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A comparative study of the MaSC/progenitors committed to the mammary development at three physiological stages in bovine

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PHYSIOLOGIE, ENVIRONNEMENT ET GÉNÉTIQUE POUR L'ANIMAL ET LES SYSTÈMES D'ÉLEVAGE (PEGASE)

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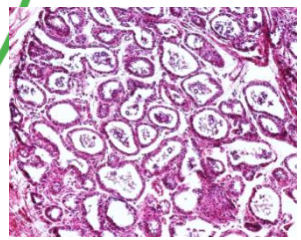
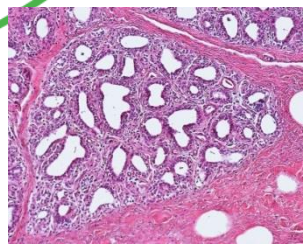
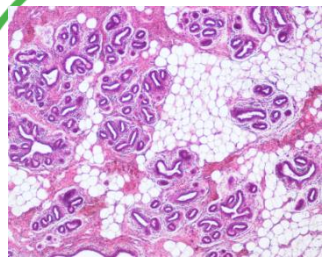
Background

The mammary development in bovine







Background

The mammary adult stem cells, key regulators of the epithelial tissue (re)generation

Existence of the mammary adult stem cells (MaSC) was highlighted:

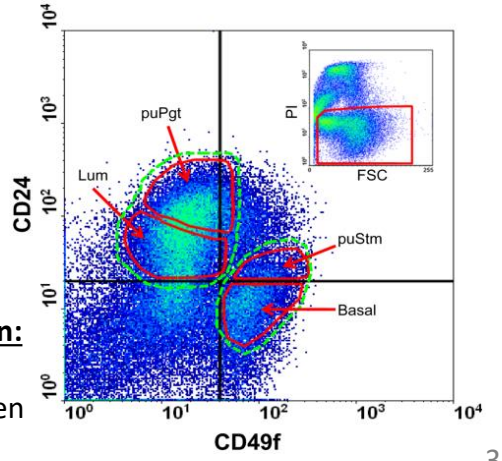
- In the murine model : using cleared mammary fat pad transplantation assay consisting in transplanting mammary tissue isolated from a donor into cleared fat pads (native epithelium removed) of a mice recipient (*DeOme et al, 1959*)

- In the bovine model :

  By long-term DNA labeling of epithelial cells with BrdU (*Capuco, 2007*) the putative MaSC being the label-retaining epithelial cells (or LREC)

  By flow cytometry (on 7 months age heifers)

  The putative MaSC were identified as : $CD49_f^{high}CD24^{pos}$ cells, regenerating epithelial tissue in xenotransplantation assay and forming mammosphere when cultured *in vitro* (*Rauner and Barash, 2012*)



Cluster of Differenciation:
$CD49_f$: α6 Integrin
CD24 : Heat stable antigen

Background

The mammary adult stem cells, a hot topic in agronomy

A good knowledge of the MaSC would be beneficial to the implementation of new **rearing strategies (as nutrition) in early life** to produce robust animals (with improved mammary development and performances) through MaSC expansion

bovine MaSC/Progenitors

Known

- The MaSC pool is thought to be defined at early life of animals.
- The number of MaSC ($CD49_f^{high}CD24^{pos}$ cells) does not vary during a lactation cycle in primiparous cows (*Perruchot et al, 2016*).

Unknown

- **In bovine, how do the MaSC/progenitors populations evolve at main physiological stages (puberty, lactation and dry off periods)?**

Experimental strategy

Identify and characterize the MaSC/progenitors committed to the mammary development at puberty in order to compare these at each targeted physiological stages (puberty, lactation and dry off periods)

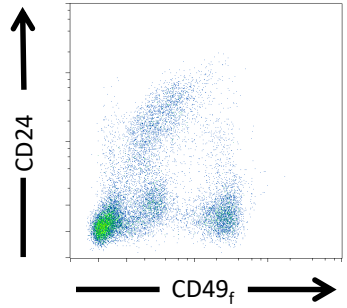
Materiel and Methods (1/2)

Animals :

Heifers	Lactating cows	Dried cows
		
17 months old age n = 4	4th lactation n= 4	6 years dry off n = 3

Methods : Mammary gland explants were dissociated to single cells using an enzymatic (collagenase/hyaluronidase/trypsin) dissociation protocol

Single cells were stained with anti-CD49$_f$ and anti-CD24 antibodies and co-expression of these markers was assessed by flow cytometry.

