Cellular and transcriptional diversity over the course of human lactation

Sarah K. Nyquist et al. ,PNAS , 2022

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10/01/2023

INTRODUCTION AND CONTEXT

Human breast milk (hBM) : What is it ?

- A dynamic fluid with millions of cells
- Water / Fat carbohydrates / protein / vitamins / minerals
- Blood cells / antibodies / enzymes

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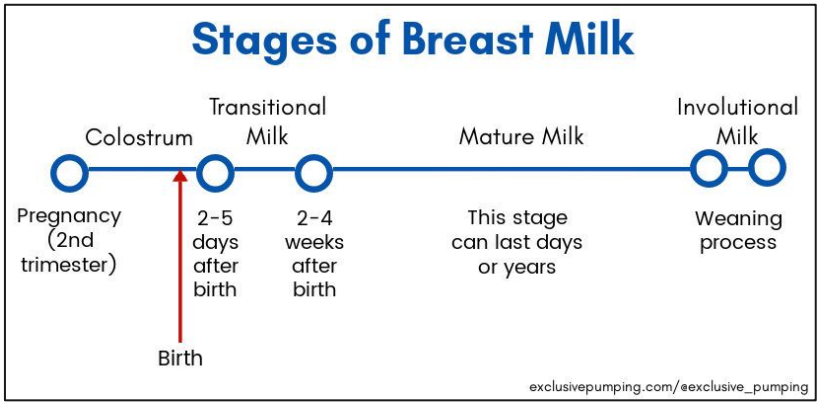
Is there only one type of hBM along lactation time ?

Human breast milk (hBM) : Different types over lactation



1) Colostrum
2) Transitional
3) Mature
4) Involution

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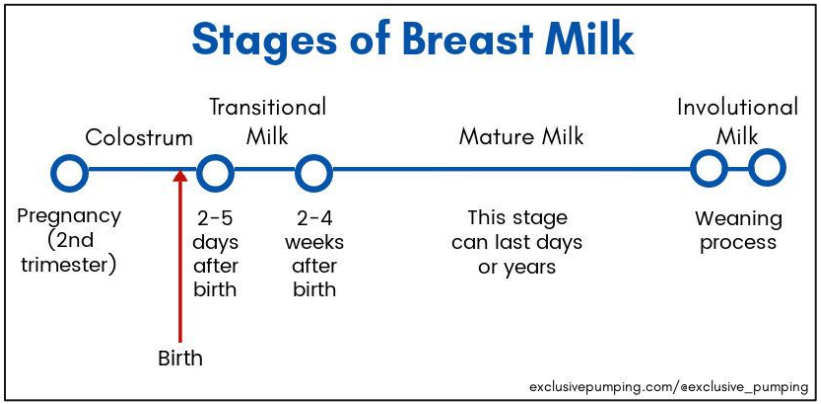

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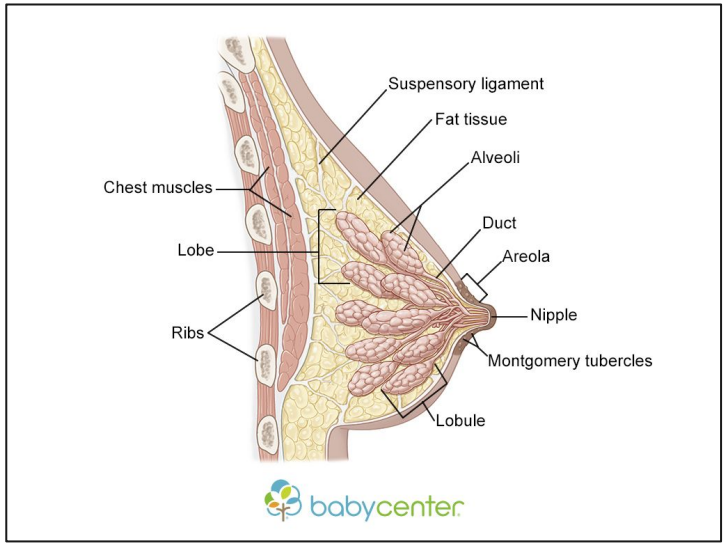
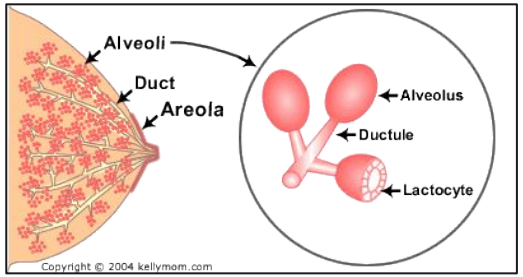
How is hBM produced in the human body?

Human breast milk (hBM) : How is it produced ?





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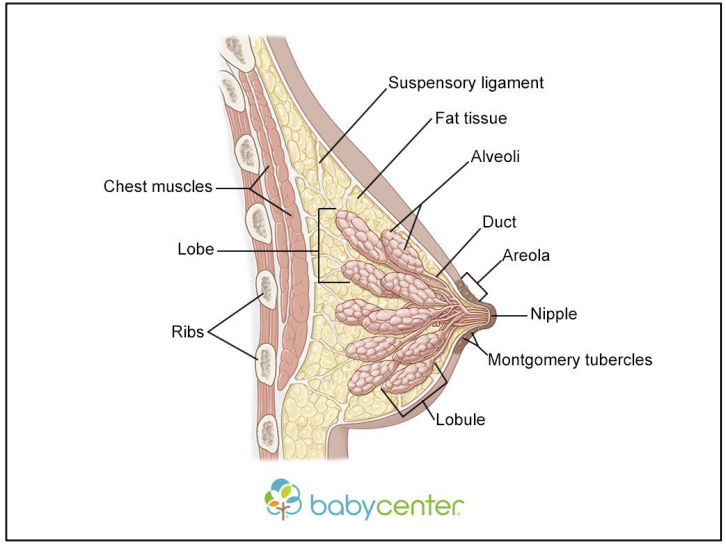

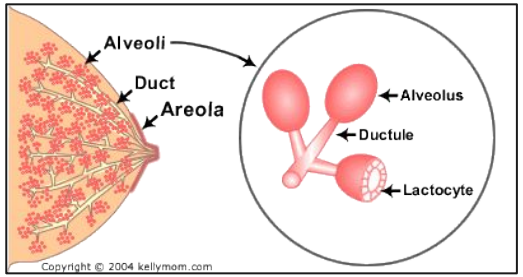

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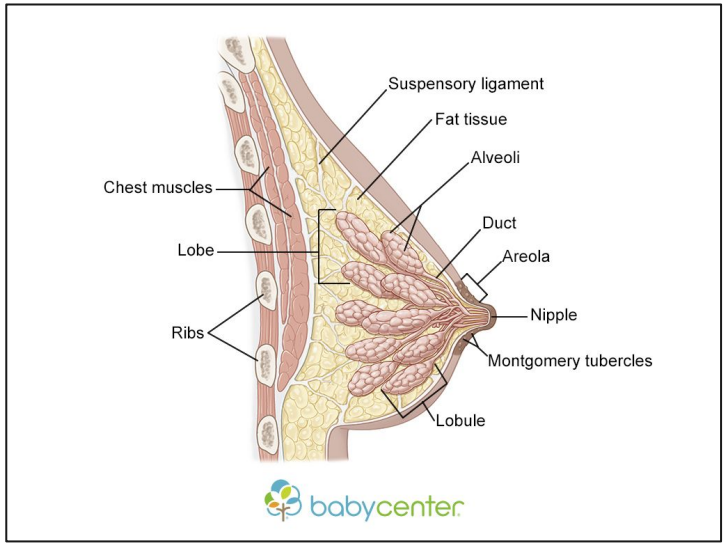

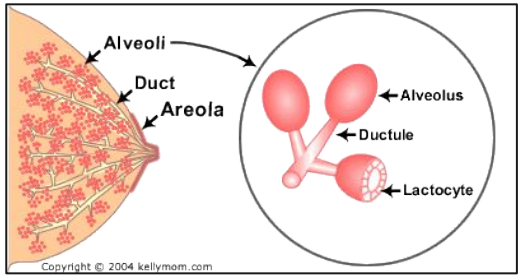

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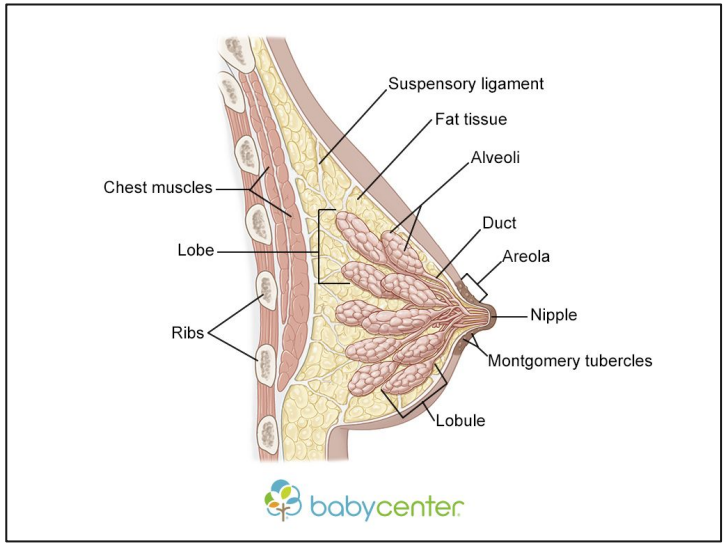

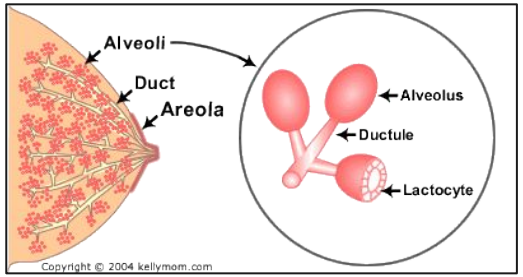

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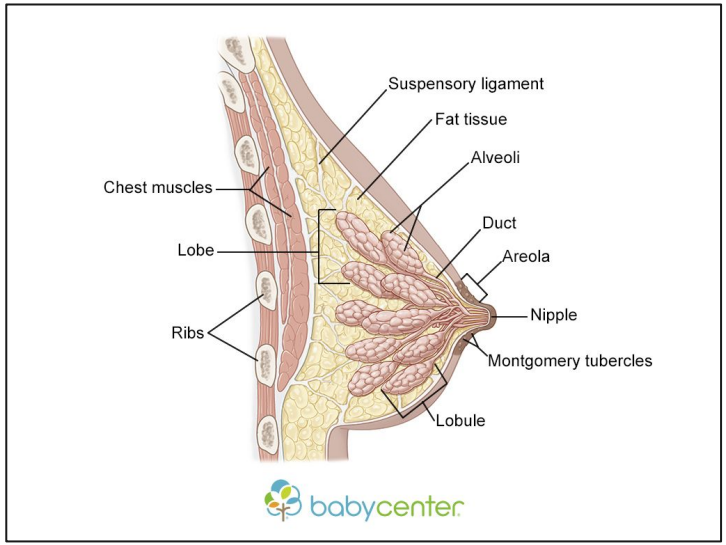

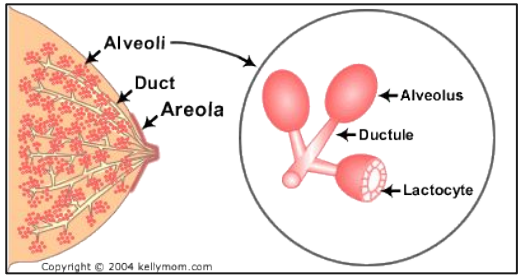

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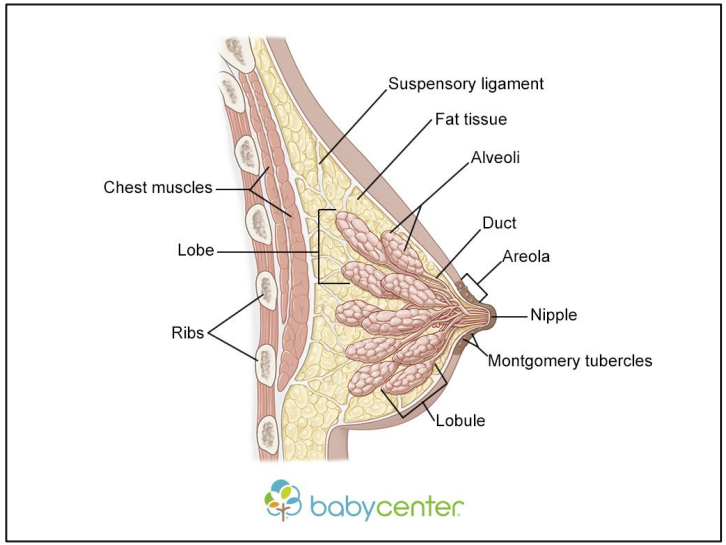

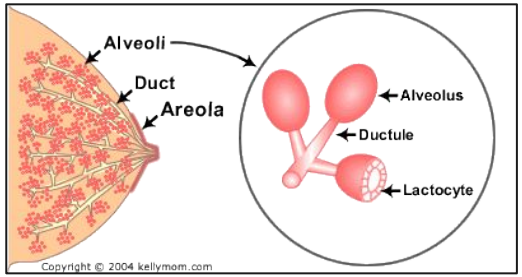

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How is hBM released for the infant?

