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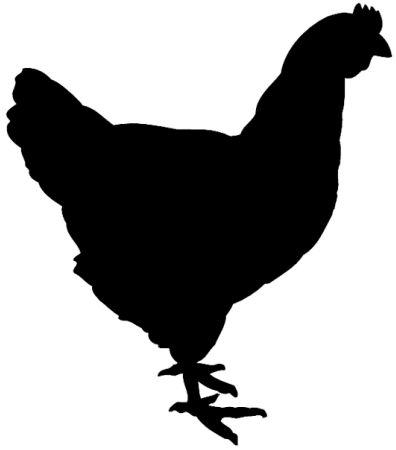
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# Avian eggshell: A model of calcium carbonate biomineralization

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uOttawa



# Potential Conflicts of Interest - Disclosure



NAME:...GAUTRON Joël

AFFILIATION: INRAe

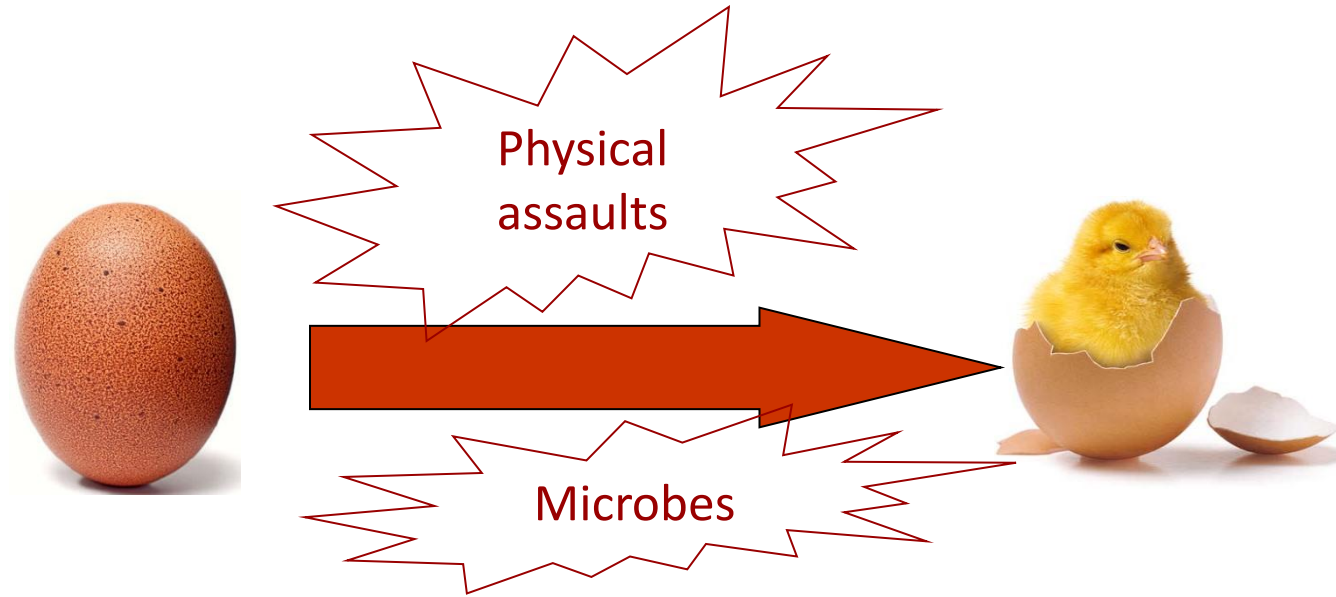
I have no potential conflict of interest to report

I have the following potential conflict(s) of Interest to report:

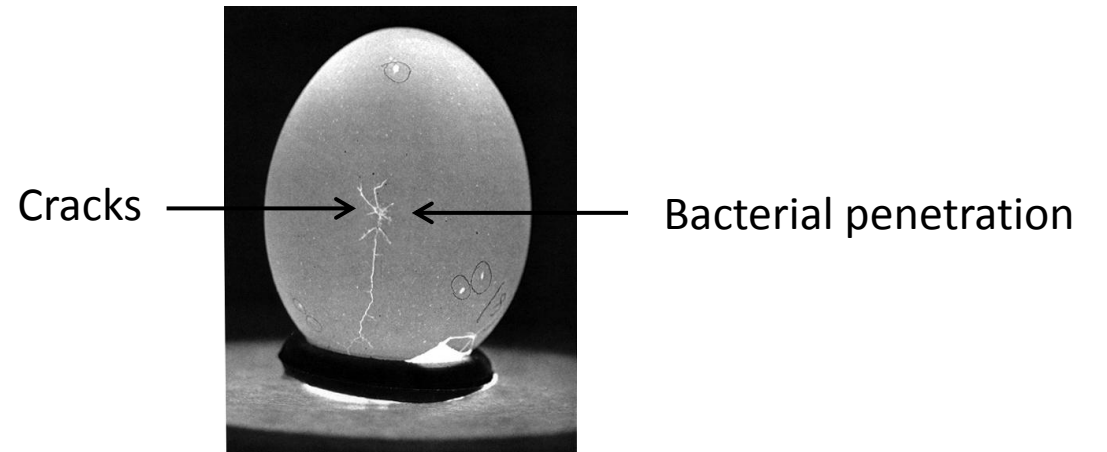
*In accordance with criterion 24 of document UEMS2012/30 "Accreditation of Live Educational Events by the EACCME", all declarations of potential or actual conflicts of interest, whether due to a financial or other relationship, must be provided to the EACCME® upon submission of the application. Declarations also must be made readily available, either in printed form, with the programme of the LEE, or on the website of the organiser of the LEE. Declarations must include whether any fee, honorarium or arrangement for reimbursement of expenses in relation to the LEE has been provided.*

# The chicken egg

The egg is basic ingredient for human diet  
Close chamber to allow the development of the embryo



Eggshell, a natural envelope to ensure physical defence of egg  
- *Protects the developing embryo*  
- *Ensures that table eggs remains free of pathogens*