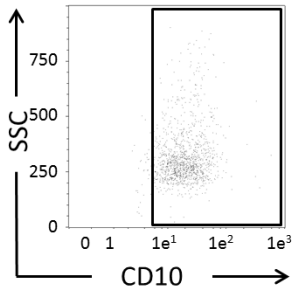


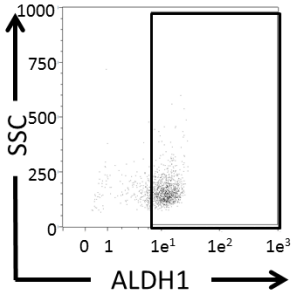
- **The proportion of epithelial sub-populations** : number of cells expressing CD49$_f$ ± CD24 were determined from the cytometric profiles

Materiel and Methods (2/2)

Determining the phenotyping characteristics and molecular signature of the epithelial sub-populations

- **A phenotyping characterization :** measure of CD10 (basal protein) expression and ALDH1 activity (MasC/progenitor marker) by flow cytometry within each sub-populations :

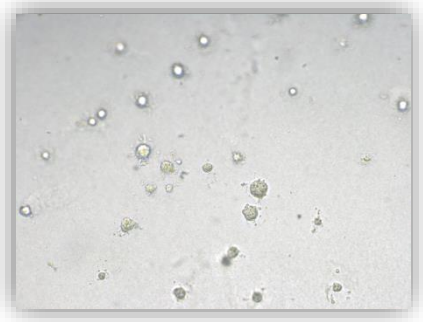


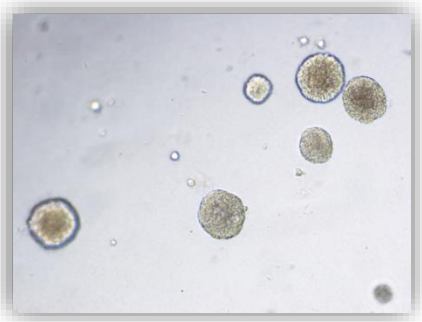


- Sorting of sub-populations expressing $CD49_f$ ± CD24 for :

- A functional test for MaSC : the mammosphere-formation assay

✓ No mammosphere formation ✓ Mammosphere formation



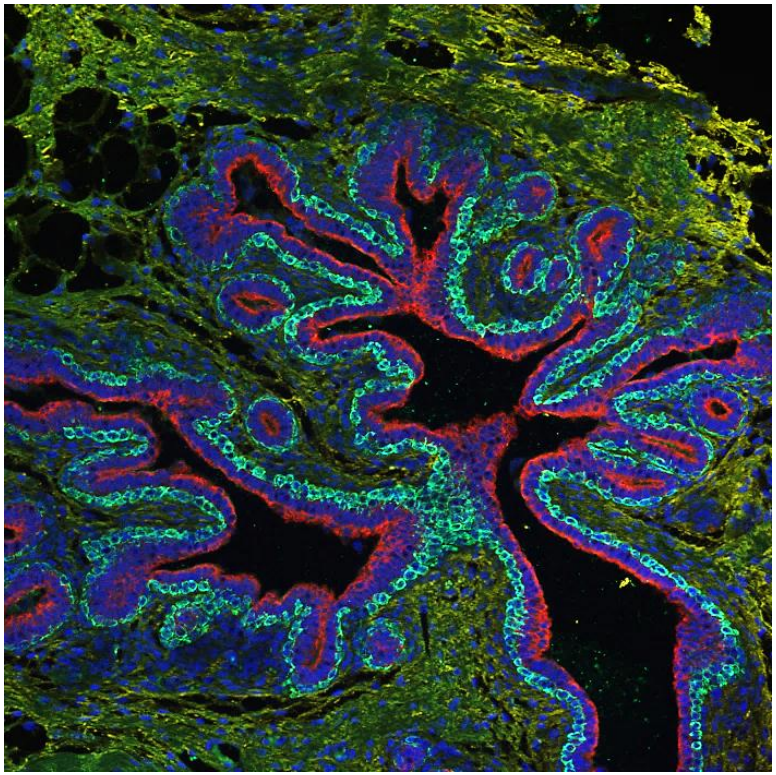


- Establishment of the molecular signature of each epithelial sub-populations: determination of the gene expression profiles by RT-qPCR