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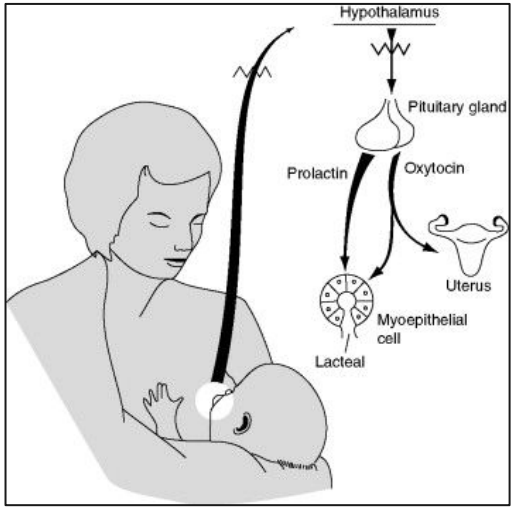

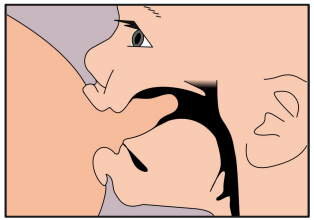

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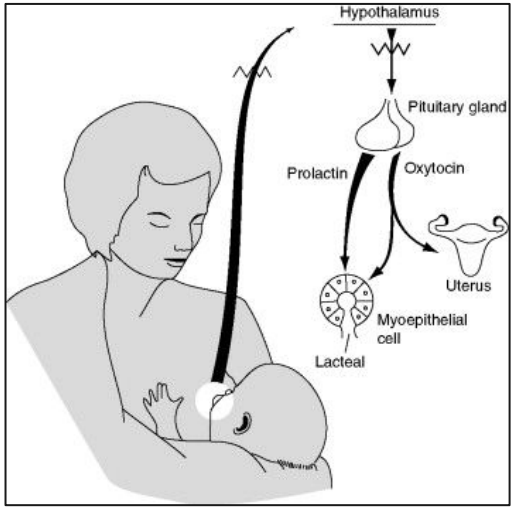

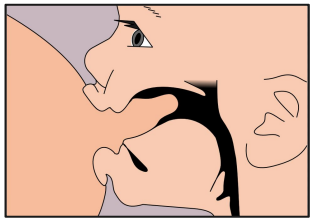

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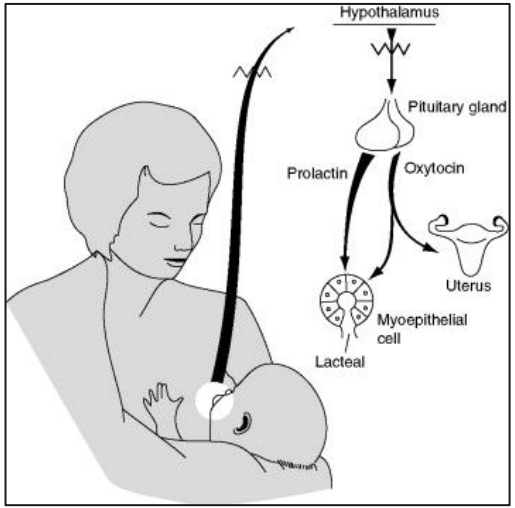

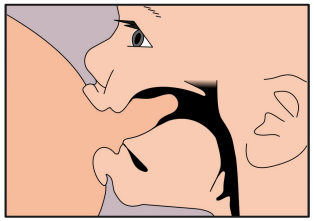

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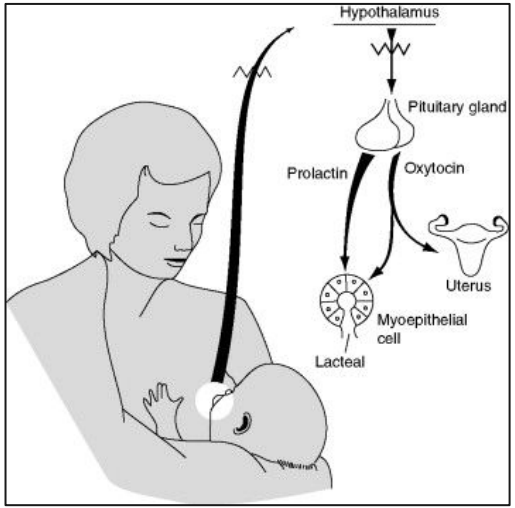

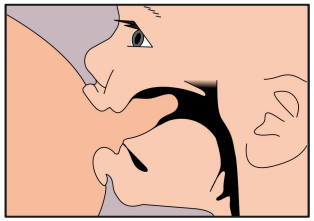

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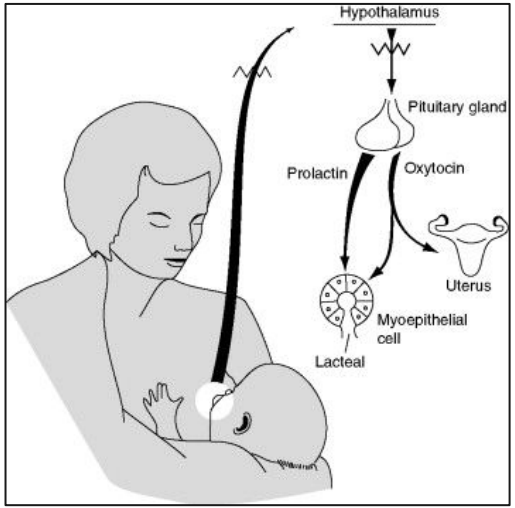

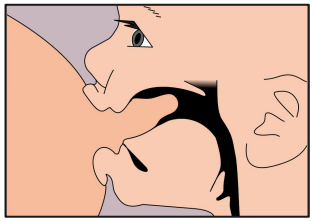

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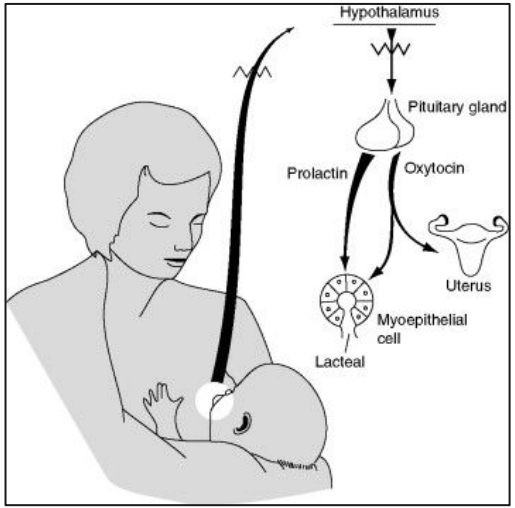

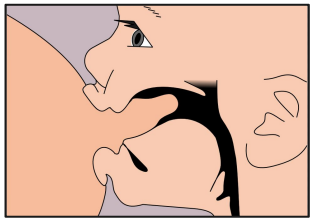

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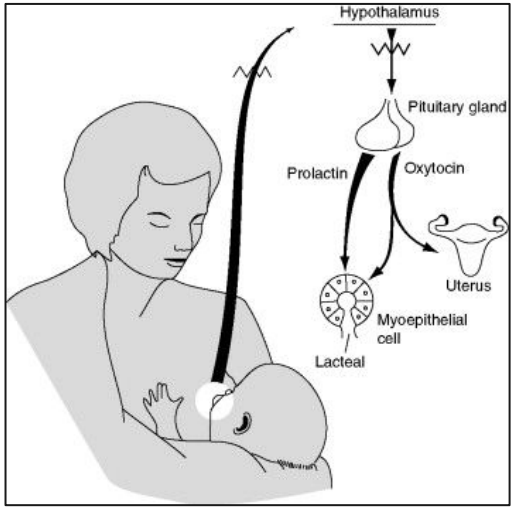

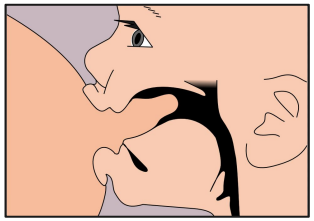

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What are hBM benefits ?

Human breast milk (hBM) : Its benefits

Long-term health benefits for both infants and nursing mothers

Infants
- decreased infection
- improved intestinal development
- improved regulation of weight

Nursing mothers
- decreased risk of ovarian and breast cancers

→ With all these benefits, it is important to better understand molecular and cellular features of hBM.

“ How a better understanding of cellular components and transcriptional diversity of the hBM across lactational time might help us to improve therapeutic support of healthy lactation and milk production ?”

Challenges of the study

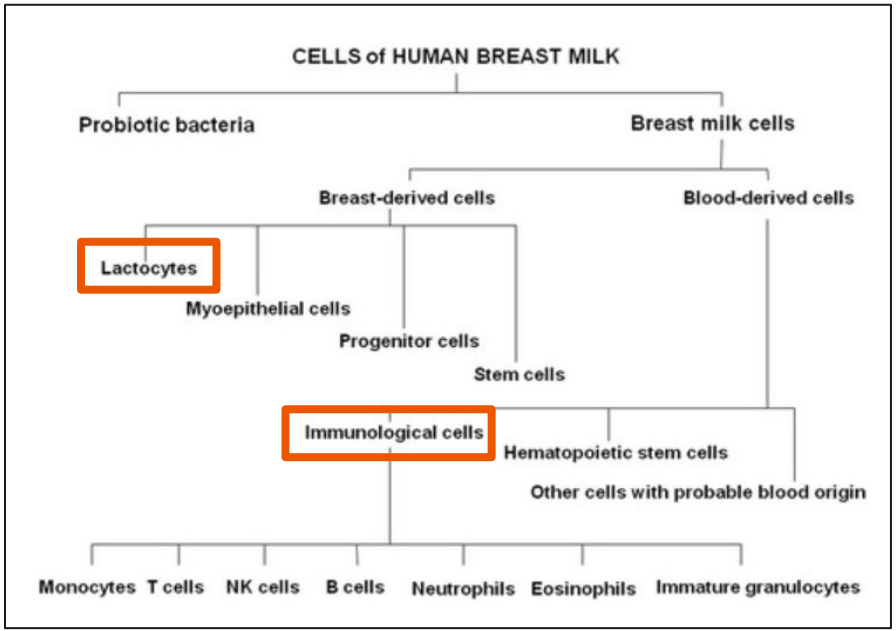
1) **Provide a valuable single-cell characterization of hBM cells longitudinally (over the course of lactation)**

2) **Influence of health and lifestyle on cells during lactation**

3) **Genes that influence lactocyte functions over the course of lactation**

EXPERIMENTAL PROCESS

Cellular composition of human mammary gland



Cells of the mammary gland are responsible for synthesizing and transporting hBM

Collect cells in hBM → **non-invasive method**

Immune and Somatic cells

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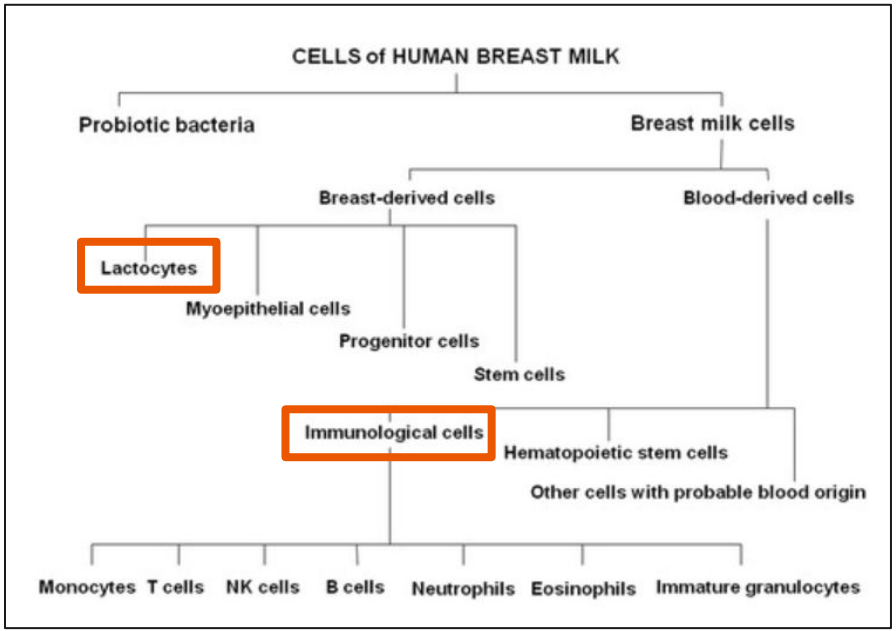

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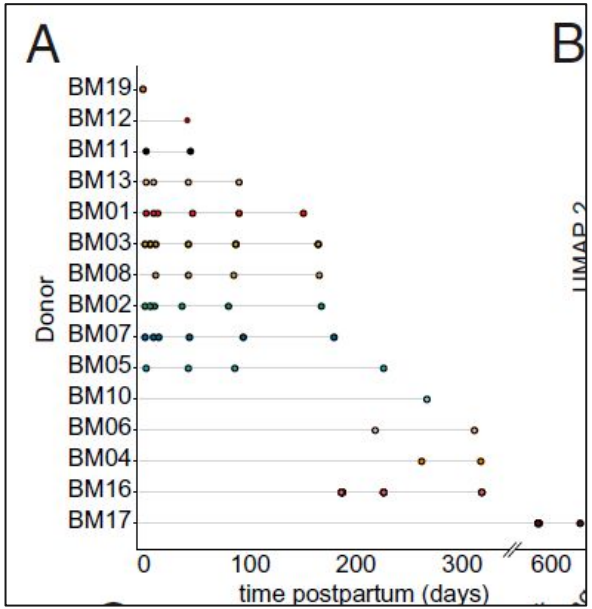
Immune and Somatic cells

→ hBM has to be collected from donors

Cohort and samples at disposal



- 15 nursing mothers (ages 25-34, median 31 y)
- Samples at several time postpartum

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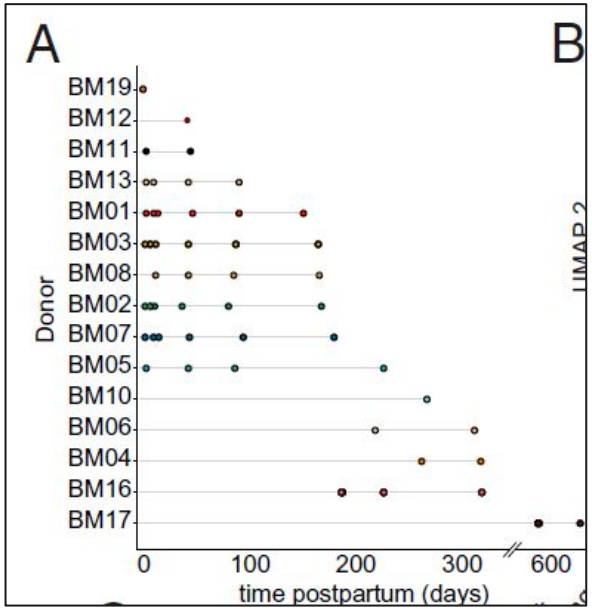

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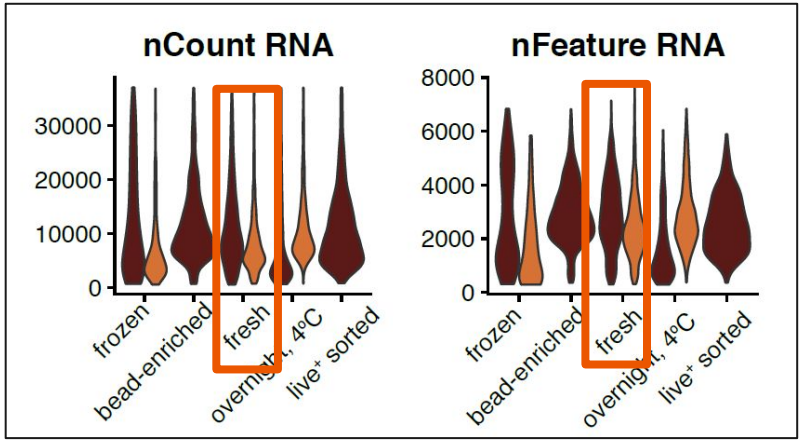
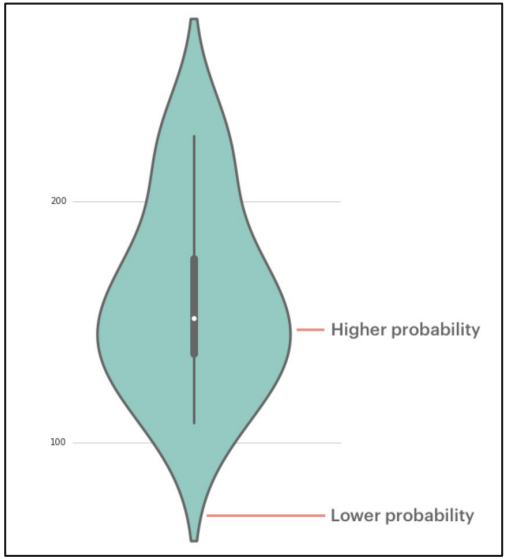
We have to isolate the cells

Cell isolation



Fresh milk → highest-quality data

Cell isolation can impact the transcriptomes of isolated cells.

