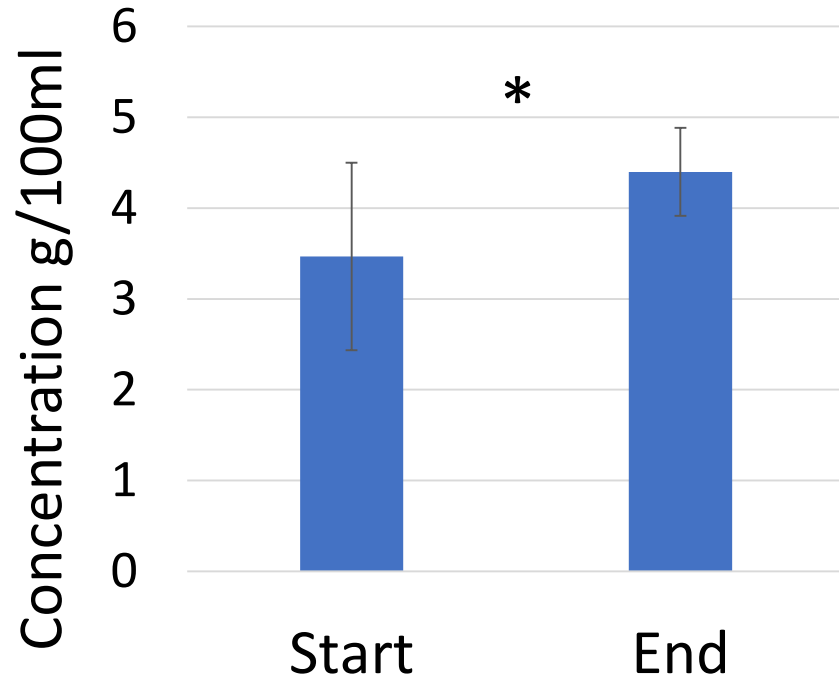


# Measuring $\alpha$ -LA level

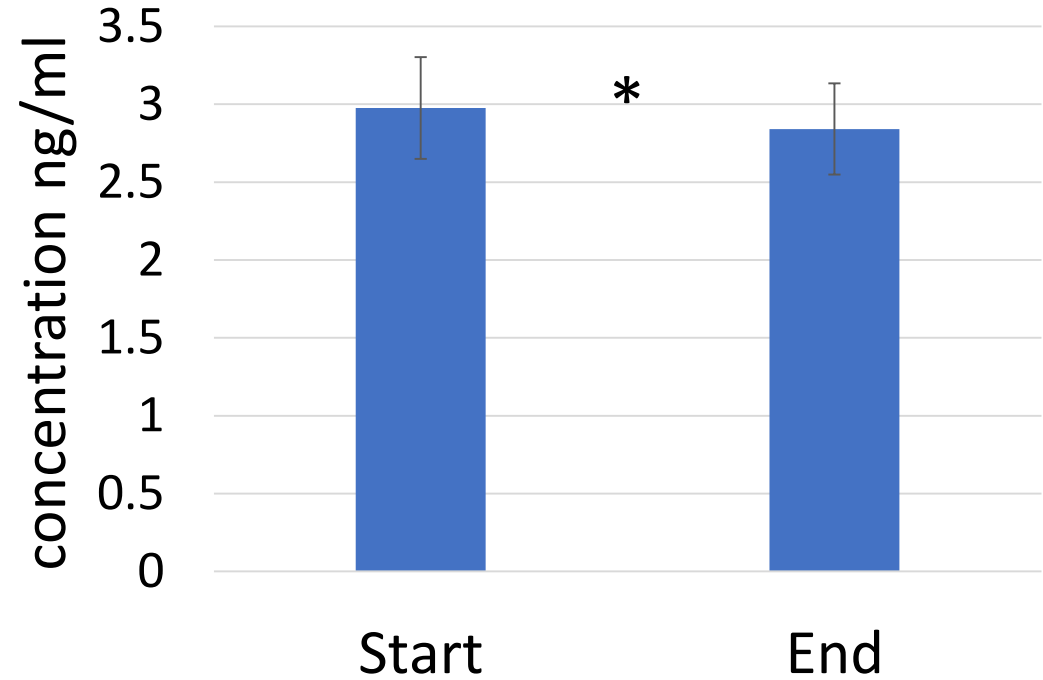


# Measuring $\alpha$ -LA level

## Bradford test on samples

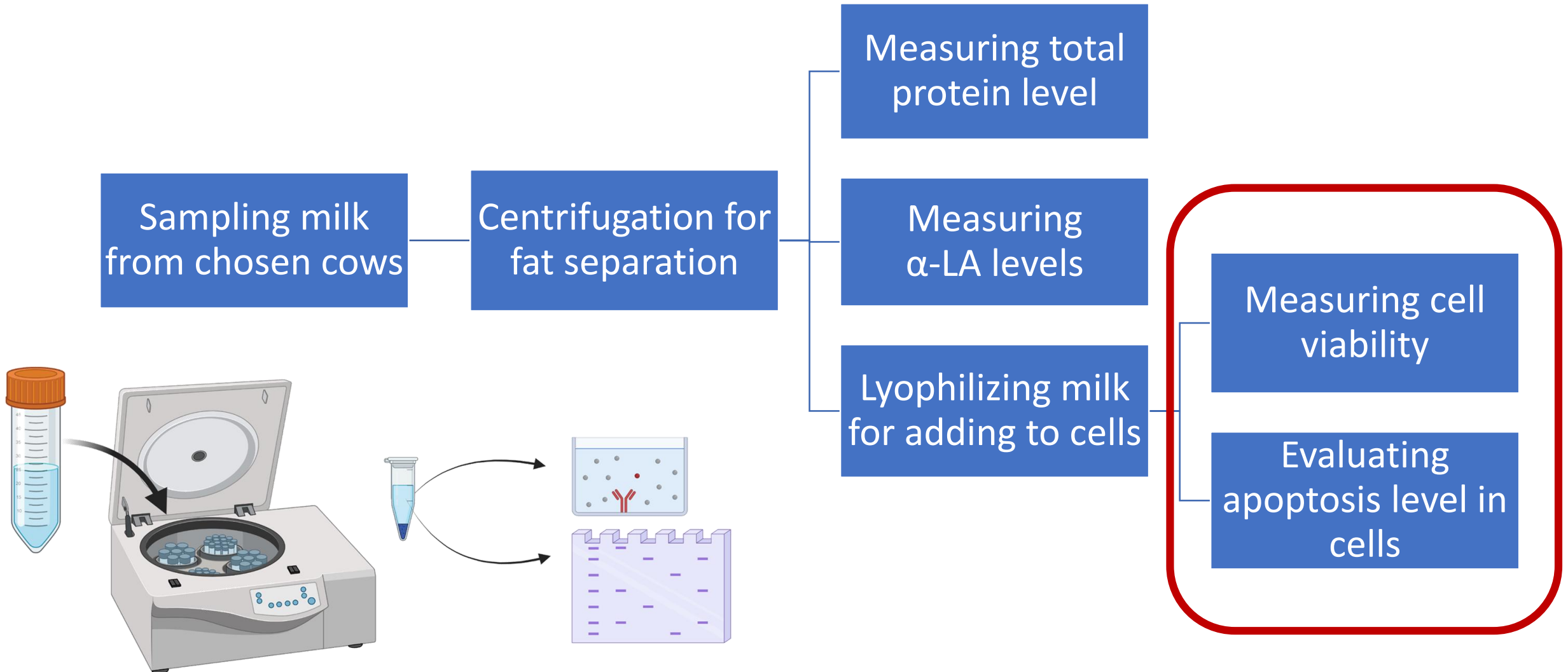


## Competitive ELISA

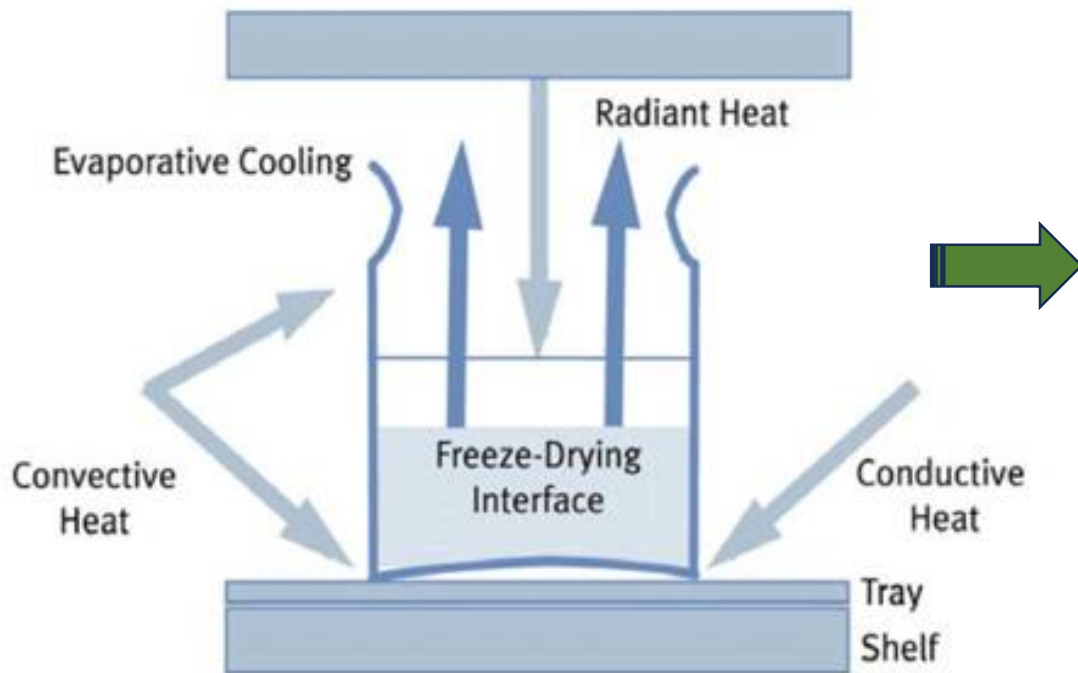




# Developing a reliable method of measuring $\alpha$ -LA levels.



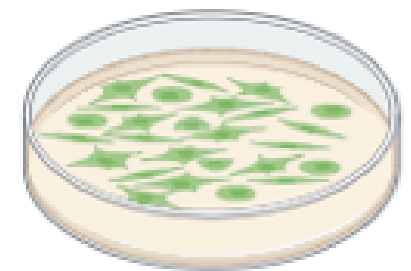
# Developing a method of adding milk to sterile cell culture