Results

At puberty, the ductal-alveolar development occurs involving various epithelial populations

- Immunofluorescence reveals ductal and terminal lobular units structures in development



Staining:

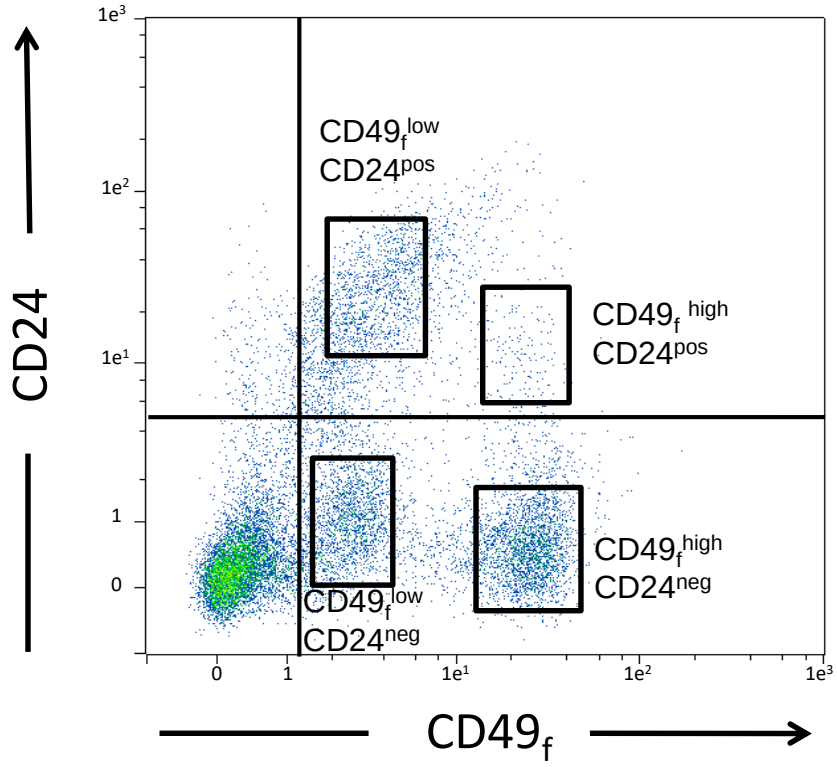
KRT14 : basal protein

KRT8 : luminal protein

Collagen type I : stromal protein

H33342 : nuclei

- Flow cytometry analysis of mammary single cells highlighted 4 epithelial sub-populations



Cell surface markers (CD; Cluster of Differenciation):

$CD49_f$: α6 Integrin

CD24 : Heat stable antigen

Results

The phenotyping characteristics and molecular signature of the epithelial cells at puberty

CD49$_f^{low}$ CD24^{pos} cells

No mammosphere formation

CD10 expression : 75% ± 27

ALDH1 activity: 78% ± 8

Genes expression : *KRT7, KRT18, KI67, ELF5*

PROGENITORS CELLS (dual lineage)

