



# Breeding factors of dairy cows and milk lipolysis and consequences of milk lipolysis on semi-hard cheese and fresh cream

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# Breeding factors of dairy cows and milk lipolysis and consequences of milk lipolysis on semi-hard cheese and fresh cream

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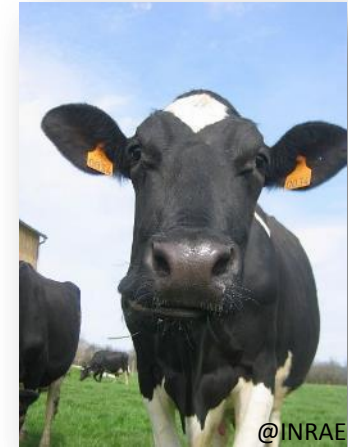
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## ➤ Presentation plan

- ❑ State of art of spontaneous lipolysis in 2023
- ❑ 3 experimentations concerning spontaneous lipolysis in dairy cows:
  - ✓ Feeding restriction
  - ✓ Milking interval
  - ✓ Milking frequency
- ❑ Impact of the extent of milk spontaneous lipolysis on the lipolysis induced during transformation
- ❑ ANR LIPOMEC project (*funding of the experiments*)



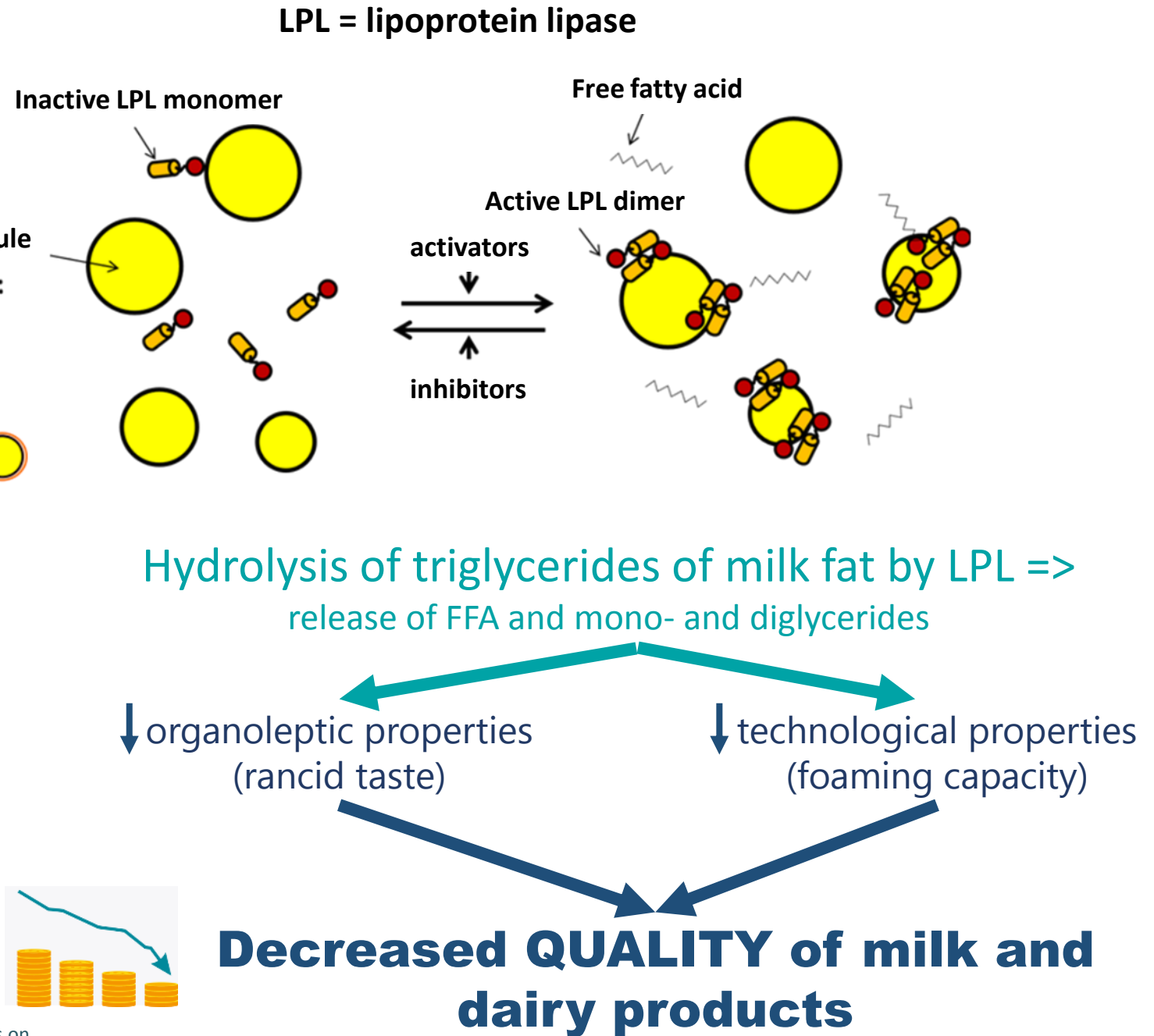
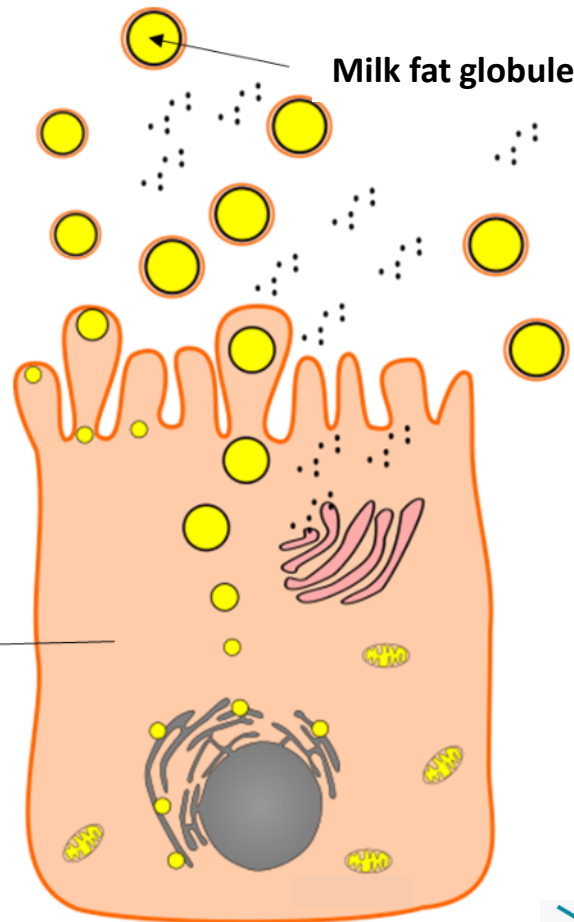
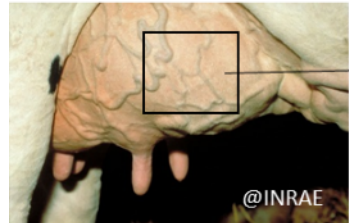
## ➤ State of art of spontaneous lipolysis in 2023