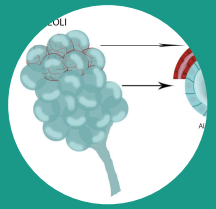


⇩

How to read a violin plot

Now, that these cells are isolated, they can be studied.

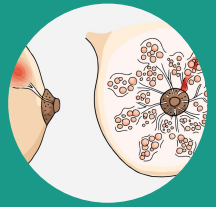
RESULTS



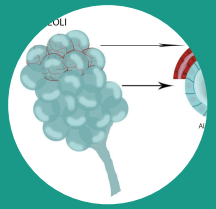
Cell types during lactation

Cell frequencies during lactation

Macrophages in hBM



Epithelial cell subclusters



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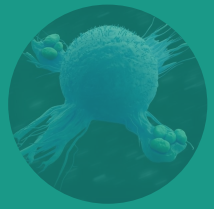

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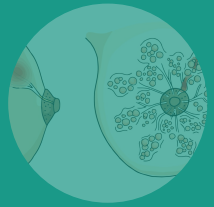
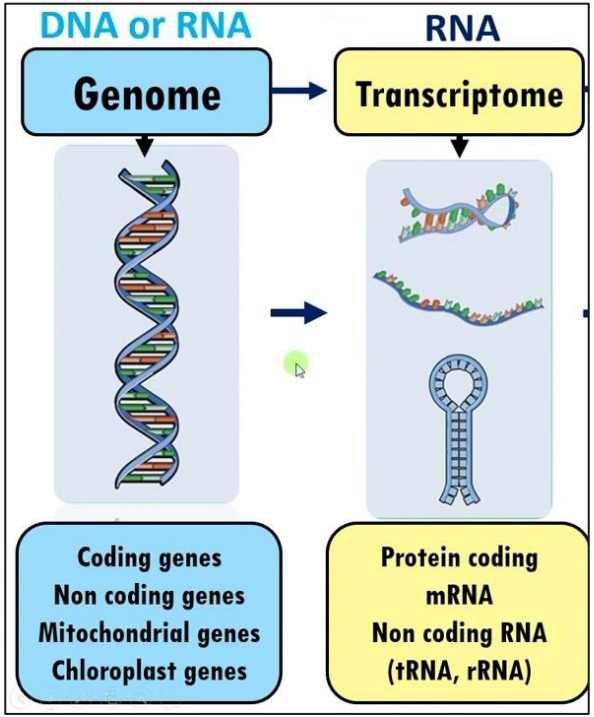


Epithelial cell subclusters

What is the transcriptome ?



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It reflects **the patterns of gene expression** that are occurring within the cell or tissue at a given time and provides **a snapshot of the cellular state**.

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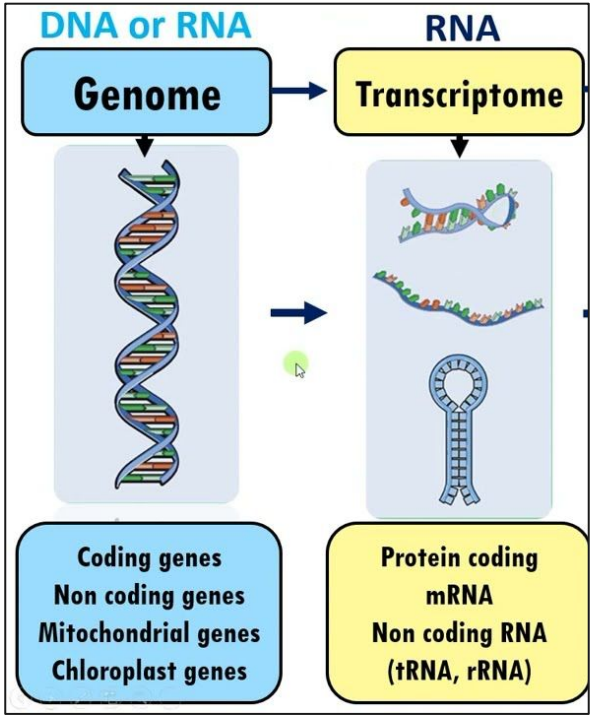

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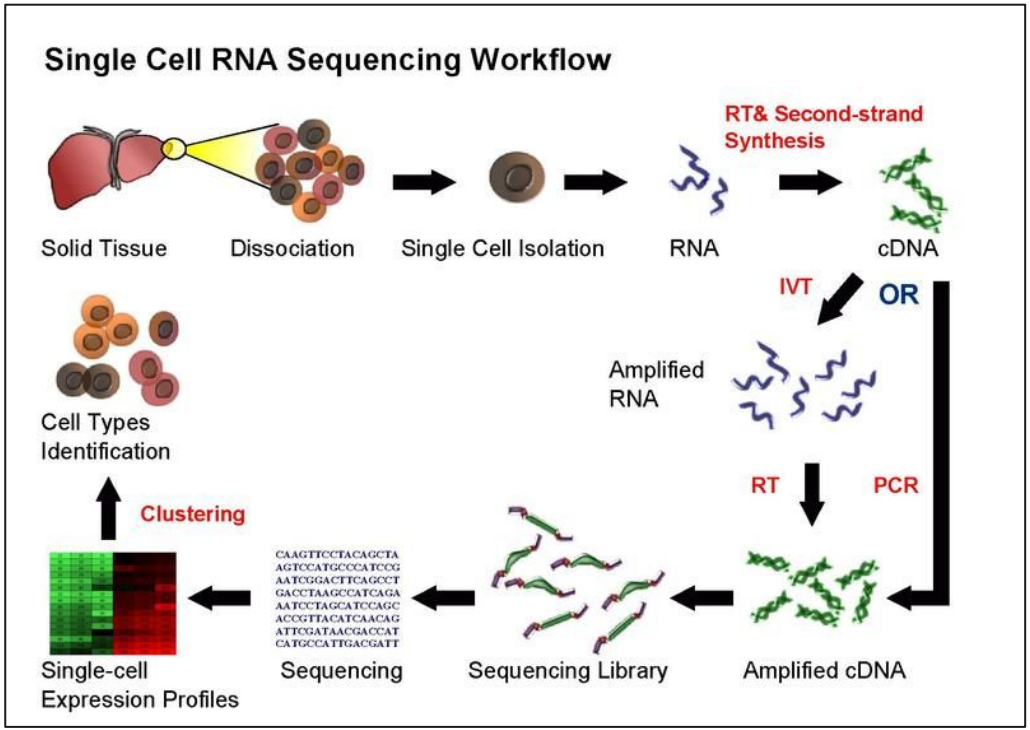
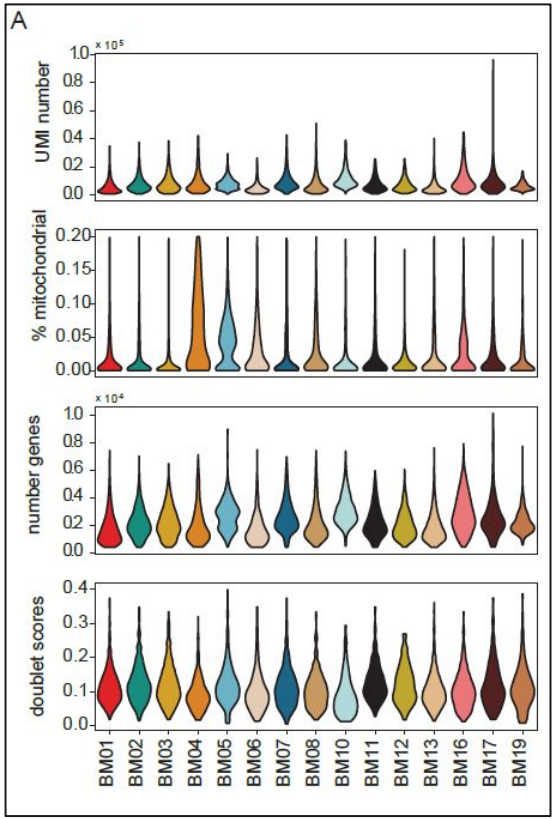
How to obtain the transcriptome of the cells ?

Generating scRNA-seq



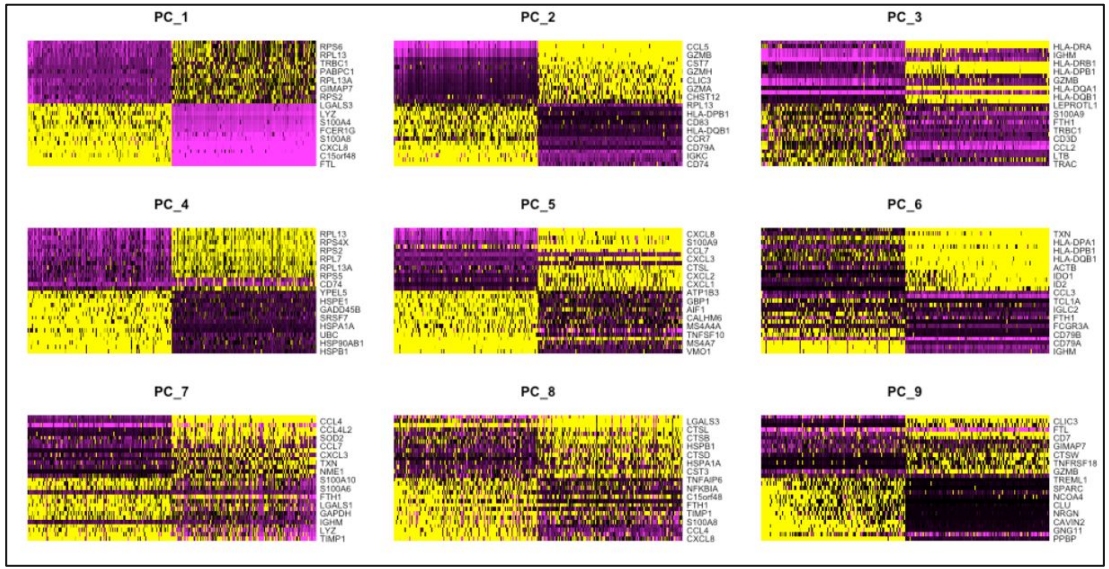
scRNA-SEQ Workflow



Results from our samples

How do you do clustering ?

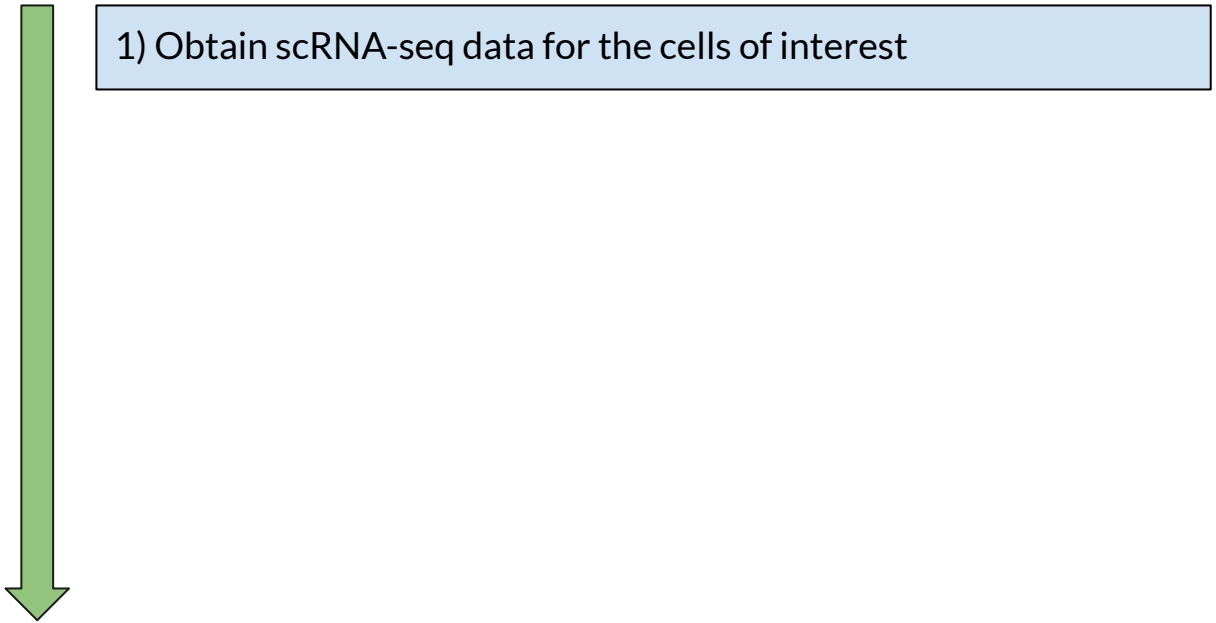
Clustering is the fact to divide cells into distinct groups based on gene expression



Use principal components

Adapted from HBCtraining

Uniform Manifold Approximation and Projection (UMAP)



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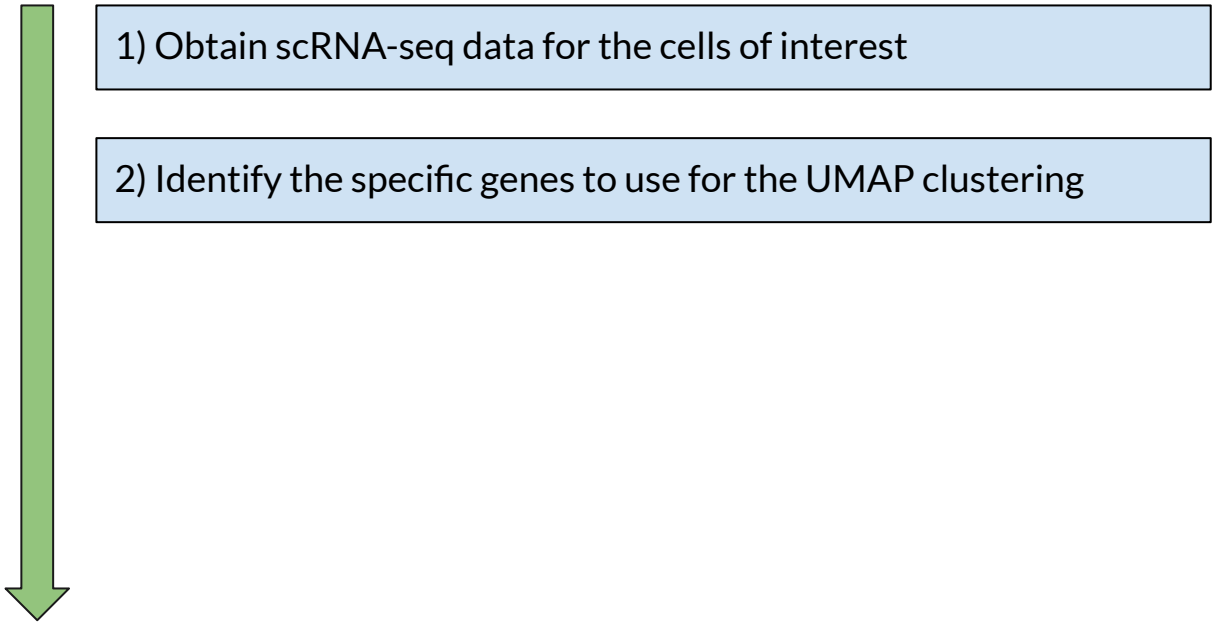

