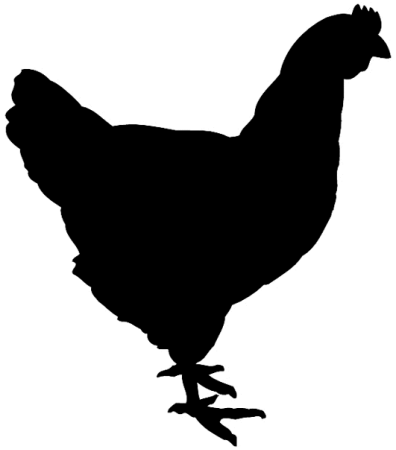
Avian eggshell: A model of calcium carbonate biomineralization

Joël Gautron, Lilian Stapane, Alejandro Rodriguez-Navarro, Yves Nys, Maxwell T Hincke



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Avian eggshell: A model of calcium carbonate biomineralization

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uOttawa

Défenses de l'œuf DOVE Valorisation Evolution

UMRBOA Biologie des Oiseaux & Aviculture

Potential Conflicts of Interest - Disclosure

NAME:…GAUTRON Joël

AFFILIATION: INRAe

x I have no potential conflict of interest to report

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

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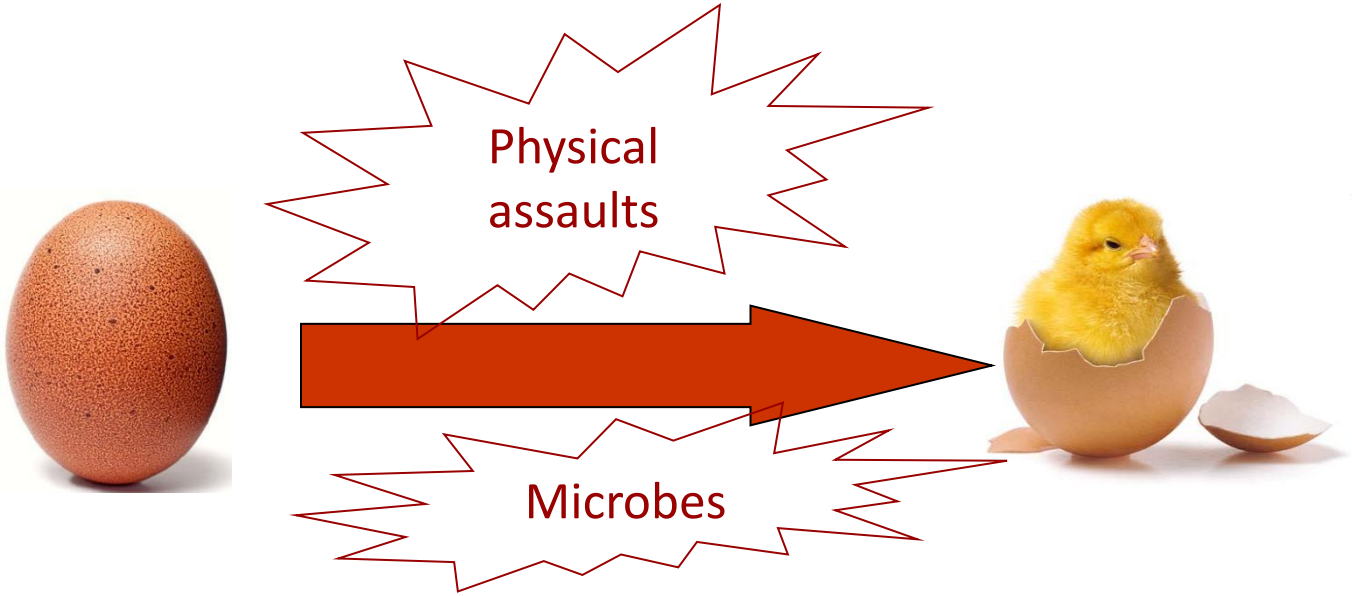
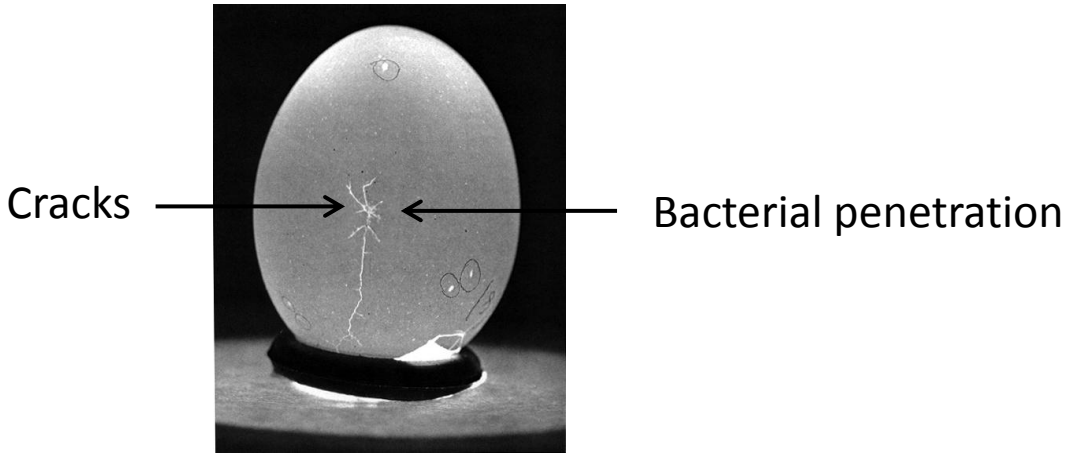
The chicken egg

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*



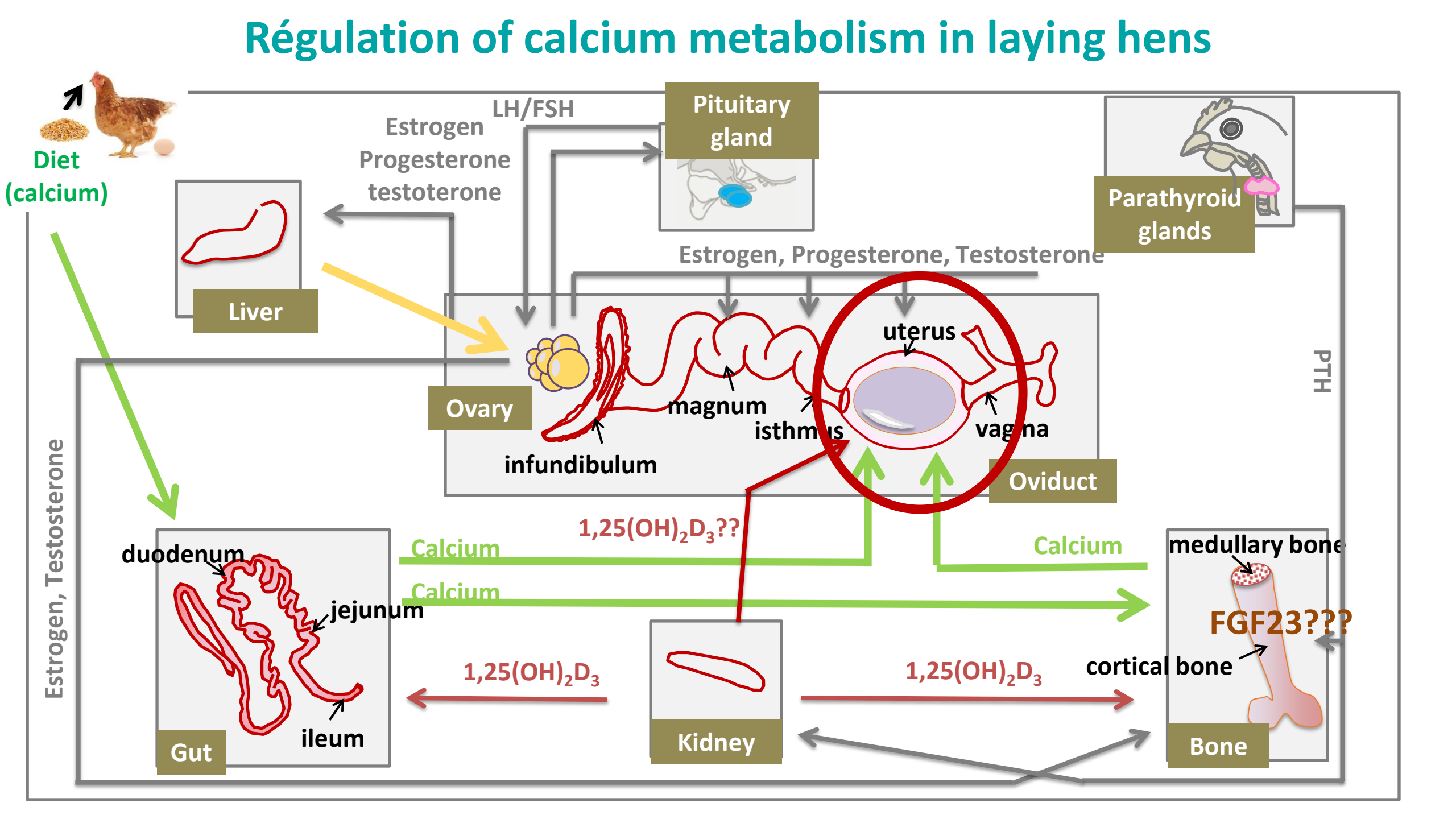
Desynchronization between calcium intakes and requirements