CD49$_f^{high}$ CD24^{pos} cells

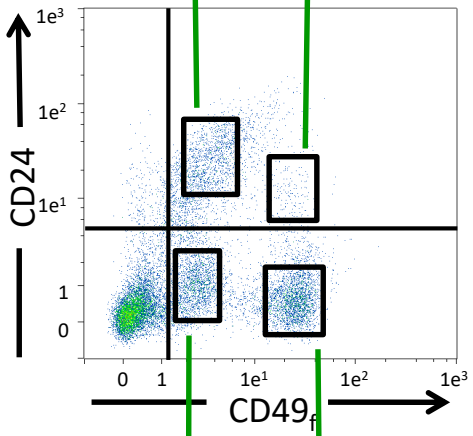
Mammosphere formation

CD10 expression : 92% ± 5

ALDH1 activity: 68% ± 9

Genes expression : *KRT14, KRT7, KRT18, ELF5*

STEM CELLS



CD49$_f^{low}$ CD24^{neg} cells

No mammosphere formation

CD10 expression : 7% ± 4

ALDH1 activity: 87% ± 13

Genes expression : *KRT7, KRT18, KRT19, PR, PRLR*

LUMINAL CELLS

CD49$_f^{high}$ CD24^{neg} cells

Mammosphere formation

CD10 expression : 93% ± 6

ALDH1 activity: 0,3% ± 0,4

Genes expression : *KRT14, Vimentin, PROCR*

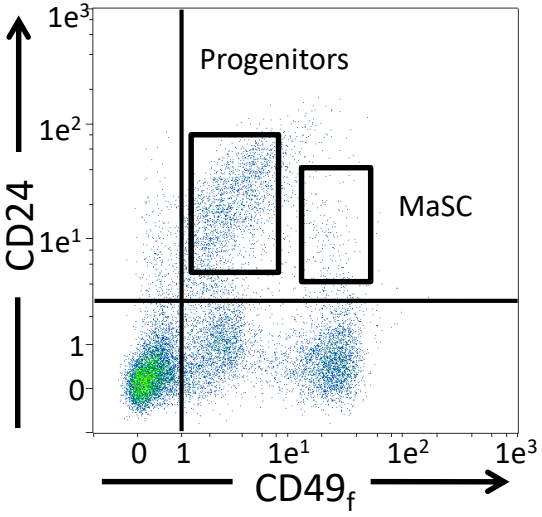
BASAL CELLS

<u>Abbreviations</u> : Aldehyde dehydrogenase (ALDH1); keratin (KRT); progesterone receptor (PR); prolactin receptor (PRLR); E74 like ETS transcription factor 5 (ELF5); protein C receptor (PROCR)

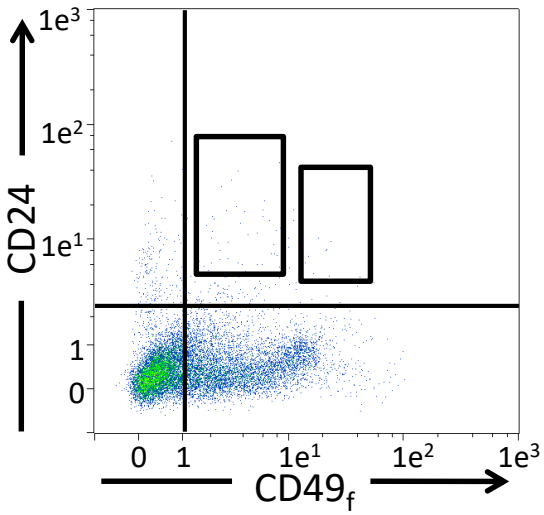
Results

Comparison of the epithelial sub-populations assessed by flow cytometry at key physiological stages