Lyophilizer



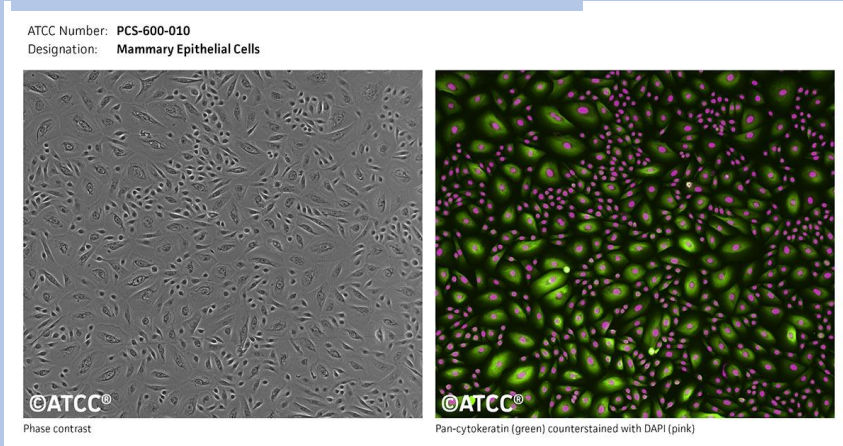
0.45 nm -> 0.22 nm



Diff. milk concentrations

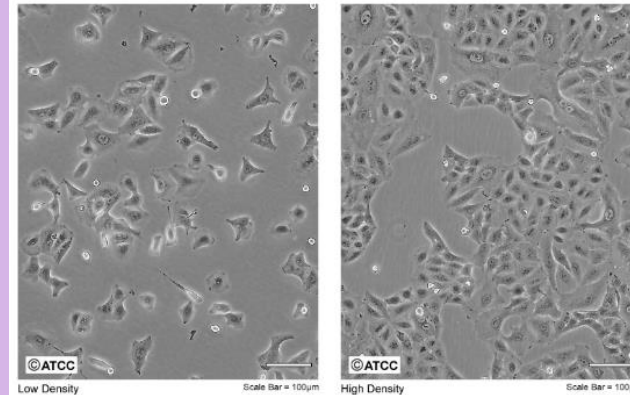
# Our cell types

**L1** cell line-  
bovine mammary  
epithelial cells



**L1** Cells of interest

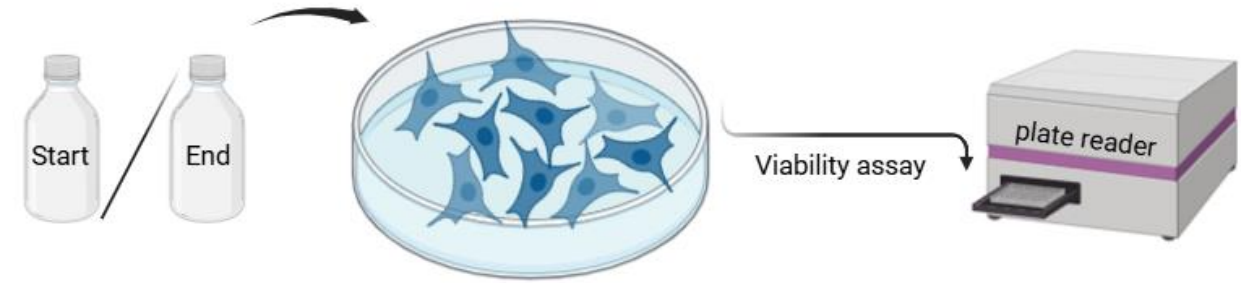
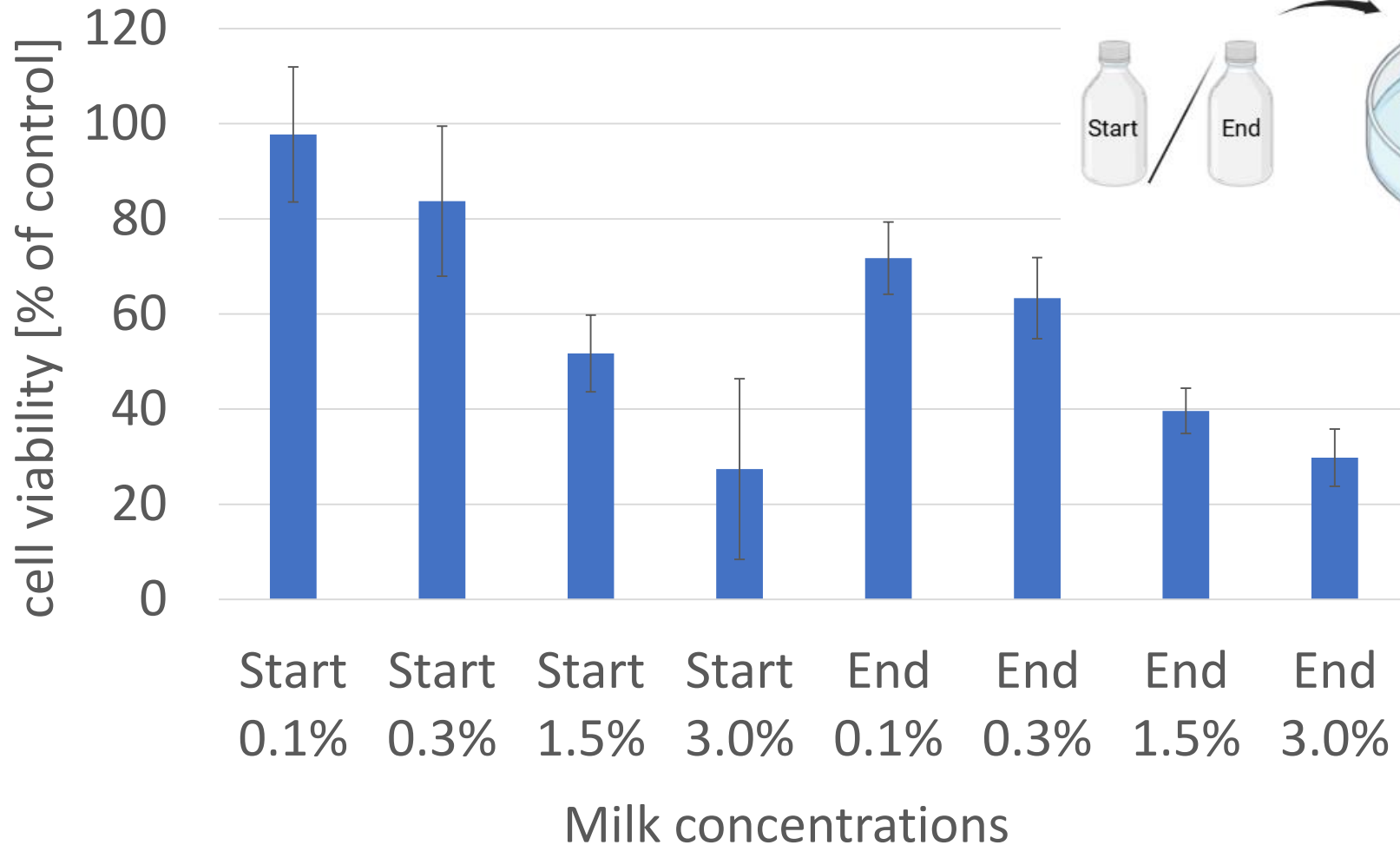
ATCC Number: CCL-185  
Designation: A-549



**A549** cell line-  
adenocarcinomic human  
alveolar basal epithelial cells

**A549** Sensitive to BAMLET

# System calibration with A549 cells

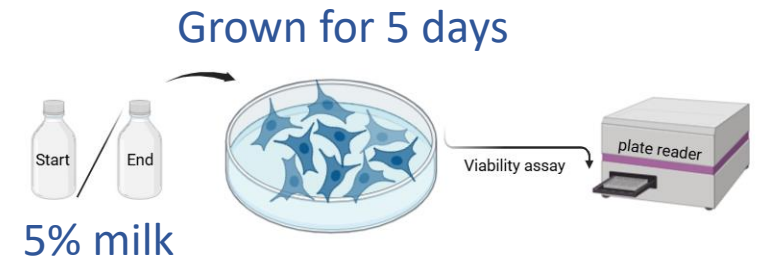


Grown for 3 days

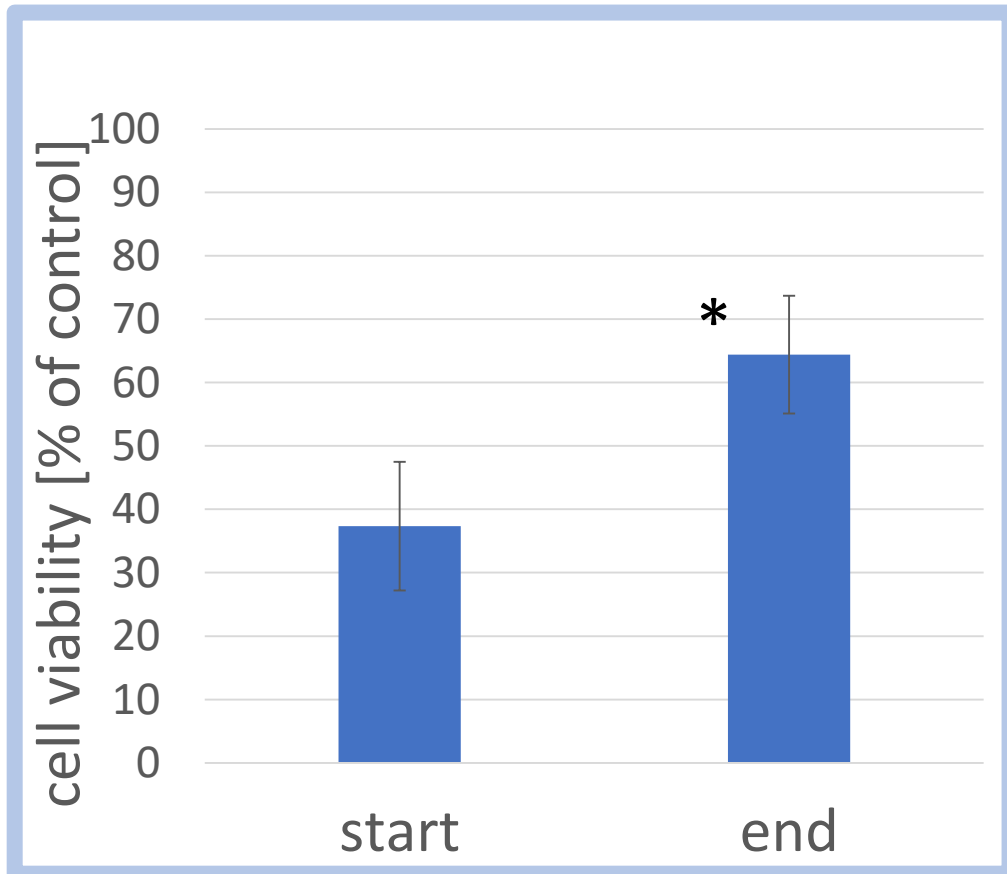
Milk from **start** and **end** of lactation effects **significantly** different on **A549** cell viability

