Understanding eggshell formation to maintain its quality in laying hens

Joël Gautron, Michel J Duclos, A.J. Rodriguez-Navarro

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Understanding eggshell formation to maintain its quality in laying hens

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USV 1842

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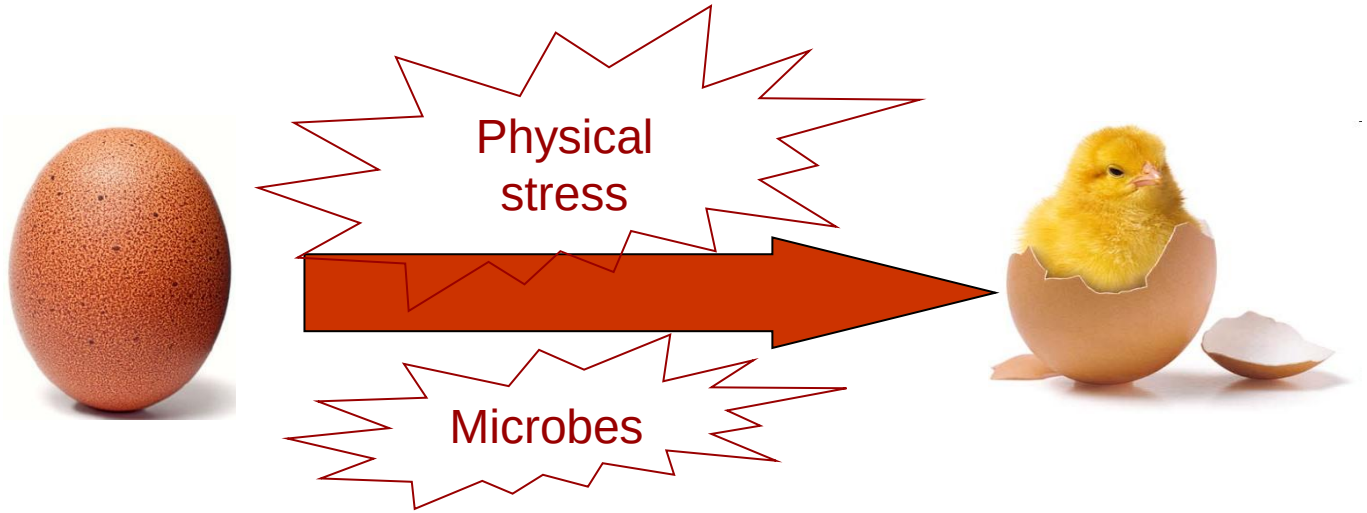
Socio-economic context

Eggs

An autonomous close chamber to allow the embryo development



Must contains the entire components necessary for embryo

- Well-balanced nutritious ingredients
- Lot of compound (> 1000) with a broad range of biological activities
- Protective systems (natural defenses)
  - Physical defense (Mainly shell)
  - Chemical defense (Proteins with antimicrobial activities)

Table eggs

A basic ingredient for human food

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Socio-economic context

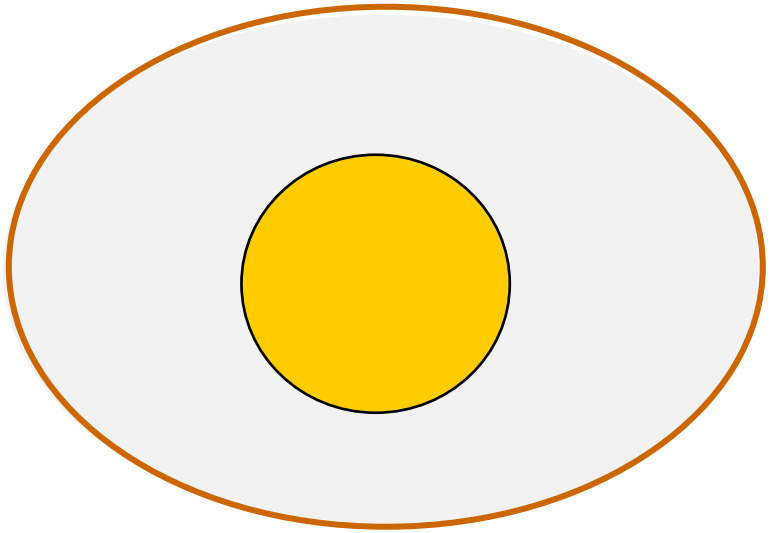
Table eggs

A basic ingredient for human food

- The cheapest animal food of high nutritional quality
- No religious prohibition
- 68.2 MT of egg produced each year in the word > 1400 billions eggs each year
- 14,7 billions eggs in France each year

Eggshell in the socio-economic context of the egg

Eggshell is the only non-consumable part of an egg…..



… Nevertheless, its quality is crucial for the marketing of the egg

Eggshell in the socio-economic context of the egg

Economic issues

Downgraded eggs due to deteriorated egg quality

Dirty, cracked or broken shells

Hatchability of the chick

Shell allows gas exchanges during embryo development

Health issues

Risks of toxi-infections for the consumer (Salmonellosis)

Eggshell as a physical barrier

Ethical issues

Housing systems and societal demand

Consumer demand for non cage and free-range systems → Lower shell quality

Long life cycle (shell quality decreases with age of birds)