# Desynchronization between calcium intakes and requirements

Calcium come from the diet

Mineralisation occurred during the night when there is no diet available



Need for shell calcification

0

--+

+++

+++

+++

+++

Availability of calcium

+++

+++

0

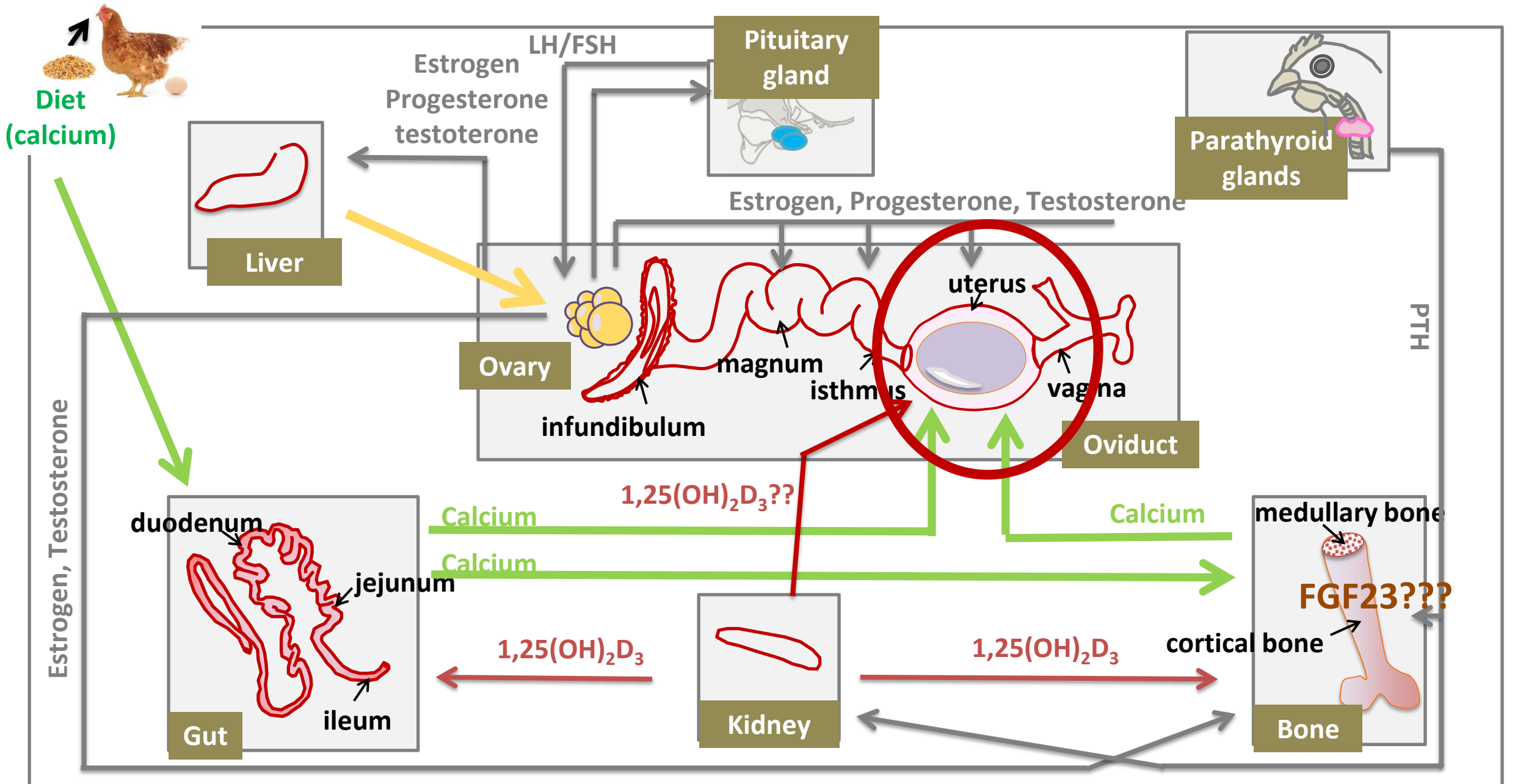
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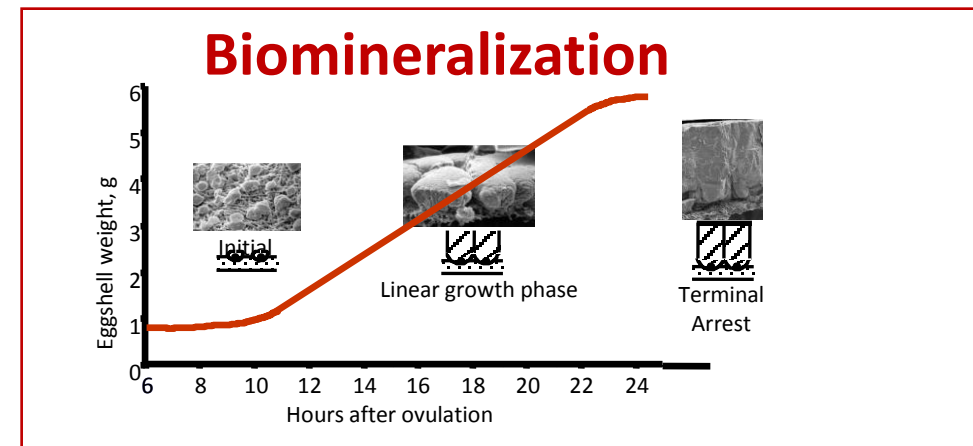
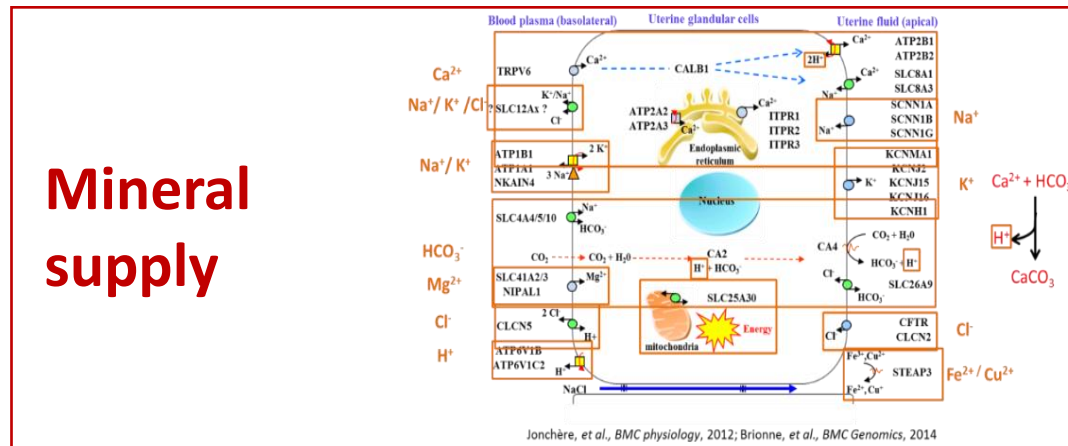
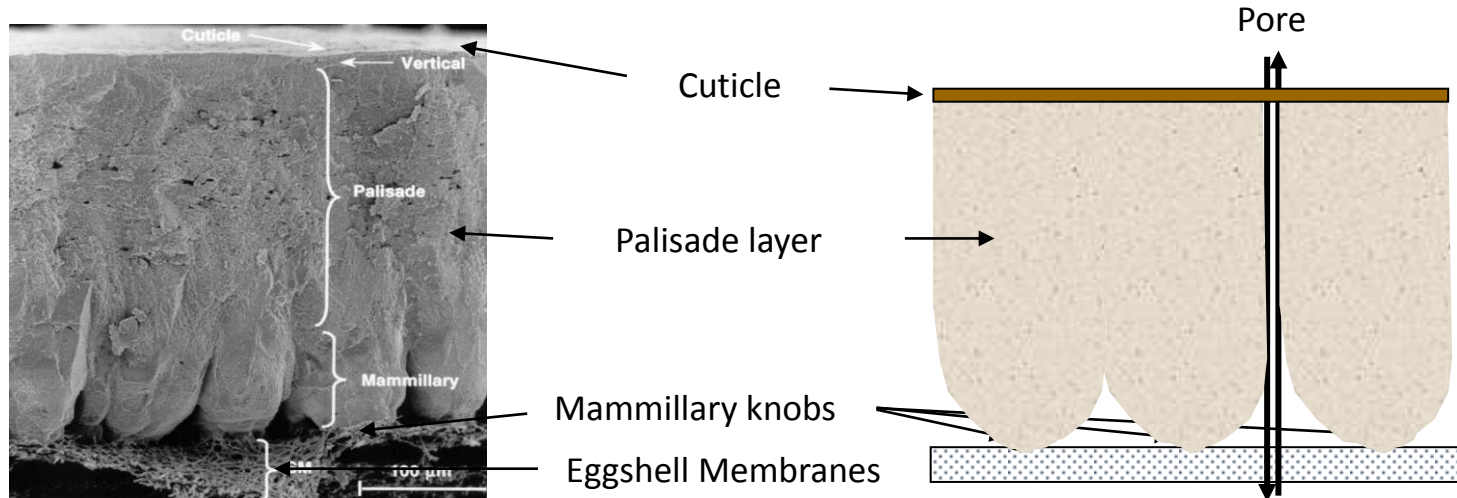
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# Régulation of calcium metabolism in laying hens



# The eggshell formation











- ✓ Eggshell biomineralization in uterus (fast process)
- ✓ 5-6 g of mineral (calcium carbonate) are deposited within a 20 h period






### 3 Potential pathways

# Mineral supply

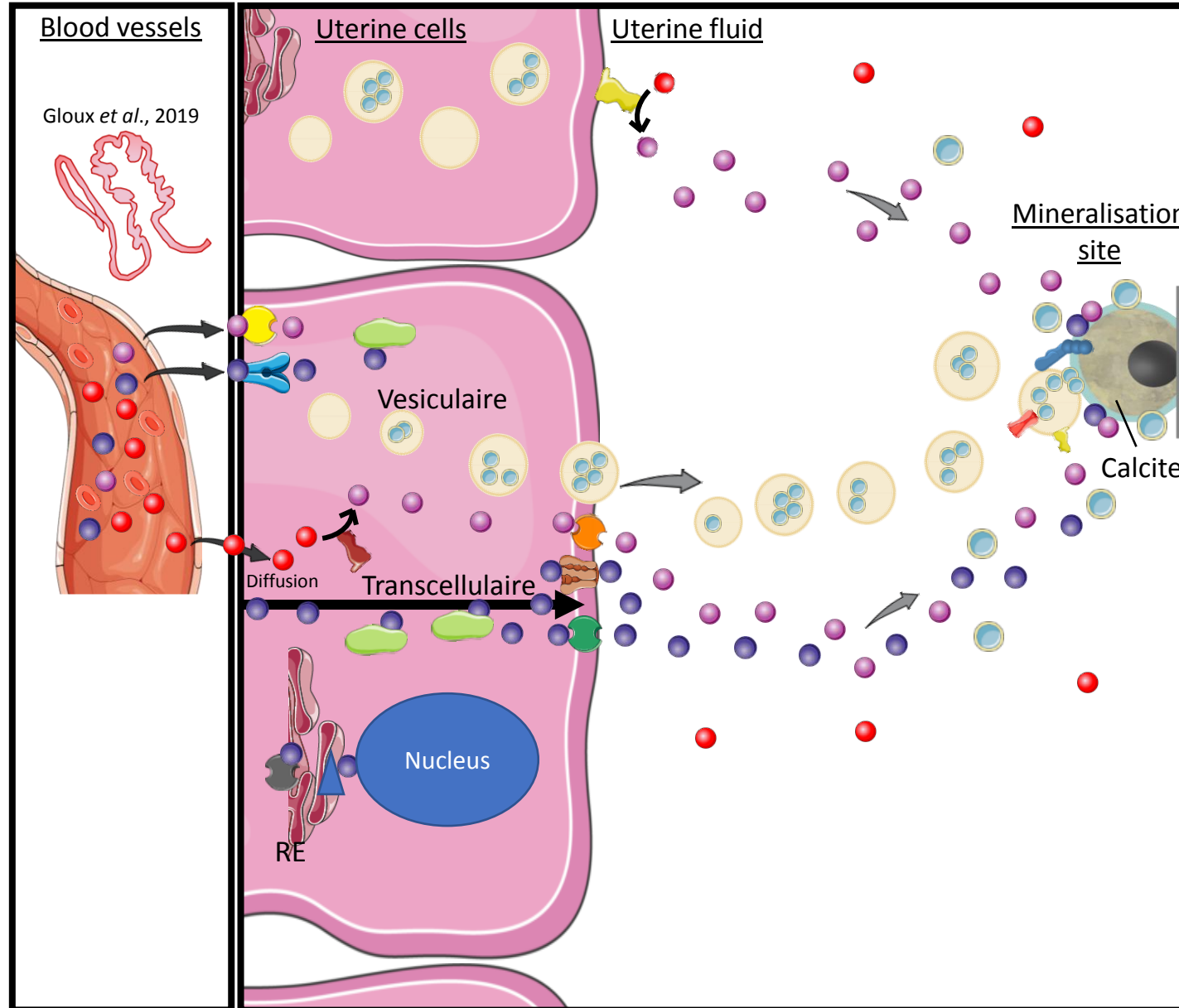
#### Transcellular

-  Carbonic Anhydrase 2
-  Carbonic Anhydrase 4
-  SLC4A4-A5-A10
-  SLC26A9
-  TRPV2-3
-  Calbindin-1
-  ATPA2/3
-  ITPR1/2/3
-  ATP2B1-B2
-  SLC8A1-A3