A definition: spontaneous lipolysis is initiated by the simple act of cooling raw milk to  $<10^{\circ}\text{C}$  after it is taken from the cow (Deeth, 2006)

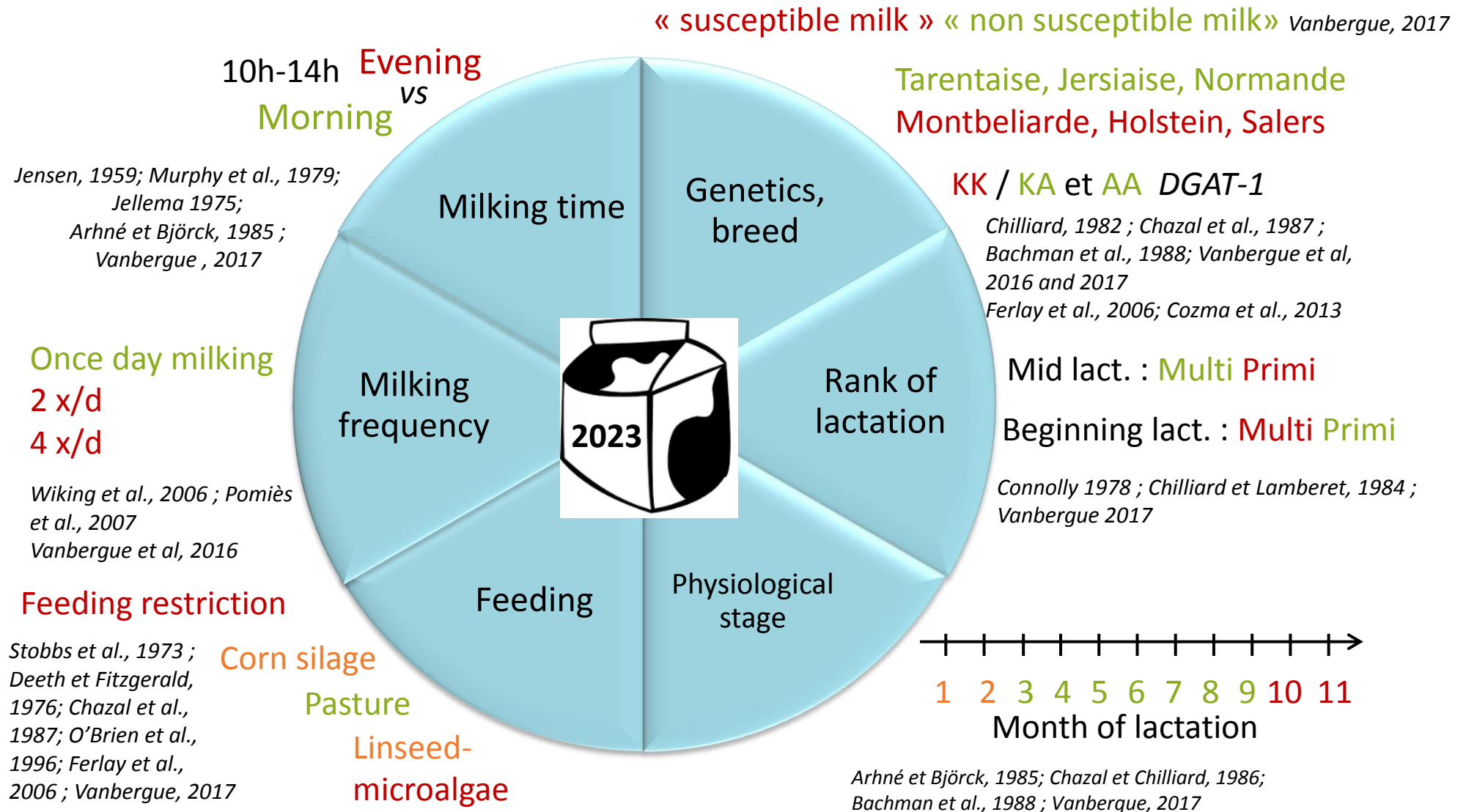




# > Milk lipolysis ?



# ➤ State of art of spontaneous lipolysis



## ➤ 3 experimentations concerning spontaneous lipolysis in dairy cows







# Feed restriction as a tool to describe and understand the mechanisms underlying lipolysis in milk in dairy cows

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## ➤ Objectives of the present study


 STATEMENT: Lipolysis is higher in milks from feed restricted cows

ASSUMPTION:



Changes in MFG composition could explain greater sensitivity of milk fat to lipolysis