Maintenance and improvment of SHELL QUALITY

Shell mechanical properties

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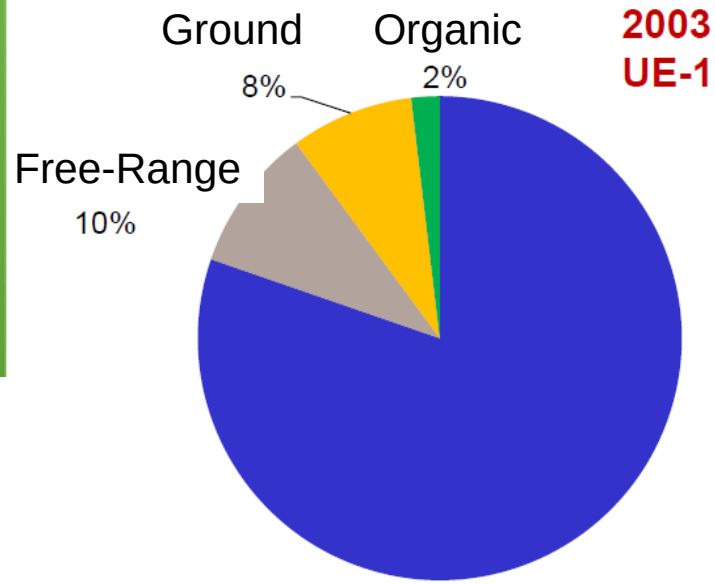
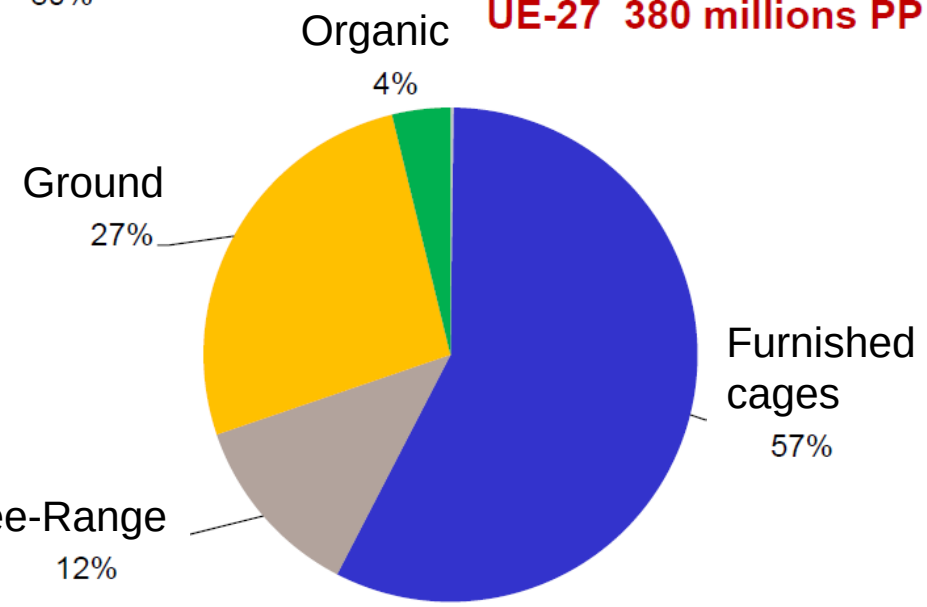
Evolution of egg production systems in UE

Evolution of egg production systems in Europe

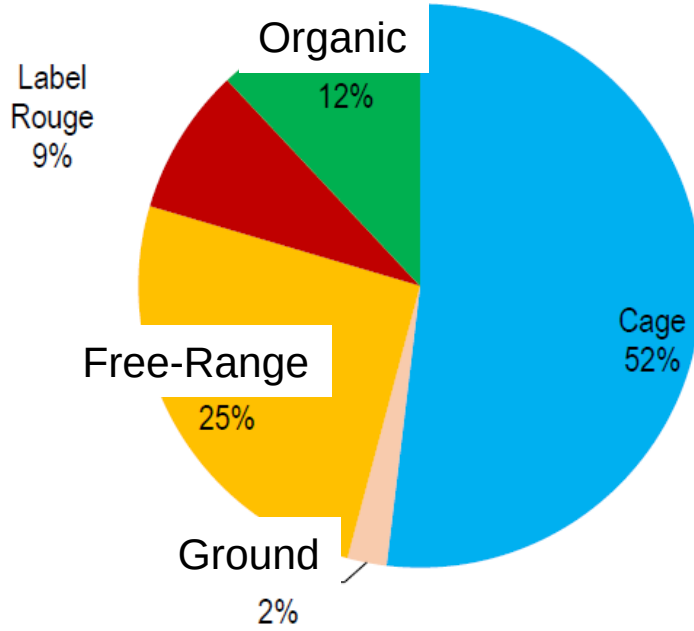


2003
UE-15 244 millions PP



2013
UE-27 380 millions PP

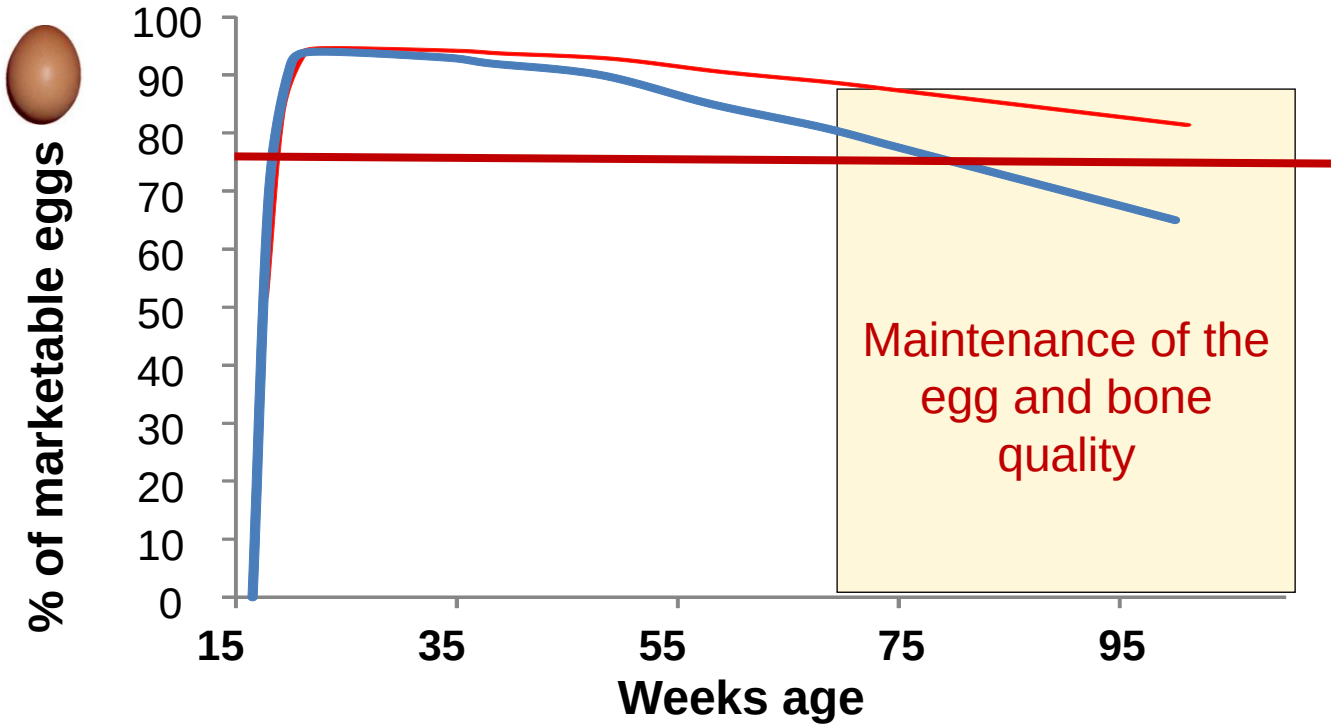
France 2017



Source Commission européenne

Increasing persistency of laying hens

Breeding companies claim that they will have developed the « long life » layer, which will be capable of producing 500 eggs in a production cycle lasting 100 weeks by 2020 (Van Sambeek, 2010)