# L1 and A549 cells viability

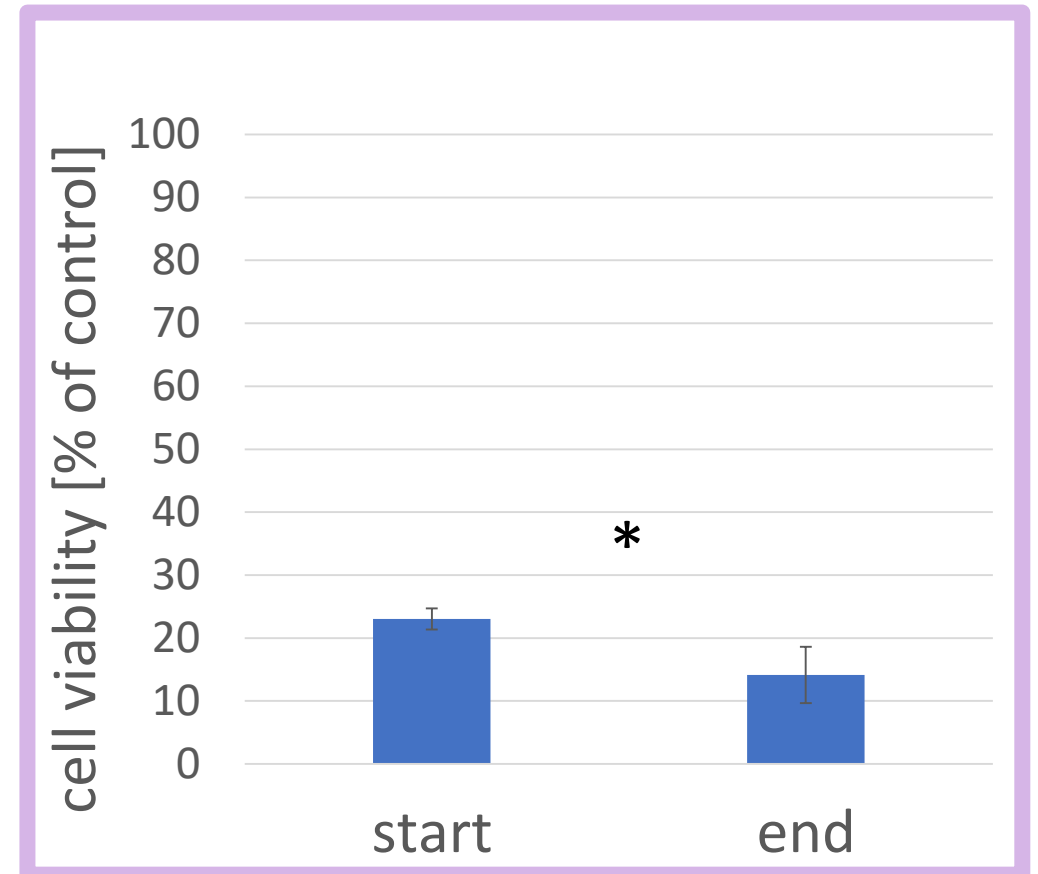
Milk from **start** and **end** of lactation effects **significantly** different on cell viability