Calcium come from the diet

Mineralisation occured during the night when there is no diet available

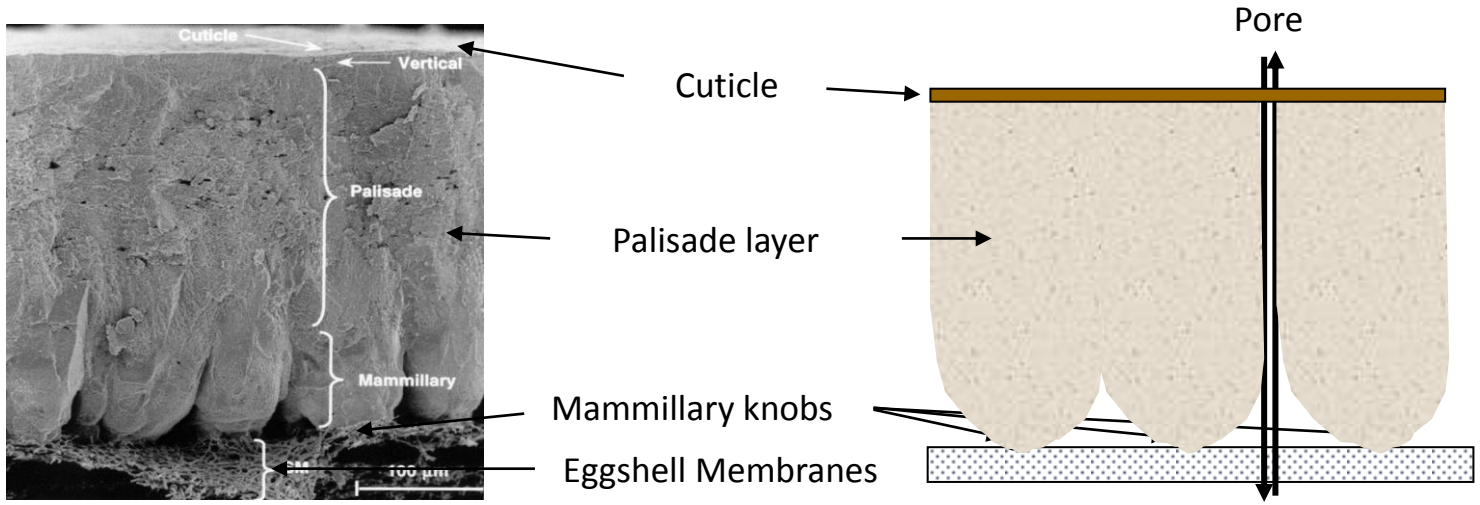
Need for shell calcification	**0**	**--+**	**+++**	**+++**	**+++**	**+++**
Availability of calcium	**+++**	**+++**	**0**	**0**	**0**	**0**

Régulation of calcium metabolism in laying hens

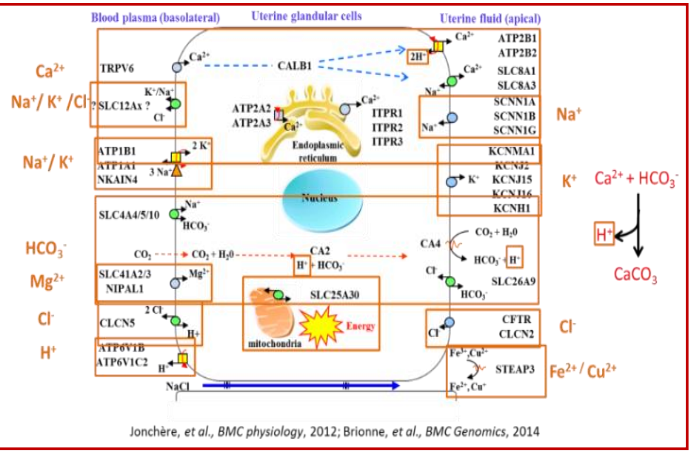


The eggshell formation

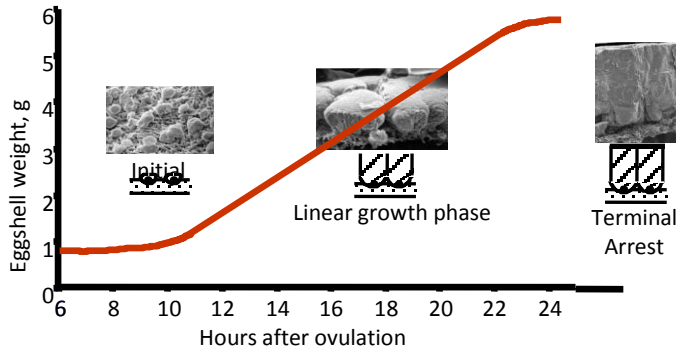
- ✓ Eggshell biomineralization in uterus (fast process)
- ✓ 5-6 g of mineral (calcium carbonate) are deposited within a 20 h period



Mineral supply



Biomineralization



Mineral supply

3 Potential pathways

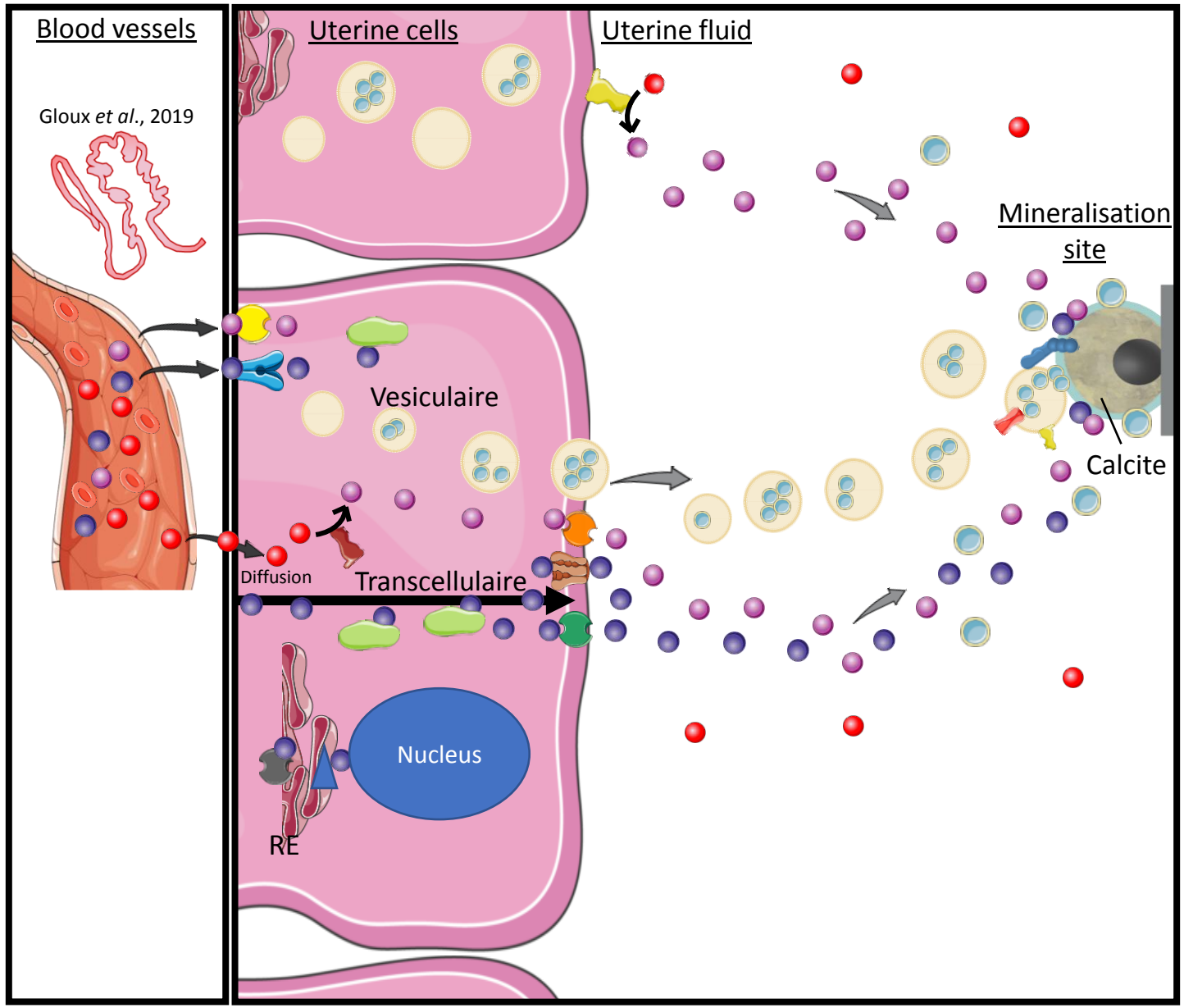
Transcellular

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

Vésicular

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

?