#### Vésiculaire

-  Extra and intra cellular vesicles
-  Annexines
-  EDIL3/MFGE8

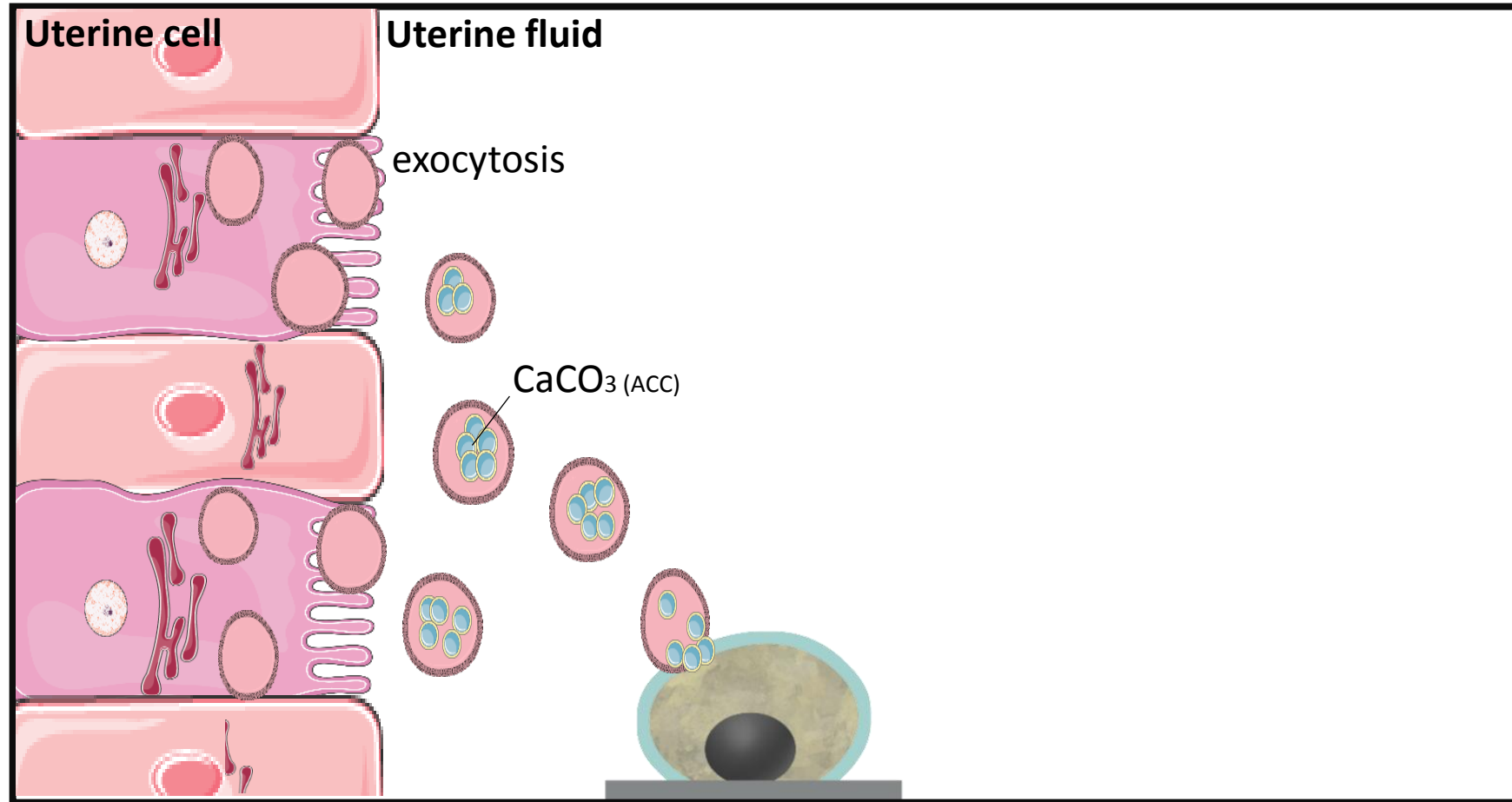
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# Eggshell biomineralization

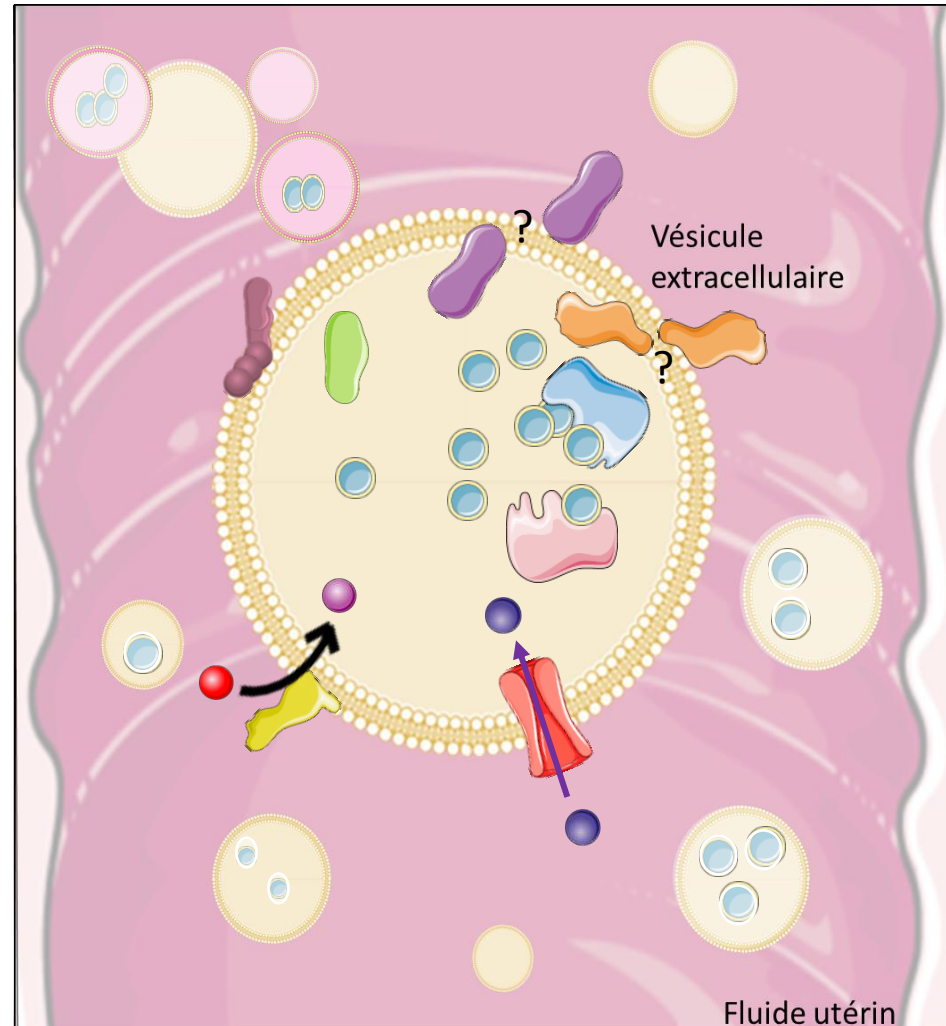
Involvement of vesicular system to transport and stabilize Amorphous calcium carbonate (ACC)



# Eggshell biomineralization

## Involvement of vesicular system to transport and stabilize Amorphous calcium carbonate (ACC)

- EDIL3
- ANXA1
- ANXA2
- ANXA8
- CA4
- PDCD6IP
- Syntenin-1
- Ovalbumin
- Ezrin
- Lysozyme



ANX supplied  $\text{Ca}^{2+}$  ?

CA4 supplied  $\text{HCO}_3^-$  ?

LYZ and OVA stabilized ACC ?  
EDIL3 adress vésicles ?

- $\text{CO}_2$
- $\text{HCO}_3^-$
- $\text{Ca}^{2+}$
- ACC

### 3 Potential pathways

# Mineral supply

#### Transcellular

- Carbonic Anhydrase 2
- Carbonic Anhydrase 4
- SLC4A4-A5-A10
- SLC26A9
- TRPV2-3
- Calbindin-1
- ATPA2/3
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- ATP2B1-B2
- SLC8A1-A3