L1

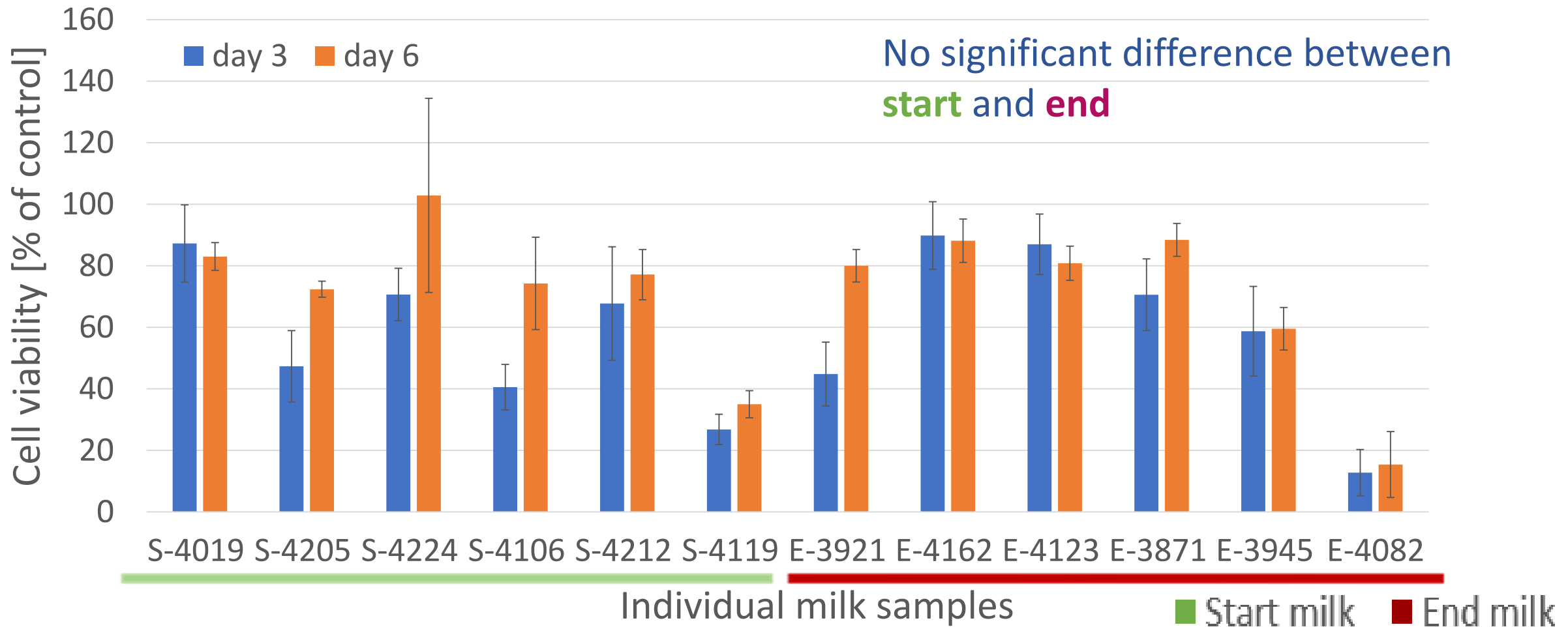


A549

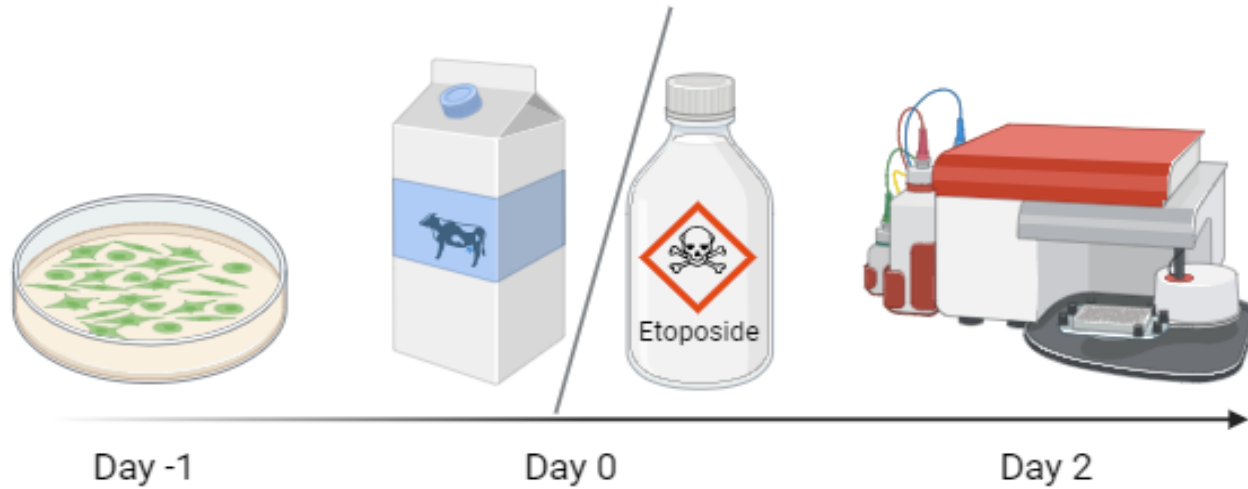


# L1 cells viability with 3% milk

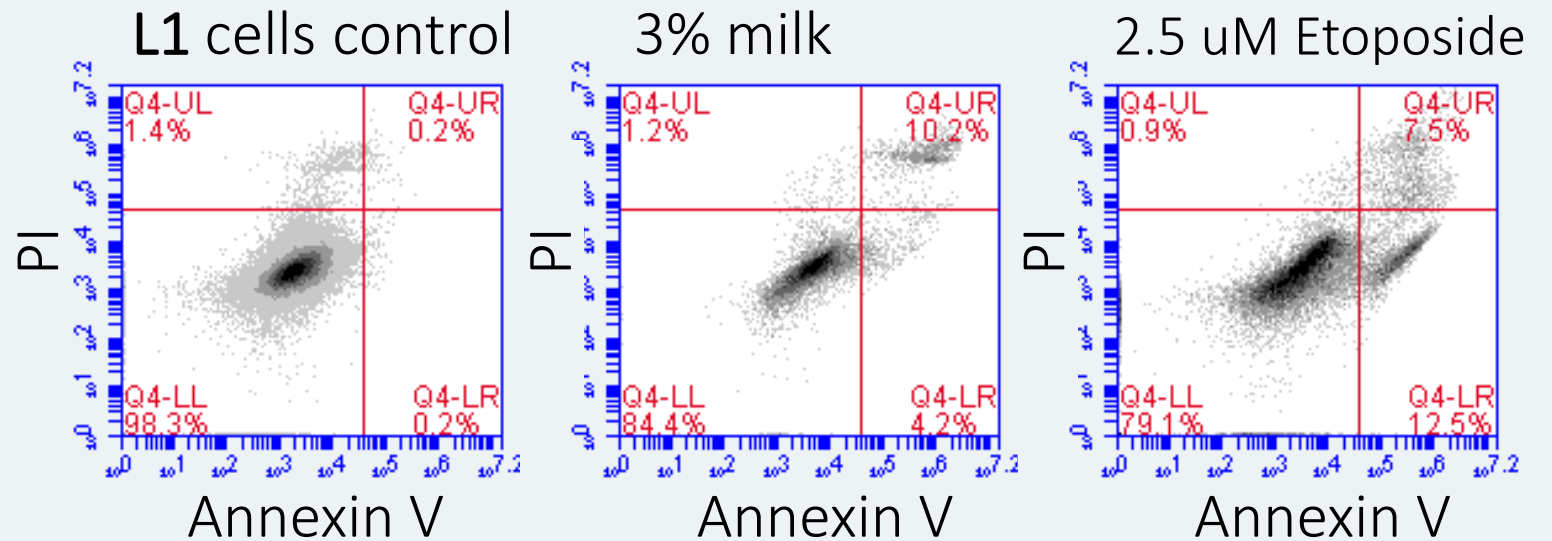