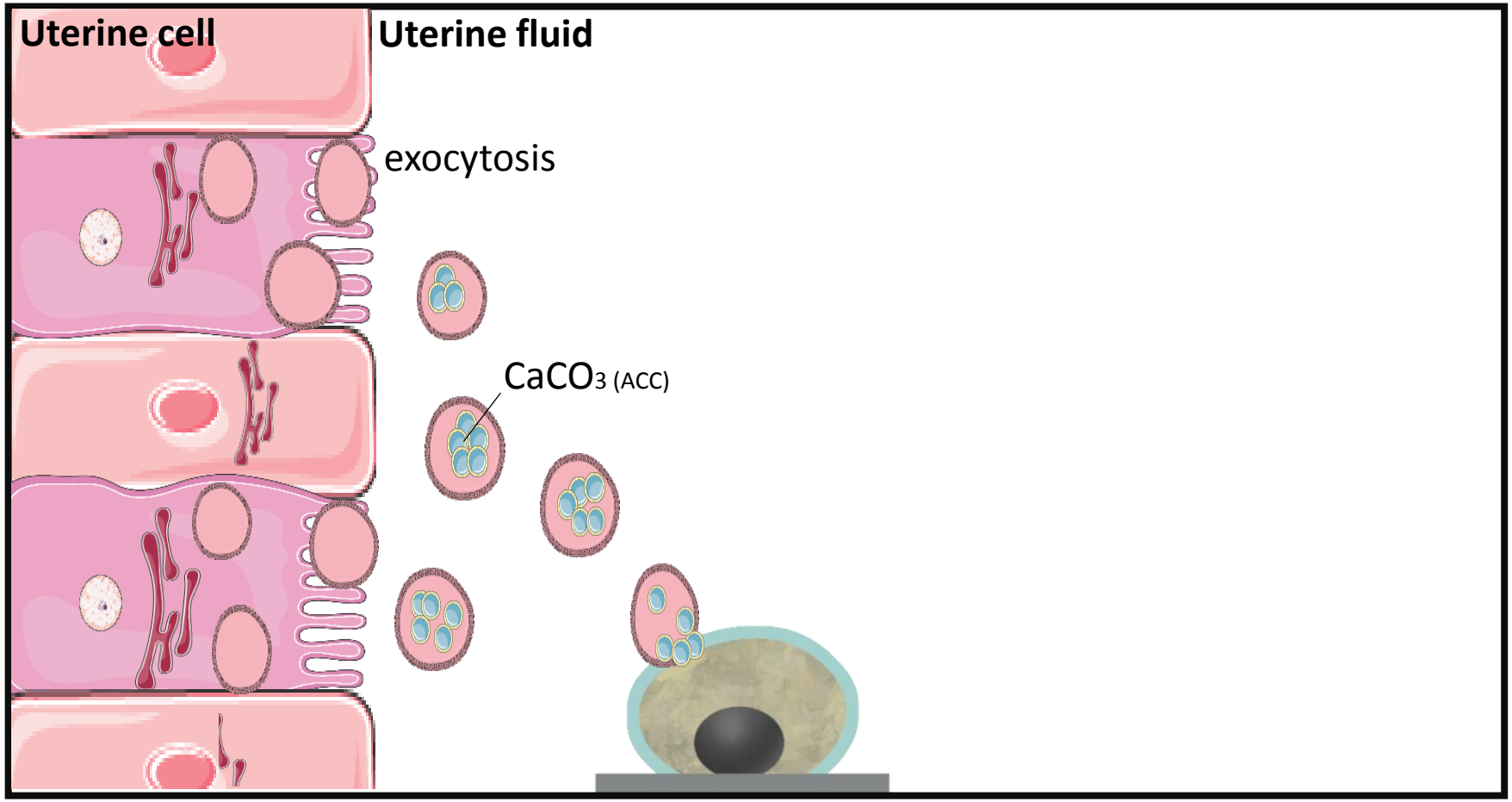


Adapted from L..Stapane-D-17/12/19

Hodges et Lörcher 1967; Jonchère *et al.*, 2012; Brionne *et al.*, 2014; Nys et Le Roy, 2018

Eggshell biomineralization

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



Stapane 2019

Eggshell biomineralization

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

EDIL3

ANXA1

ANXA2

ANXA8

CA4

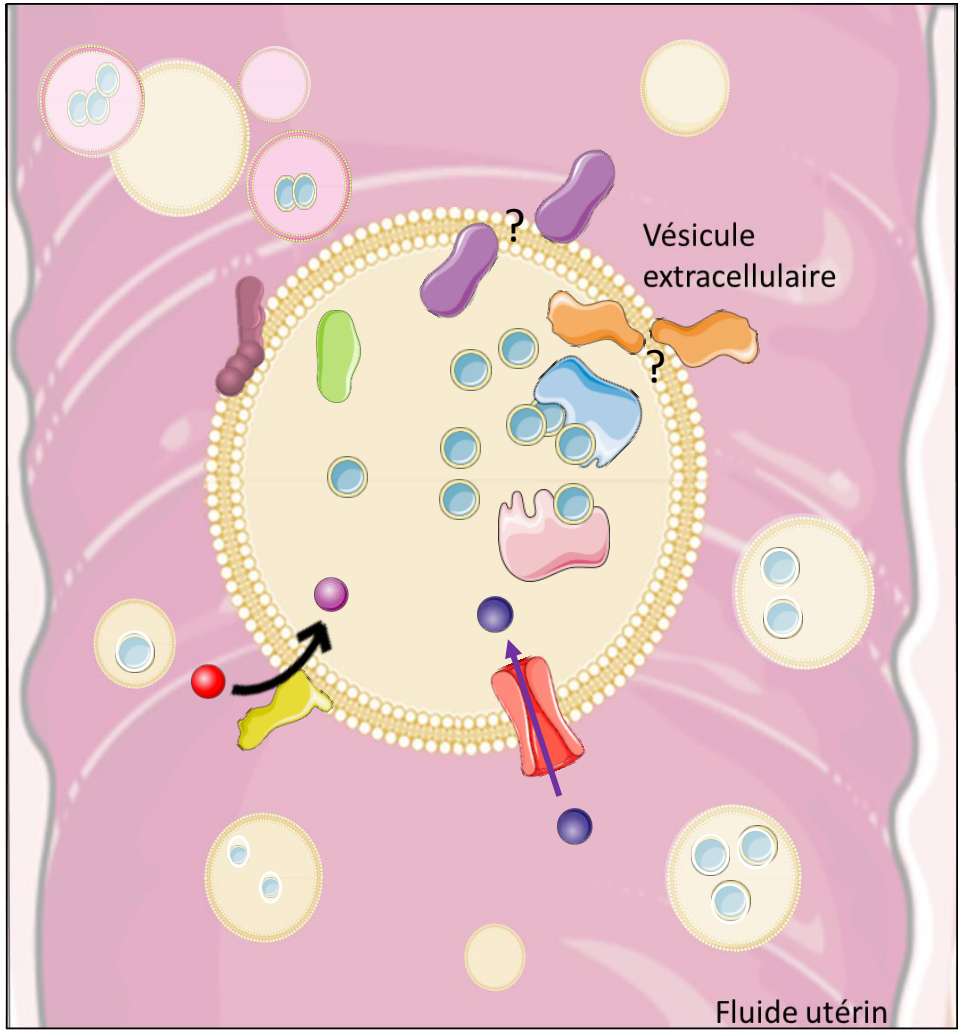
PDCD6IP

Syntenin-1

Ovalbumin

Ezrin

Lysozyme



ANX supplied Ca^{2+} ?

CA4 supplied HCO^{3-} ?

LYZ and OVA stabilized ACC ?
EDIL3 adress vésicles ?

CO_2

HCO_3^-

Ca^{2+}

ACC

Adapté de L.Stapane-Doctorat-17/12/19

Mineral supply

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Transcellular

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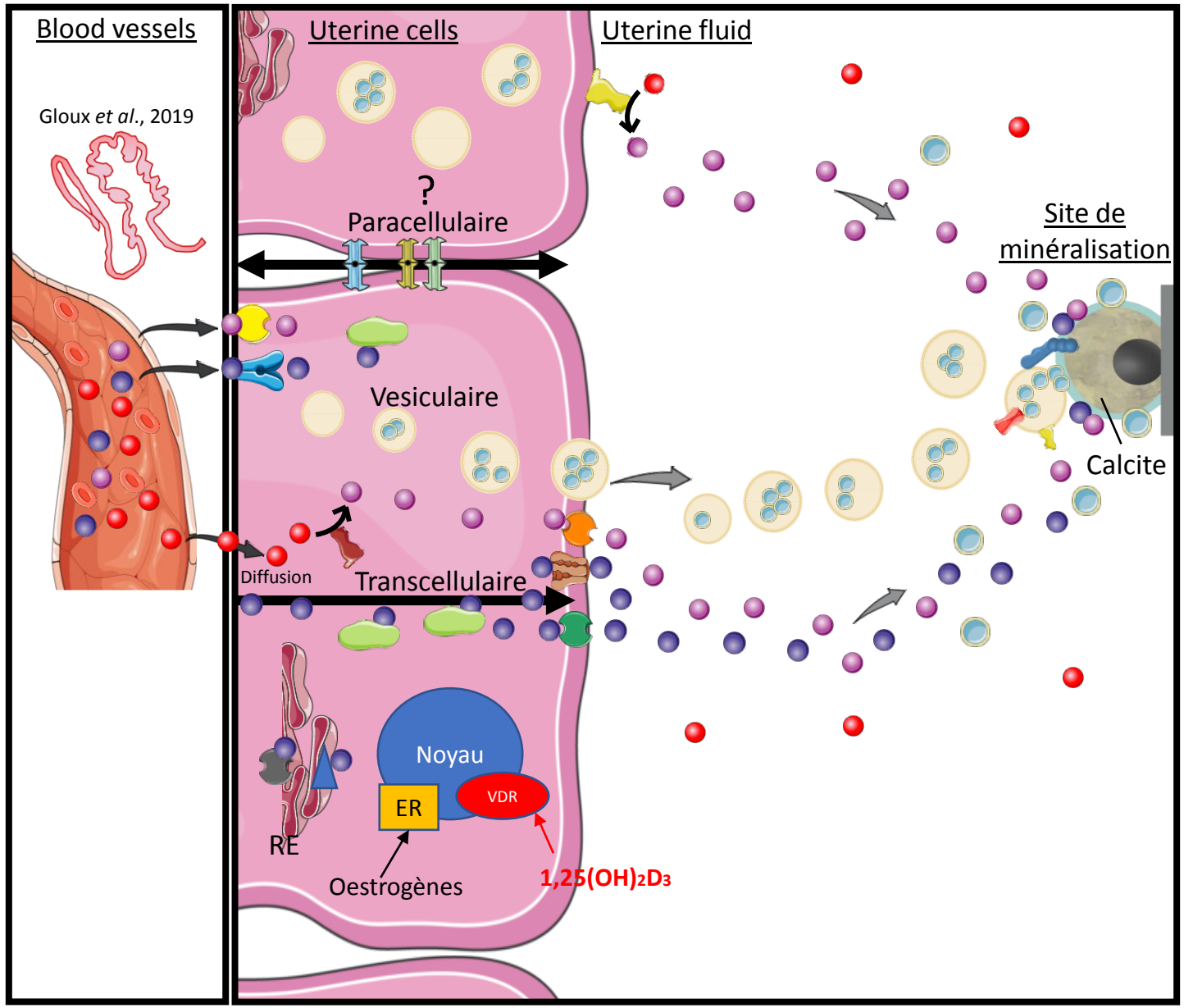
Vésicular

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

Paracellular ?