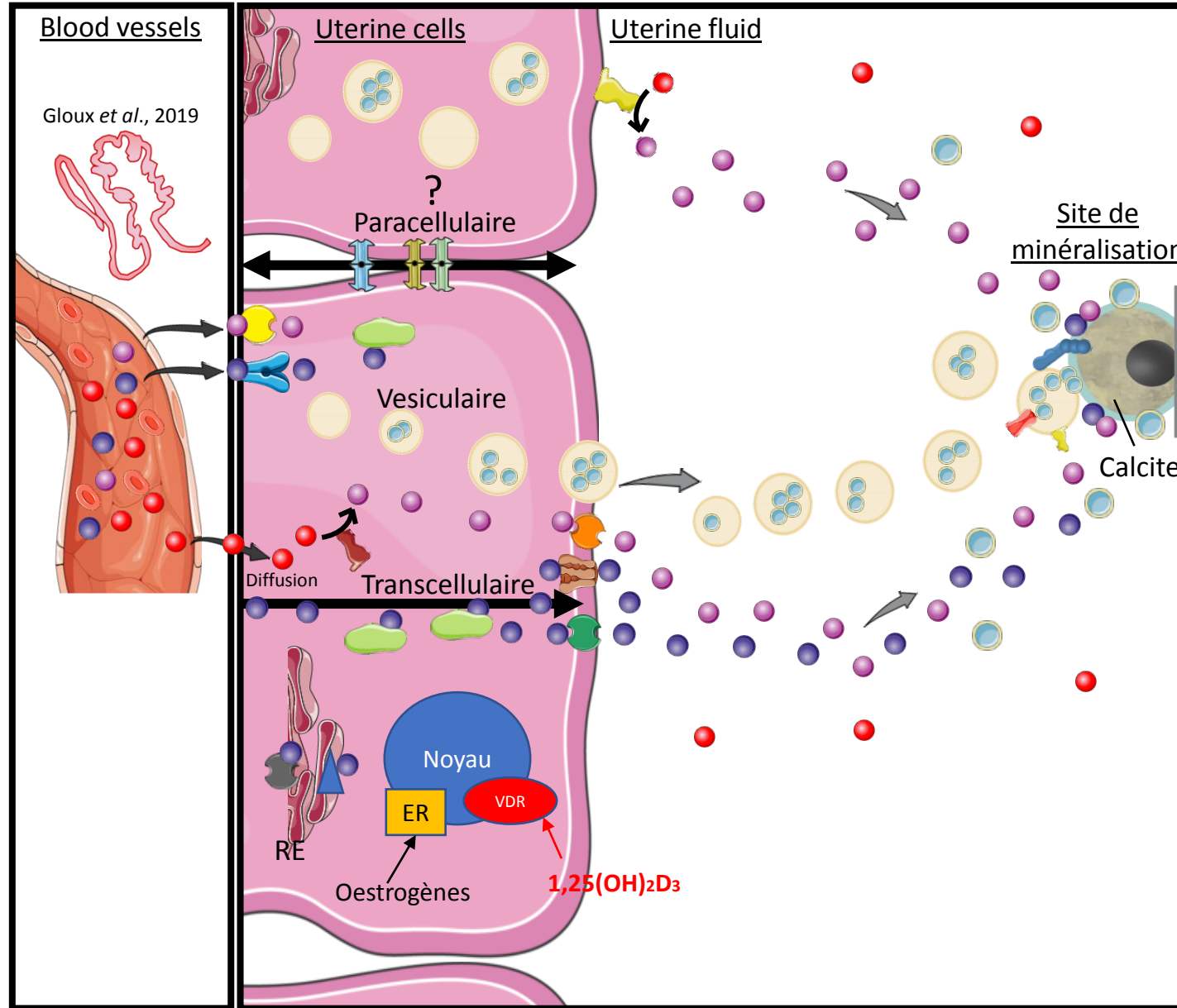
#### Vésiculaire

- Extra and intra cellular vesicles
- Annexines
- EDIL3/MFGE8

#### Paracellular ?

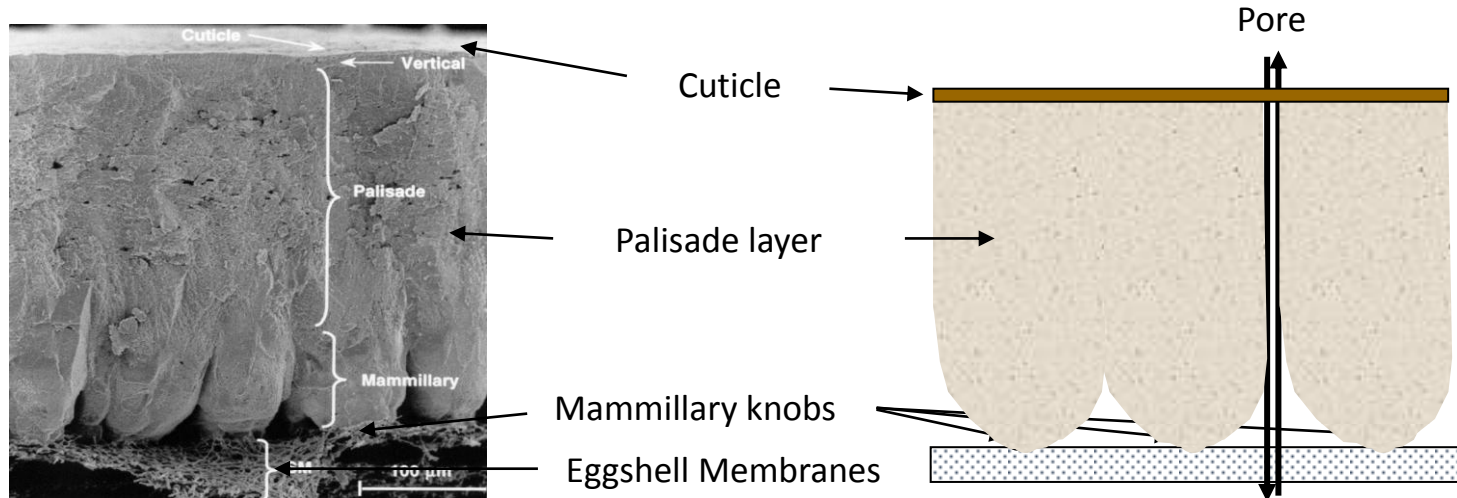
- Claudins
- JAM
- Occludin/TJP

- $\text{HCO}_3^-$
- $\text{Ca}^{2+}$
- $\text{CO}_2$
- ACC

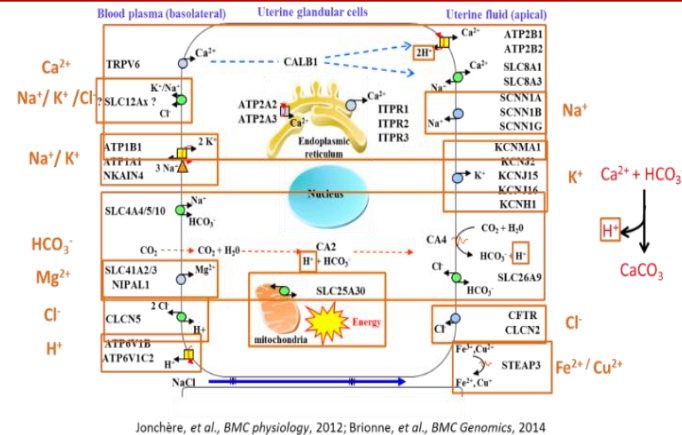


# The eggshell formation

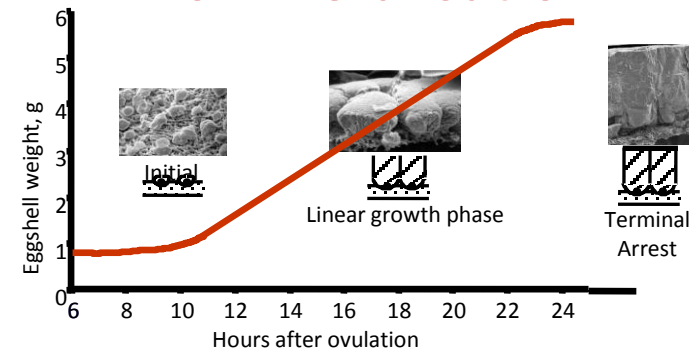
- ✓ Eggshell biomineralization in uterus (fast process)
- ✓ 5-6 g of mineral (calcium carbonate) are deposited within a 20 h period



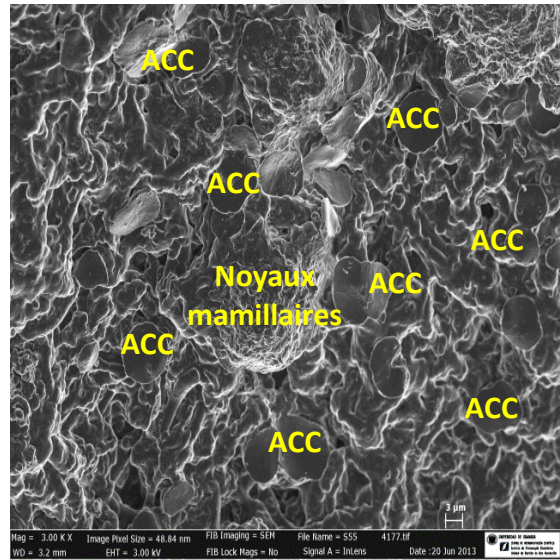
## Mineral supply



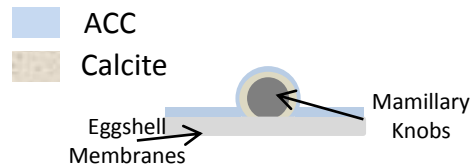
## Biomineralisation



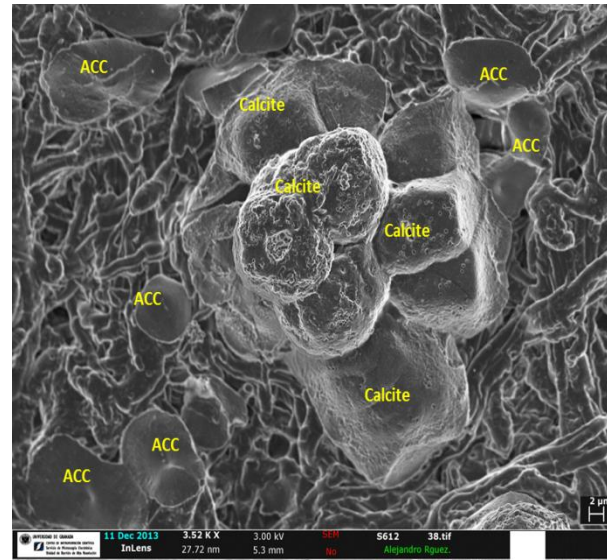
# Eggshell biomineralization



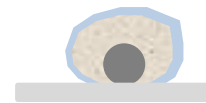
First events of nucléation



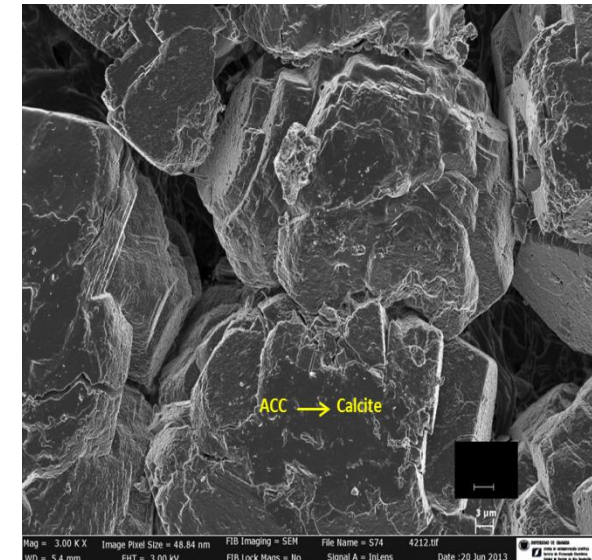
**Time 1 (5-6 h Post ovulation):**  
ACC particles nucleate on the whole eggshell membranes.  
Form massive deposits



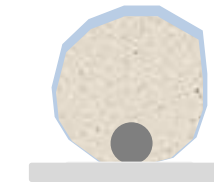
Calcite formation



**Time 2 (6-7 h post ovulation):**  
Interface-coupled dissolution precipitation process  
Direct transformation of ACC into calcite aggregates on mamillary knobs