Objectives =

- ☐ to induce spontaneous lipolysis in milk by **reducing the amount of feed** supplied to dairy cows
  - ☐ to study the mechanisms of milk lipolysis
-  a better understanding of the molecular mechanisms controlling the degradation of milk fat through integrative biology approaches

## ➤ Material and methods: experimental design



44 dairy cows ( $2 \times 2 \times 11$ )  
 $165 \pm 16$  days in milk

2 weeks

1 week

2 weeks

Corn silage + conc. 100 % ad lib

Corn silage + conc. 65 % ad lib

Corn silage + conc. 65 % ad lib

Corn silage + conc. 100 % ad lib



**Once at the end of each period:**

- ✓ Milk sampling, morning and evening milking
- ✓ Blood sampling after morning milking
- ✓ Mammary biopsies



## ➤ Material and methods: Analyses

### ☐ Measurements and laboratory analysis

- ✓ Milk yield, fat and protein contents
- ✓ Lipolysis
- ✓ Plasma metabolites
- ✓ Milk fat globules and casein micelles size
- ✓ Casein and minerals contents

### ☐ Statistical analysis

Effects of feeding levels on parameters : SAS MIXED procedure

$$Y_{ijkl} = \mu + \text{Group}_i + \text{Milking Time}_j + \text{Period}_k + \text{Feeding level}_l + \text{Milking Time}_j \times \text{Feeding level}_l + \text{CovY}_{ijkl} + e_{ijkl}$$

## ➤ Milk production and composition

	100% <i>ad lib</i>	65% <i>ad lib</i>
Milk, kg/d	30.6	<b>25.5</b>
Milk protein content, g/kg	31.4	<b>30.1</b>
Milk fat content, g/kg	40.0	40.1
Milk lactose content, g/kg	49.3	49.5

❑ Feed restriction was accompanied by

- a decrease in milk yield (- 5.1 kg) and in protein content (-1.3 g/kg)
- no effect on fat and lactose contents: surprising for lactose content considered as

biomarker of energy deficit

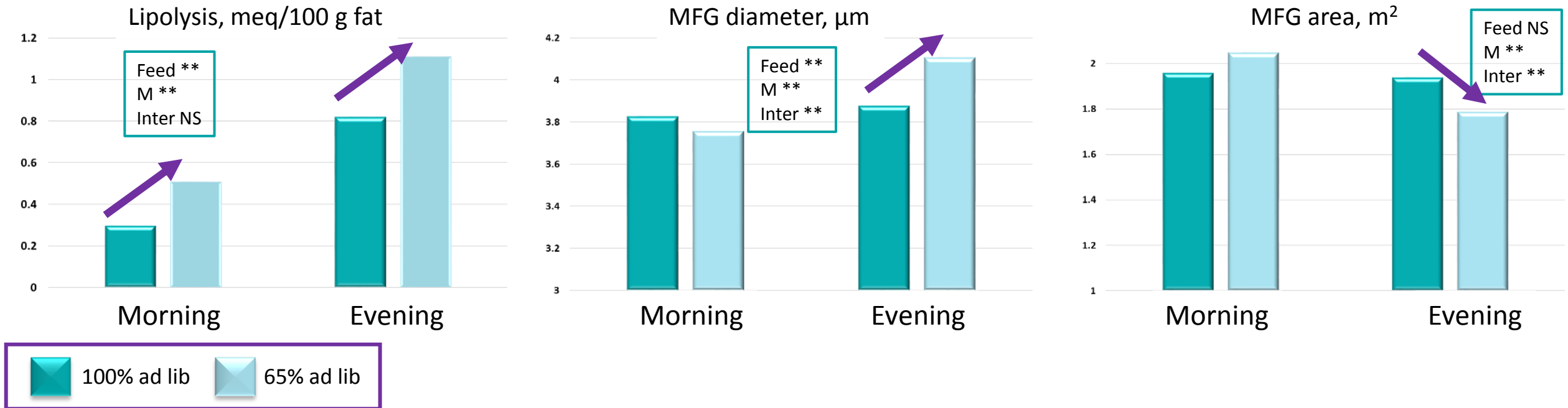
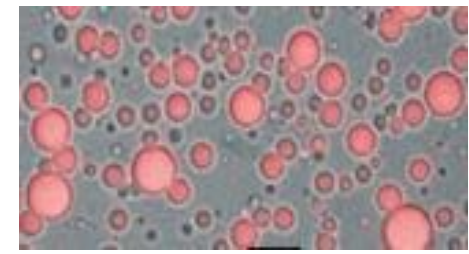
*Vanbergue et al, 2018*



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## ➤ Milk lipolysis



☐ Increase of lipolysis with feeding restriction (*Vanbergue et al, 2017 and 2018*)

☐ Larger fat globules associated with higher degree of lipolysis, higher fat/protein ratio (*Couvreur and Hurtaud, 2017*) without variation of milk fat content



## ➤ Assumptions & Interpretation



- ❑ Change in protein composition and polar lipids of MFG membrane (biomarkers of high lipolysis)  
With an effect on the size of the MFG and on the solidity of its membrane => accessibility of the LPL to its substrate.
- ❑ ↗ LPL activity measured in morning milk with feeding restriction (708.8 vs. 501.7  $\eta$ mol/min/mL)
- ❑ ↗ LPL expression (mRNA measured on mammary tissue (biopsies) and on epithelial cells isolated from milk)? No result for the moment
- ❑ Action on inhibitors (PP5,...) or activators (apolipoproteins, Glycam1,...) of LPL

## > Conclusion

- ❑ Objective of the experiment achieved: induce lipolysis by restricting feed to dairy cows
- ❑ Milk samples and biopsies performed on cows with extreme lipolysis: genomic, proteomic, lipidomic analyzes in progress.
  - ➡ Integrative biology approach, linking milk proteomics to mammary gland transcriptomics
- ❑ What's next?
  - ➡ 2 experiments with the same feeding strategy on ewes and on goats in 2021 for a better understanding of the response and mechanisms regulating the lipolytic system among species (results published soon).Ultimately, a comparative analysis between the 3 species will be also done.