Uniform Manifold Approximation and Projection (UMAP)

1) Obtain scRNA-seq data for the cells of interest

2) Identify the specific genes to use for the UMAP clustering

3) Compute the expression levels of the specific genes for each cell

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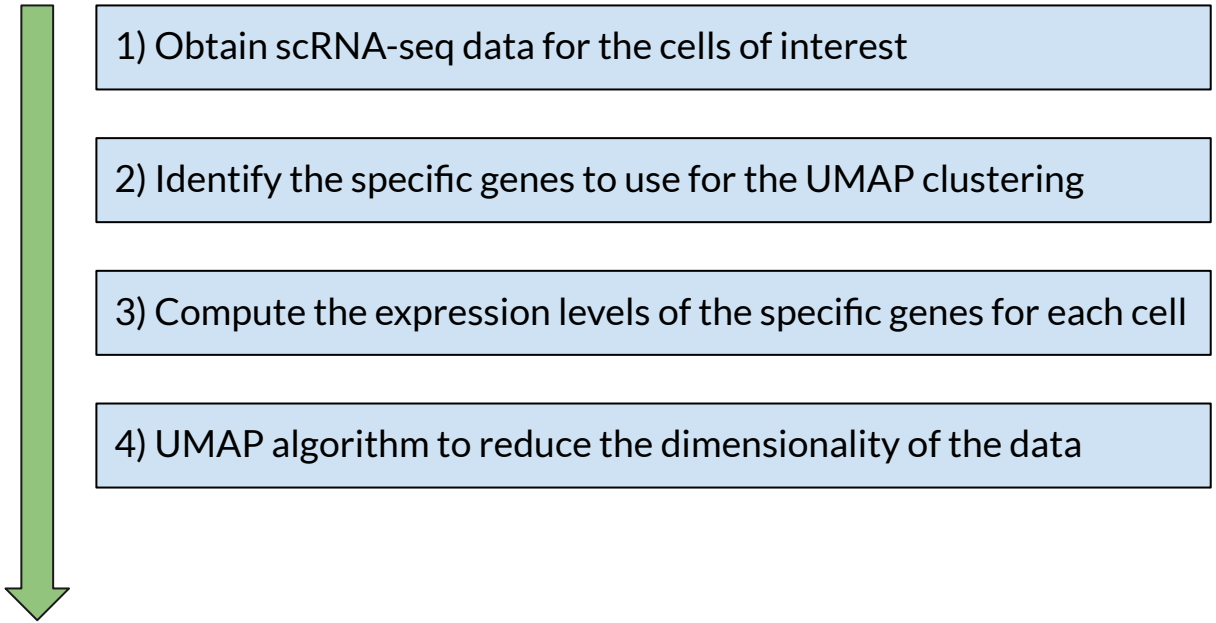

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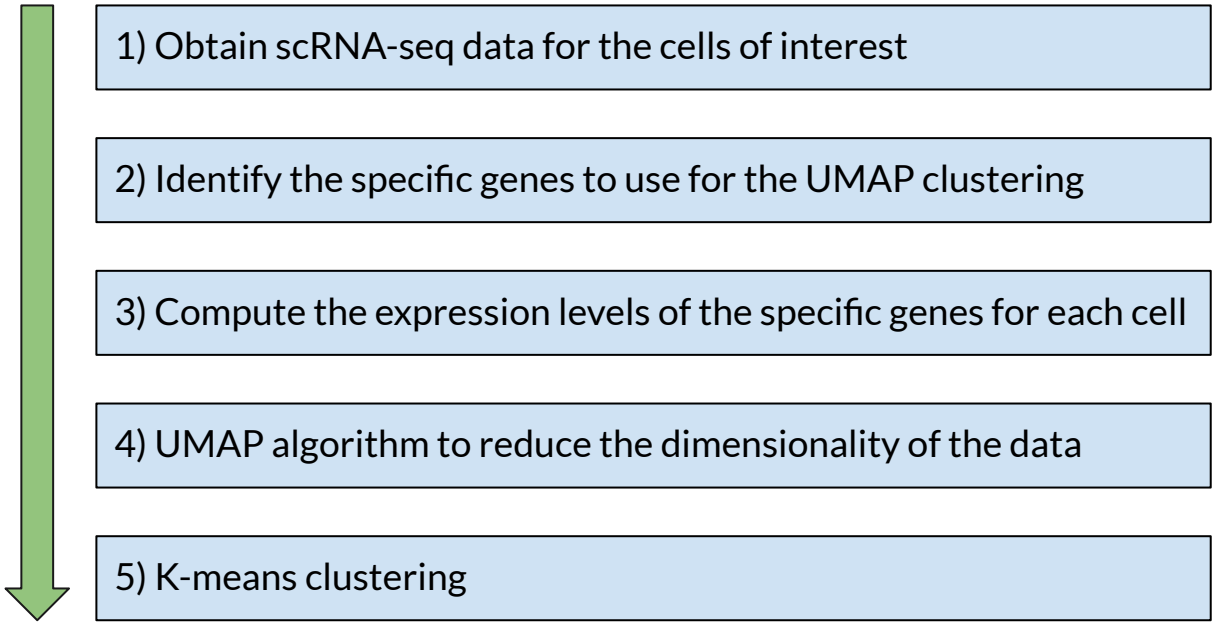
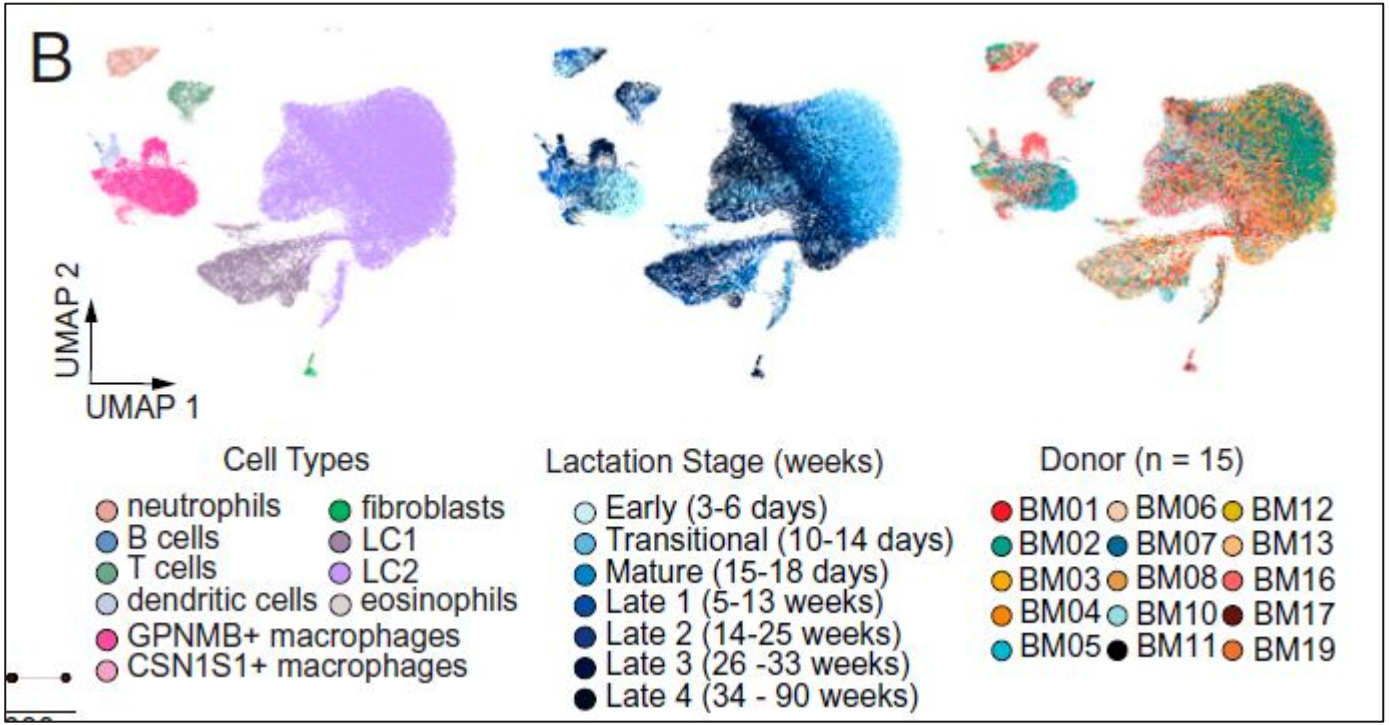
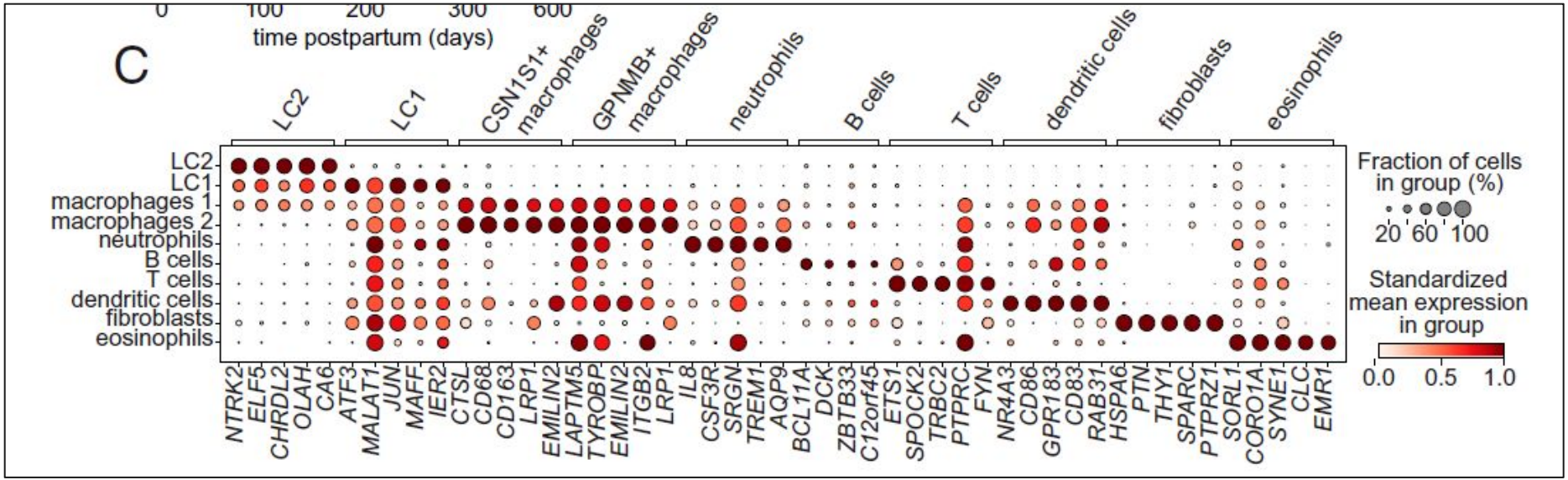


Clustering cell types with marker genes



Clustering cell types with marker genes



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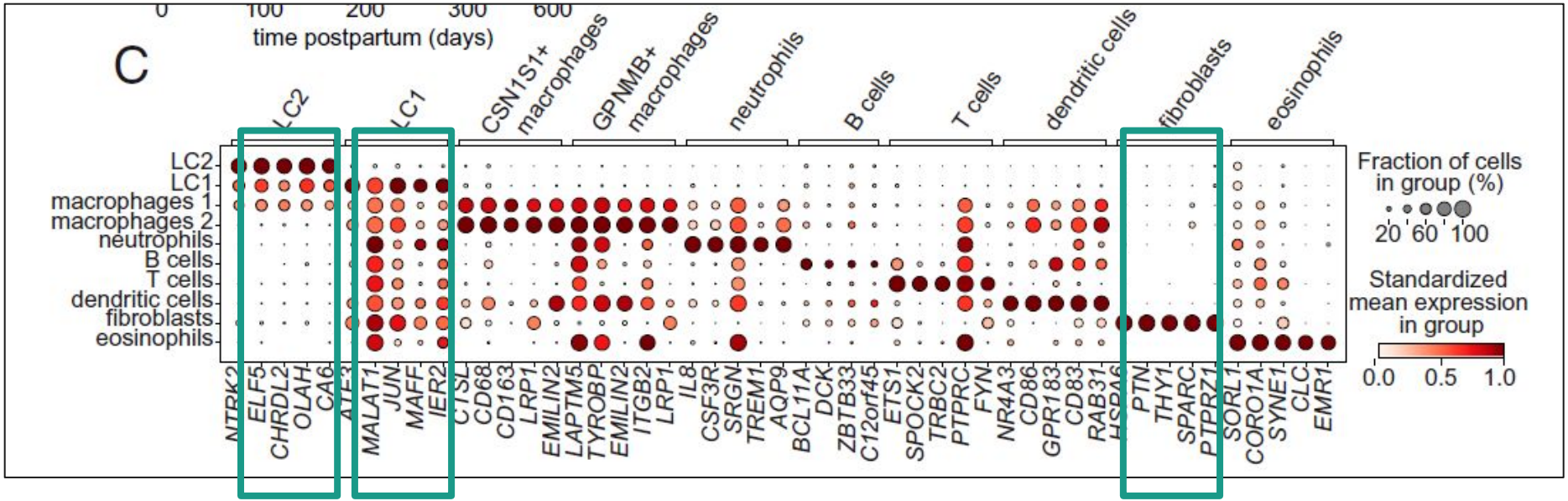
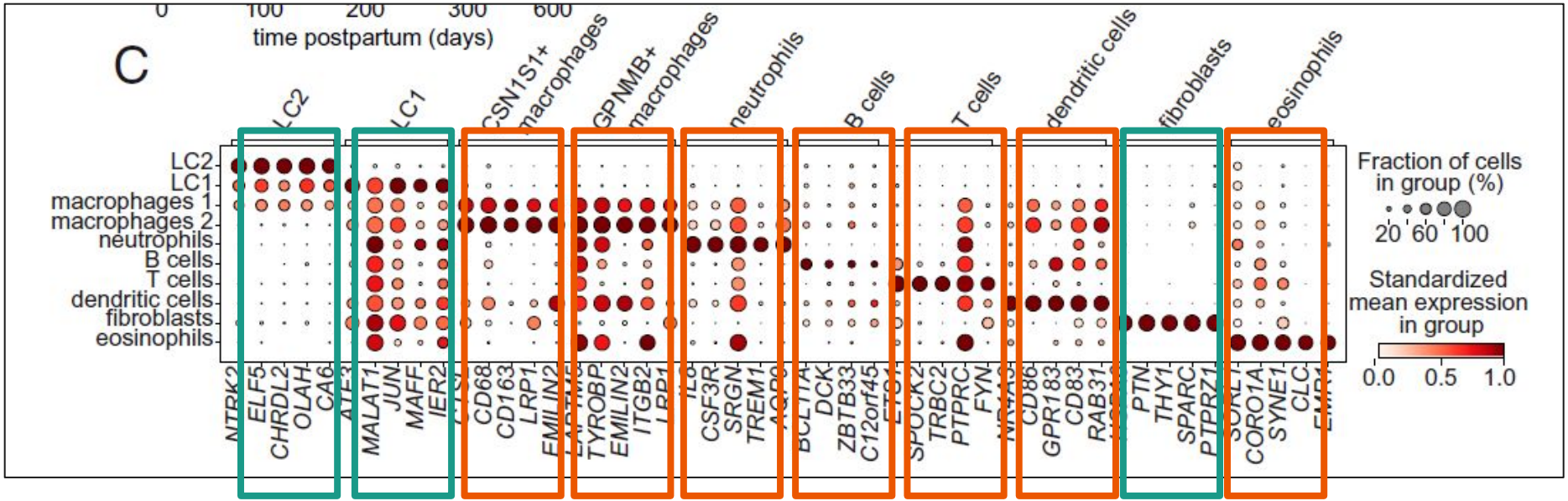