- *Claudins*
- *JAM*
- *Occludin/TJP*

- HCO_3^-
- Ca^{2+}
- CO_2
- ACC



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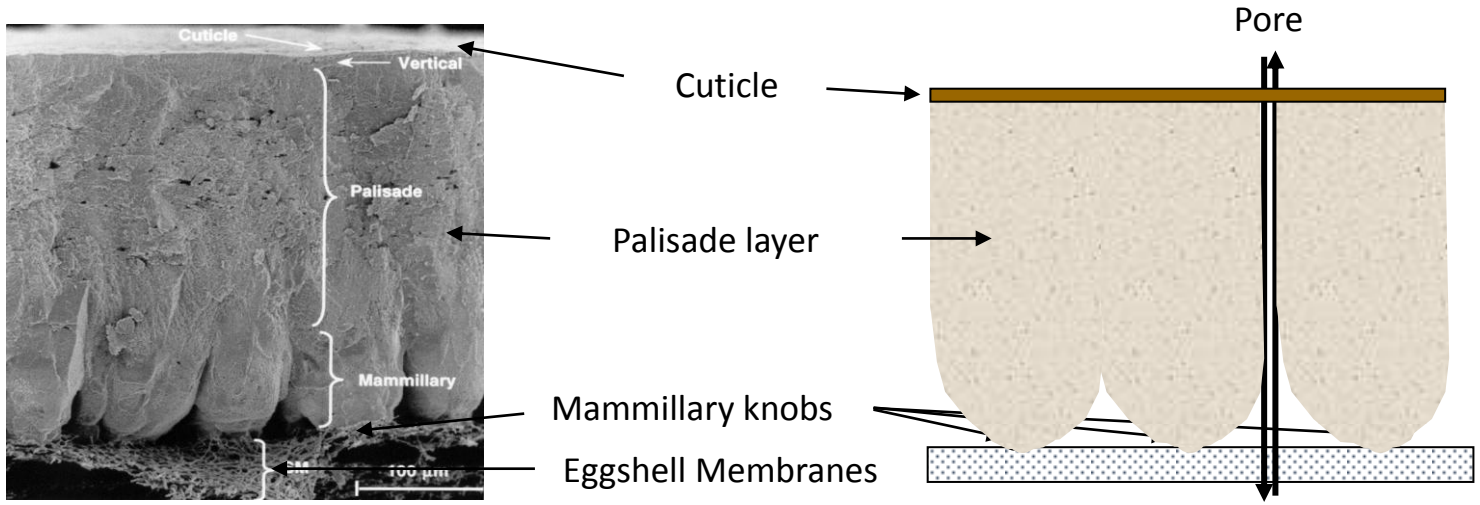
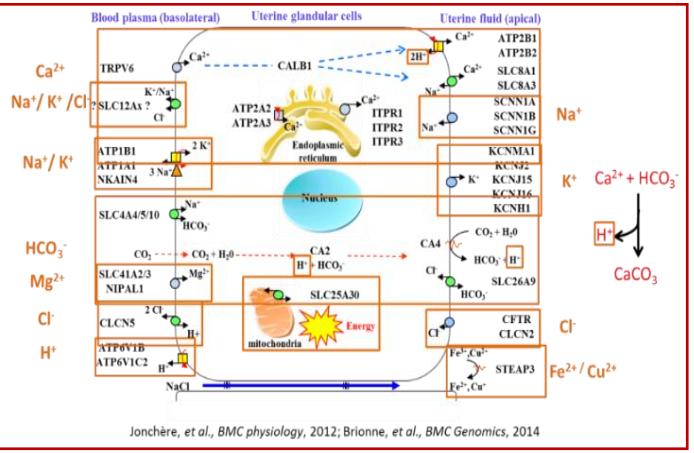
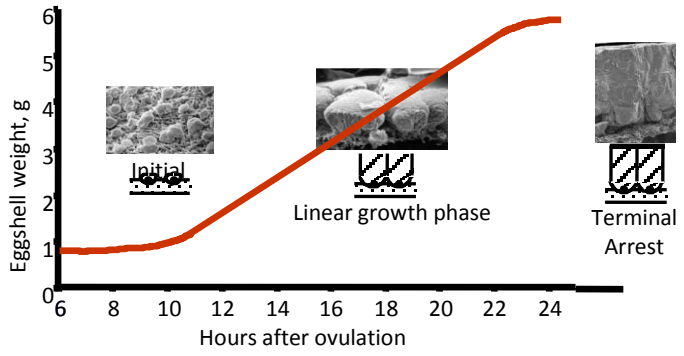


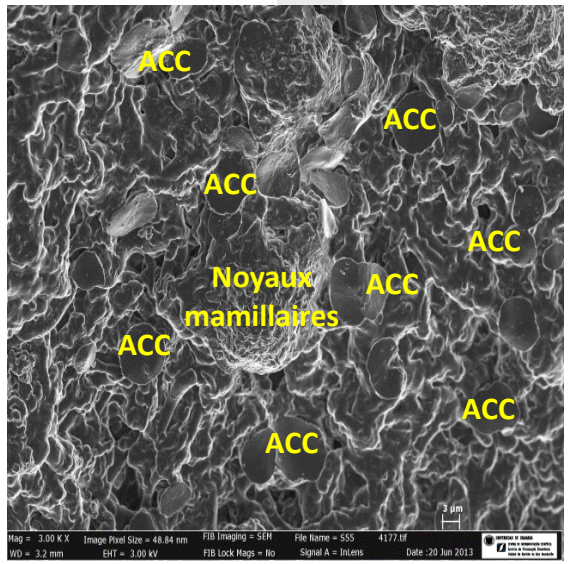
Mineral supply



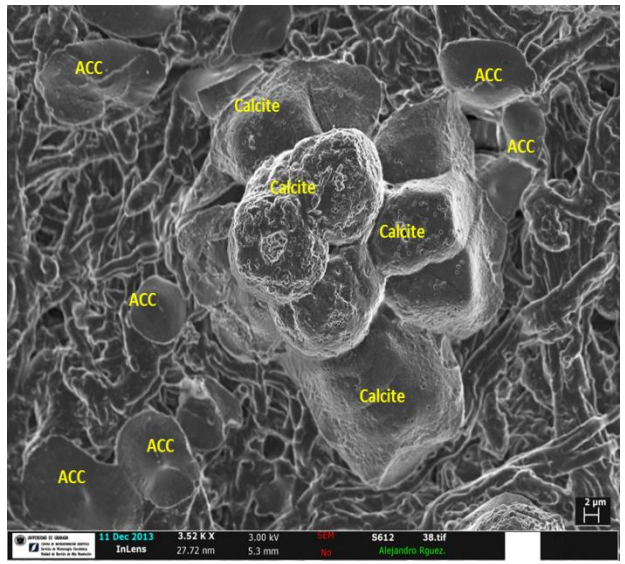
Biomineralisation



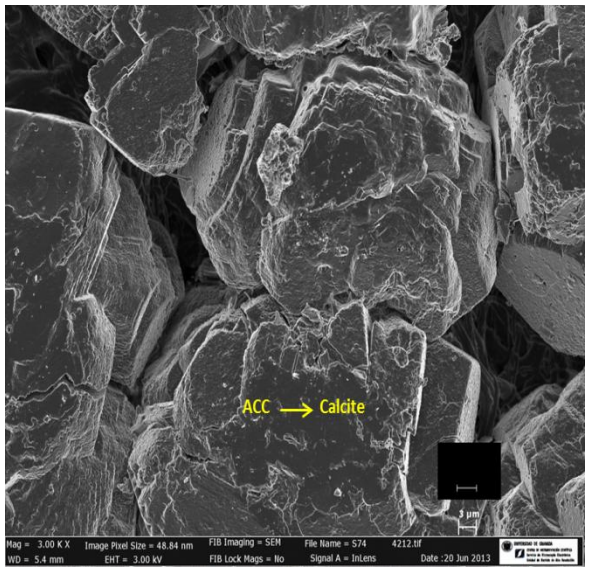
Eggshell biomineralization



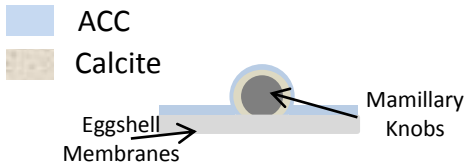
First events of nucléation

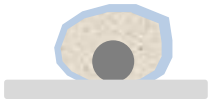


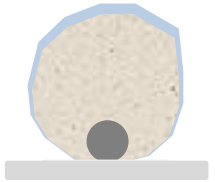
Calcite formation



Larger calcite crystal units deposition







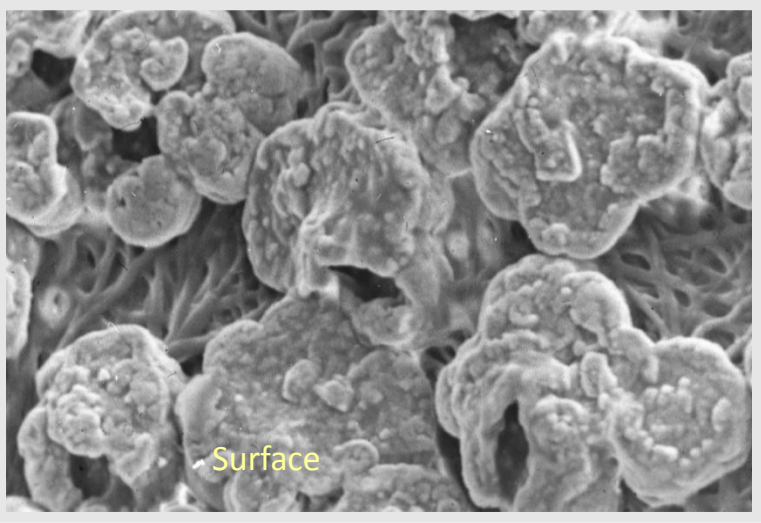
Time 1 (5-6 h Post ovulation):
ACC particles nucleate on the whole eggshell membranes.
Form massive deposits