# Impact of milking interval on milk spontaneous lipolysis in dairy cows

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## ➤ Objectives of the present study



STATEMENT: Lipolysis is higher in milks from the evening milking than in those from the morning milking with a 10-14 h interval

ASSUMPTION: levels of lipolysis in milk depends on:



[ the length of the intervals between milkings ?  
the nycthemeron ?

Objectives = to study the impact of the interval duration between 2 consecutive milkings on the spontaneous lipolysis of milk



## ➤ Material and methods: Experimental design

❑ 21 primiparous and multiparous dairy cows chosen according to their susceptibility to lipolysis (measured by MIR)

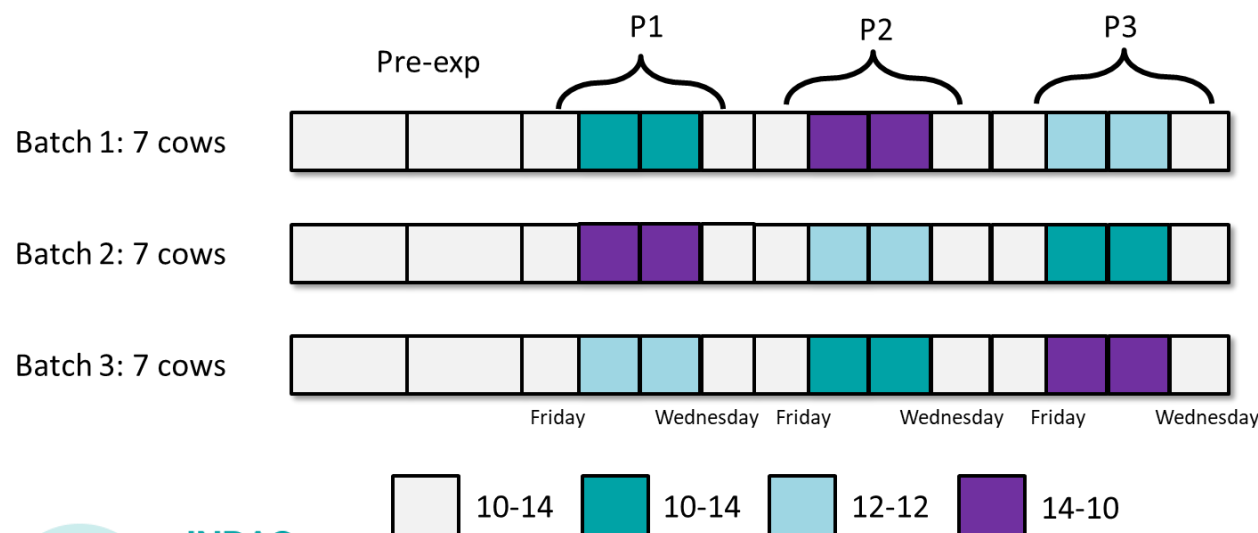
❑ 3 treatments :

- ✓ 10–14 (milking 6:30 am, 4:30 pm)
- ✓ 12–12 (milking 6:30 am, 6:30 pm)
- ✓ 14–10 (milking 6:30 am, 8:30 pm)

### Latin Square Design

❑ 3 periods of 2 weeks:

- ✓ 5 days of adaptation to treatment
- ✓ 1 sampling day
- ✓ 8 return days to 10 h – 14 h



## ➤ Material and methods: Analyses

### ☐ Measurements and laboratory analysis

- ✓ Milk yield, fat and protein contents
- ✓ Lipolysis
- ✓ Milk fat globules and casein micelles size
- ✓ Casein and minerals contents

### ☐ Statistical analysis

Effects of duration of milking intervals on parameters : SAS MIXED procedure

$$Y_{ijk} = \mu + \text{Cow}_i + \text{Period}_j + \text{MilkingInterval}_k + \varepsilon_{ijk}$$



## ➤ Results-Discussion: Milk production



		10-14	12-12	14-10	Interval effect
Milk, kg	Morning	19.2	17.5	15.5	***
	Evening	13.9	16.0	17.8	***
	Day	33.1	33.6	33.2	NS
Fat content, g/kg	Morning	39.2	40.4	43.4	***
	Evening	42.9	40.5	39.1	***
	Day	40.7	40.2	41.1	NS
Protein content, g/kg	Morning	30.9	30.2	29.1	***
	Evening	30.2	31.4	31.6	***
	Day	30.5	30.7	30.4	NS

