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COMPREHENSIVE PROTEOMIC ANALYSIS OF *GALLUS GALLUS* UTERINE FLUID

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Domestic hens are able to keep spermatozoa in their genital tract for long periods, and can so produce fertilized eggs for up to 3 weeks after one insemination. An extensive description of the avian uterine fluid proteome will help to provide the basis for a better understanding of a number of diseases and processes, including sperm survival but also female infertility and cell storage. Uterine fluid was collected (n=10) into a plastic tube placed at the entrance of the everted vagina 10h after oviposition. Bottom up proteomic approach using SDS-PAGE and nano LC-MS/MS (ultimate 3000 RSLC system coupled to LTQ Velos Orbitrap mass spectrometer) was performed with a high-low resolution MS strategy. Data were matched against NCBIInr database using Mascot 2.3 and identifications were validated by the peptide and protein Prophet algorithm using Scaffold 4.0 software. Bioinformatics treatments of data set was carried out to refine annotation of proteins using NCBIInr database, and to describe uterine fluid proteins using SecretomeP 2.0 and SignalP 4.1 tools, InterproScan software, and, Exocarta, KEGG and UniprotKB databases. Among a total of 922 proteins that were identified, 836 (91%) were identified in *Gallus gallus* databases, whereas 86 (9%) were identified in others species, indicating unknown chicken isoforms. Deepens analysis of cellular component revealed three categories of proteins. The secreted proteins (165) known to be secreted with a peptide signal or by an unconventional pathway, the exosomal proteins (644) which match against exosomal databases (Exocarta, UniprotKB, KEGG) and the last category refers to proteins which are not annotated as exosomal or secreted (113). Secreted proteins are composed of protease inhibitors (11), cytoskeletal and extracellular matrix proteins (22), enzymes (metabolic, proteases etc.) (49) and others proteins implied in calcification of eggshell (OC-17, OC-116). Exosomal proteins mainly consist in enzymes (metabolic, oxidoreductase) (225), chaperon proteins (HSPA8, HSP90AA1,...) (26) and proteins implied in MVB biogenesis (Alix, TSG101, Clathrin,...) (25). We have isolated exosomes and confirmed the presence of exosomal markers (CD63, HSPA8) by western blot in the avian uterine fluid. The presence of exosomal proteins in avian uterine fluid may represent a novel and exiting mechanism of cell-cell interactions, that may explain at least in part, the long term sperm survival. We believe that the thorough catalogue of proteins presented here can serve as a valuable reference for the study of sperm interaction with the female genital tract. Moreover, it could be an interesting tool for biomarkers discovery involved in fertility.