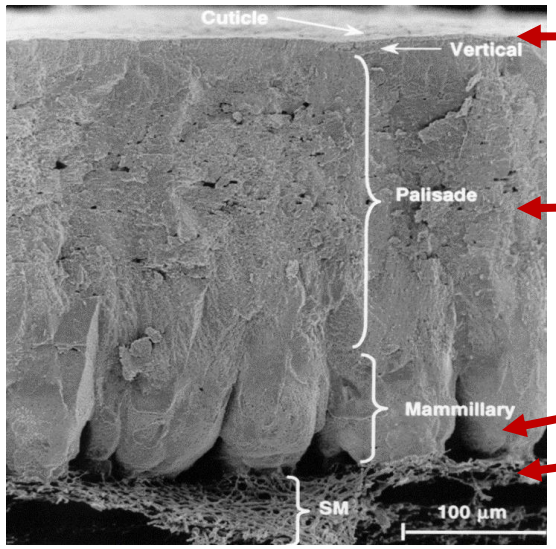


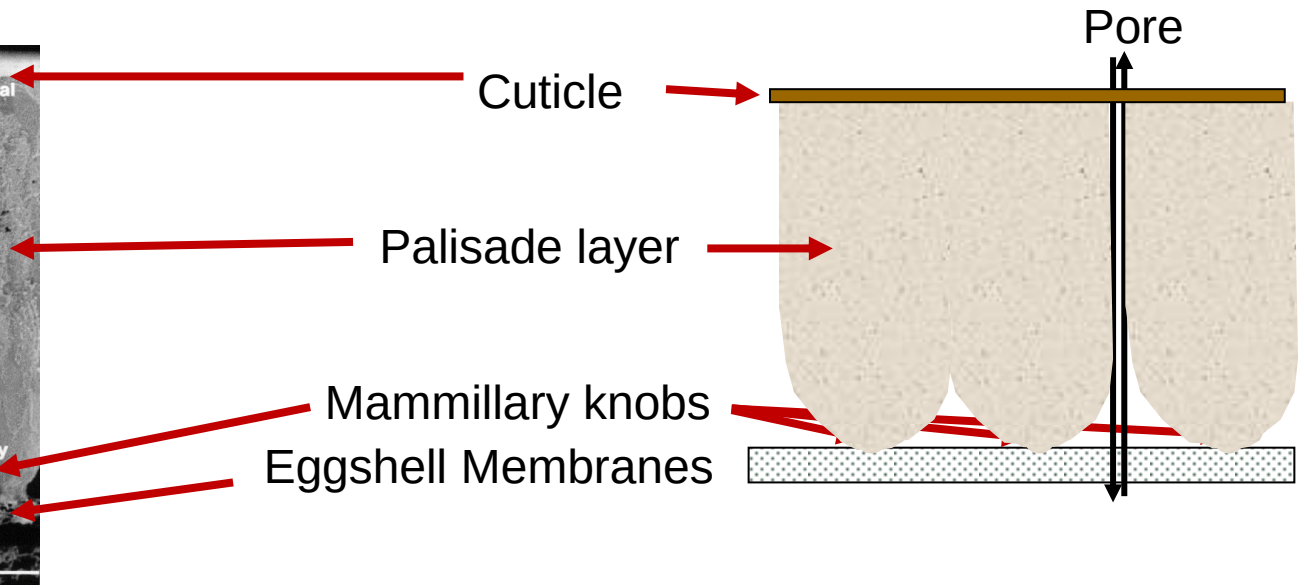
75 % of marketable eggs is the beneficial limit

Bain et al., 2016 estimated « than even 25 more eggs per hen could potentially reduce the UK flock, including breeding hens by 2,5 millions birds per annum. »

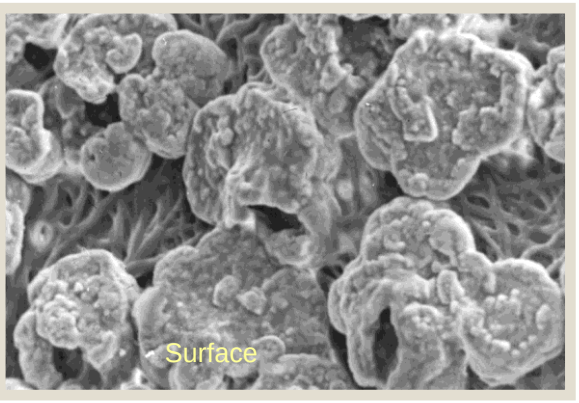
The eggshell formation

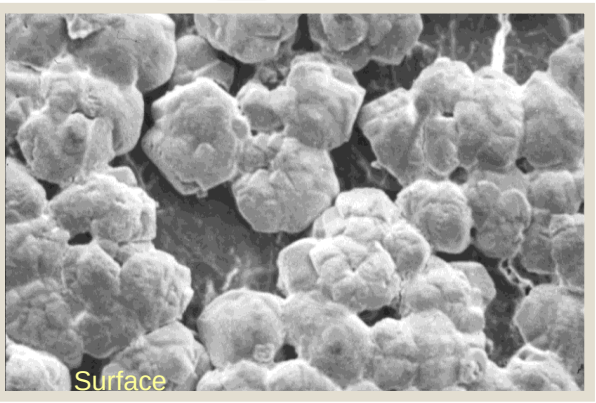
- ✓ Eggshell biomineralization in uterus (fast process)
- ✓ 5-6 g of mineral (calcium carbonate) are deposited within a 20 h period