- 7 immune cell clusters (green)
- 3 non-immune cell clusters (orange)

Clustering cell types with marker genes



- 7 immune cell clusters (green)
- 3 non-immune cell clusters (orange)

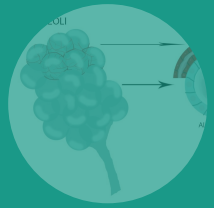
Cell abundance

- Lactocyte epithelial cells are the most abundant cell types (LC1 and LC2) - **81.7 % of all cells per sample**

- Macrophages are the most abundant immune cell type - **50.5 % of immune cells**

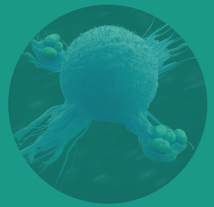
- Identification of more immune clusters than previous studies

How these cells proportion evolves during the lactation, and how are they associated with maternal-infant metadata ?

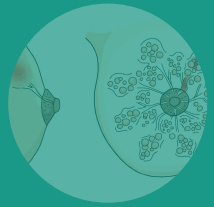


Cell types during lactation

Cell frequencies during lactation

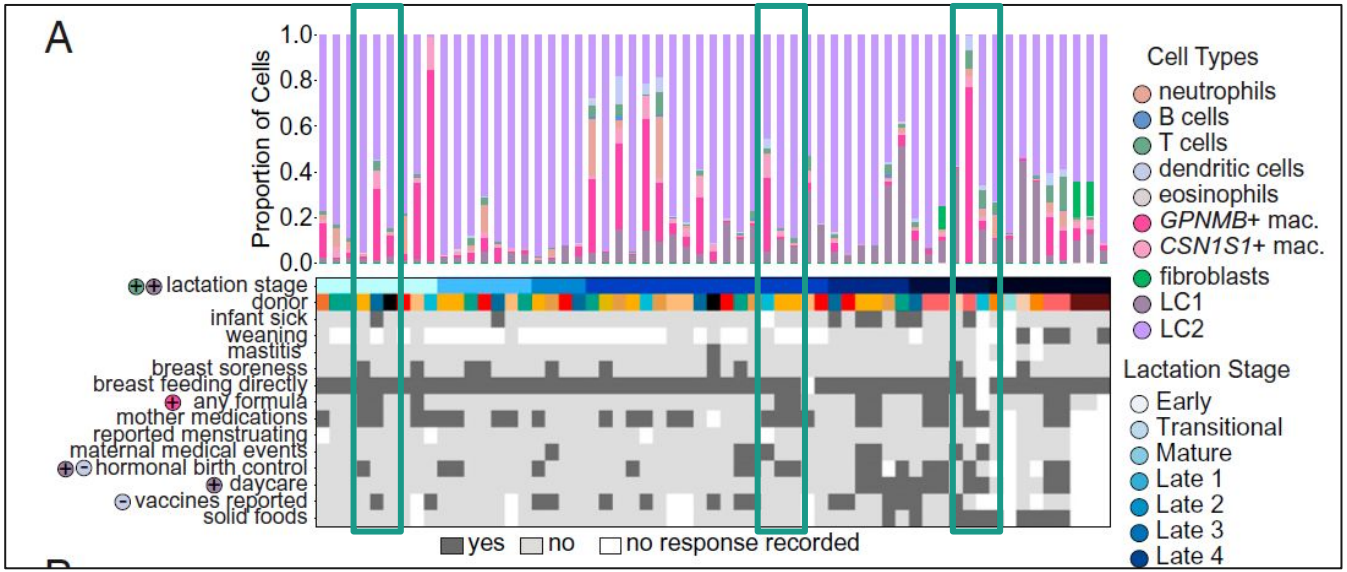


Macrophages in hBM



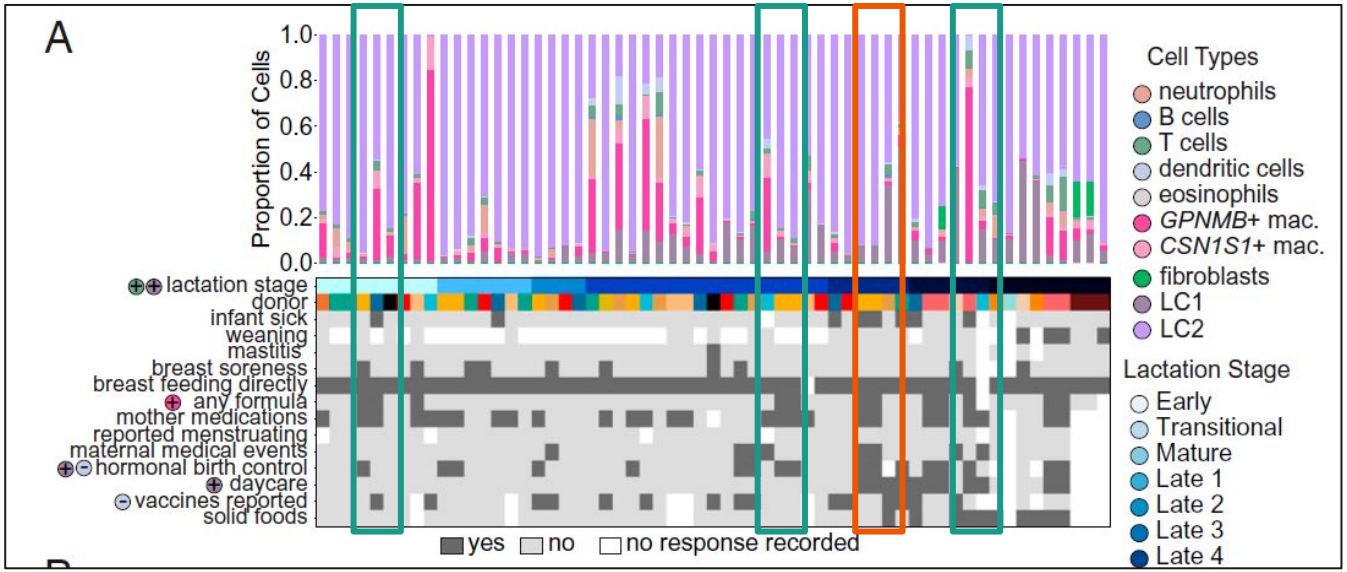
Epithelial cell subclusters

Maternal-Infant Metadata



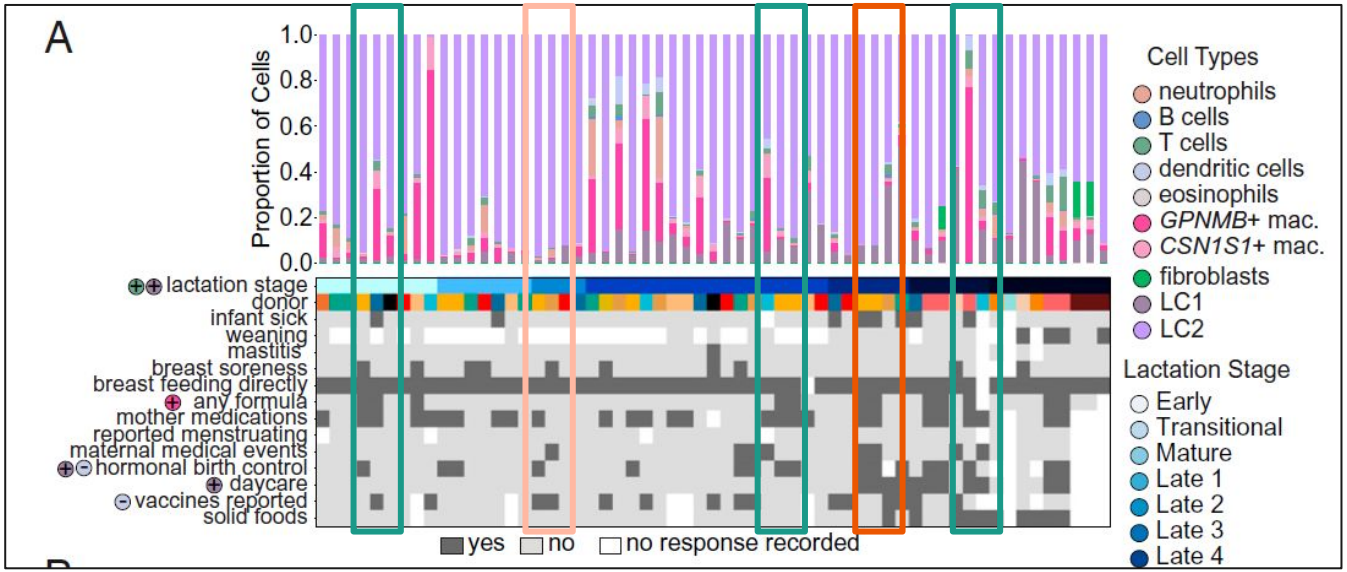
- GPNMB+ associated with formula supplementation

Maternal-Infant Metadata



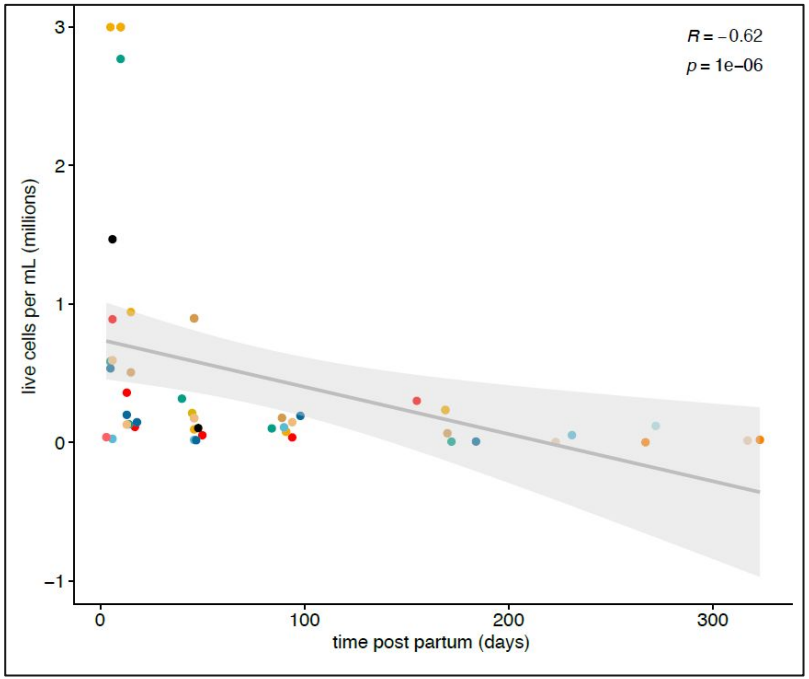
- GPNMB+ associated with formula supplementation
- LC1 associated positively with daycare and hormonal birth control

Maternal-Infant Metadata



- GPNMB+ associated with formula supplementation
- LC1 associated positively with daycare and hormonal birth control
- Dendritic cells negatively associated with hormonal birth control and infant vaccinations

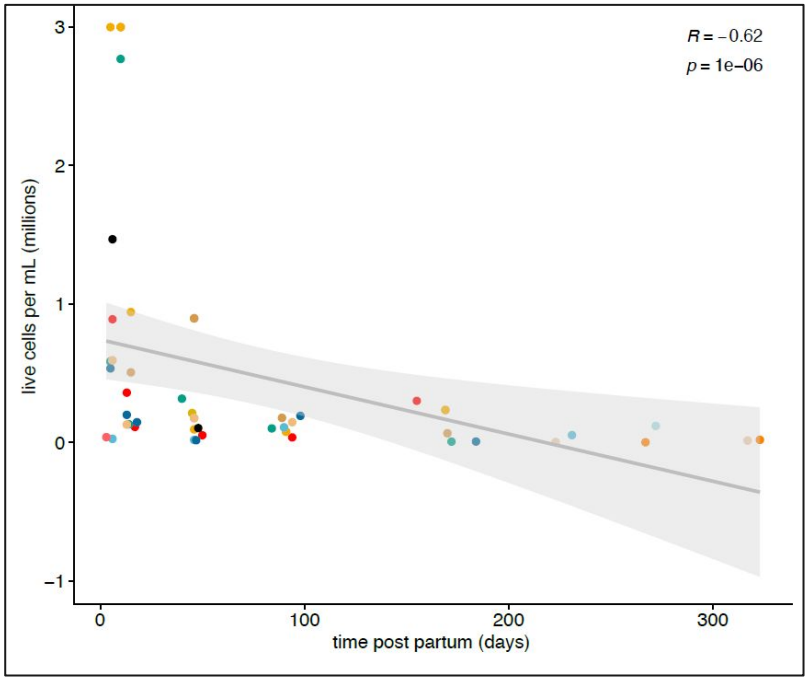
Cell-count evolution



- Total cell counts decrease over the course of lactation.