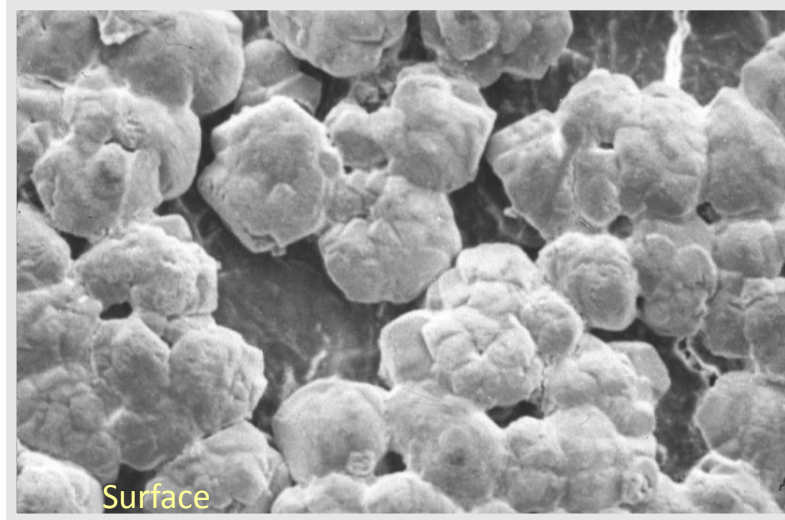
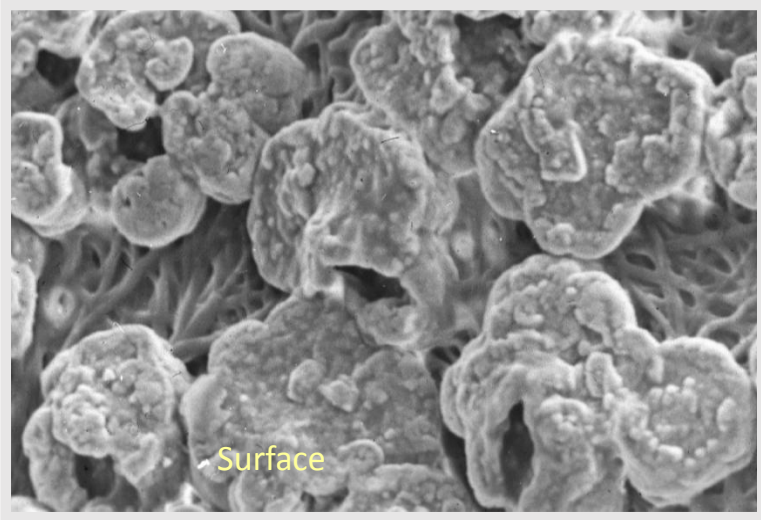


Larger calcite crystal units deposition

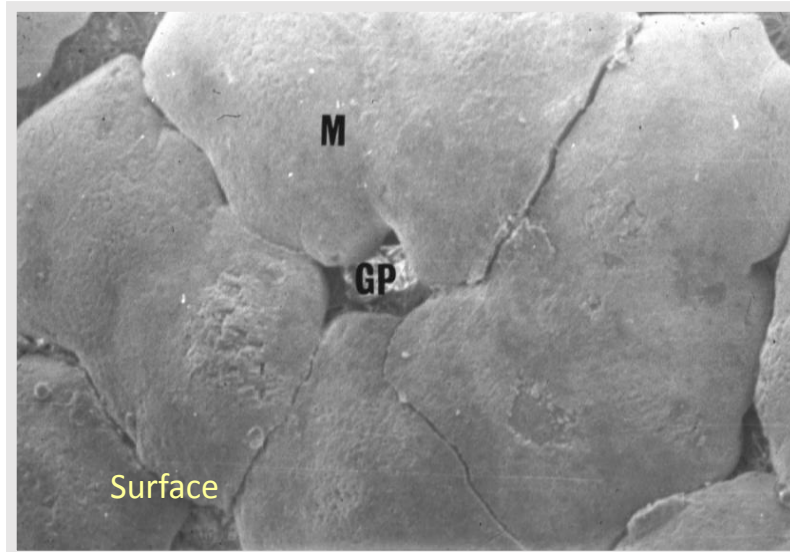
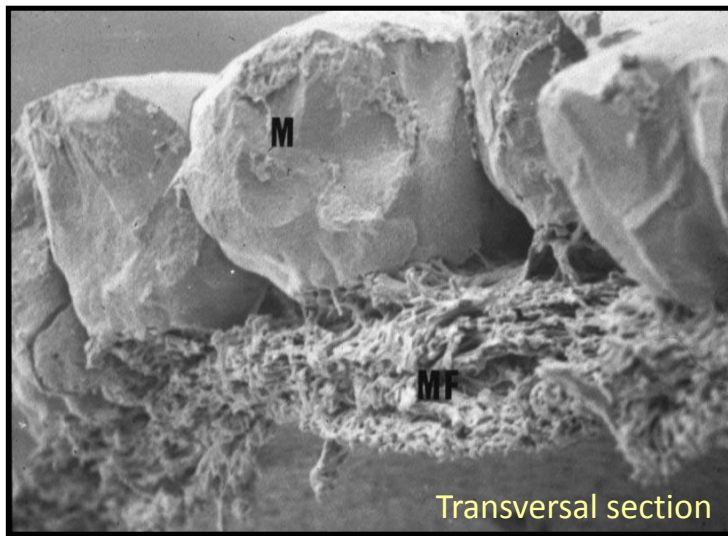


**Time 3 (>7h post ovulation):**  
Additional cristallisation events on calcite template

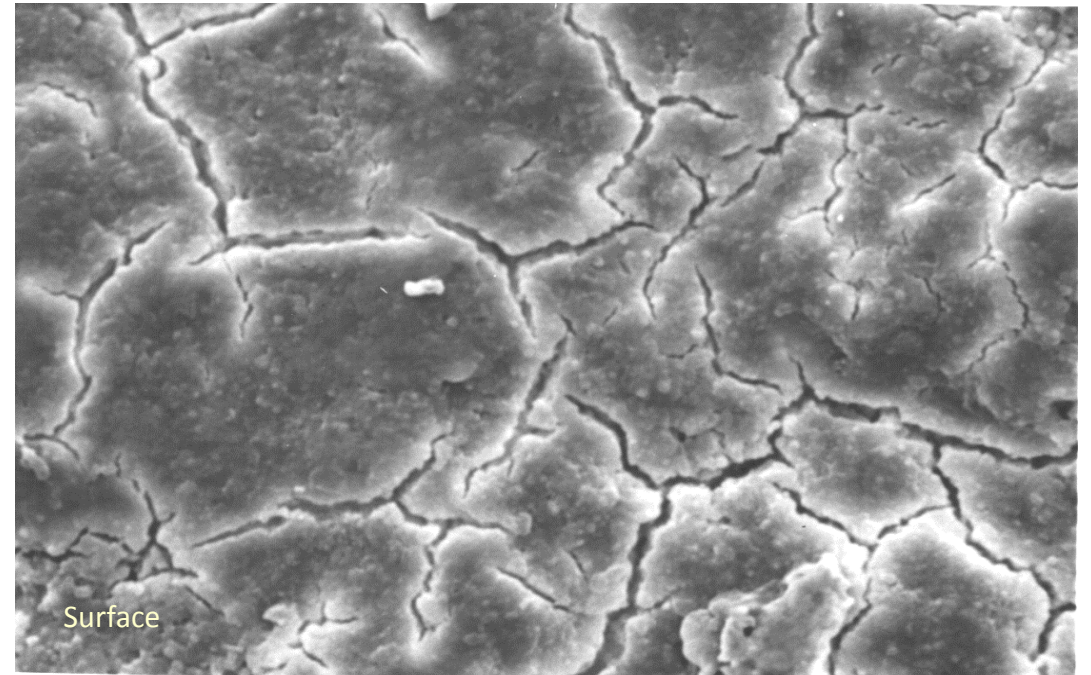
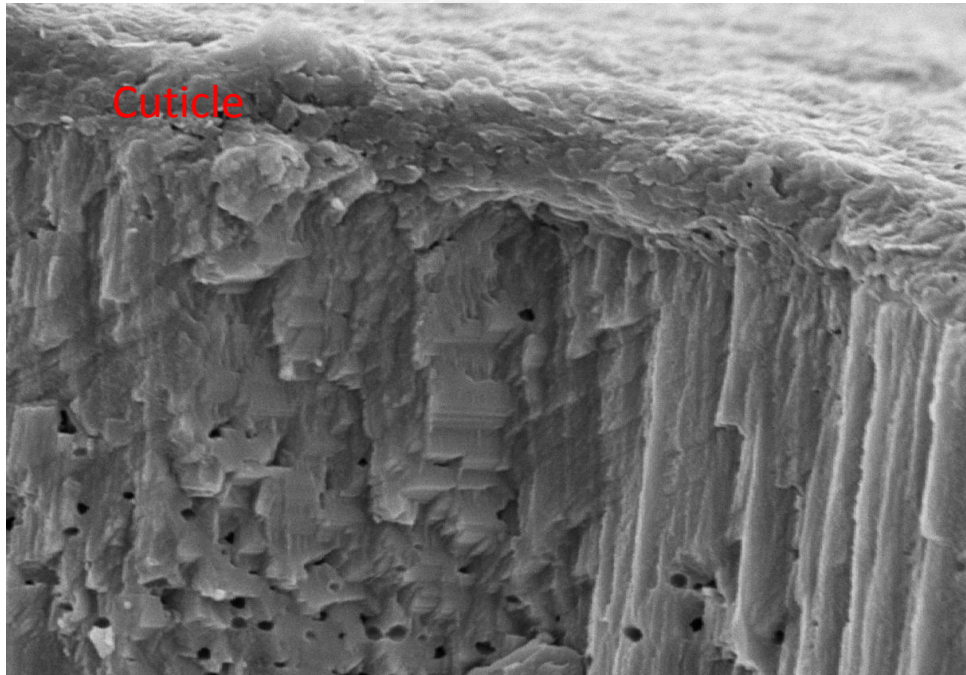
# Eggshell biomineralization



