



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

Introduction

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.

Materials & Methods

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). The following semen quality parameters were recorded: volume (mL), concentration ($\times 10^9/\text{mL}$), viability (%), total motility (TM, %), progressive motility (PM, %), VCL ($\mu\text{m/s}$), VSL ($\mu\text{m/s}$), VAP ($\mu\text{m/s}$), LIN (VSL/VCL), STR (VSL/VAP), ALH (μm), BCF (Hz) and WOB (VAP/VCL). Sperm 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.

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Aim

The aim of the present study was to assess sperm proteomic profile and semen quality parameters to characterize between breed variability in chickens.



Results

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, as reported in Figure 1, 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 (Table 1).

Figure 1 – Venn diagrams of proteins identified in sperm cell samples of different chicken breeds.

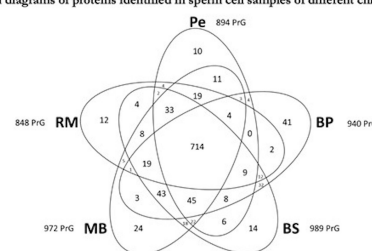


Table 1 – Sperm quality parameters recorded in Italian chicken breeds

Breed*	Viability %	PM %	VCL %	VSL %	VAP %	LIN %	STR %
Bionda	86.6 \pm 1.76a	22.2 \pm 1.99ab	70.6 \pm 3.86ab	27.1 \pm 1.74ab	43.5 \pm 2.58ab	40.3 \pm 1.09ab	63.2 \pm 0.87a
Bianca	84.0 \pm 1.89a	20.1 \pm 2.24a	69.1 \pm 4.14ab	25.0 \pm 1.94b	41.8 \pm 2.83ab	37.5 \pm 1.14a	60.7 \pm 0.89b
Mericanel	95.6 \pm 3.63b	30.4 \pm 4.26bc	78.2 \pm 8.68ab	33.6 \pm 3.75ac	52.1 \pm 5.56bc	43.2 \pm 2.52bc	64.5 \pm 2.14ab
Pepoi	94.2 \pm 1.43b	24.1 \pm 1.64ab	62.4 \pm 2.95a	25.3 \pm 1.41b	39.8 \pm 2.04a	41.1 \pm 0.79b	64.1 \pm 0.60a
Robusta	96.2 \pm 1.82b	32.8 \pm 2.06c	76.6 \pm 3.96b	35.0 \pm 1.79c	51.5 \pm 2.64c	47.2 \pm 1.11c	69.1 \pm 0.90c

*Bionda: Bionda piemontese; Bianca: Bianca di Saluzzo; Mericanel: Mericanel della brianza; Robusta: Robusta maculata

Conclusions

The performed analysis described the occurring correlation between the proteomic landscape characterising the uniqueness of heritage chicken breeds and their sperm qualitative traits: motility in particular.