### SUMMARY

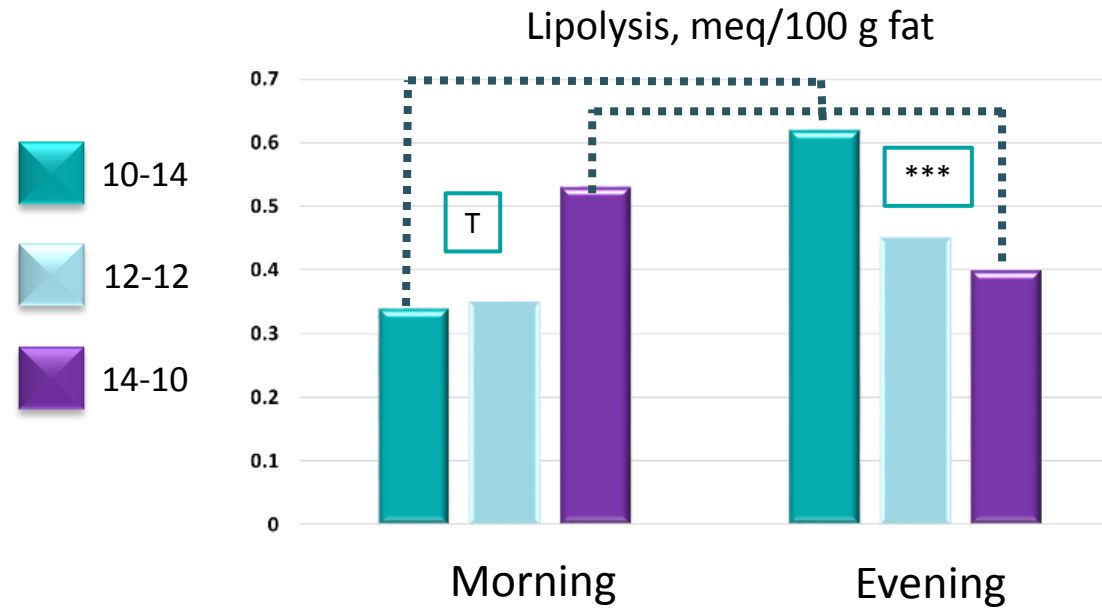
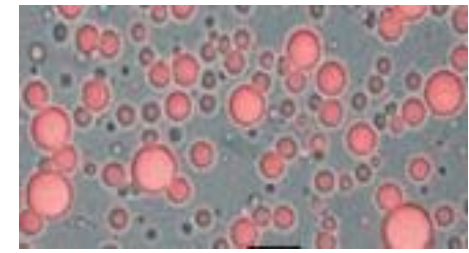
- ✓ No effect of milking intervals on milk yield, fat and protein contents
- ✓ Opposite effects between 10-14 and 14-10 on morning and evening milks
- ✓ 12-12 : slight differences in milk yield and protein content between morning and evening
- ✓ For 12-12 and 14-10, increase of synthesis of milk constituents during the night, not for 10-14!

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# ➤ Results-Discussion: Lipolysis and milk fat globules



No significant effect of milking intervals on MFG diameter and area

SUMMARY

Fluctuation of lipolysis between morning and evening milking ↔ interval between milkings (*Wiking et al, 2006*), not to nycthemeron

↗ LPL activity with 10-14 especially in evening milk (737 vs 642 vs 572  $\eta$ mol/min per mL)  
12-12 14-10

Penalization of the reconstitution of the membrane which surrounds the triglycerides (*Connolly, 1978*)

No correlation between LPL activity and lipolysis (*Cartier and Chilliard, 1990*)



INRAE

## ➤ Conclusion

- ❑ This original study
  - ✓ shows that milking interval prevails over nycthemeron in affecting milk lipolysis
  - ✓ still raises a number of questions about the mechanisms of lipolysis





# Impact of milking frequency on milk spontaneous lipolysis in dairy cows

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## Objective of the present study



👁️👁️ Statement: Lipolysis is higher in milks from automatic milking systems (AMS) in relation to milking frequency and lower in once day milking

HYPOTHESIS: levels of lipolysis in milk depend on:



- ✓ the length of the intervals between milkings?
- ✓ the milk yield?

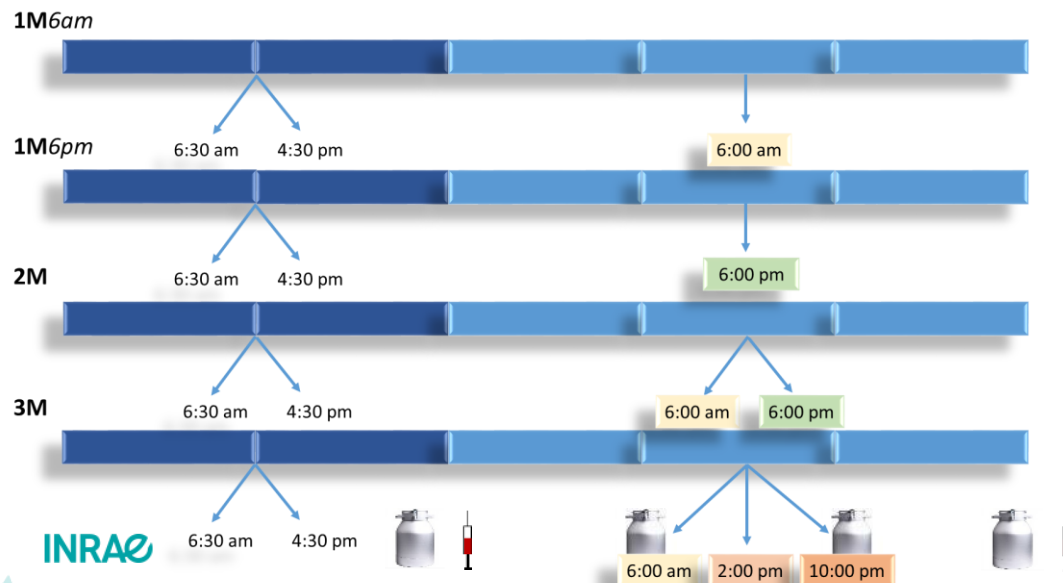
Objectives = to study the impact of **milking frequency** on the spontaneous lipolysis of milk

## ➤ Material and methods: Experimental design

- ❑ 32 primiparous and multiparous dairy cows chosen according to their susceptibility to lipolysis (measured by MIR and copper soap method) (4 susceptible dairy cows (**Sus** > 0.6 mEq/100 g fat) and 4 non-susceptible dairy cows (**Nsus** < 0.6 mEq/100 g fat) by batch)
- ❑ 4 treatments :
  - ✓ **1M6am**: one milking/ day at 6:00 am
  - ✓ **1M6pm**: one milking/ day at 6:00 pm
  - ✓ **2M**: 2 milkings / day at 6:00 am and 6:00 pm
  - ✓ **3M**: 3 milkings / day at 6:00 am, 2:00 pm and 10:00 pm