Donor
- BM01
- BM02
- BM03
- BM04
- BM05
- BM06
- BM07
- BM08
- BM10
- BM11
- BM12
- BM13
- BM16
- BM17
- BM19

Cell-count evolution



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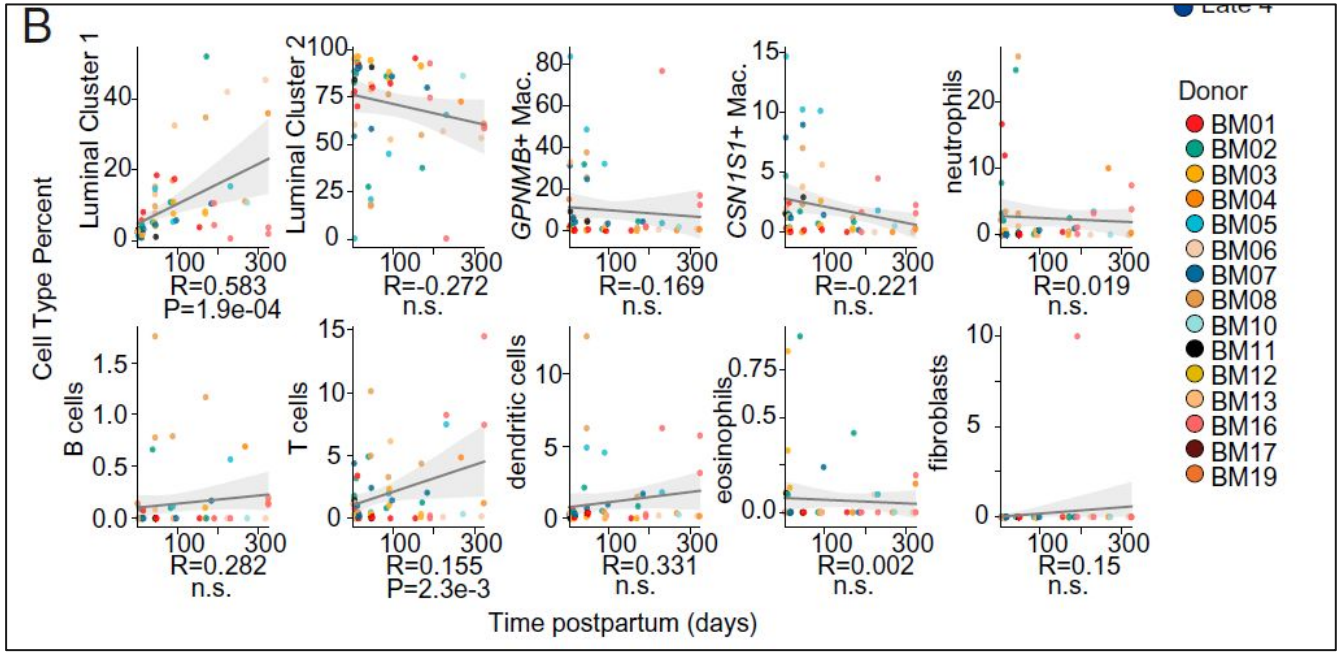
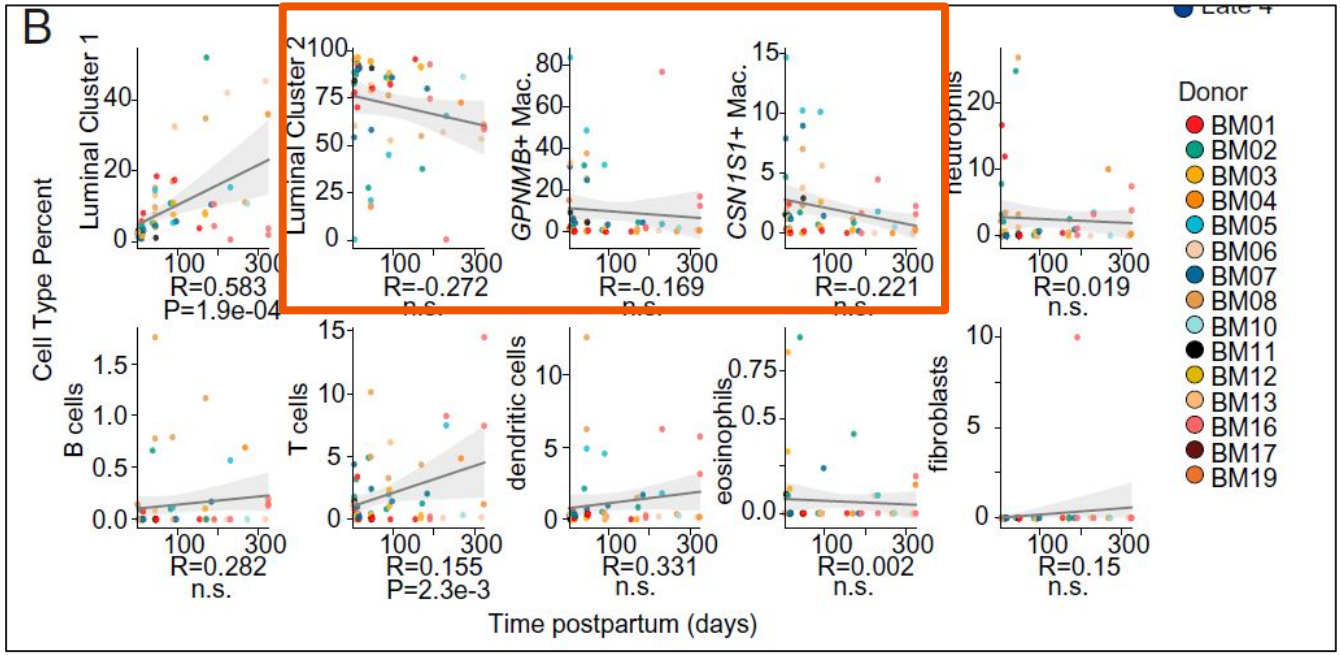
- BM17
- BM19

How do they specifically evolve ?

Cell-count per clusters

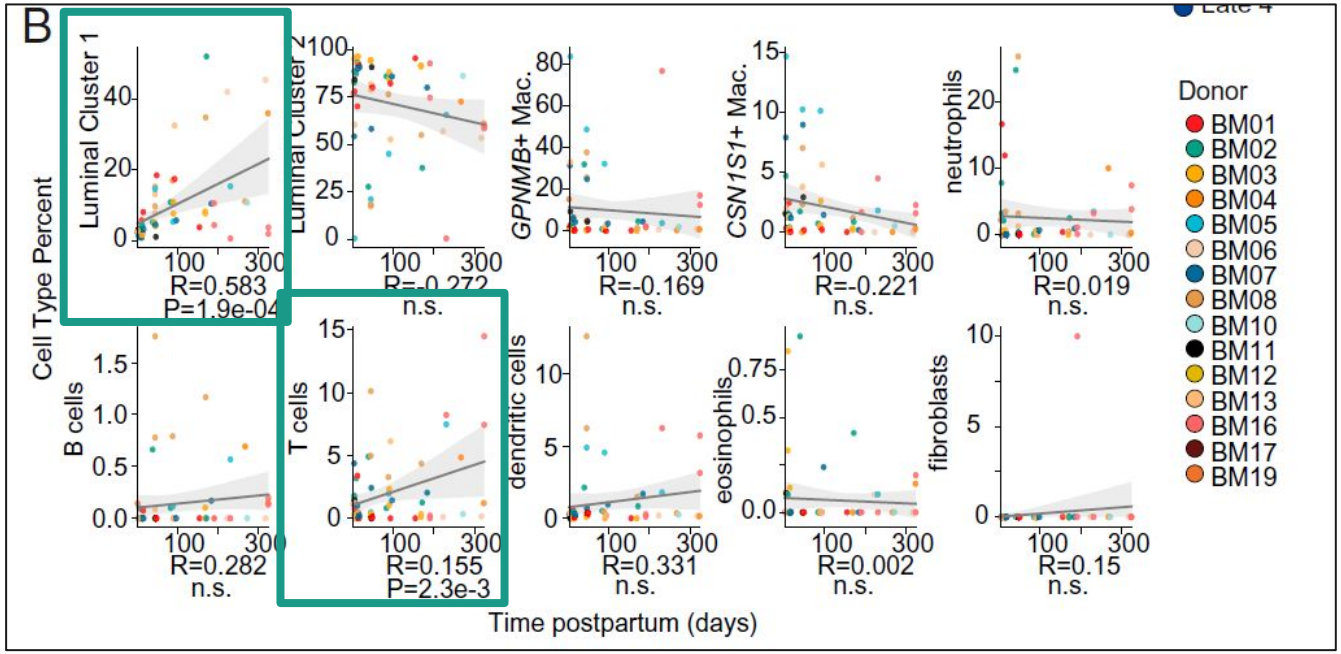


Cell-count per clusters



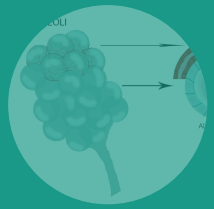
LC2 and macrophages remains constant

Cell-count per clusters



LC1 and T-cells are associated with postpartum as they increase.

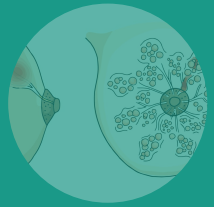
Macrophages appears as very important in the immune cells. It is therefore essential to understand better the functions of this cell in hBM.



Cell types during lactation

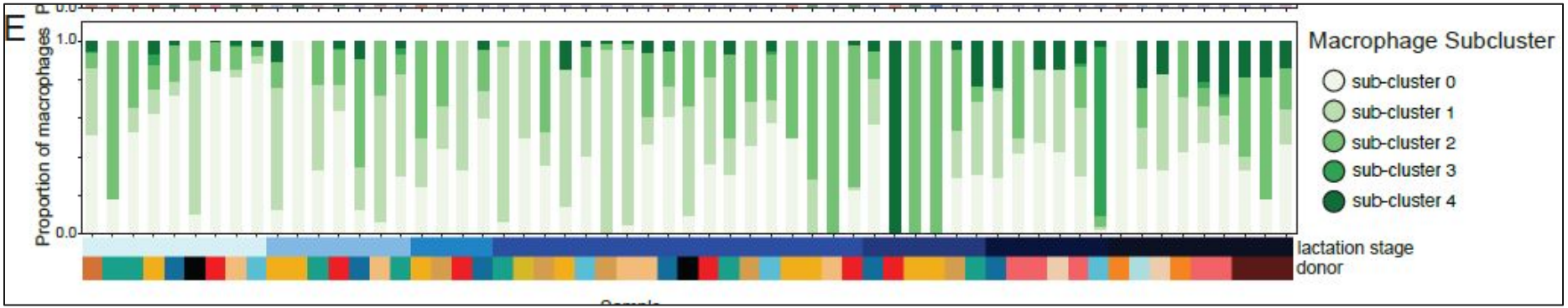
Cell frequencies during lactation

Macrophages in hBM



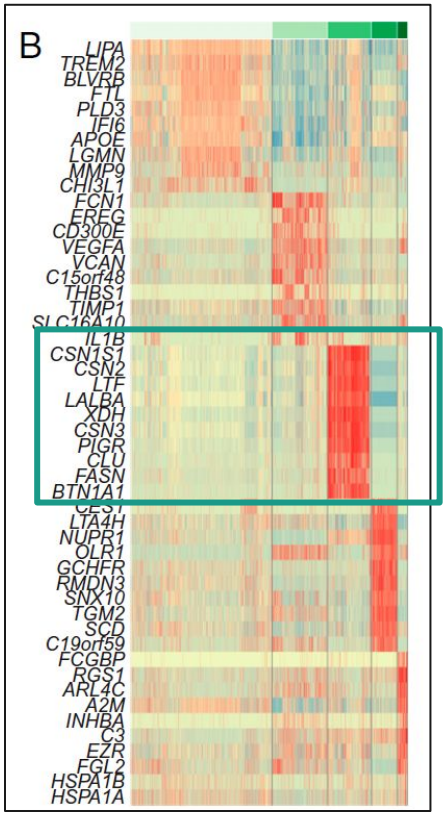
Epithelial cell subclusters

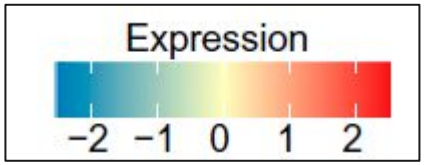
Identification of macrophages sub-clusters



- 5 macrophages subclusters

Functional enrichment of marker genes

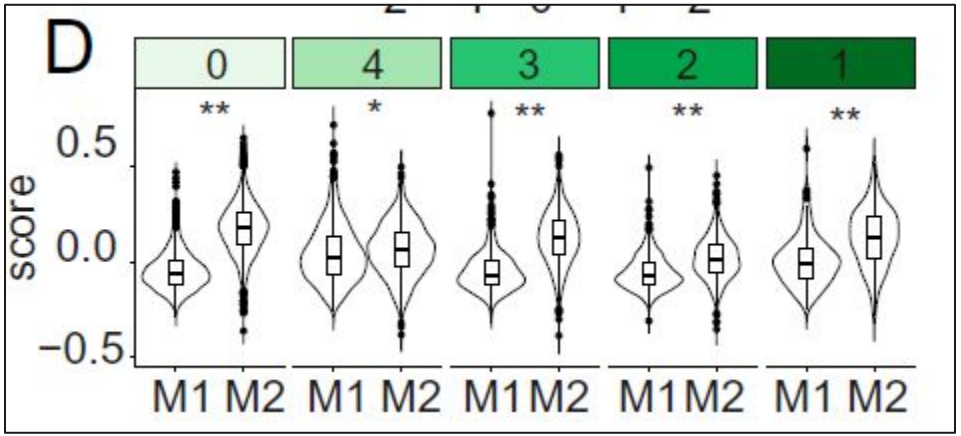




- Heatmap of top marker genes for each identified macrophage subscluster

- Subcluster 2 is comprised almost entirely of CSN1S1+ related to structural pathways.
- Subcluster 2 might be important for **structural maintenance**

Identification of inflammatory clusters

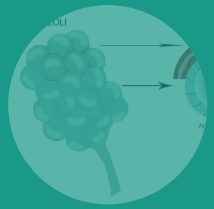


- M1 inflammatory
- M2 anti-inflammatory

Subclusters (except subcluster 1) scored higher for M2-gene sets

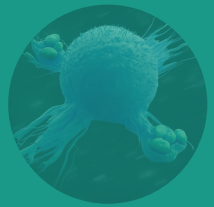
Macrophages seems to serve **immunosuppressive** and **tissue maintenance functions**

Now that we sought into macrophages and immune cells, let's focus on somatic cells and more especially epithelial cells that are primordial in the lactation process.