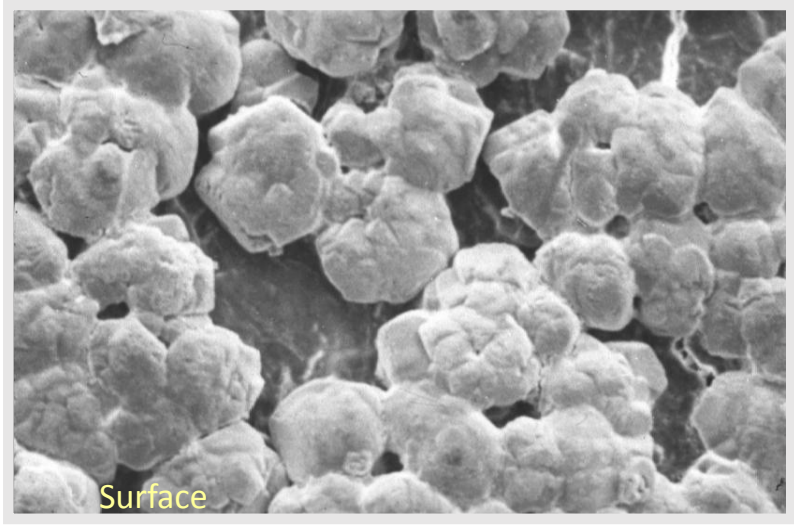
Time 2 (6-7 h post ovulation):
Interface-coupled dissolution precipation process
Direct transformation of ACC into calcite aggregates on mammillary knobs

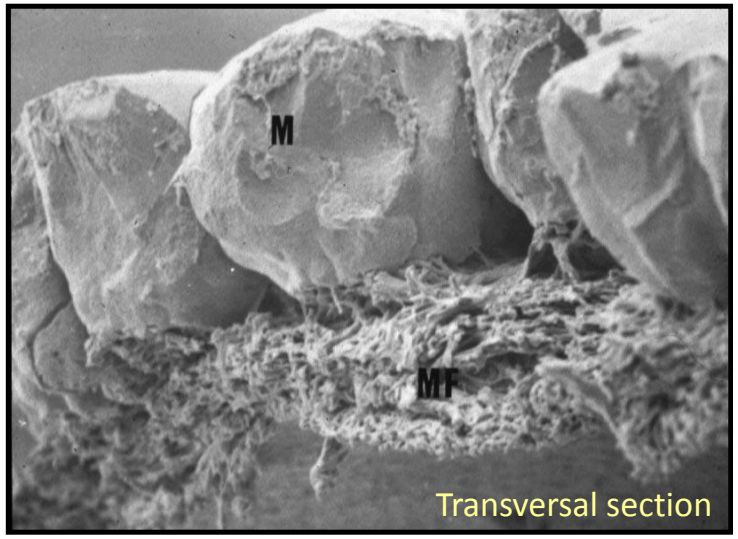
Time 3 (>7h post ovulation):
Additional cristallisation events on calcite template

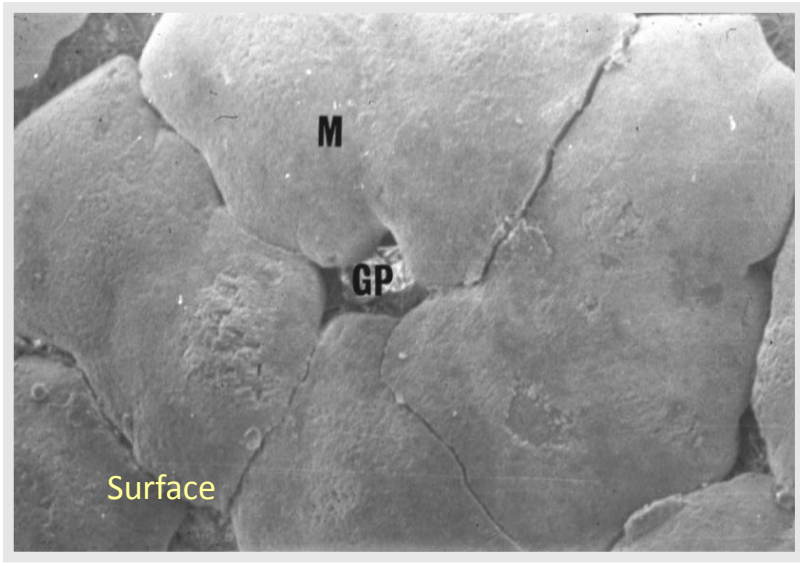
Rodriguez-Navarro et al., Journal of structural Biology, 2015