Continuous design

- ❑ 2 weeks of pre-experiment
- ❑ 3 experimental weeks



**At the end of the pre-experiment and week 3**

- ✓ Milk samples at each milking
- ✓ Blood samples



## ➤ Material and methods: Analyses

### ☐ Measurements and laboratory analysis

- Milk yield, fat and protein contents
- Lipolysis
- Milk fat globules and casein micelles size
- Casein and minerals contents

### ☐ Statistical analysis

Effects of milking frequencies on parameters in wk 3 : SAS MIXED procedure

$$Y_{ij} = \mu + \text{Milking}_i + \text{LipoSens}_j + \text{LipoSens}_j \times \text{Milking}_i + \text{Cov } Y_{ij} + \varepsilon_{ij}$$

## ➤ Results-Discussion: Milk production

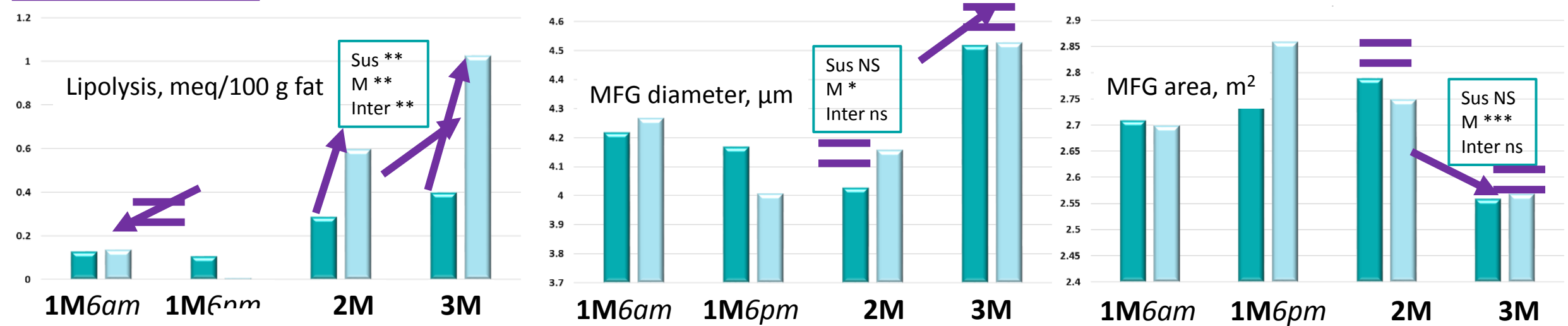
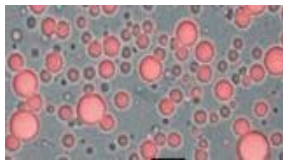


						Effect		
		1M6am	1M6pm	2M	3M	SEN	M	SEN*M
Milk, kg/d	NSus	27.3	18.3	39.5	37.0	NS	***	NS
	Sus	25.4	23.2	42.3	38.2			
Milk fat content, g/kg	NSus	45.1	40.2	35.7	40.4	NS	T	NS
	Sus	47.2	41.5	40.7	40.6			
Milk protein content, g/kg	NSus	33.3	31.8	31.3	32.5	NS	NS	NS
	Sus	32.7	31.9	32.3	31.4			

### SUMMARY

- ✓ No significant effect of milking frequencies on milk fat and protein contents
- ✓ Higher milk production for **2M** and **3M**, intermediate for **1M6am** and lower for **1M6pm**. For **1M6pm**, consequence of a large decrease for 2 dairy cows (more than 20 kg !)
- ✓ No effect of the individual susceptibility to lipolysis on these parameters

# ➤ Results-Discussion: Lipolysis and milk fat globules



❑ Compared to **2M**, decrease in lipolysis with **1M** and increase with **3M**

❑ With **3M**, MFG diameter ↗ and MFG specific area ↘

❑ No effect of dairy cow susceptibility to lipolysis with **1M**, but increase of lipolysis with **2M** and **3M** for susceptible dairy cows, without any effect on MFG diameter and area.

Penalization of the reconstitution of the membrane which surrounds the triglycerides (*Connolly, 1978*): not enough time between milkings to synthesize MFGM (*Wiking et al, 2019*)  
 ↗ LPL activity with ↘ interval (*Sundheim, 1988*)



## ➤ Conclusion

- ❑ We confirmed the effects of milking frequencies: less lipolysis in once day milking and more lipolysis with 3 milkings per day (automated milking systems)
  
- ❑ Original result:
  - ✓ Similar decrease in lipolysis in susceptible and non-susceptible cows with once day milking
  - ✓ On the other hand, marked increase in lipolysis in susceptible cows with 2 or 3 milkings per day compared to non- susceptible cows



## ➤ Impact of the extent of milk spontaneous lipolysis on the lipolysis induced during transformation





# Impact of the extent of milk spontaneous lipolysis on the lipolysis induced during transformation



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[https://www6.rennes.inrae.fr/plateforme\\_lait](https://www6.rennes.inrae.fr/plateforme_lait)