## individual samples



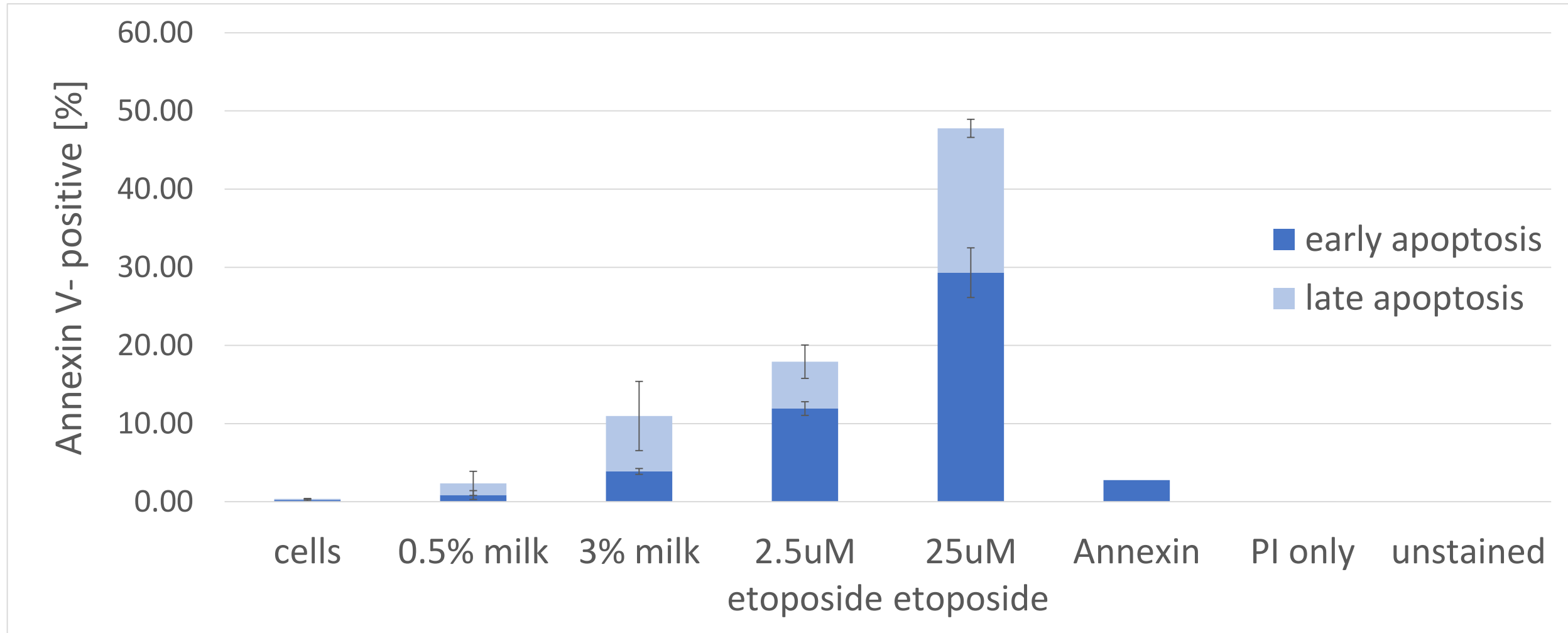
# Evaluating apoptosis level in cells



FACS and  
Apoptosis detecting dyes:  
Annexin v - early apoptosis  
PI- late apoptosis.