Eggshell biomineralization



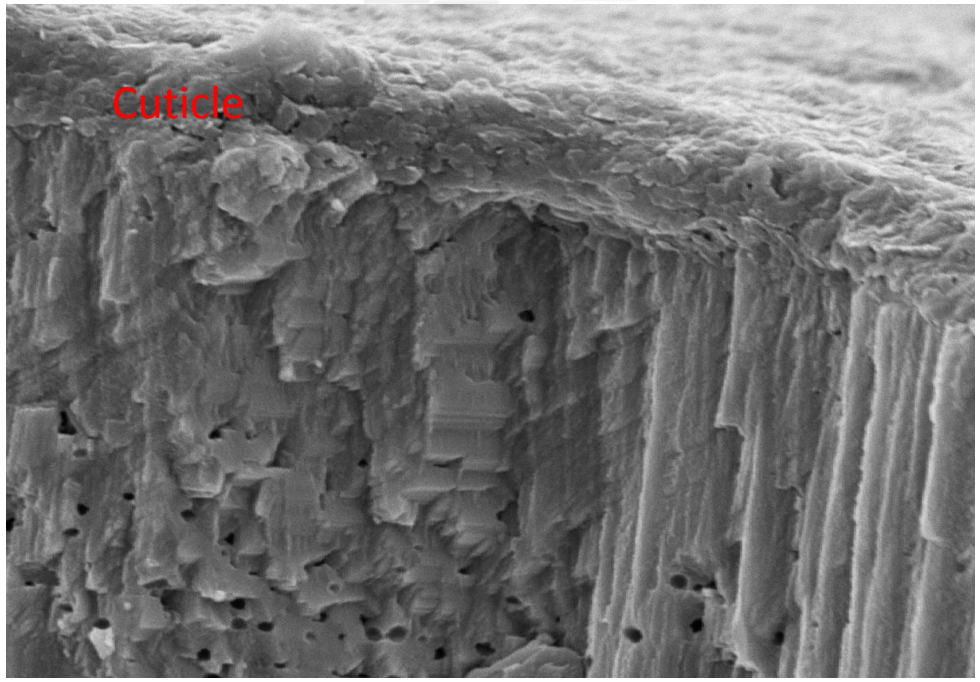


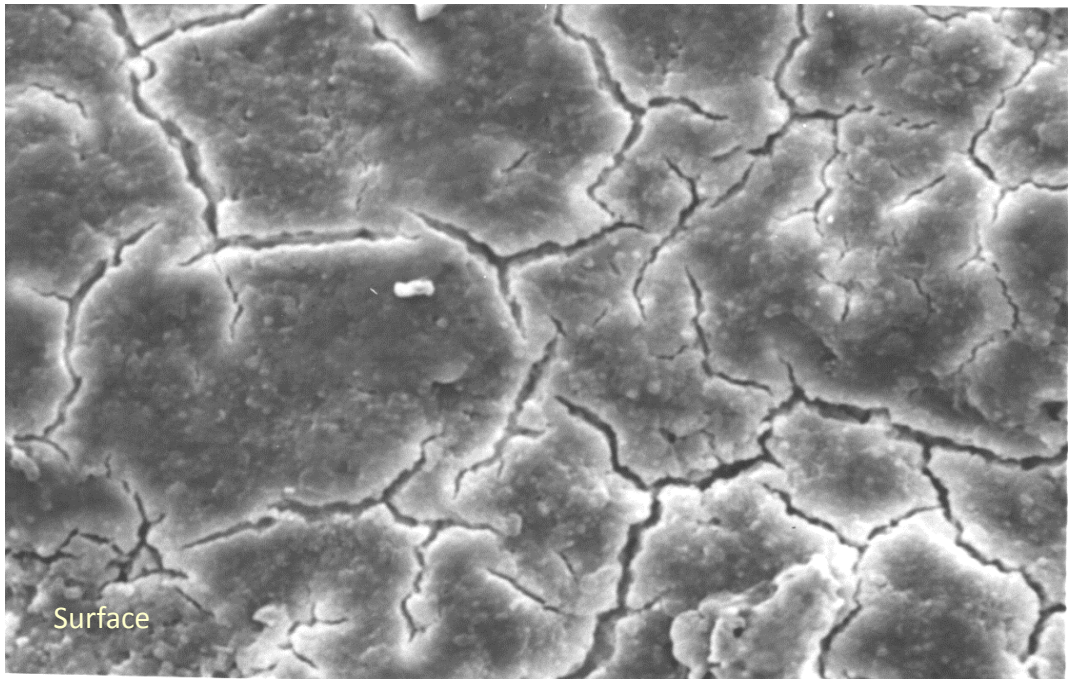




Time 4 (7-10h post ovulation):
Caclcite deposition and fusion of adjacent cônes

Eggshell biomineralization

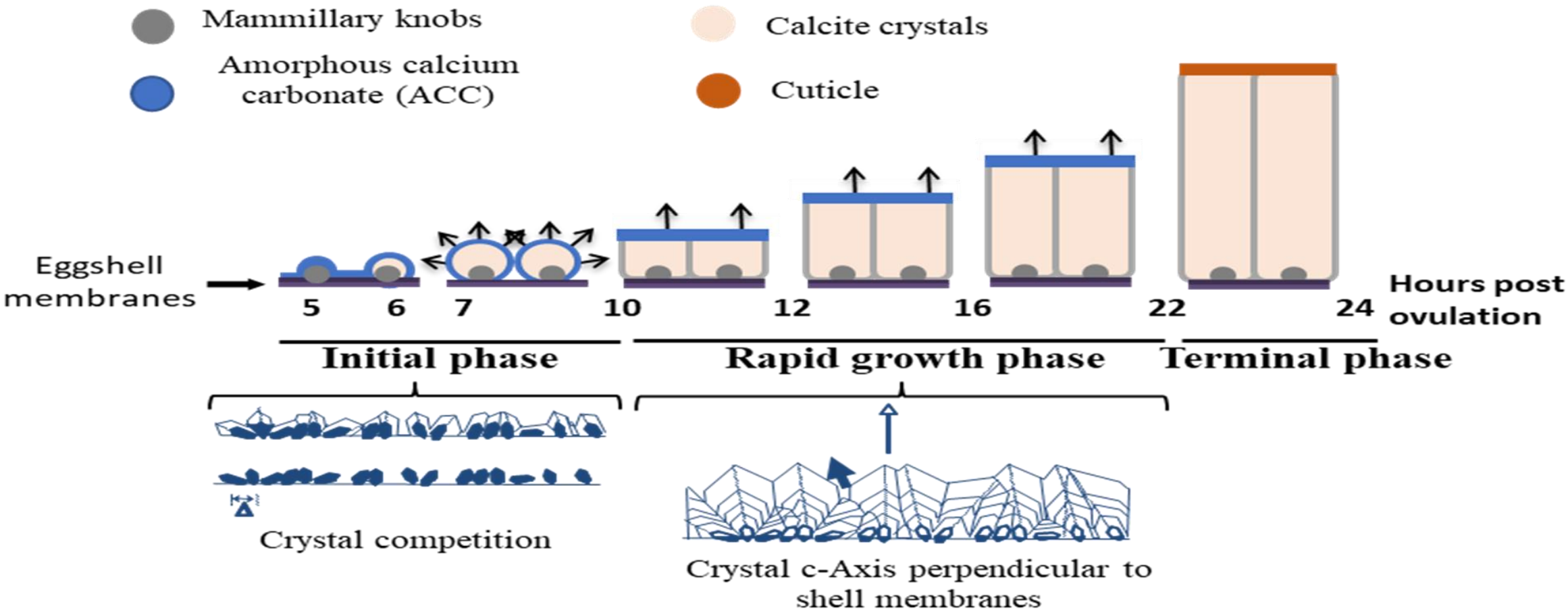




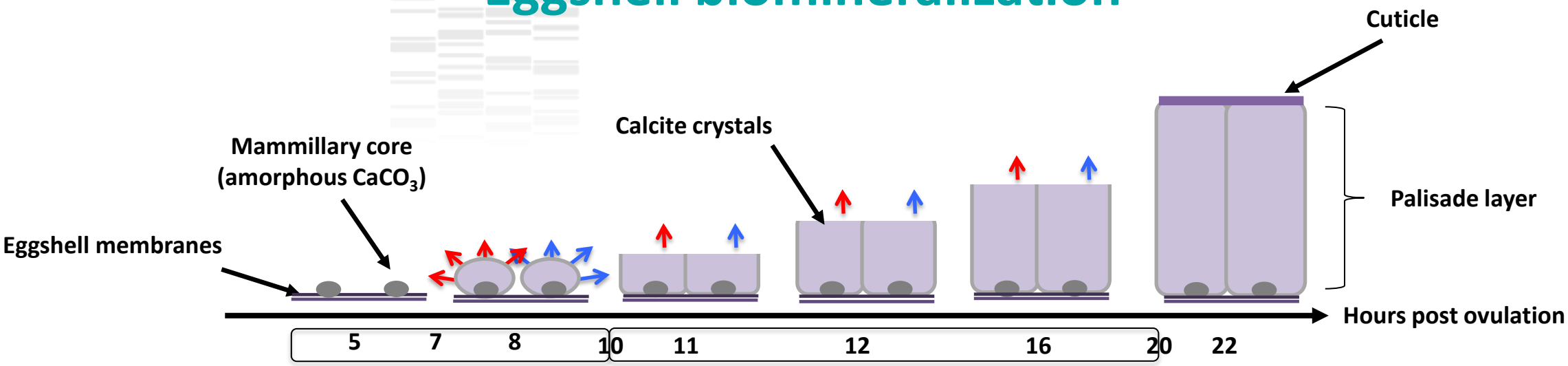
Time 5 (11 to Oviposition):

- 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)**

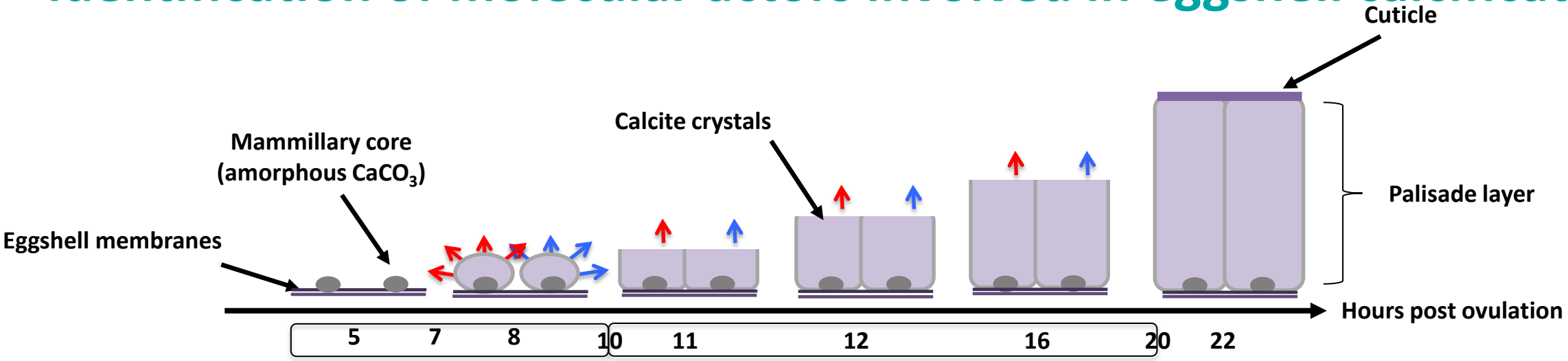
Ultrastructure, Mechanical properties

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

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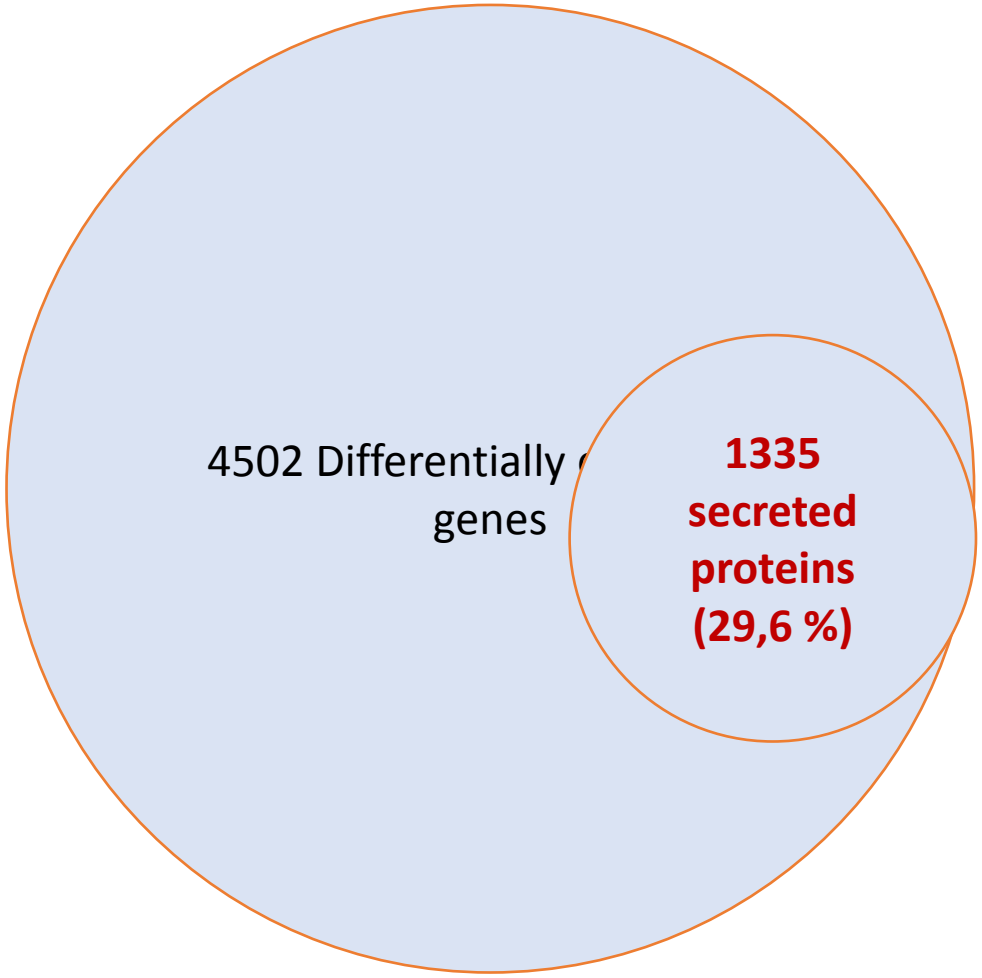
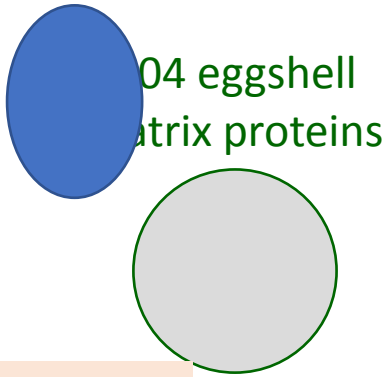
ANR
Impact
(2013-2017)