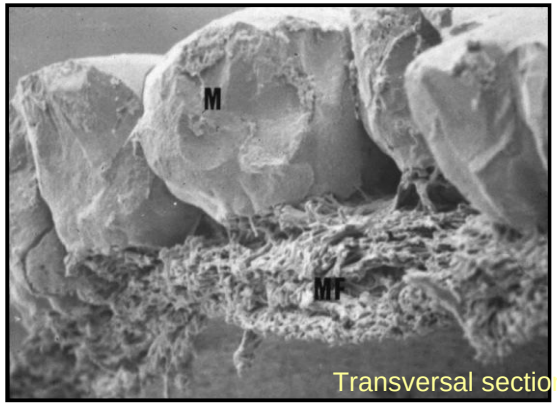


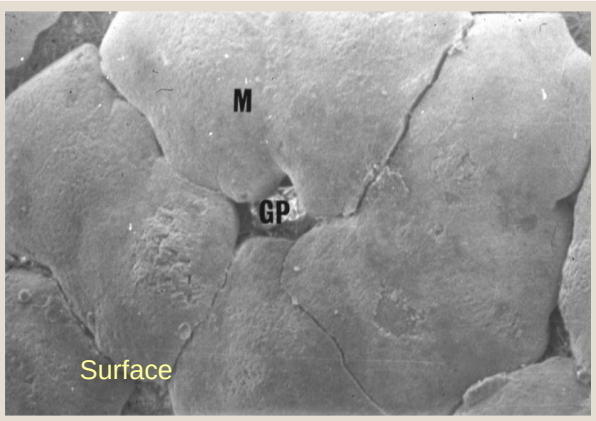


The eggshell minéralization

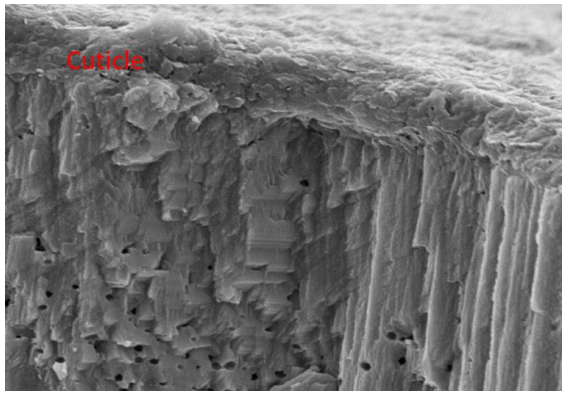




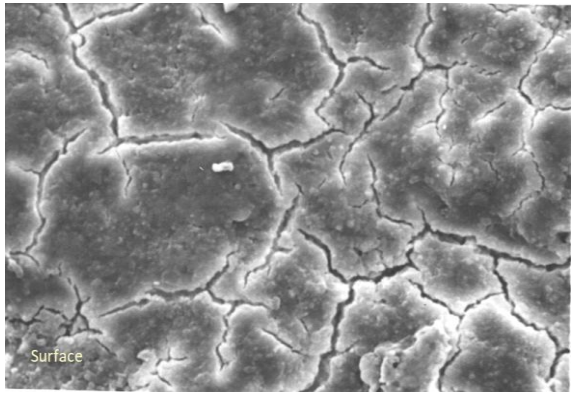




7-10h post ovulation : Calcium carbonate (calcite) deposition and fusion of adjacent cones



From 12 h post ovulation to lay (oviposition)
Formation of the compact layer (palisadic layer)
Cuticle deposition



Oviposition
Drying of cuticle and formation of cracks allowing gaz exchanges

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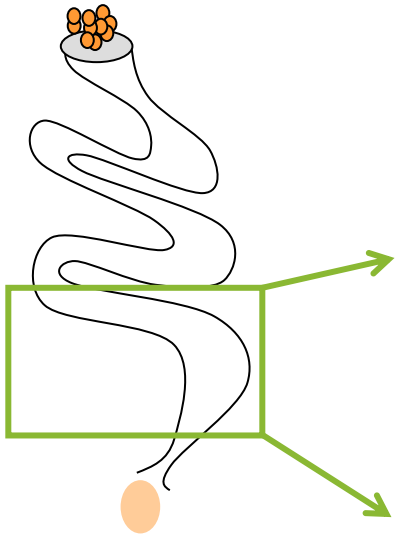
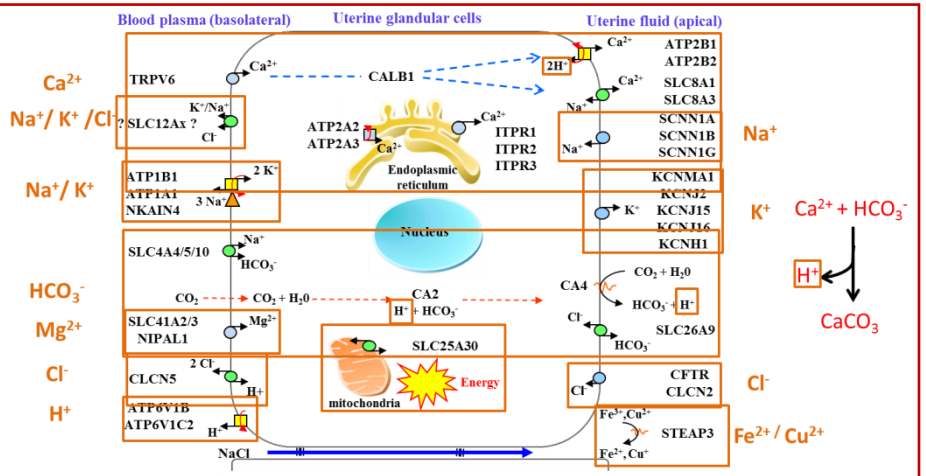
Eggshell formation in uterus

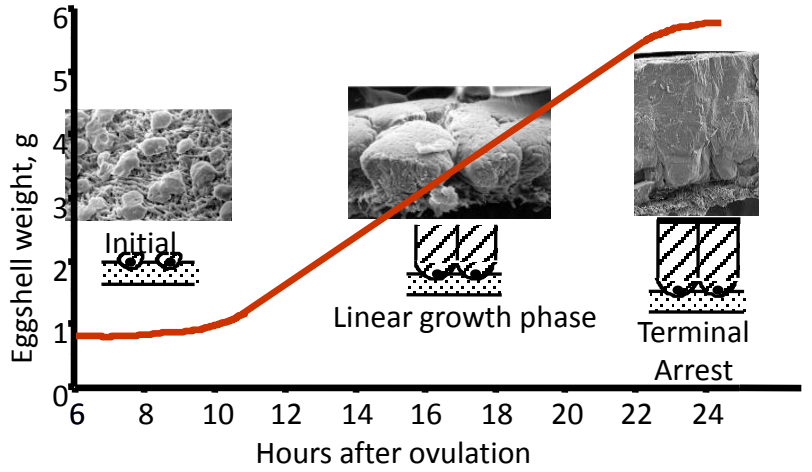


Supply of minerals for shell mineralization

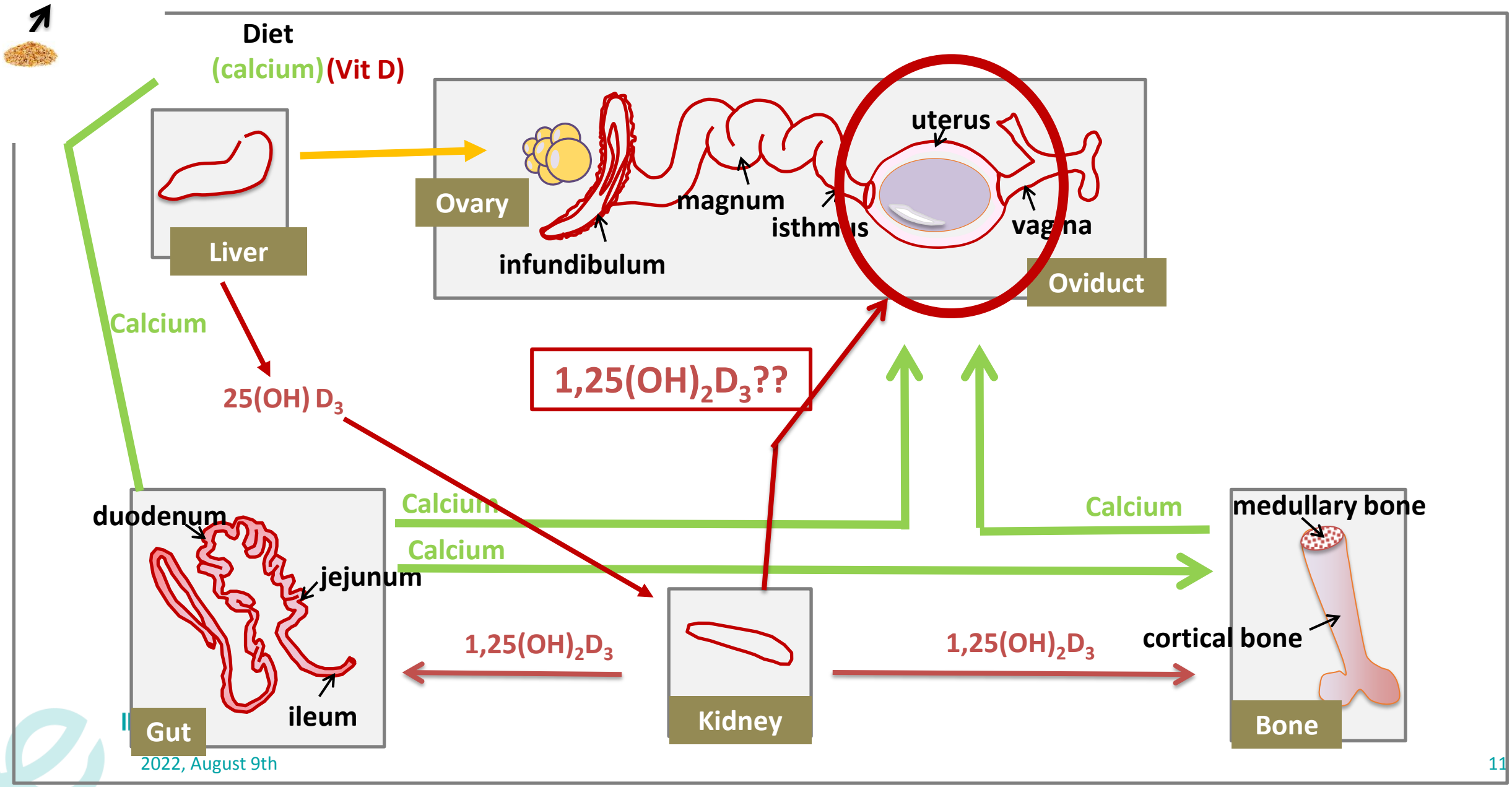


Jonchère, *et al., BMC physiology*, 2012; Brionne, *et al., BMC Genomics*, 2014

Shell calcification (biomineralization)



Vitamin D and Ca metabolism during shell calcification



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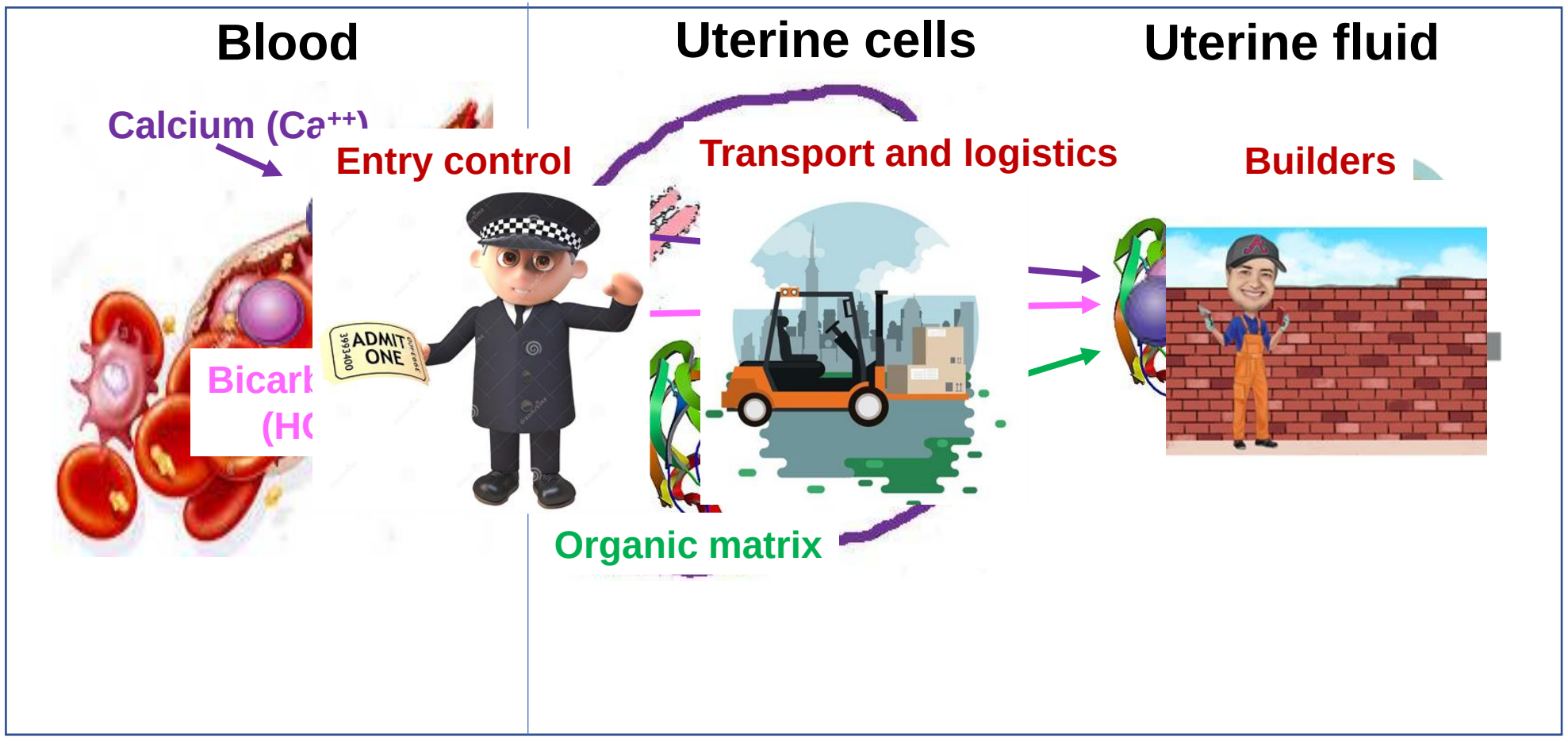
Regulation of shell calcification

$Ca^{++} + HCO_3^- \rightarrow CaCO_3 + H^+ + \text{Organic matrix} \rightarrow \text{Shell}$



Regulation of 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*

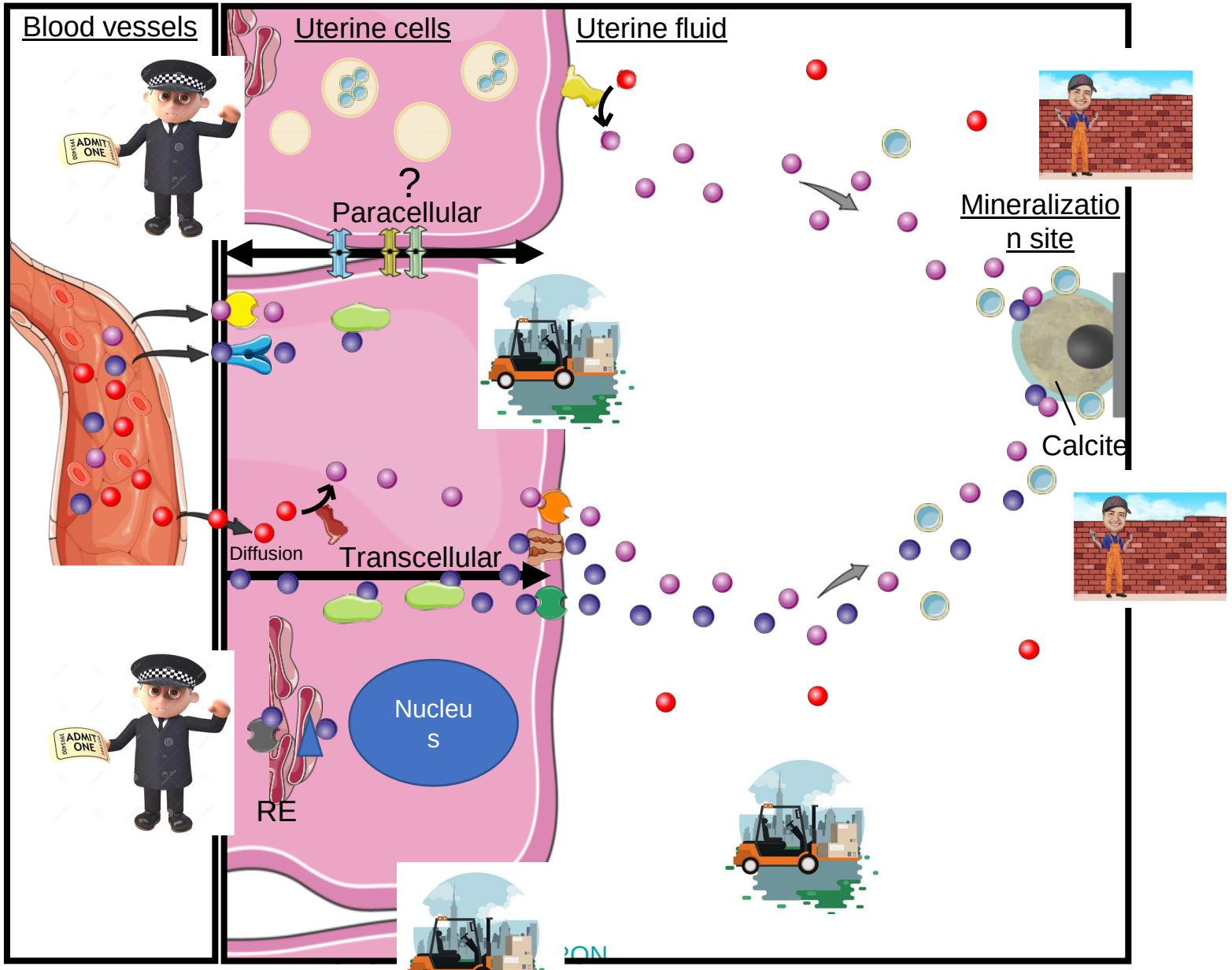
Vesicular

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

Paracellular ?

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

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



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

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

3 Potential pathways

Regulation of mineral supply

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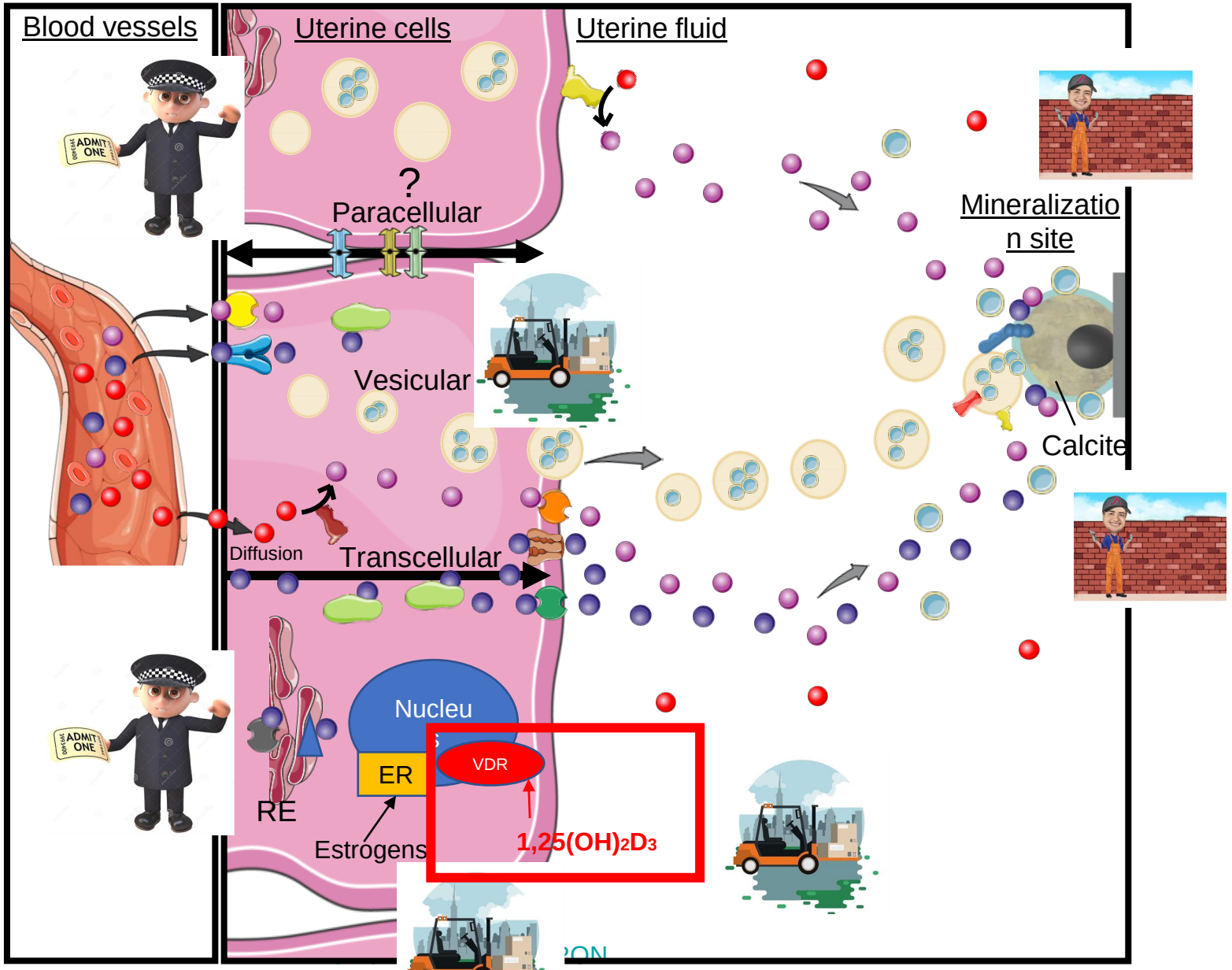