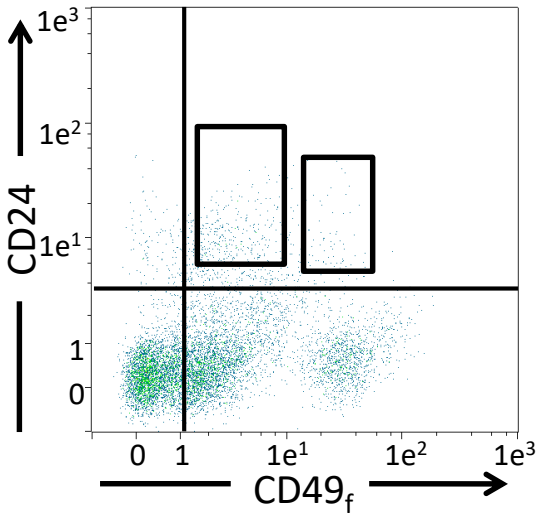
Heifers



Lactating cows



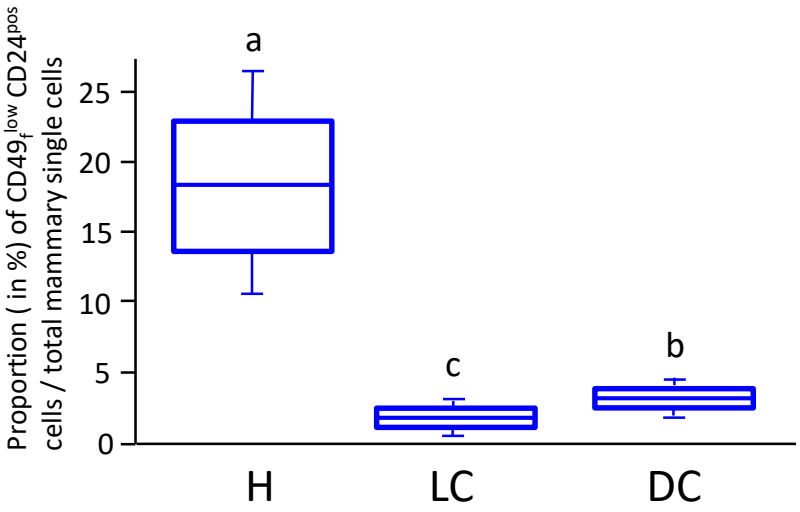
Dried cows



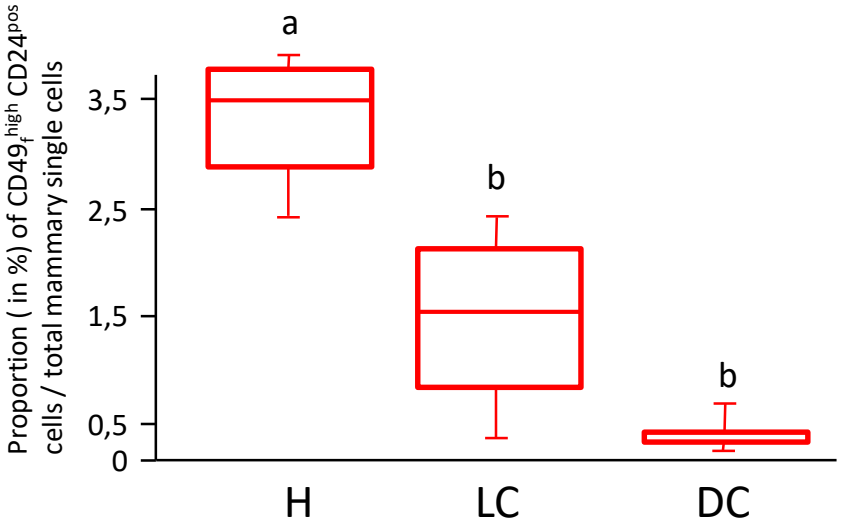
Results

The proportion of the MaSC/progenitors cells at the 3 physiological stages

The Progenitors cells



The MaSC



The progenitors are abundant at puberty but poorly present during the lactation and dry off periods

The proportion of MaSC decreases from puberty to lactation and at the dry off period

Conclusions & perspectives

How do the MaSC/progenitors populations evolve at main physiological stages in bovine?

Conclusions :

At puberty, the MaSC/progenitors proportion is more abundant than at lactation and dry off

The MaSC proportion decreases gradually as the animal ages and goes through lactations : from puberty to dry off

Perspectives:

Validating the stemness of sorted cells by the xenotransplantation assay

In-depth characterization of epithelial cell sub-populations (after sorting) using RNAseq

If we increase the MaSC pool in early life (before puberty), may we optimize the future long-term performances (milk production, mammary tissue robustness and/or infectious resistance) ?


Thank you...

The proportion of the MaSC/progenitors cells at 3 physiological stages

		Heifers	Lactating cows	Dry cows
Luminal cells	$CD49_f^{low}$ $CD24^{neg}$	19.8% ± 1.2	21.2% ± 2.4	28.7% ± 7.0
	$CD49_f^{med}$ $CD24^{neg}$	1.4% ± 0.2 ^{b}	7.2% ± 1.6 ^{a}	7.1% ± 1.6 ^{a}
Basal cells	$CD49_f^{high}$ $CD24^{neg}$	22.1% ± 1.6 ^{a}	2.4% ± 0.9 ^{c}	17.7% ± 1.8 ^{b}
Progenitors	$CD49_f^{low}$ $CD24^{pos}$	18.6% ± 3.4 ^{a}	1.6% ± 0.4 ^{c}	3.0% ± 0.3 ^{b}
MaSC	$CD49_f^{high}$ $CD24^{pos}$	3.3% ± 0.3 ^{a}	1.5% ± 0.5 ^{b}	0.4% ± 0.1 ^{b}

- The Progenitors cells are present at puberty then almost nonexistant at lactation and dry off periods

- The proportion of MaSC decreases gradually at the lactating period then at dry off