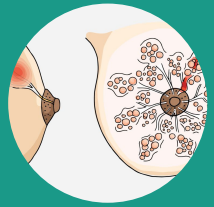


Cell types during lactation

Cell frequencies during lactation

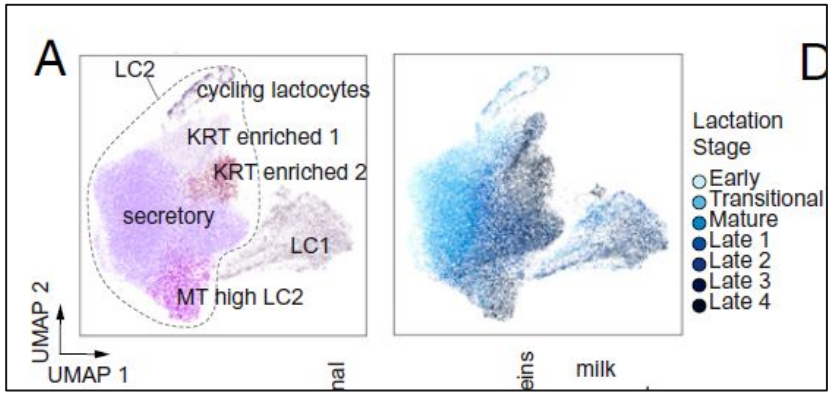


Macrophages in hBM



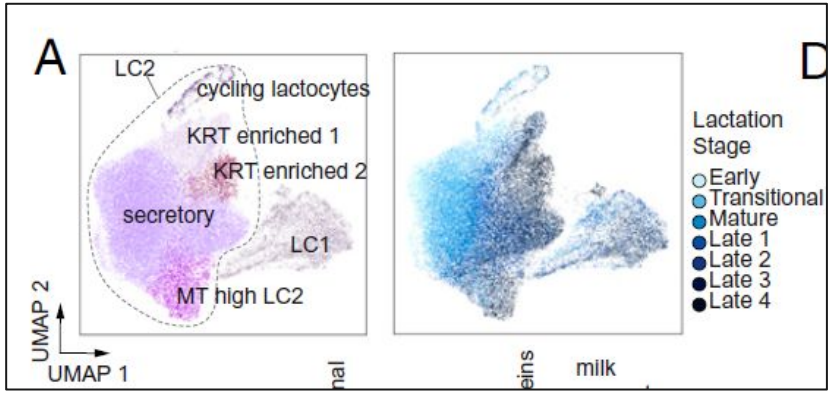
Epithelial cell subclusters

Subclusters of epithelial cells



- 6 subclusters of epithelial cells

Subclusters of epithelial cells



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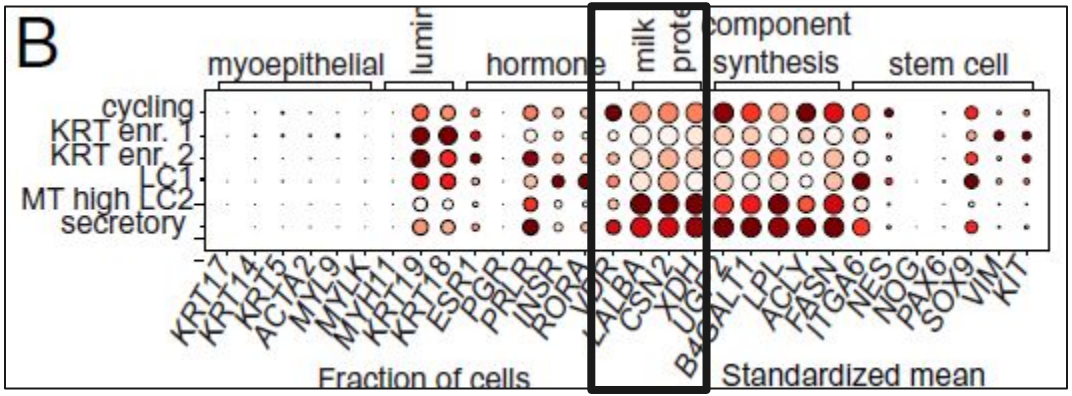
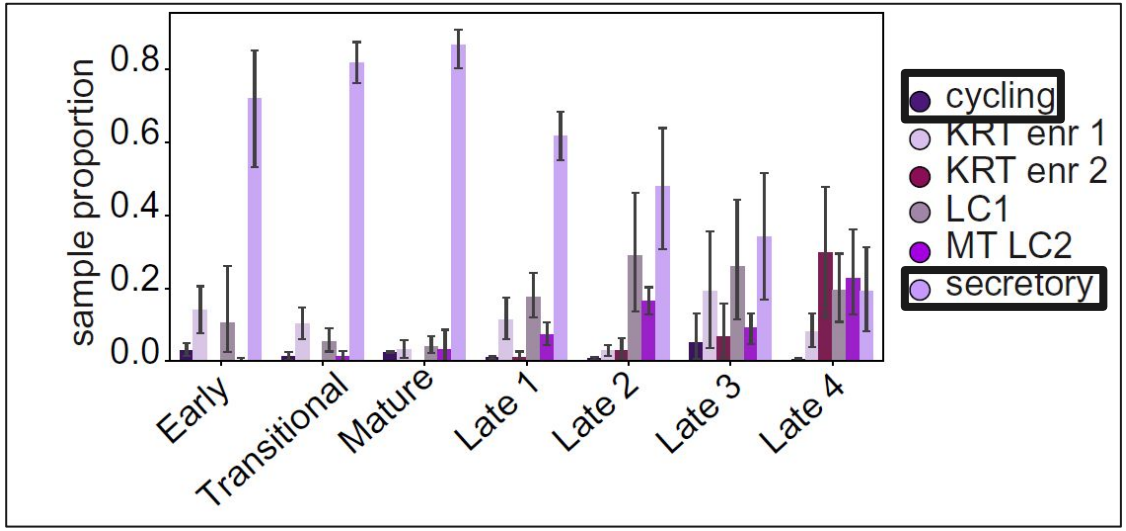


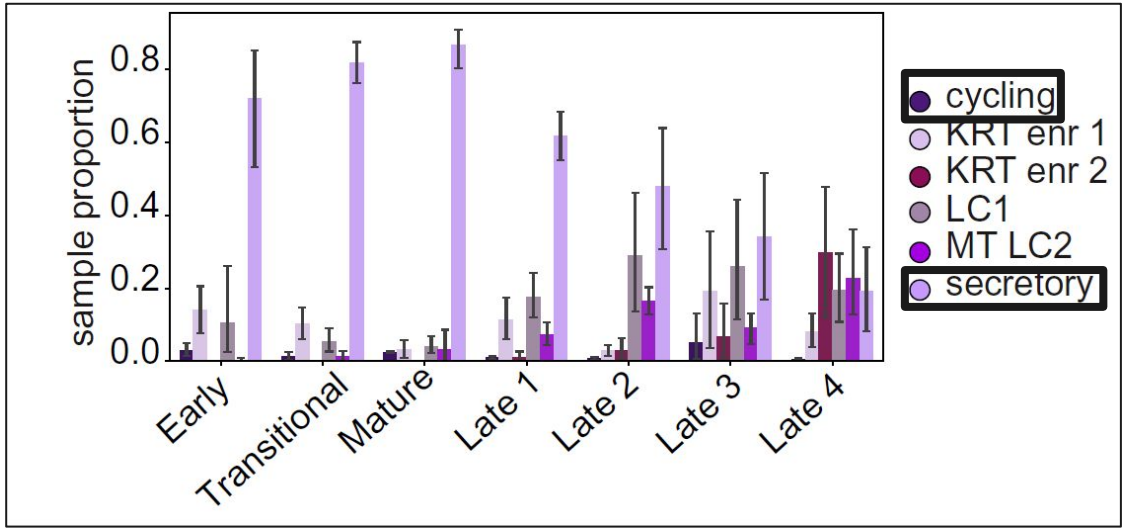
- Genes related to milk synthesis
- LALBA, CSN2, XDH

Subclusters over the course of lactation



- All subclusters except secretory and cycling lactocytes increase over the course of lactation

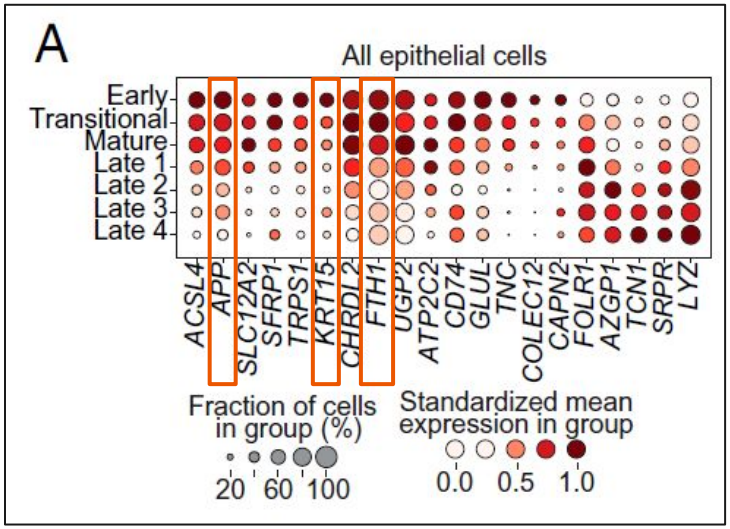
Subclusters over the course of lactation



- All subclusters except secretory and cycling lactocytes increase over the course of lactation

⇒ Is there also changes in gene expression over the course of lactation ?

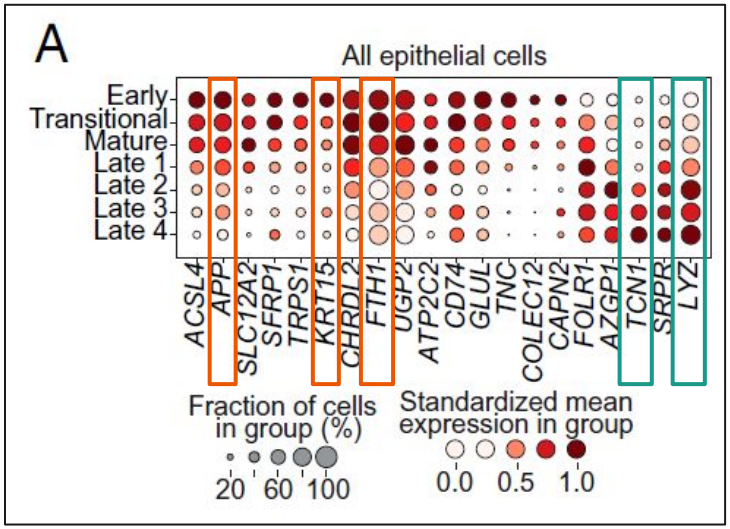
Gene expressions over the course of lactation



- Genes are differentially expressed over time

- ***APP, KRT15, FTH1* decrease**

Gene expressions over the course of lactation

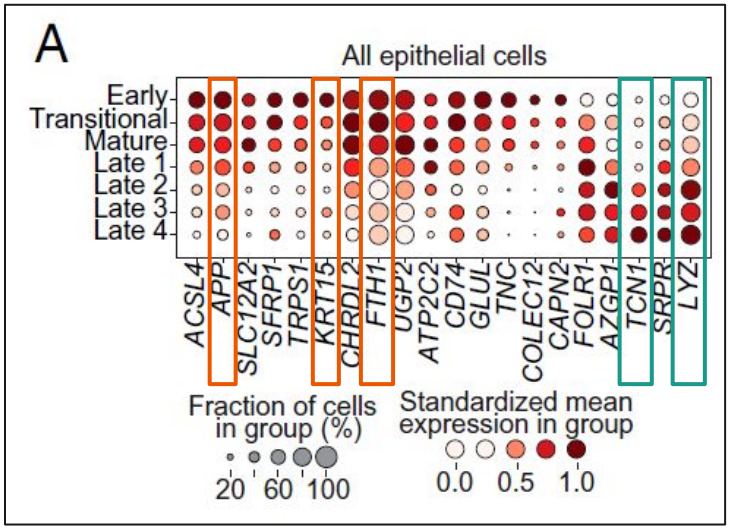


- Genes are differentially expressed over time

- ***APP, KRT15, FTH1*** **decrease**

- ***LYZ, TCN1*** **increase**

Gene expressions over the course of lactation



- Genes are differentially expressed over time

- ***APP, KRT15, FTH1* decrease**

- ***LYZ, TCN1* increase**

- Genes related to **metabolism** and **milk component biosynthesis** decrease

- Genes related to **structural pathways** and **signaling** increase

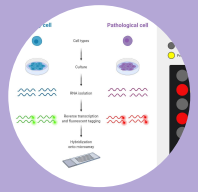
Now that we saw how the expression of epithelial cells over the course of lactation, which simple experiment could be led to pursue the study ?

EXPERIMENT

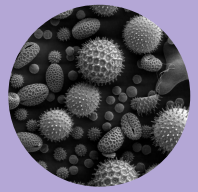
My thoughts

1) **Improve the characterization of the cell transcriptomes**

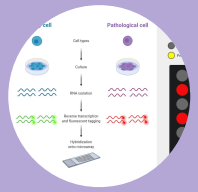
2) **Obtain more quantitative data on the cells over the course of lactation**



Improve cell transcriptome



Quantitative data on the cells



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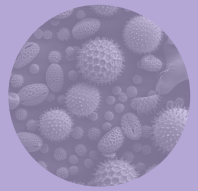
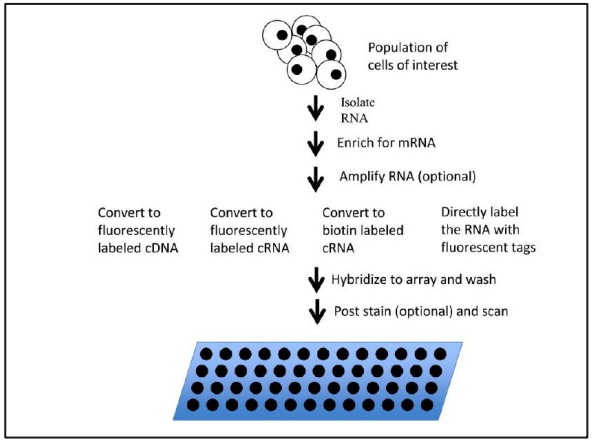
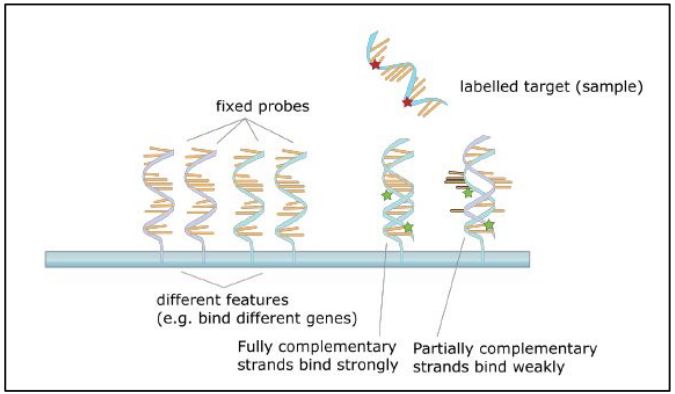


Quantitative data on the cells

DNA Micro-Array presentation





- DNA microarrays measure the expression levels of large number of genes simultaneously.