**Time 4 (7-10h post ovulation):**  
Calcite deposition and fusion  
of adjacent cônes



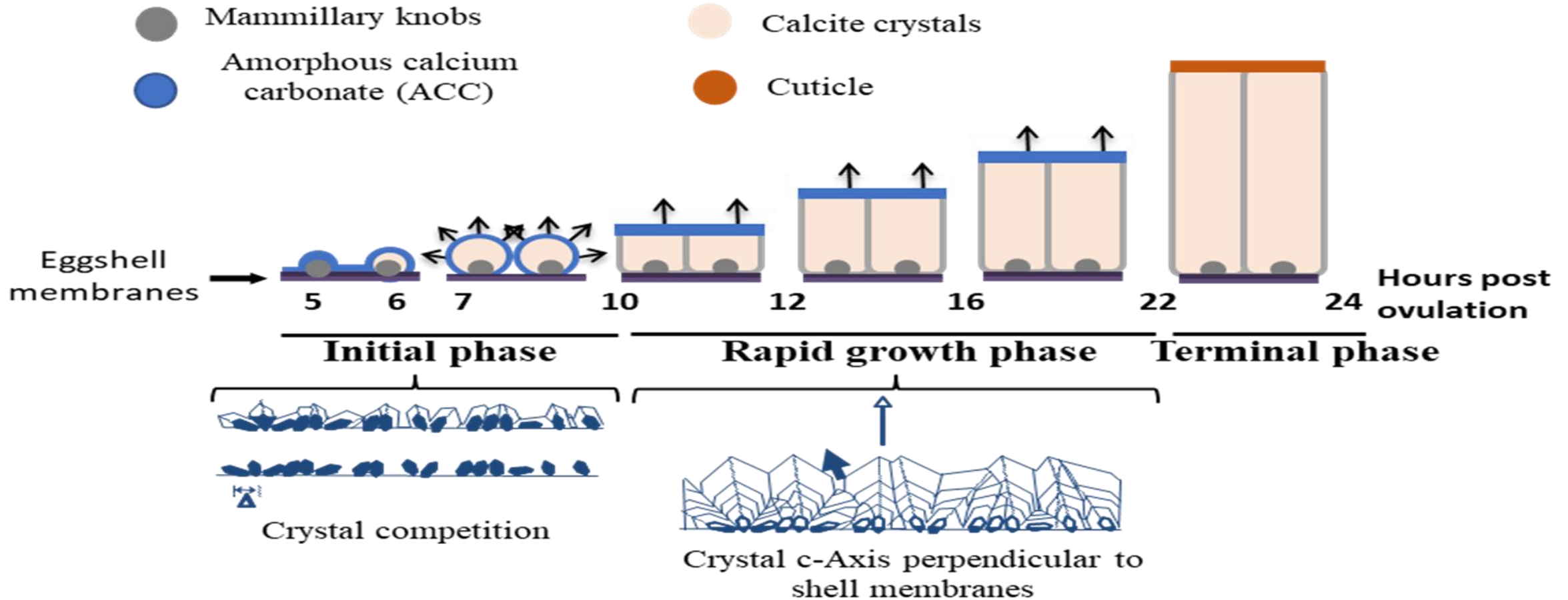
# Eggshell biomineralization



## Time 5 (11 to Oviposition):

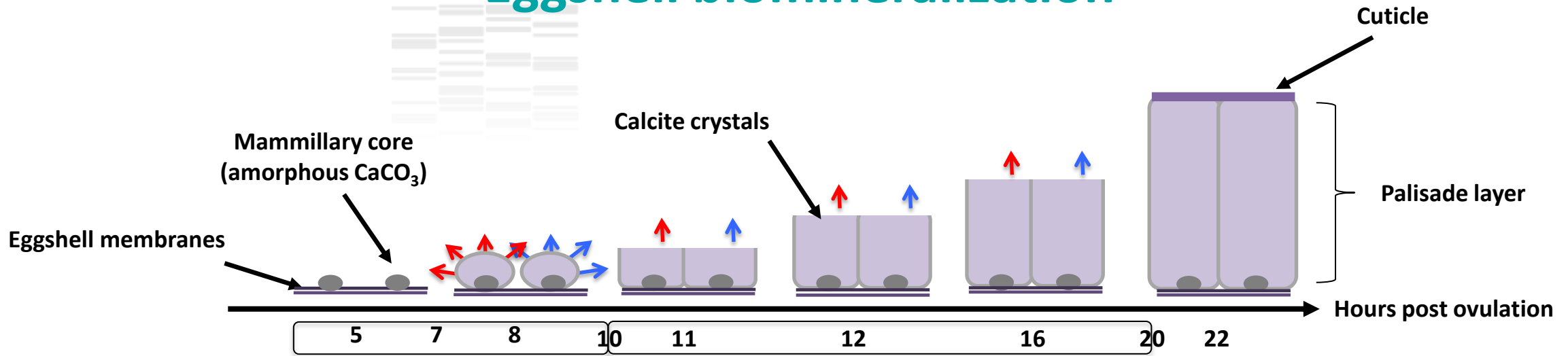
- Formation of palisade layer. Generation of a compact layer with crystals all oriented perpendicular to the surface
- Deposition of a thin layer of vertical structure
- Cuticle deposition
- Oviposition, drying and cracking of cuticle

# Eggshell biomineralization





# Eggshell biomineralization



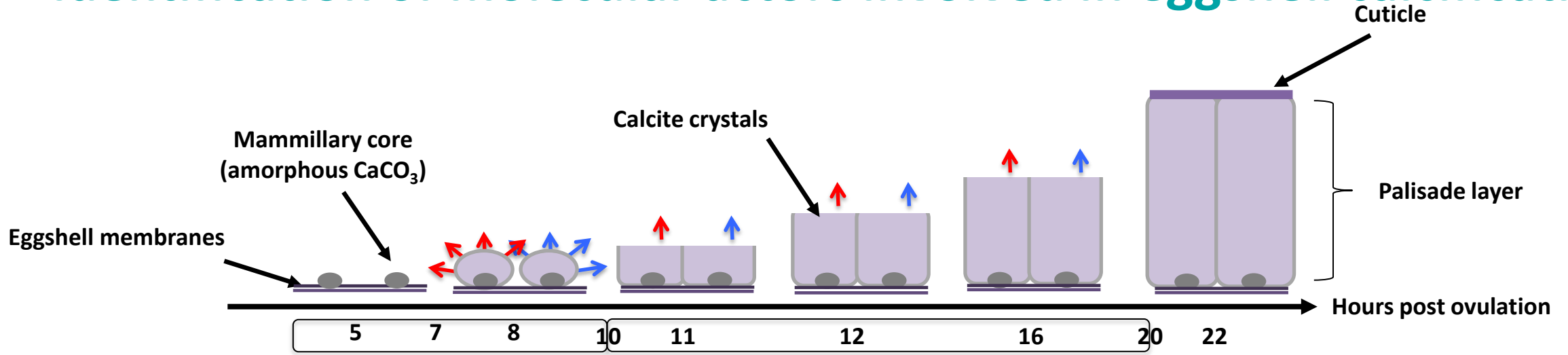
95 % of calcium carbonate (calcite) ← Interaction → 3.5 % organic matrix (protéines, protéoglycanes)

**Ultrastructure, Mechanical properties**  
First events of shell mineralisation are crucial

**Role of organic matrix proteins at pivotal events**

- ✓ *Stabilization of amorphous calcium carbonate (ACC)*
- ✓ *Polymorphs, morphology and size of crystals*

# Identification of molecular actors involved in eggshell calcification

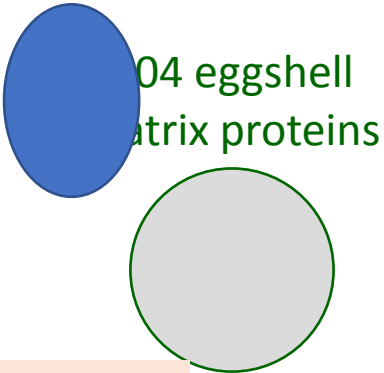
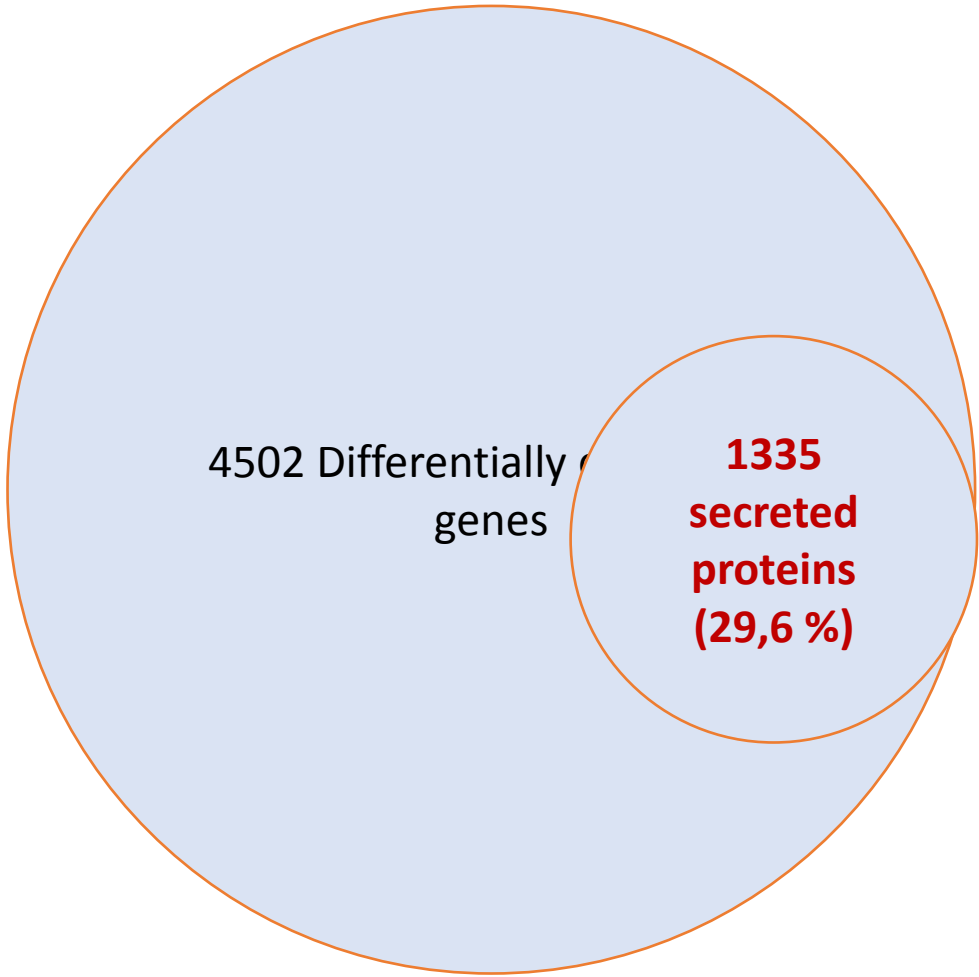


High-throughput quantitative proteomics, Uterine RNA-seq, statistical and bioinformatic functional analyses of matrix proteins



To sort major protein candidates involved in particular key points of the eggshell mineralization