<sup>2</sup>PEGASE, INRAE, Institut Agro, 35590, Saint Gilles, France



# Objective of the present study



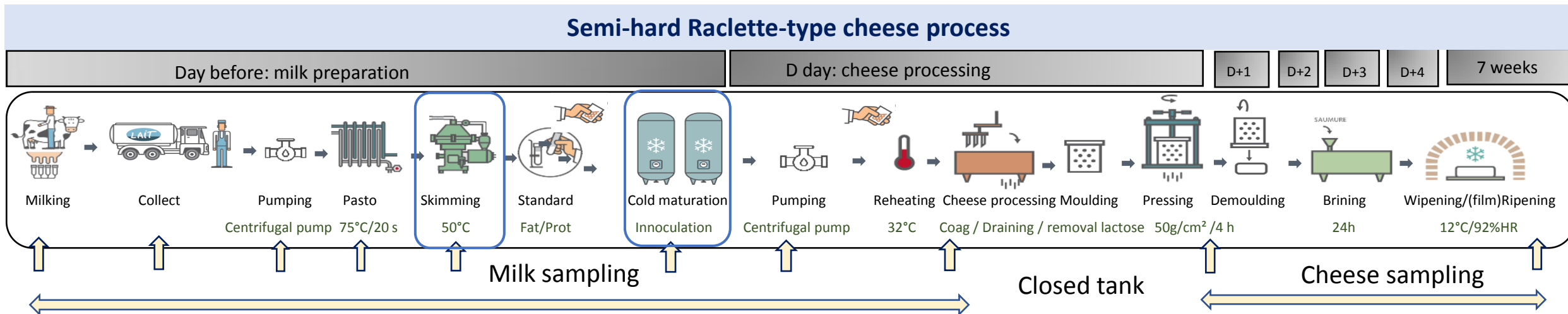
Statement: milk processing induce lipolysis due to fat globule damage

HYPOTHESIS: the induced lipolysis could be more pronounced in milk sensitive to spontaneous lipolysis

Objective: to compare the changes in lipolysis induced during milk processing operations between susceptible and non-susceptible milks



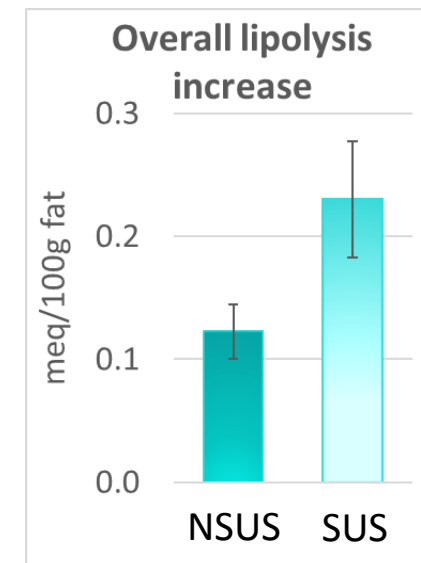
## ➤ Main results: example of semi-hard cheese



✓ We measured lipolysis evolution all along the process from milking to renneting (by sampling)

✓ The most impactful steps were **skimming** and **cold maturation**

✓ We observed a significantly greater overall increase for **susceptible milks**, compared to **non-susceptible milks** (regardless the initial lipolysis degree)



## ➤ Main results: sensory evaluation for semi hard cheese and sour cream

- ✓ We used Triangular tests to evaluate sensory differences
- ✓ No significant differences were perceived between products made from sensitive or non sensitive milks (regardless the product)



2 month-ripened  
cheeses



sour cream stored  
for 2 months at 4°C

## ➤ Conclusion

- ✓ We measured a significantly greater lipolysis induced in sensitive milks during some key steps during milk transformation in cheese and sour cream
- ✓ However, these differences of induced lipolysis were not sensorially perceived in the two dairy products tested

➤ And the full story of the adventure INRAE “Lipolysis”...  
... the ANR LIPOMECE project



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# ➤ The ANR project LIPOMECE

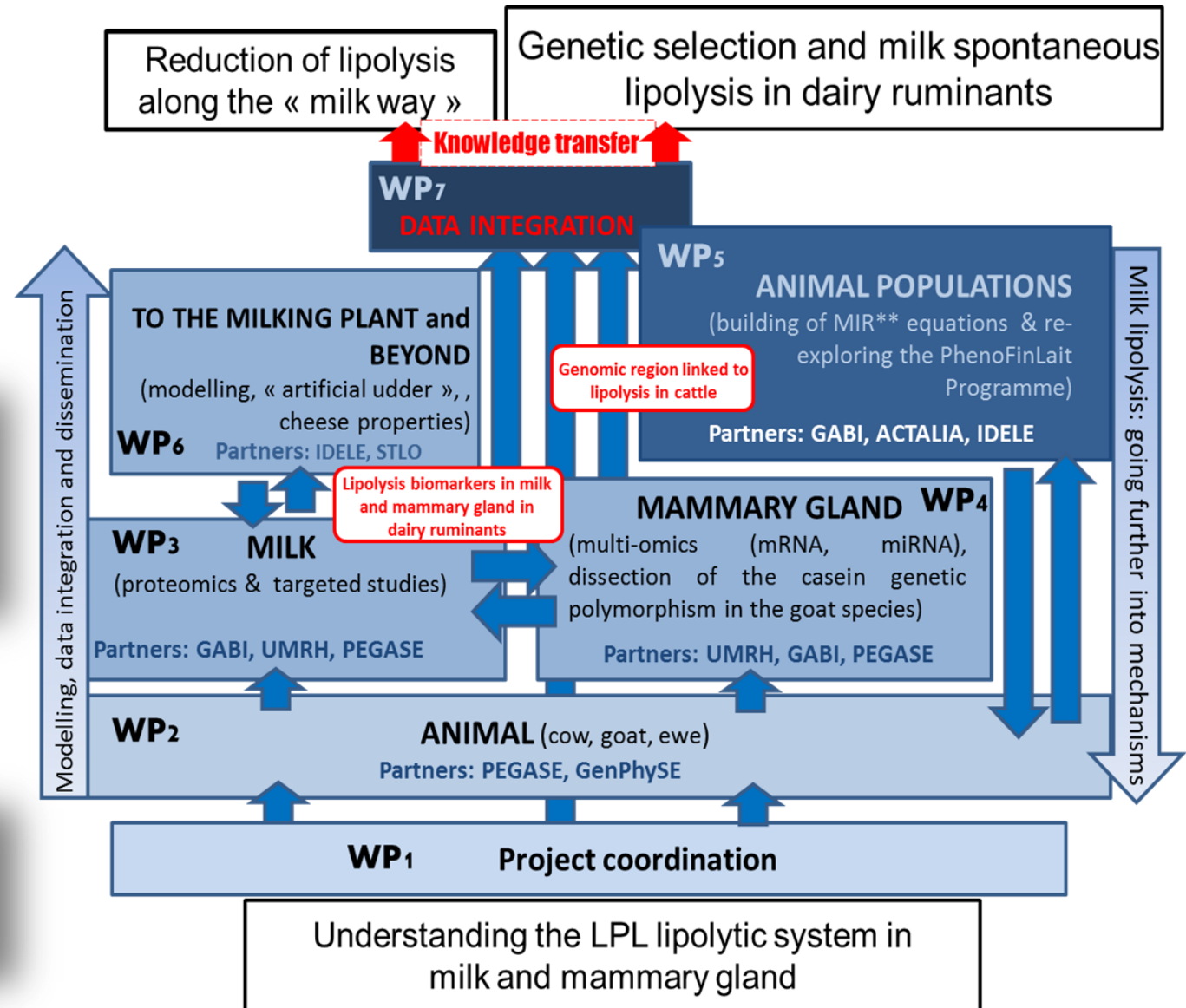
Coordination C. Cebo (INRAE)