Identification of molecular actors involved in eggshell calcification





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

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Impact (2013-2017)

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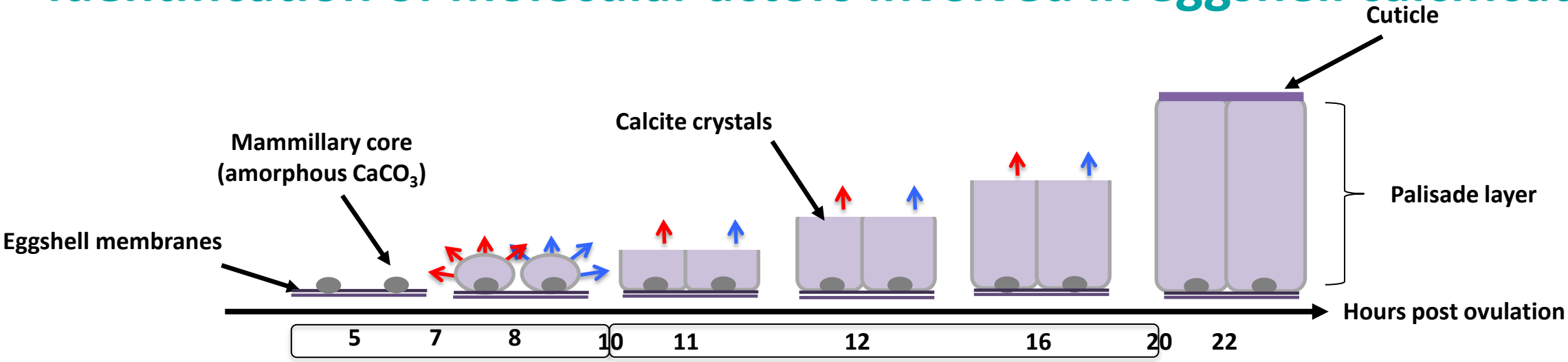

444 Main candidate proteins to be involved in the shell biomineralization process

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

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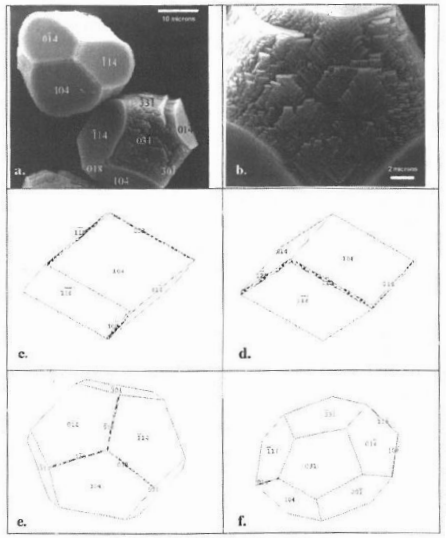
Impact (2013-2017)

Eggshell biomineralization

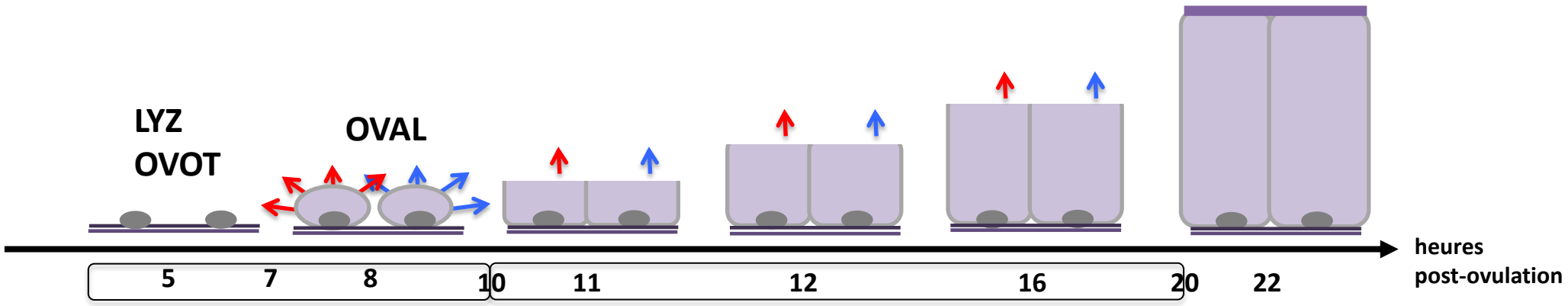
❑ Proteins having a direct involvement in eggshell mineralization

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



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

J. GAUTRON[a], M.T. HINCKE[b], M. PANHELEUX[a], J.M. GARCIA-RUIZ[c], T. BOLDICKE[d] and Y. NYS[a,*]



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Freeman et al, 2010

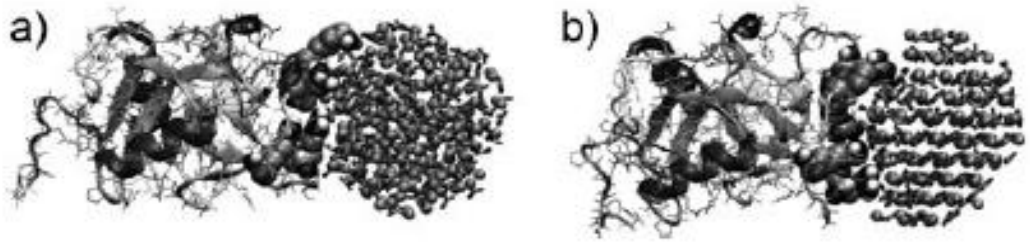
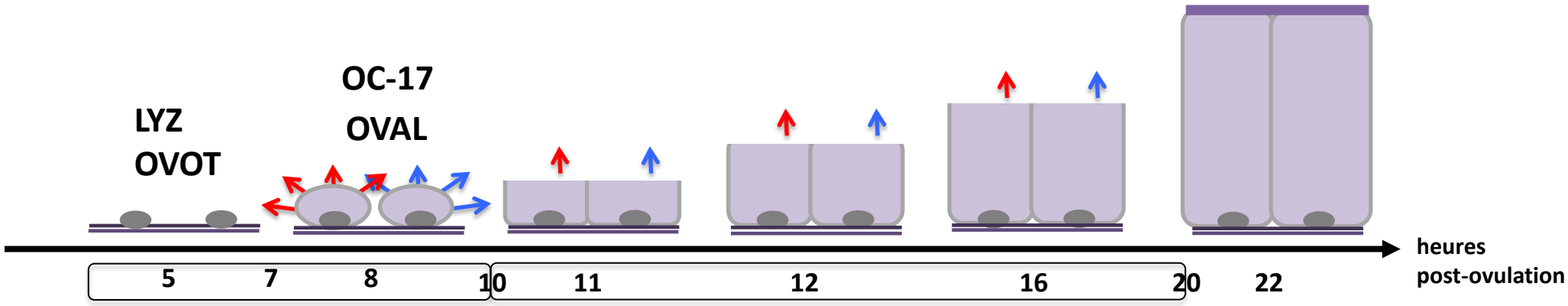


Figure 1. Ovocleidin-17 bound to an amorphous (a) and a crystallized (b) calcium carbonate nanoparticle containing 192 formula units. The



Eggshell biomineralization

❑ Proteins having a direct involvement in eggshell mineralization

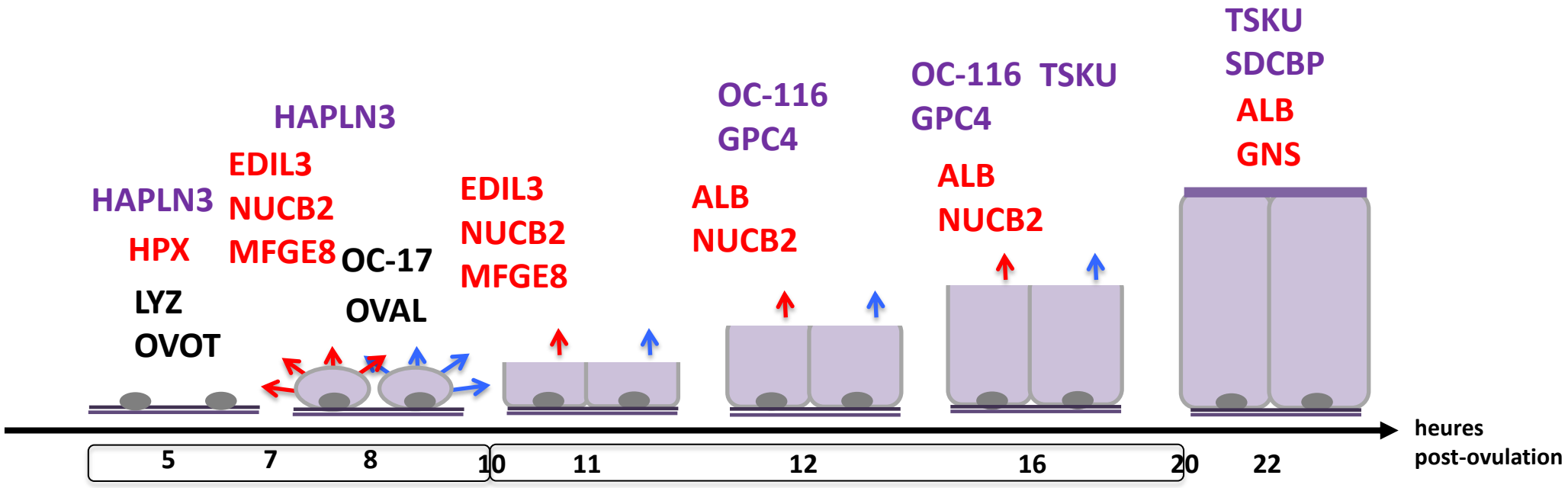
✓ Proteins with established role in the **biomineralisation**