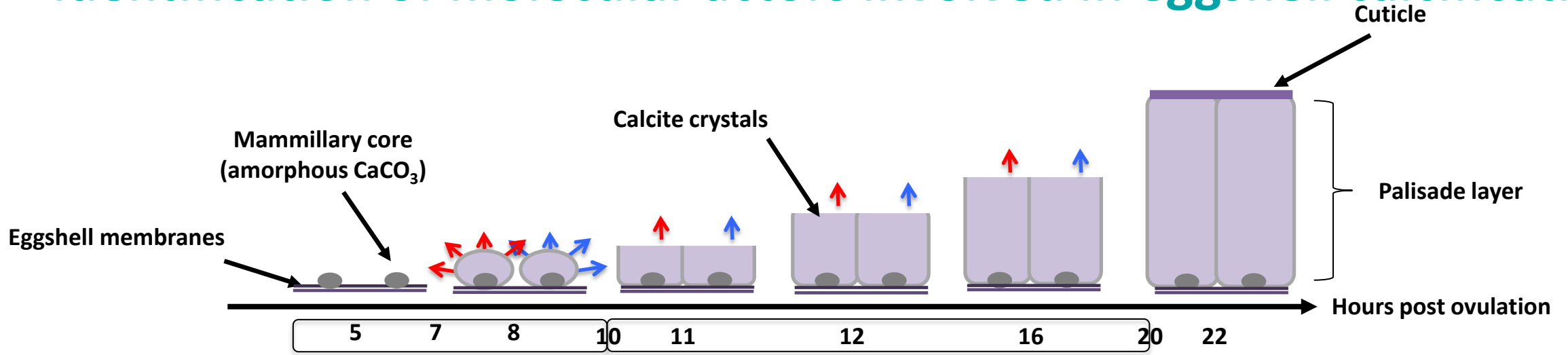
# Identification of molecular actors involved in eggshell calcification



**444 Main candidate proteins to be involved in the shell biomineralization process**



# Identification of molecular actors involved in eggshell calcification



**444 Main candidate proteins to be involved in the shell biomineralization process**

## Predicted functional activities of the identified matrix proteins ?

(Marie et al., 2014, 2015a,b)

Classification in 3 different groups according to their potential functions

**Associated to mineralization process**

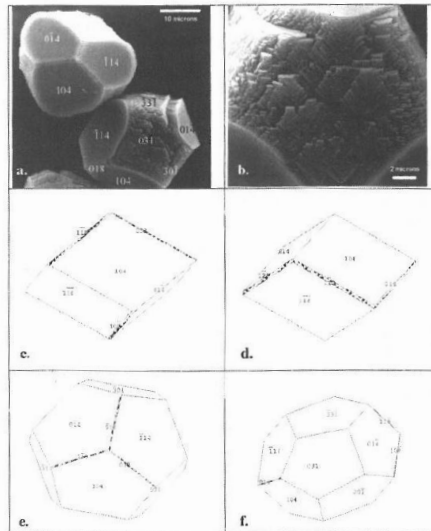
**Involved in the regulation of activity of proteins**

**Antimicrobial and other proteins**

# Eggshell biomineralization

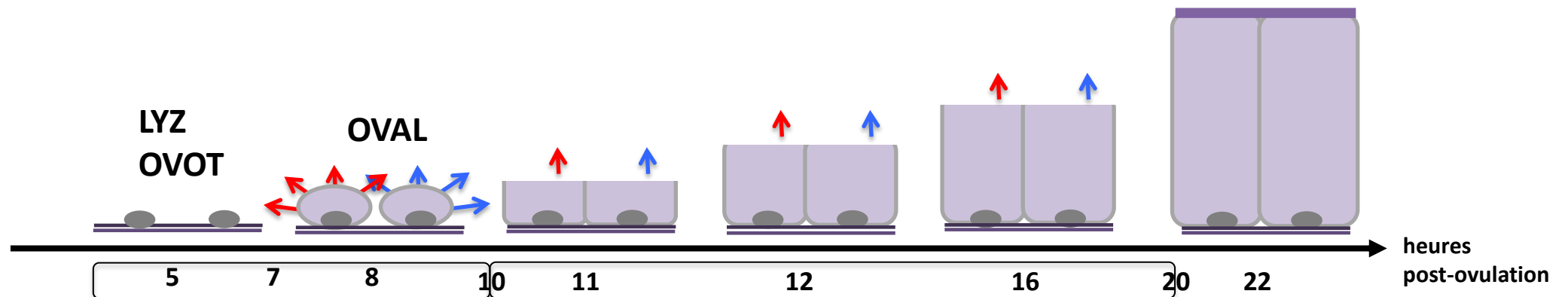
## □ Proteins having a direct involvement in eggshell mineralization

- ✓ Proteins with established role in the **biomineralisation**



## Ovotransferrin is a Matrix Protein of the Hen Eggshell Membranes and Basal Calcified Layer

J. GAUTRON<sup>a</sup>, M.T. HINCKE<sup>b</sup>, M. PANHELEUX<sup>a</sup>, J.M. GARCIA-RUIZ<sup>c</sup>, T. BOLDICKE<sup>d</sup> and Y. NYS<sup>a,\*</sup>

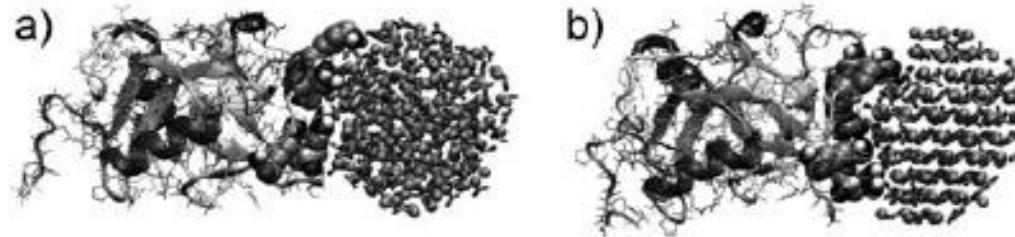


# Eggshell biomineralization

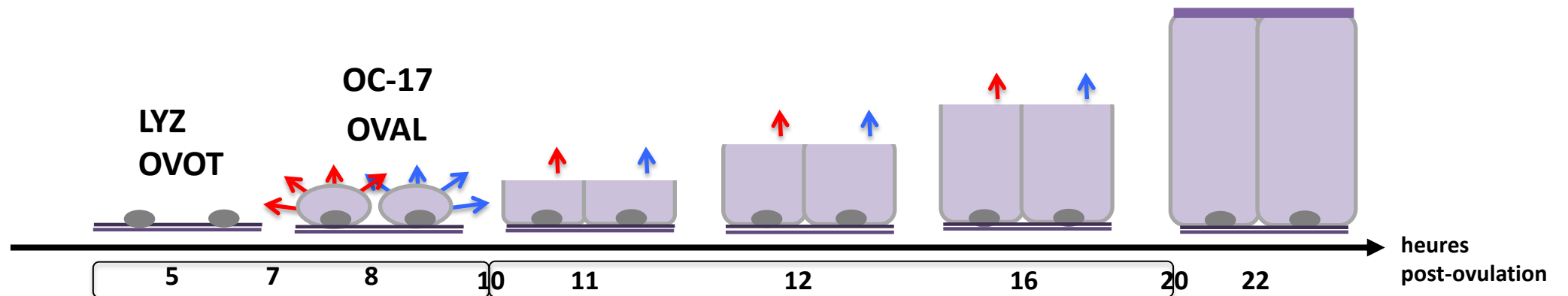
## □ Proteins having a direct involvement in eggshell mineralization

- ✓ Proteins with established role in the **biomineralisation**

Freeman et al, 2010



*Figure 1.* Ovocleidin-17 bound to an amorphous (a) and a crystallized (b) calcium carbonate nanoparticle containing 192 formula units. The



# Eggshell biomineralization

## □ Proteins having a direct involvement in eggshell mineralization

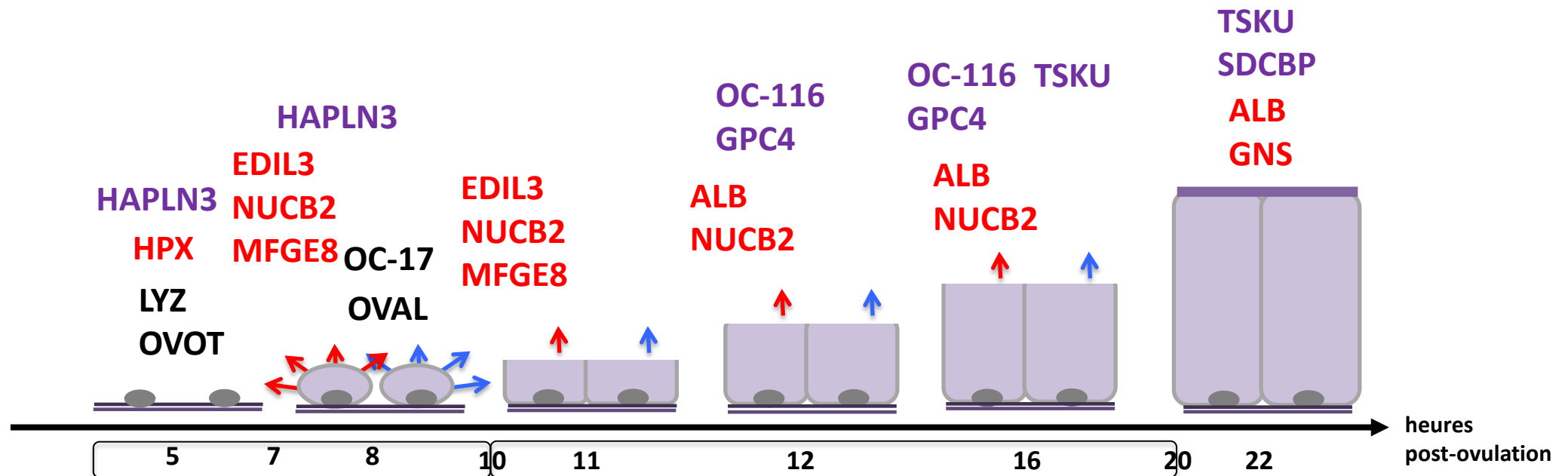
✓ Proteins with established role in the **biomineralisation**

✓ **Calcium binding proteins (CaBPs)** interacting with calcium, favoring crystal nucleation and driving the morphology of crystals

