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## **PROTEOME AND BIOLOGICAL FUNCTIONS OF INNER AND OUTER PERIVITELLINE LAYERS IN THE CHICKEN EGG**

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The chicken egg perivitelline layer (PL), also known as the vitelline membrane, is an extracellular proteinaceous structure surrounding the egg yolk, in contact with the albumen. In avian reproduction, PL is involved in the sperm-egg binding and acrosome reaction, in the early embryonic development and in the physical and antimicrobial protection of the embryo. It thus contributes to the quality of both hatching and table eggs. It consists of two sublayers: the inner PL (IPL) and the outer PL (OPL), that are in contact with the yolk content and the egg white, respectively. About 140 different proteins were identified in the PL to date; however, the distribution of most of these proteins between IPL and OPL remains to be elucidated. The specific sites of synthesis, namely the ovary for the IPL and the oviduct for the OPL, suggest that these two sublayers possess different biological functions. The present study aims at characterizing the proteome composing IPL and OPL, and identifying the intrinsic putative functions of each perivitelline sublayer. For this purpose, PLs were harvested from ISA-Brown eggs collected on the day of lay and carefully washed to remove traces of yolk and egg albumen. IPL and OPL were carefully manually separated from isolated PLs and proteins composing these two layers were independently solubilized, fractionated by SDS-PAGE and analyzed by tandem mass spectrometry coupled to nanoliquid chromatography (GeLC-MS/MS). The proteomic analysis revealed 412 proteins in the PL including 314 and 239 proteins that were identified in IPL and OPL, respectively. Among these, 141 were common to both sublayers and encompass the most abundant proteins of the PL. The tissue origin of some of these shared proteins was investigated by the analysis of gene expression (RT-qPCR) in different reproductive tissues assumed to be involved in the formation of IPL (liver, theca, granulosa) and OPL (oviduct segments). The functional annotation (gene ontology/bibliography) of secreted proteins revealed that IPL is mainly involved in fertilization and in early embryonic development, while OPL seems to be rather involved in physical and antimicrobial defense but also in embryogenesis. Altogether, the data generated from this study give new insights into the structure and composition of the chicken egg PL and provide an integrative overview of the respective physiological functions of IPL and OPL.