✓**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 Ca2+ ions



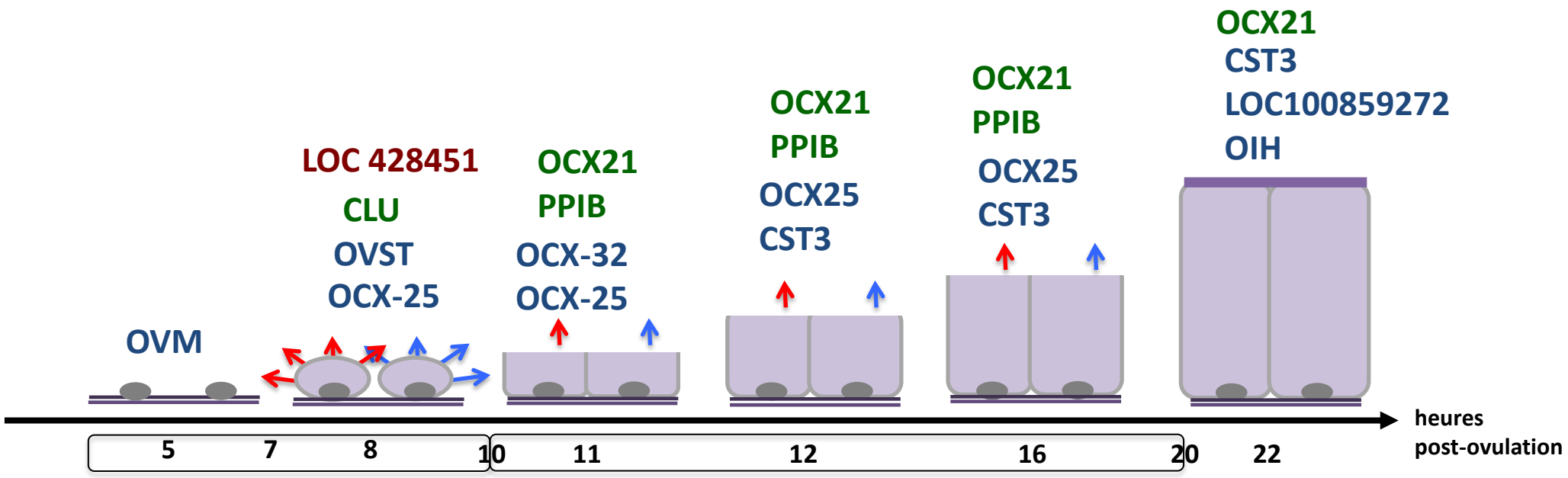
Eggshell biomineralization

❑ 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

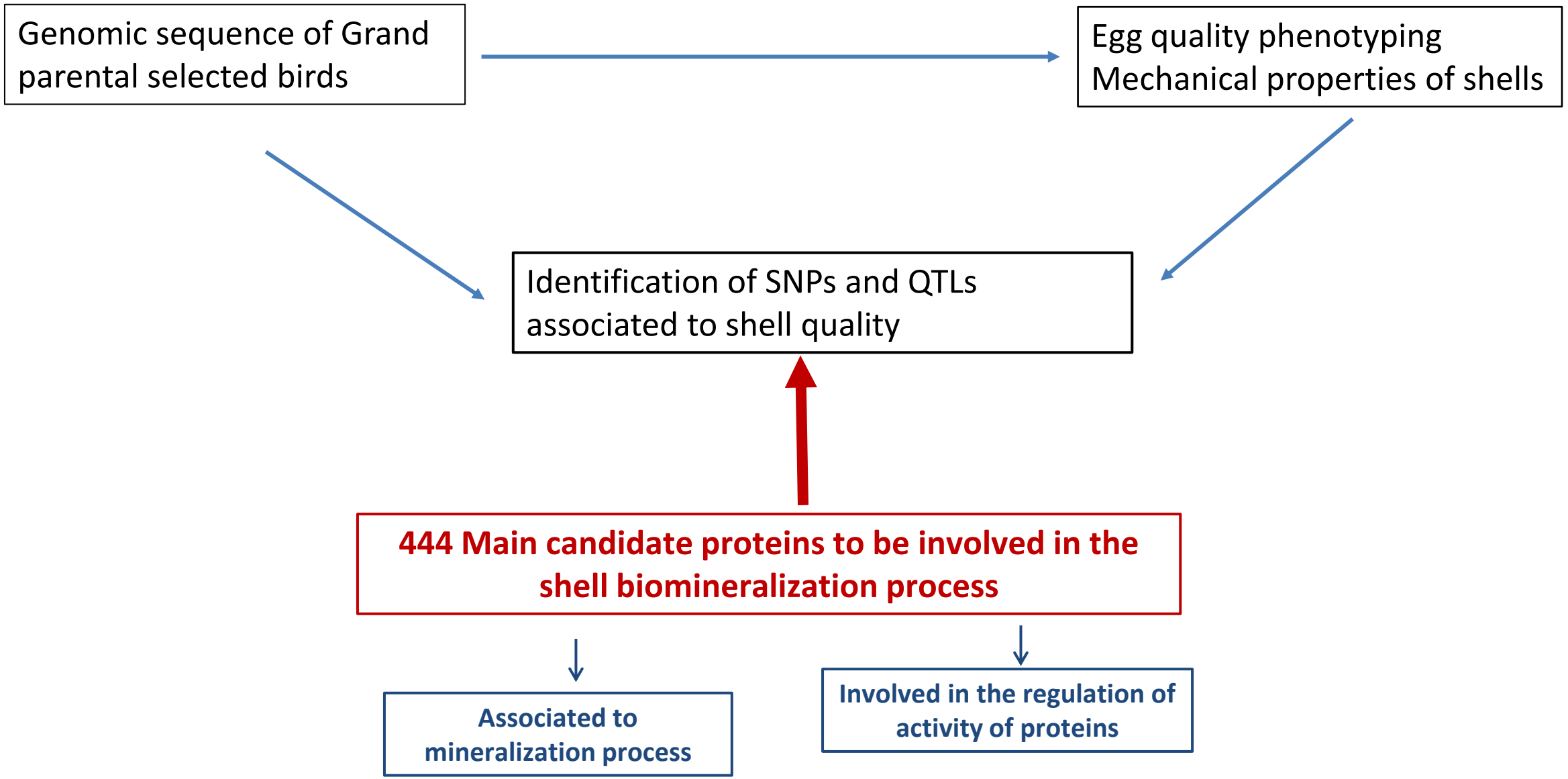
- Mapping genes coding matrix proteins to detect polymorphisms and haplotype related to good quality shell

Candidate Genes of eggshell calicification in laying hens (CACAO)

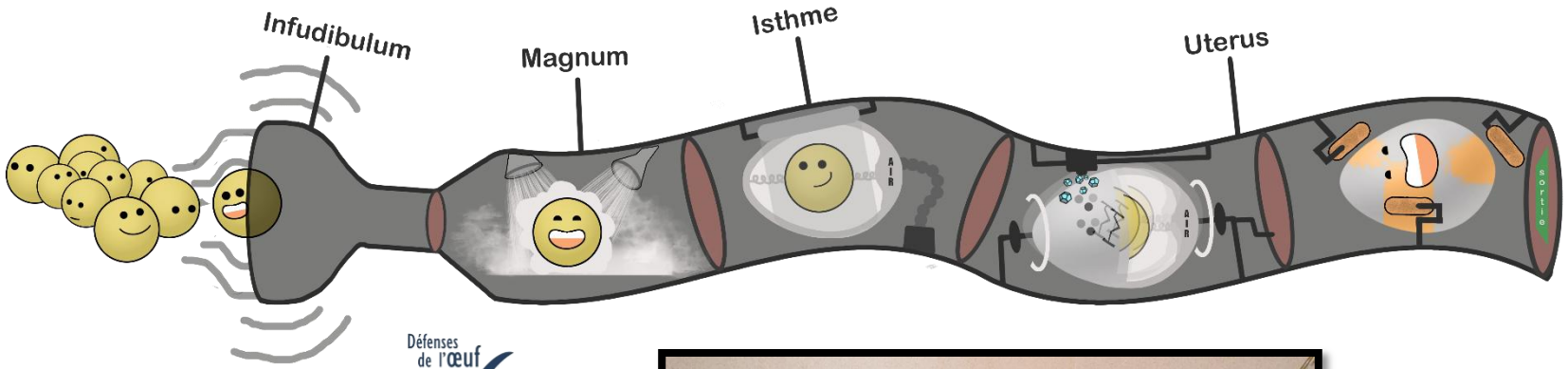
Défenses de l'œuf
DOVE
Valorisation Evolution

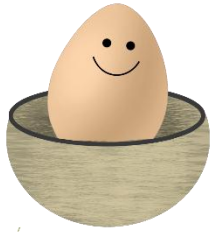
Eggshell Calcification Polymorphism Candidates (POLCACAO)

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