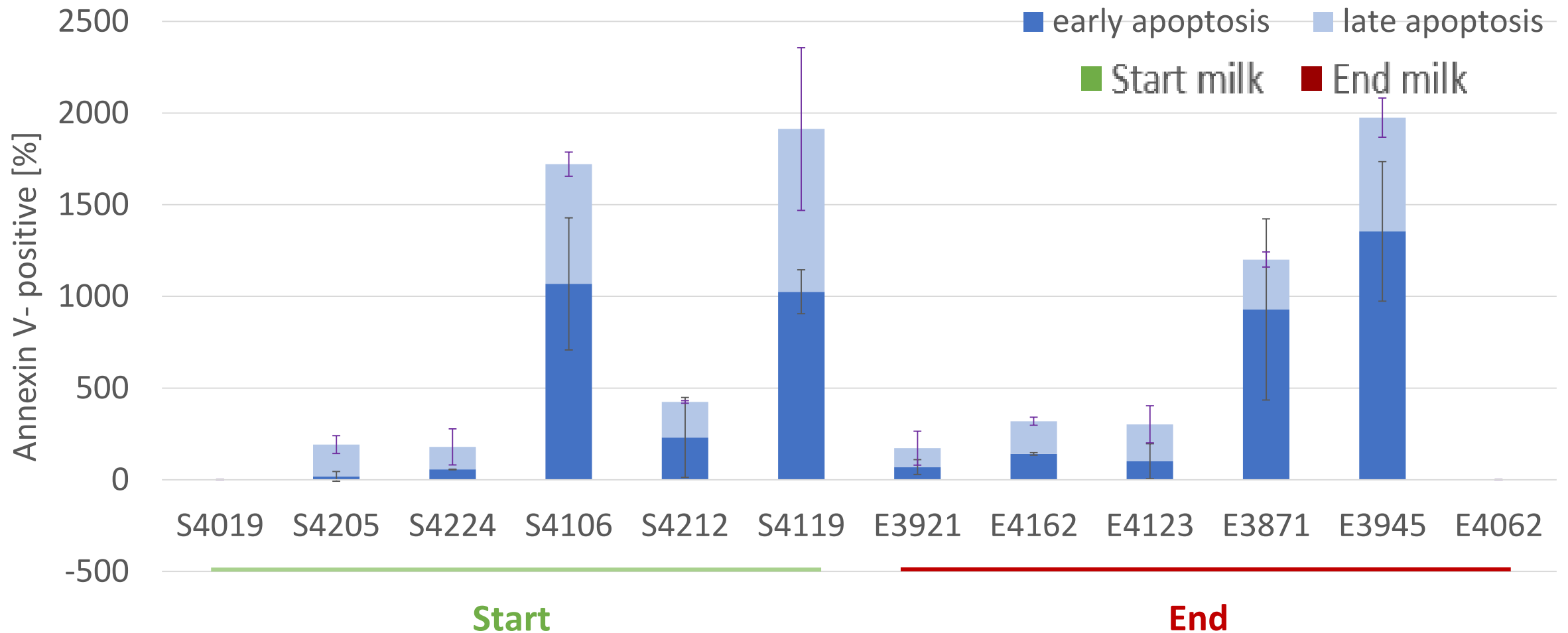
# Apoptosis of L1 cells





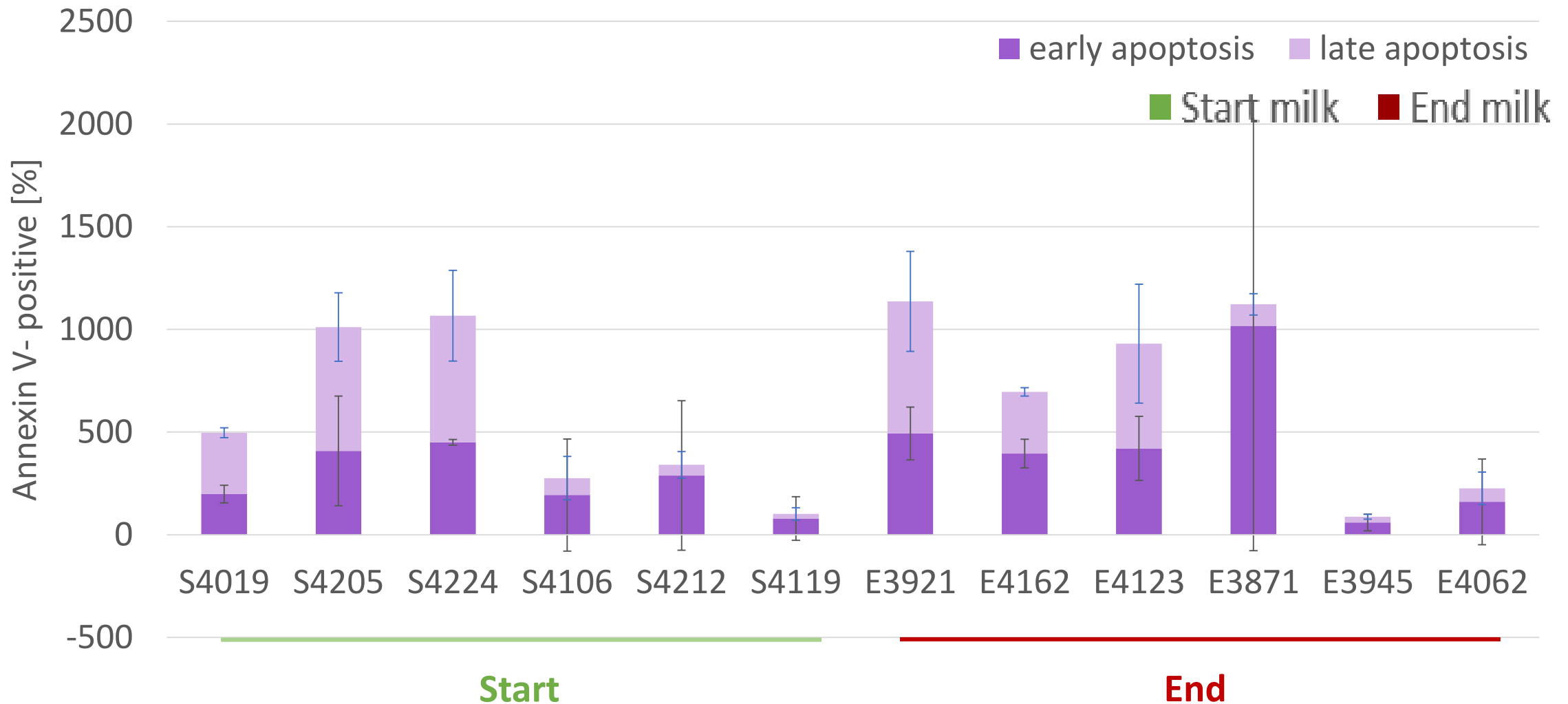
# Apoptosis rates of L1 cells

Induced by 3% Start and End milk in Individual samples



# Apoptosis rates of A549 cells

## Induced by 3% Start and End milk in Individual samples

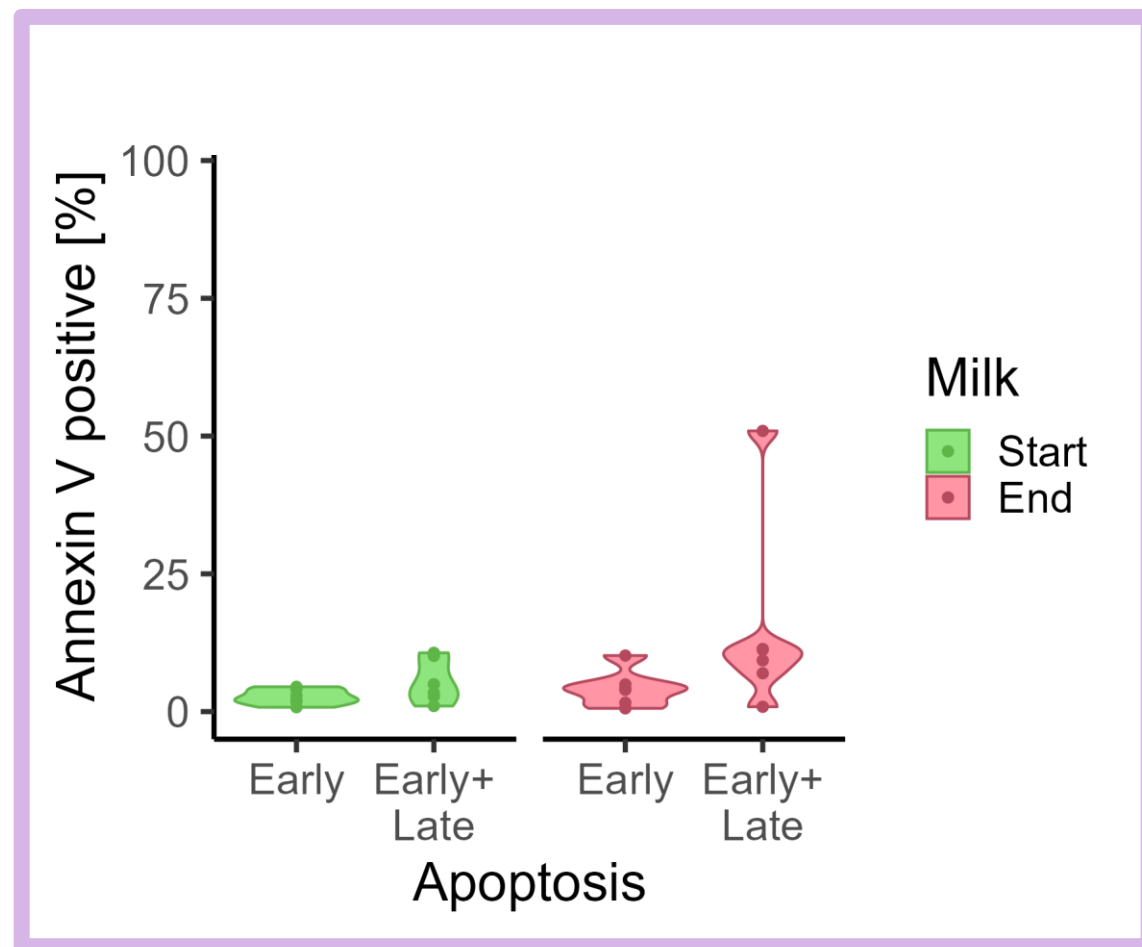
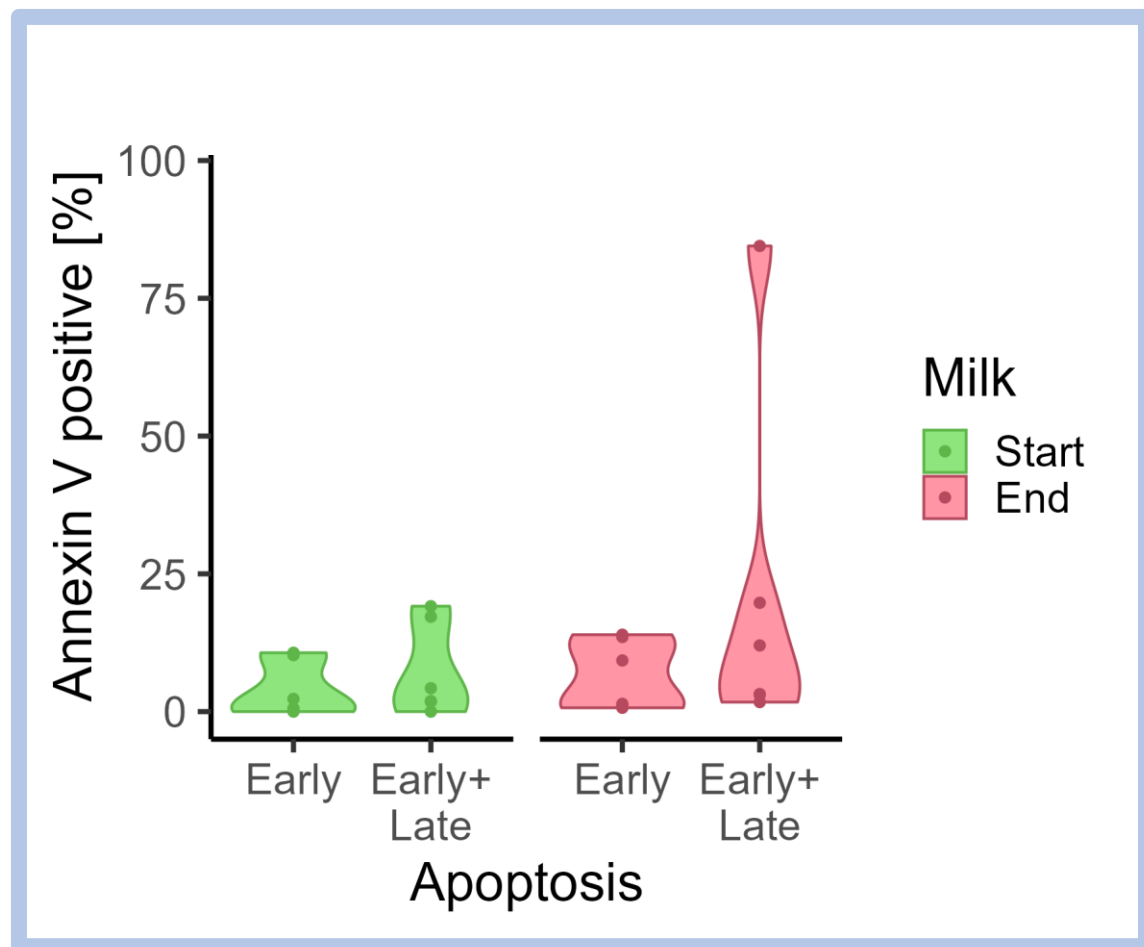


# Apoptosis rates of L1 and A549 cells

Induced by 3% Start and End milk in Individual samples

L1

A549



# Summary and Conclusions

- The total protein concentration of start milk is lower
- **$\alpha$ -LA** levels in start milk is higher than in end milk
  - Thus, the diff. in  **$\alpha$ -LA** concentration is higher
- Milk decreases cell viability and enhances apoptosis in **L1** and **A549** cells
  - But when looking at individual samples- big variability

# Future plans

- Take new samples from “Start milk” cows, in order to compare start and end milk from same individuals.
- Checking  **$\alpha$ -LA** concentration in milk, with and without oleic acid.
- After establishing the system, we want to use  **$\alpha$ -LA** as a predictor of efficiency of milk producing.
- Developing methods for controlling the milking period span.

