Titolo:
Semen quality parameters of Italian chicken breeds: characterization at the molecular level by proteomics

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Innovative proteomic studies have revealed an exceptional diversity in the protein composition of sperm, and new molecular markers have been suggested to assess sperm fertilizing ability in domestic mammals, whereas very few studies are available in birds. The aim of the present study was to assess sperm proteomic profile and semen quality parameters to characterize between breed variability in chickens.

Semen quality parameters and proteomic profile were assessed in the following Italian chicken breeds: Mericanel della Brianza (n=4), Bionda Piemontese (n=9), Bianca di Saluzzo (n=9), Pepoi (n=10) and Robusta Maculata (n=5). Semen was routinely collected twice weekly and the following quality parameters recorded: volume (mL), concentration ($\times 10^9$/mL), viability (%), total motility (TM, %), progressive motility (PM, %), VCL (µm/s), VSL (µm/s), VAP (µm/s), LIN (VSL/VCL), STR (VSL/VAP), ALH (µm), BCF (Hz) and WOB (VAP/VCL). Sperm pellets were separated by centrifugation and frozen at -20°C until further analyses. The proteomic profile of sperm was evaluated by a shotgun label-free proteomic approach. The entire protein content was extracted from each sample, reduced, alkylated and digested with trypsin. The obtained tryptic digest mixture was subjected to nano LC-MS/MS analysis using a high-resolution mass spectrometer (Orbitrap Fusion Tribrid, Thermo Fisher Scientific). Acquired raw files were analysed by MaxQuant software (version 1.6.1.0) for protein identification and relative quantification in each dataset.

The good reproducibility of the protein extraction method, the efficacy of the whole proteomics workflow, as well as the low sample variability within breed, were assessed evaluating (I) the number of identified proteins, (II) the percentage of MS/MS analyses performed, (III) the sum of the LFQ (Label Free Quantification) signal intensity values and (IV) the Pearson's coefficient values between the different datasets. This analysis allowed to choose the datasets (n=4 for each breed) to be subjected to the differential analysis, for the identification of proteins commonly and exclusively present in the different breeds. The bioinformatic analysis performed by GSEA (Gene Set Enrichment Analysis), highlights the high correlation between those proteins and sperm quality parameters above mentioned, in particular for those concerning sperm mobility.