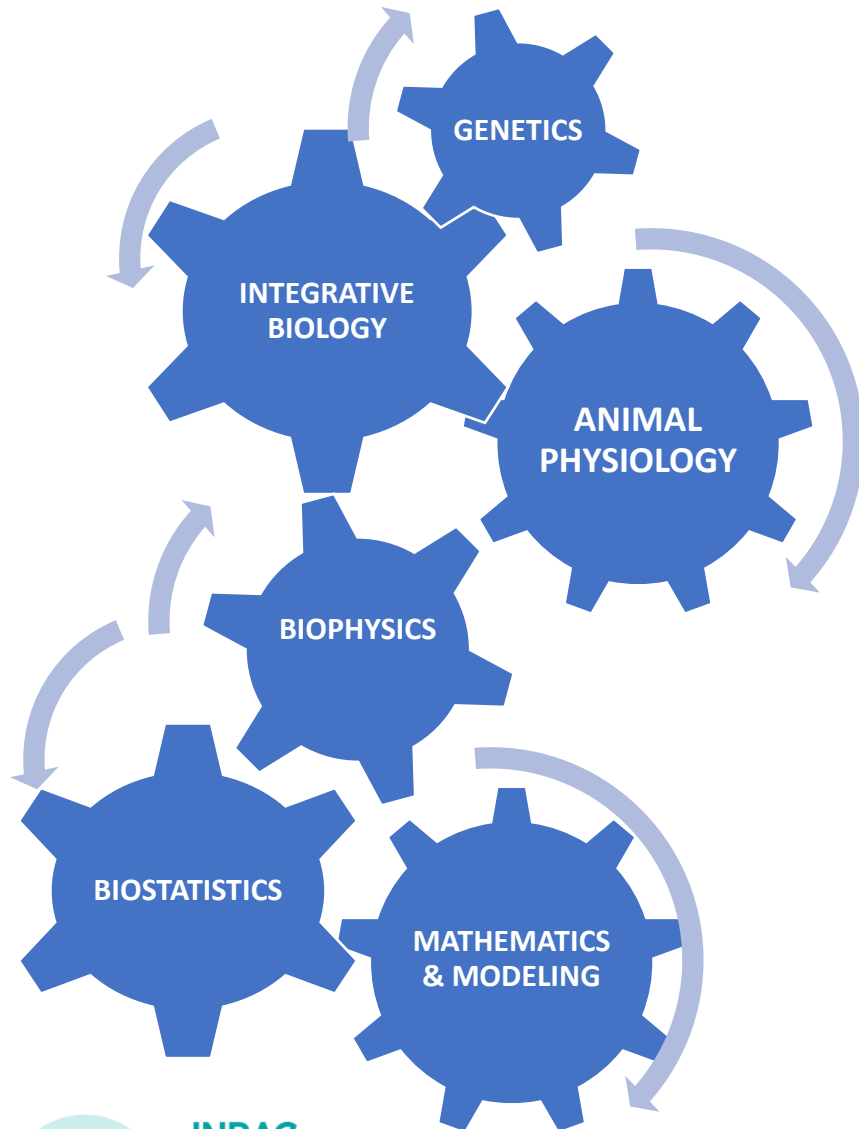
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## ➤ LIPOMECH, a transdisciplinary, multi-actors and multi-scale program



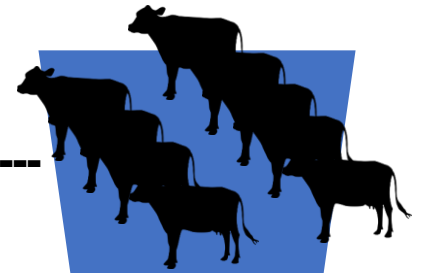
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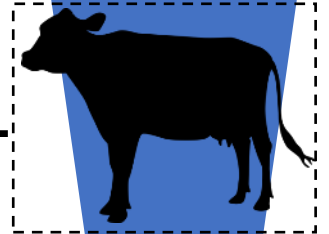
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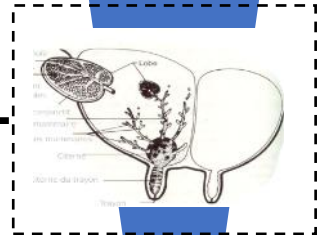
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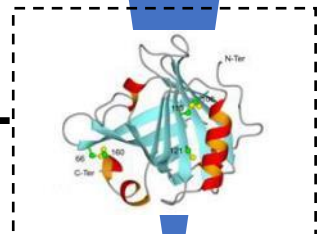
ANIMAL-----



MAMMARY GLAND-----



LPL & OTHERS MOLECULES-----





# THANK YOU FOR YOUR ATTENTION !

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TO CO-AUTHORS AND  
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AND LABORATORY TECHNICIANS

Actors & Funders of  
LIPOMECE project:

