**Eggshell formation in hens: mechanisms of mineralization and impact on calcium homeostasis**

Nys Yves1, Joël Gautron1, Maxwell Hincke2 and Alejandro Rodriguez-Navarro3

**1**INRA, Val de Loire, Recherches avicoles, F-37380 Nouzilly, France; 2 Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada; 3 Departamento de Mineralogía y Petrología, Universidad de Granada, Granada, Spain.

The chicken egg is a giant reproductive cell protected by an eggshell, which forms daily in the distal segment of oviduct, in an acellular uterine fluid that contains inorganic and organic precursors of the shell. Its distinctive features, as compared to bone or teeth, are the nature of the mineral and the absence of cell-directed assembly during its fabrication on eggshell membranes. Eggshell is a biomineral made of 95% calcium carbonate (calcite) and 3.5% organic matrix (proteins and proteoglycans). This structure is the consequence of interactions between both mineral and organic matrix constituents resulting in a highly ordered structure with unique mechanical properties. The calcification process occurs in the uterus where biomineralization follows a temporal sequence. The sequential events of mineralization correspond to the following phases: 1) the widespread deposition of amorphous calcium carbonate (ACC), 2) ACC transformation into crystalline calcite aggregates, 3) formation of larger calcite crystal units. This initial phase is followed by 4) the development of a columnar structure with preferential calcite crystal orientation during the active calcification growth phase, and 5) the termination of calcification just prior the oviposition. This process is under the control of the organic matrix as demonstrated by numerous *in vitro* and *in vivo* evidences. The uterus synthesizes many eggshell matrix proteins which either influence the process of shell mineralization or affect the activities of the matrix proteins. A large number of protein candidates are currently identified but their precise role in the process of mineralization remains under study. In *Gallus gallus*, eggshell formation takes place daily and is one of the most rapid mineralization processes known. This requires large amounts of CaCO3 as the hen exports 10% of her total body calcium (2 g) each day, and the equivalent of her body weight, as eggshell, in one year of egg production (>1.5 kg). Neither ion (Ca2+ and HCO3-) is stored in the uterus, but they are continuously supplied during eggshell formation by the blood plasma *via* trans-epithelial transport taking place across the uterine glandular cells. Such intense Ca2+ metabolism challenges calcium homeostasis in hens in the organs contributing to the supply and transfer of calcium: intestine, uterus and bone. Hens develop numerous physiological adaptations. Hen intestinal Ca absorption is increased by 6-fold following the stimulation of 1,25(OH)2D3 at the kidney level. The uterus also acquires the capacity to transfer great quantities of Ca2+ and HCO3- ions to supply the mineral precursors to the eggshell Ca CO3. As in mammals, PTH regulates calcium ﬂux by stimulating the production of 1,25(OH)2D3), which controls intestinal absorption of calcium, however, any direct effect of this metabolite on uterine tissue has not been demonstrated. Factors regulating the matrix protein synthesis remain to be identified. This review updates knowledge on the mechanism of eggshell formation including role of ionic transporters and shell matrix proteins and introduces the current knowledge on the regulating factors controlling eggshell formation and Ca metabolism in hens.