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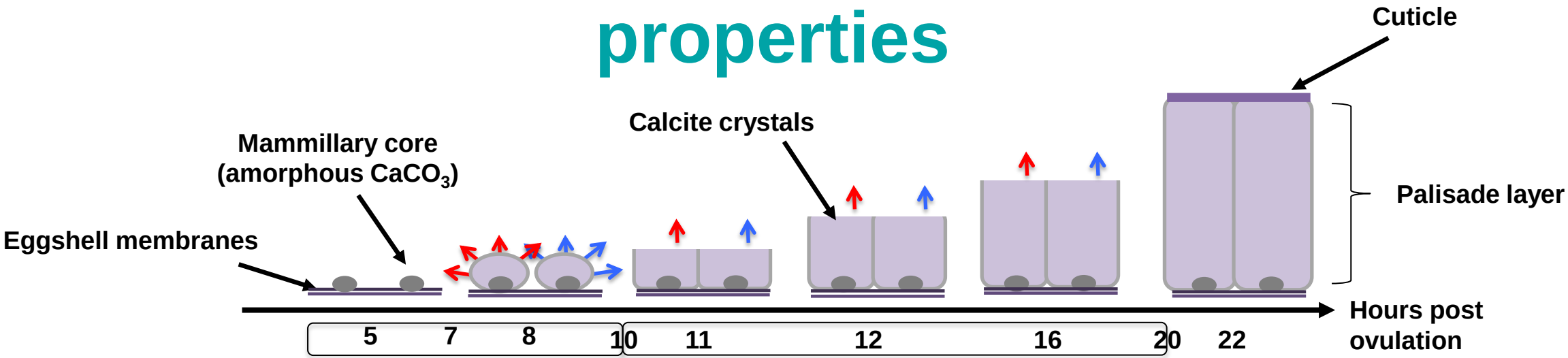
Eggshell biomineralization and mechanical properties



95 % of calcium carbonate (calcite) ← Interaction → 3.5 % organic matrix (proteins, proteoglycans)

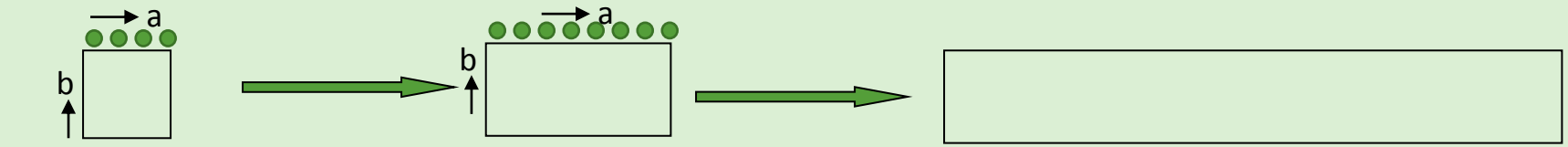
Quantity → **Mechanical properties** ← Ultrastructure

Eggshell biomineralization and mechanial properties

3.5 % organic matrix → About 900 proteins in the shell (Gautron et al., 2019)

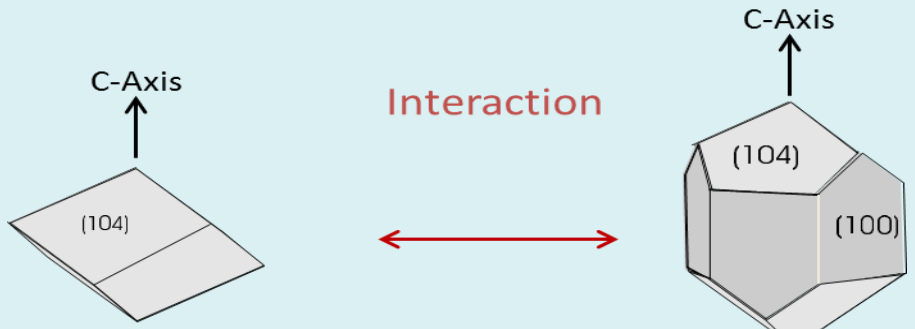
Stabilize the amorphous calcium carbonate (ACC), controls polymorphs, morphology and size of crystals