- *Proteins with EF-hand and EGF-like calcium binding domains*

✓ **Proteoglycans** and proteoglycan binding proteins

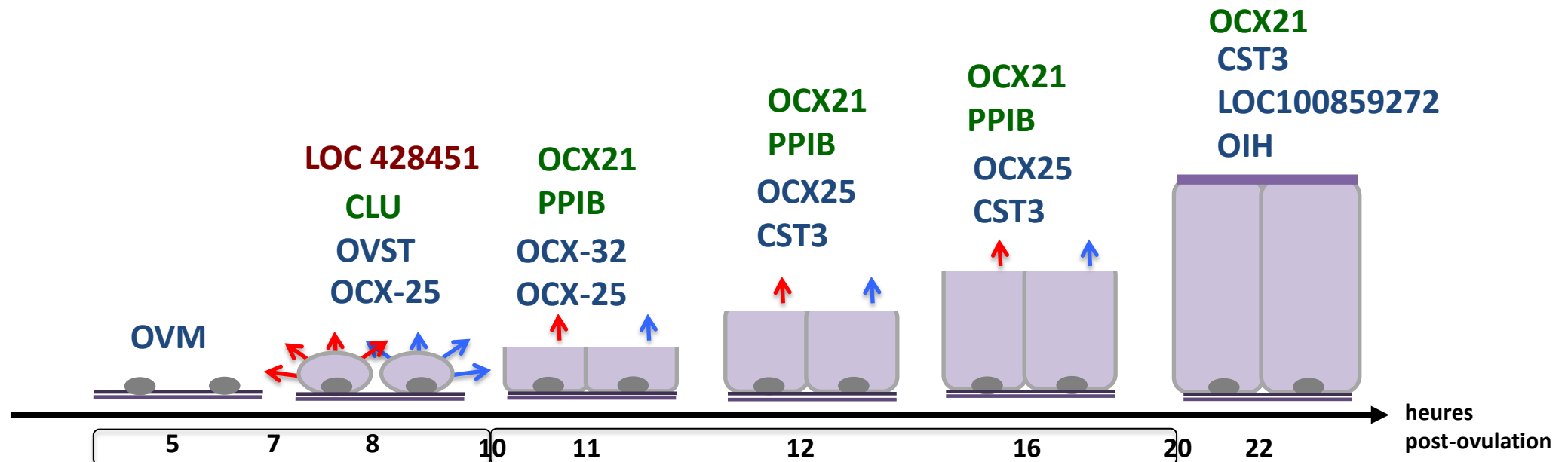
- proteoglycans have a negative charge to attract  $\text{Ca}^{2+}$  ions



# Eggshell biomineralization

## ❑ Proteins involved in the regulation of proteins driving mineralization

- ✓ Proteins involved in the **proper folding of the eggshell matrix** to ensure calcium and mineral interactions and to ensure template to the mineralized structure
- ✓ Proteins **inhibiting or activating proteins present in the mineralization milieu (non cellular)**.
  - *Direct interaction with other proteins.*
    - *Molecular chaperone interact with proteins driving mineralization*
    - *Proteases and protease inhibitors (specific and controlled role during calcification process, either by degrading proteins or regulating processing of proteins into their mature forms)*
- ✓ Mineralization depends of the **degree of protein phosphorylation**
  - *Kinases and Phosphatases*





# And now ?



## Physiology

Understand the mechanisms of shell manufacturing and determine the origin of its weaknesses

## Genetics

Classical and genomic selection

### Recent Developments and Future Prospects :

- ✓ Genomic selection (precision, taking into account the male effect)
- ✓ Taking into account scientific advances in the knowledge of mechanisms
  - ✓ Candidate gene approach

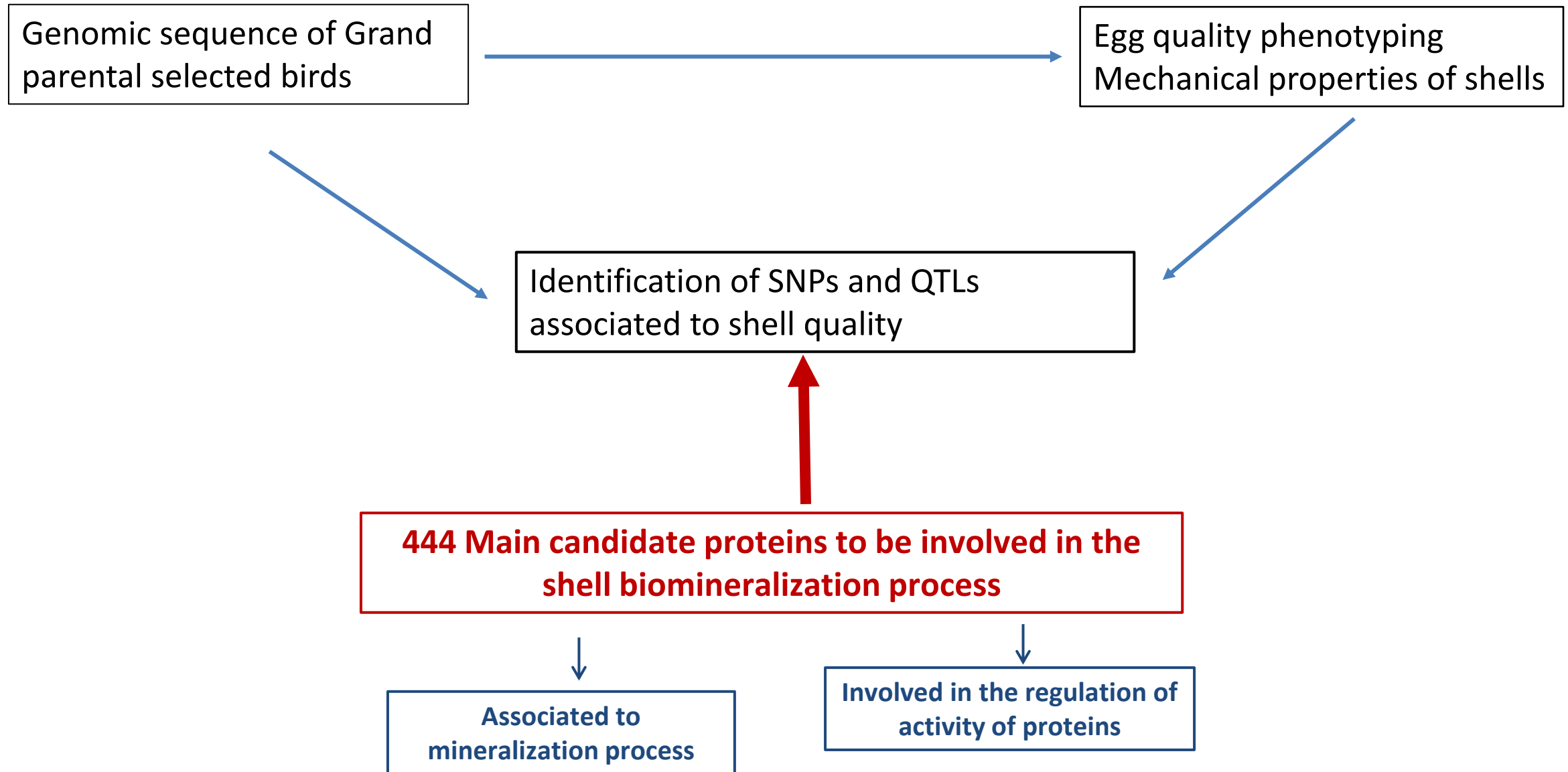
- Mapping genes coding matrix proteins to detect polymorphisms and haplotype related to good quality shell



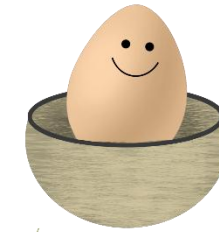
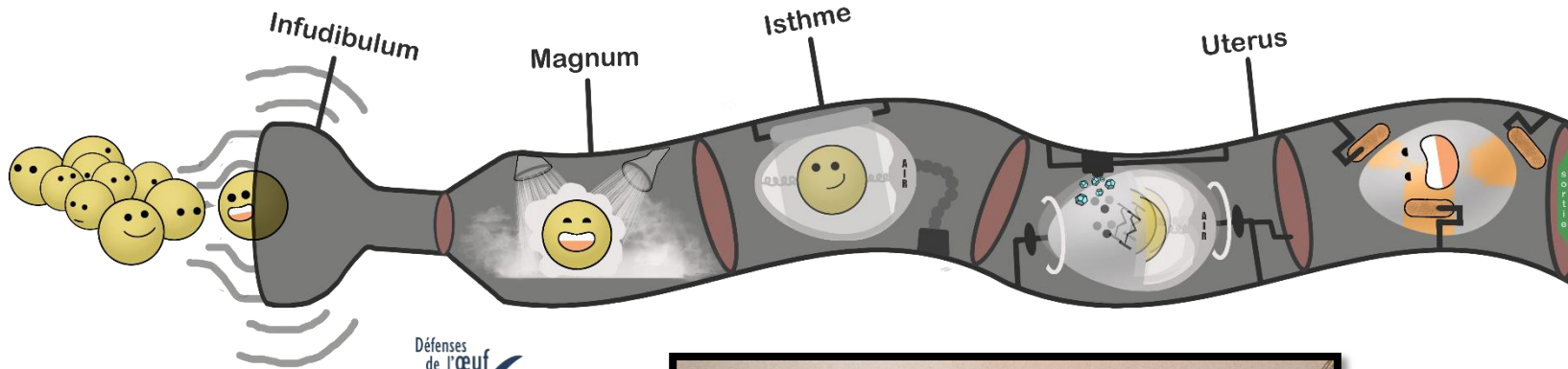
Candidate Genes of eggshell calcification in laying hens (CACAO)

Eggshell Calcification Polymorphism Candidates (POLCACAO)

# Complementarity of information between proteomics (candidate genes) and genomics results (QTL / Sequencing).



# Thank you for your attention



- DOVE
- A. Rodriguez-Navarro
- N. Le Roy, J. Ezagal
- Y. Nys et M. Hincke
- P. Leroy, F. Heraud, C. Diot
- BOA
- PAIB2
- PEAT
- Pegase
- GeT-Genotoul
- Universidad de Granada



**PEGASE**  
Au cœur de la recherche  
et de la formation  
en production animale