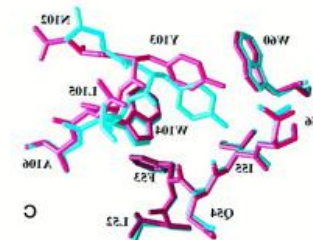
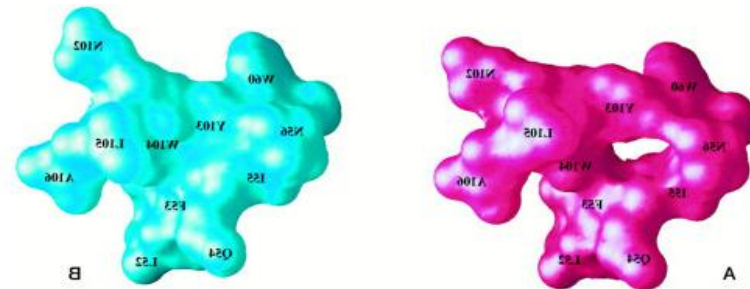
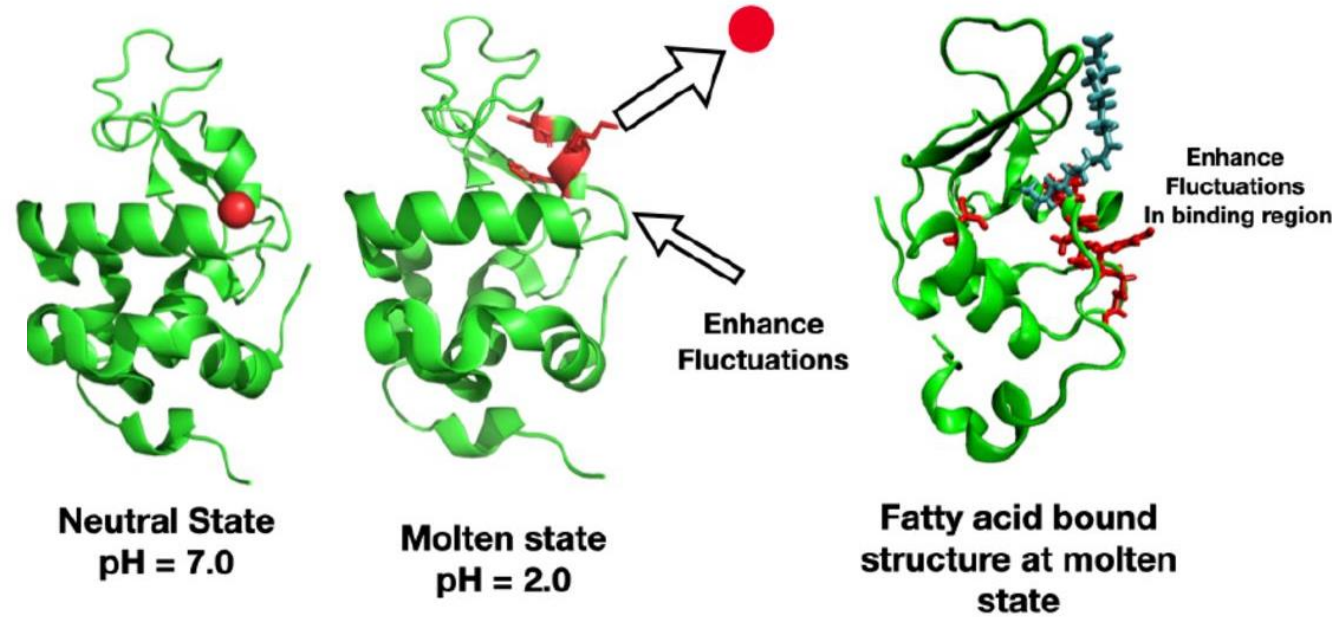
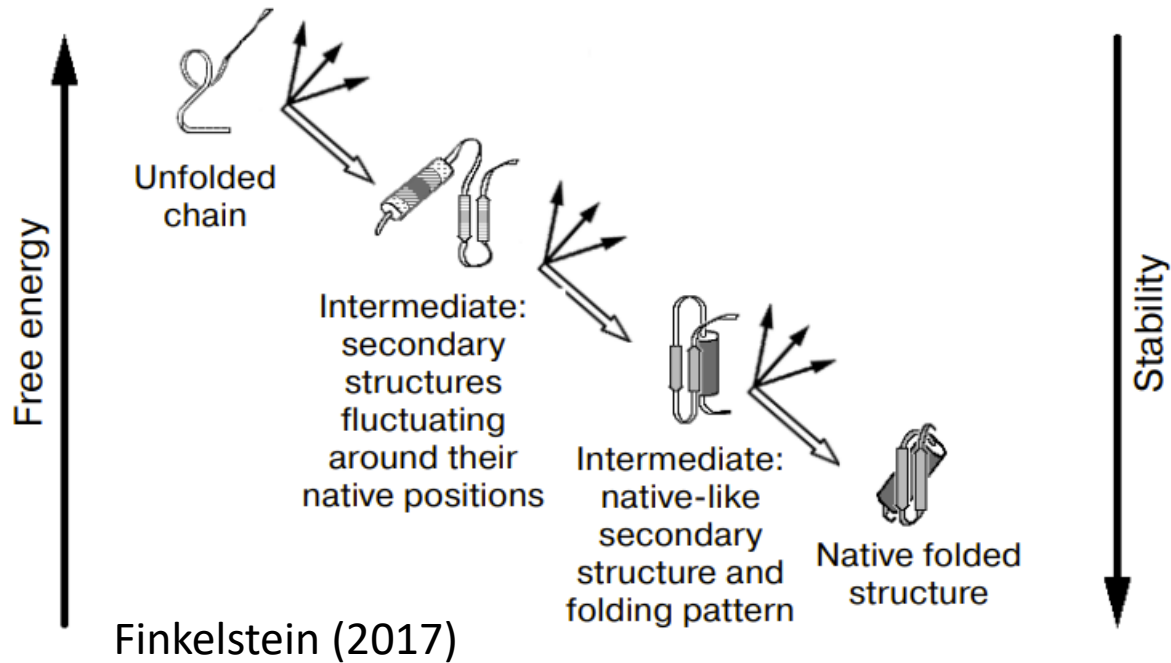


# Thank You For Listening

Funding:



# Molten globule protein MG- $\alpha$ -lactalbumin



Evangelia et al. (2000)