Theoretical example for understanding



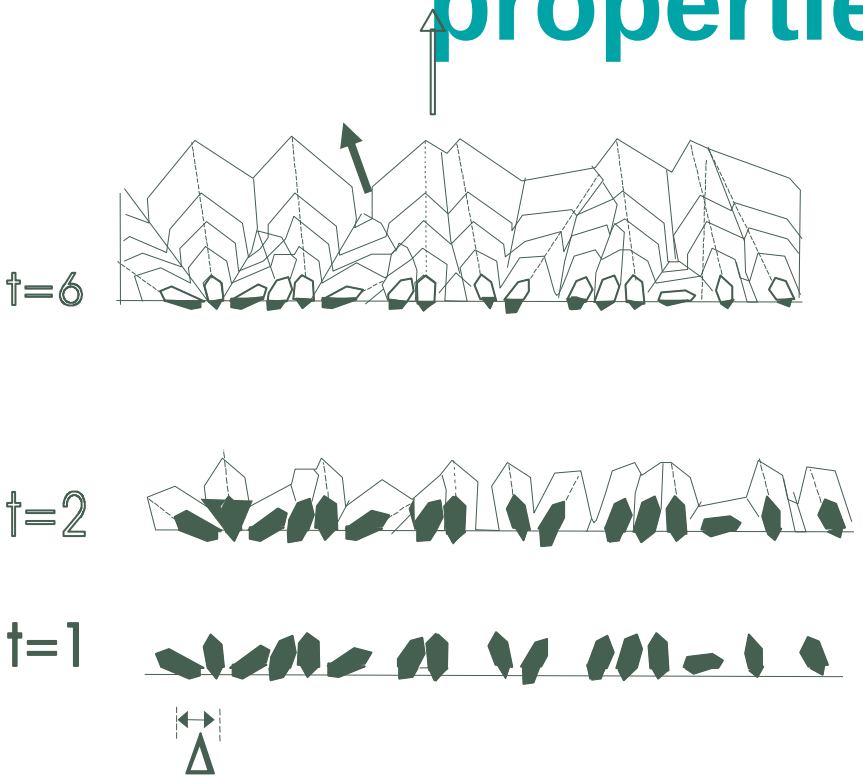
Original Crystal — Inhibition of growth along the b axis — Final shape of the crystal

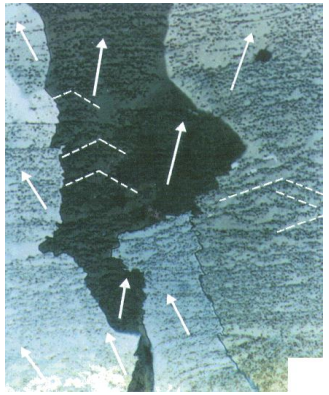
The real life of chickens: Calcite crystals in eggshell

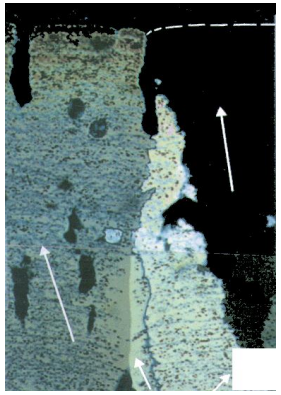


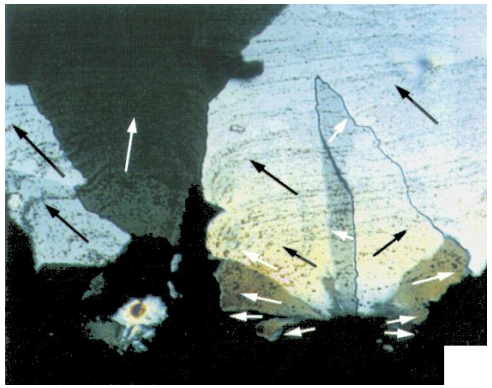
Original calcite crystal — Shape of the chicken calcite crystal

Eggshell biomineralization and mechanical properties









Establishment of the ultrastructure and a preferred crystal orientation perpendicular to the surface → **Eggshell mechanical properties**

Rodriguez-Navarro 2003

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Mechanical eggshell properties
Mass or fabric ?

95% CaCO3 (calcite) ⟷ **Interaction** ⟷ 3.5% organic matrix (proteins, proteoglycans)

Quantity → **Mass**

Control of shell calcification → Ultrastructure (fabric)

Mass → **60 %** → Mechanical Properties

Ultrastructure (fabric) → **40 %** → Mechanical Properties

Nutrition, génetics, environnement, light programms

→ **The most studied in the past**
Potential gains are slight

Polymorphisms of genes coding matrix proteins
Under control of vitamin D
→ genetics/nutrition intercation

→ **The less studied**
Potential gains are elevated

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How to maintain and improve shell quality

In the last 20 years, lot of scientific programs were performed to identify thousands of molecular actors involved in the eggshell mineralisation

These molecular players show polymorphisms associated with differences in shell quality

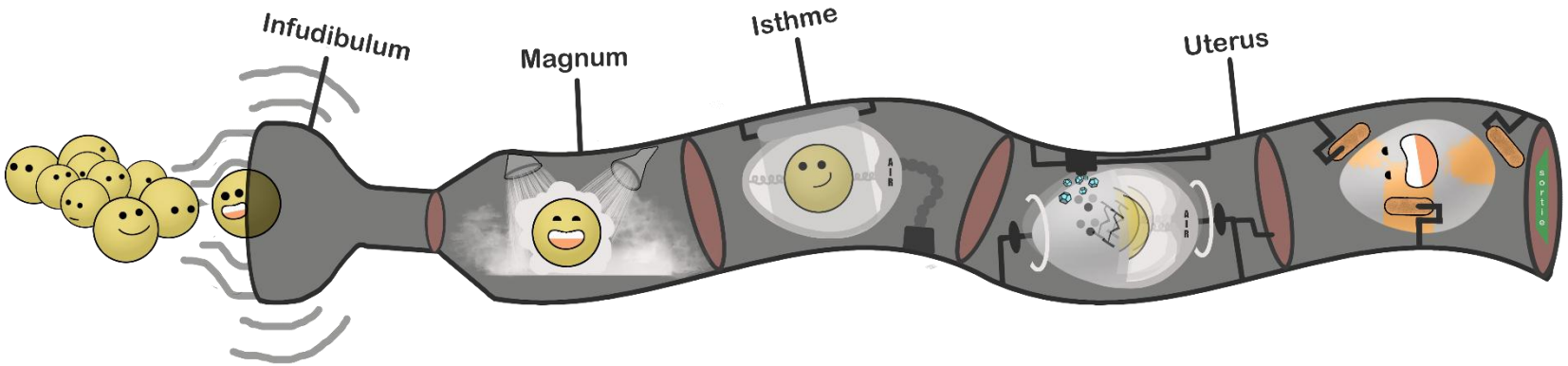
These molecular networks show differences in abundance according to vitamin D status

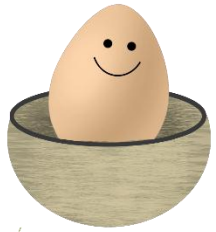
Towards genomic selection to obtain favorable haplotypes

Towards precision vitamin feeding

Shells with enhanced mechanical properties, even during extended production cycles

Ackowledgments





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