Advantages of sc SEQ-RNA compared to DNA Micro-Array

1) **Higher sensitivity;** able to detect lower levels of gene expression

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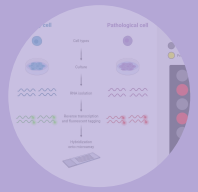
1) **Higher sensitivity;** able to detect lower levels of gene expression

2) **Greater dynamic range;**

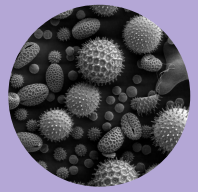
3) **Greater accuracy;**

4) **Greater flexibility;**

5) **Greater throughput;** study many samples simultaneously

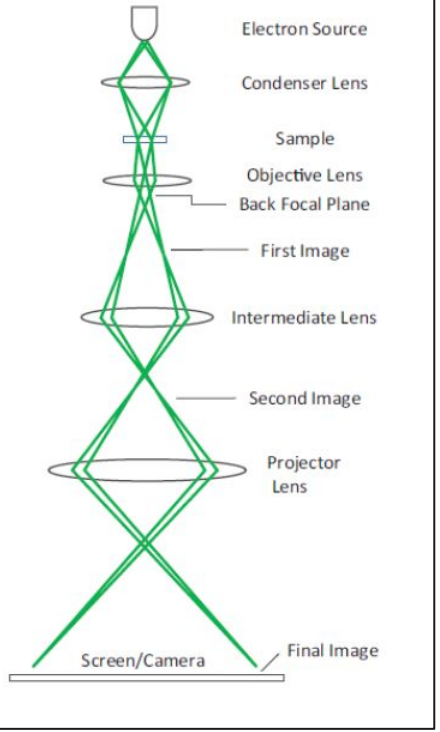


Improve cell transcriptome



Quantitative data on the cells

Transmission electron microscopy : principle



- E-beam with high acceleration voltage penetrating the sample → Ultrathin sections → Higher resolution + Ultrastructure

- **3 components**
  - **Illumination system;** electron source, condenser lens
  - **Objective lens**
  - **Imaging system;** intermediate and projector lenses

Transmission electron microscopy : sample preparation

1) **Fixation;** preserve the sample by adding chemicals that stabilize the cellular structure. Done in formaldehyde or glutaraldehyde.

Transmission electron microscopy : sample preparation

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Transmission electron microscopy : sample preparation

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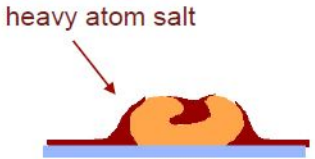
3) **Embedding;** solid material such as resin (epoxy or acrylic)

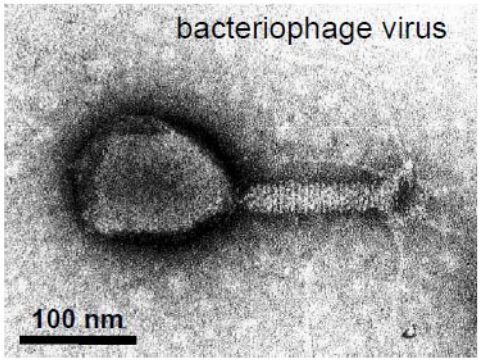
4) **Sectioning;** Cut to be thin enough (~ 70-80 nm)

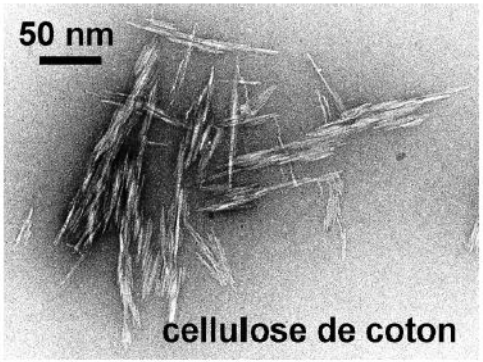
5) **Staining;** Stained with heavy metals such as lead or uranyl acetate

Transmission electron microscopy : contrast enhancement

TEM : Contrast enhancement of organic specimens

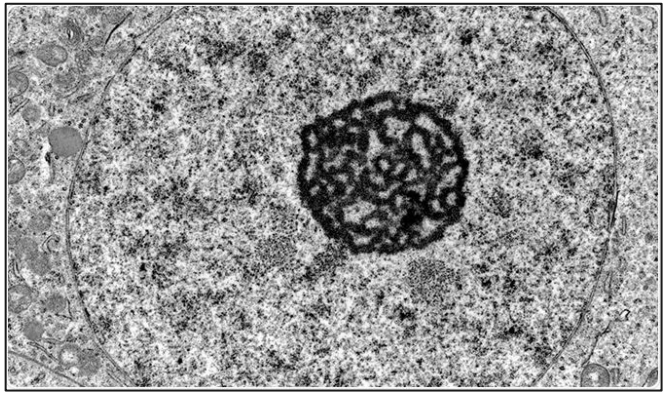






Negative staining with uranyl acetate

Transmission electron microscopy



- SEM can visualize many features of the cells

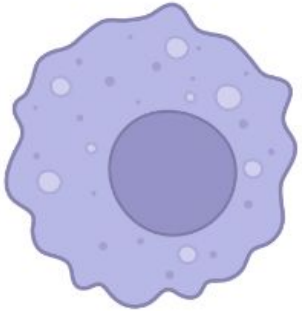
- Cell membrane / Cytoplasm / Nucleus / Cell junctions / Extracellular matrix

DISCUSSION

Size of the cohort

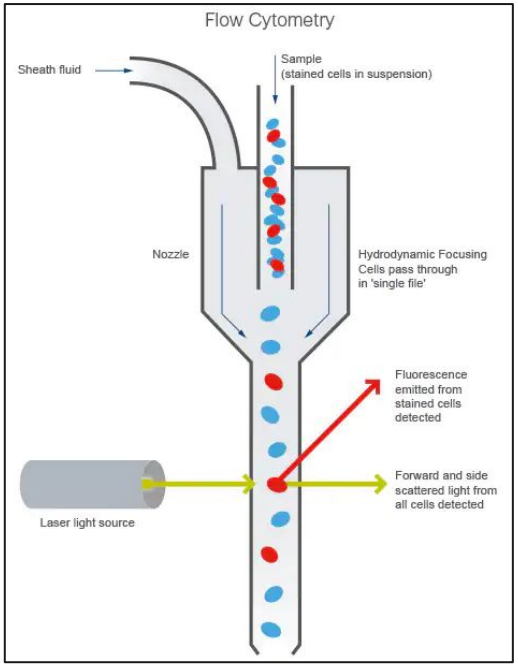
- Increase the size of the cohort to have more metadata

Macrophages characterization

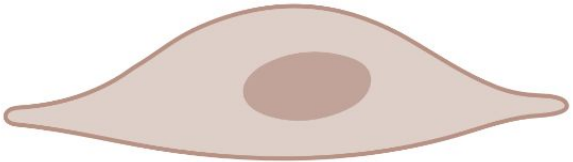


- More macrophages than previous studies

- Do more cell processing and potential use of flow cytometry



Epithelial cell characterization



- Influence of **daycare attendance** and **hormonal birth control** on epithelial cells concentration

- Identification of **key genes** in epithelial cell subclusters

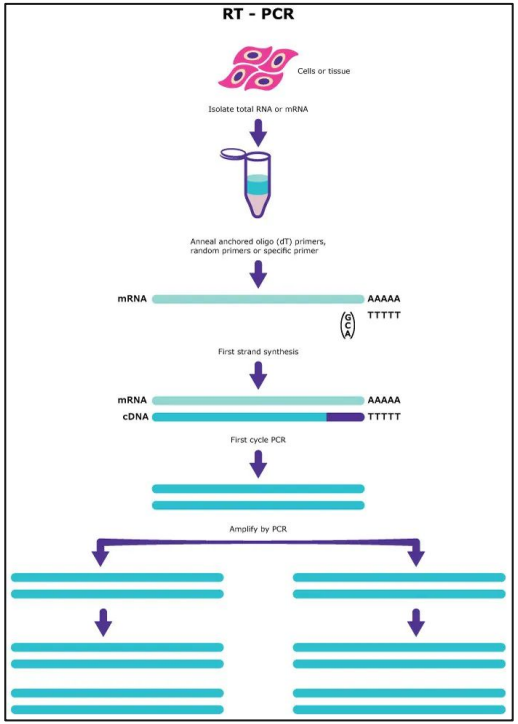
- Decrease of milk transport, synthesis, and production over time.

- **LC1 increase** (tight junction) // s**ecretory lactocyte decrease** (milk component synthesis and secretion)

- Fewer components are synthesized in the lactocytes themselves

Concerning the experiment

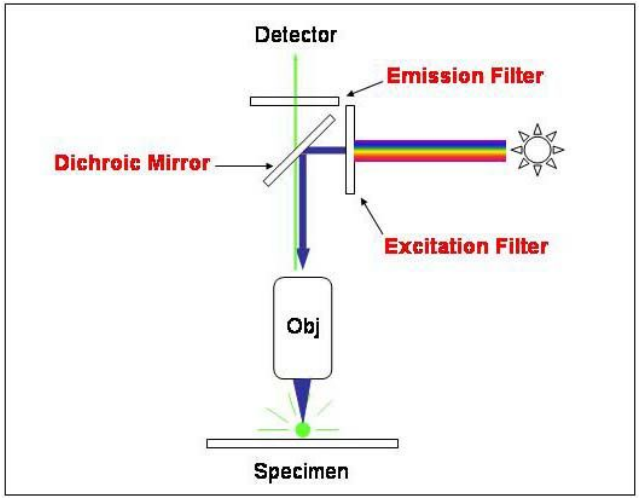
- qPCR is also an alternative to sc SEQ-RNA more precise than Micro-Array

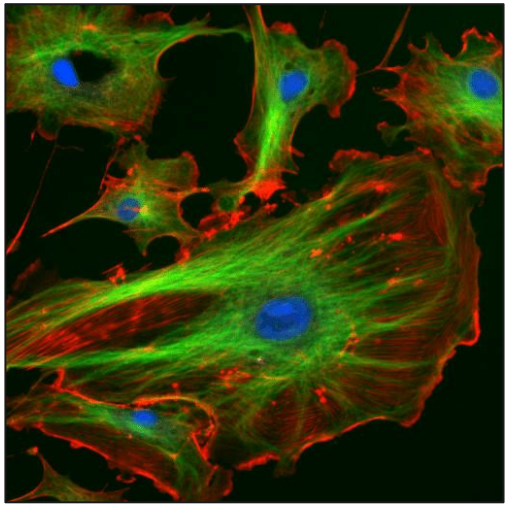


- Sensitive and specific method to detect and quantify specific genes

Concerning the experiment

- TEM might be complicated to use

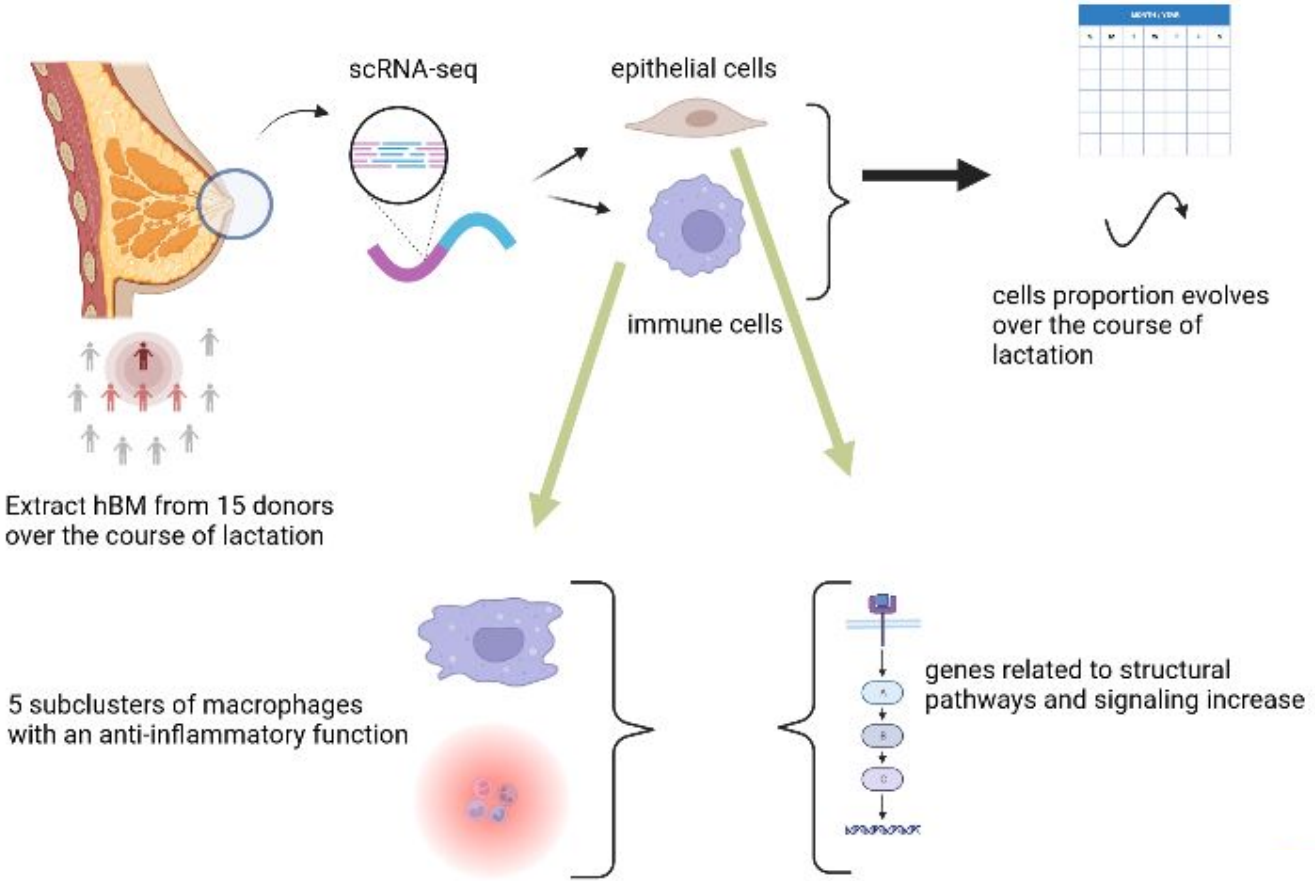




- Fluorescent microscopy or Confocal microscopy are easier to use

CONCLUSION

Conclusion


scRNA-seq
epithelial cells
immune cells
cells proportion evolves over the course of lactation
Extract hBM from 15 donors over the course of lactation
5 subclusters of macrophages with an anti-inflammatory function
genes related to structural pathways and signaling increase

Conclusion

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Nutritional supplements and mother's milk composition: a systematic review of interventional studies

Mojtaba Keikha, Ramin Shayan-Moghadam, Maryam Bahreynian & Roya Kelishadi

International Breastfeeding Journal **16**, Article number: 1 (2021) | Cite this article

9897 Accesses | **16** Citations | **4** Altmetric | Metrics

Impact of lifestyle on the hBM composition

Thank you !

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