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AGE RELATED CHANGES IN PROTEOME AND QUALITY PARAMETERS OF SONICATED DOG EPIDIDYMAL SPERMATOZOA

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Abstract

This PhD thesis was performed in two studies. The first study was provided for proteomic analysis of intracellular and membrane-associated fractions of canine (*Canis lupus familiaris*) epididymal spermatozoa and additionally to find optimal sonication parameters for the epididymal sperm morphological structure separation and sperm protein isolation. Sperm samples were collected from 15 dogs. Protein fractions (PFs): intracellular (SIPs) and membrane-associated (SMAPs) were isolated. After sonication, sperm morphology was evaluated using Spermac Stain™. The sperm PFs were analyzed using gel electrophoresis (SDS-PAGE) and nanoliquid chromatography coupled to quadrupole time-of-flight mass spectrometry (NanoLC-Q-TOF/MS). UniProt database-supported identification resulted in 42 proteins identified in the SIPs and 153 proteins in the SMAPs. Differentially abundant proteins (DAPs) were found in SIPs and SMAPs. Based on a gene ontology analysis, the dominant molecular functions of SIPs were catalytic activity (50%) and binding (28%). Hydrolase activity (33%) and transferase activity (21%) functions were dominant for SMAPs. Bioinformatic analysis of SIPs and SMAPs showed their participation in important metabolic pathways in epididymal sperm, which may suggest their potential as sperm quality biomarkers.

The second study aimed to investigate the relationship between the functional quality and proteome of epididymal spermatozoa of dogs that were differing in age. The study was conducted on 30 male dogs that were divided into three age groups. G1 – 12 to 41 months old, G2 – 42 to 77 months old, and G3 – 78 to 132 months old. The sperm samples were assessed using a computer-assisted semen analysis (CASA). The epididymal sperm proteins were analyzed using SDS-PAGE, NanoUPLC-Q-TOF/MS and bioinformatic tools. The sperm quality parameters were significantly lower in older dogs. NanoUPLC-Q-TOF/MS identification resulted in 865 proteins that were found in the G1, 472 in G2, and 435 in G3.

There were seven proteins that were present in all three age groups, and four of them (ACTB, CE10, NPC2, CRISP2) showed significant changes among the studied groups.

The studies showed, for the first time, mass spectrometry and bioinformatic analysis of intracellular and membrane-associated proteins of canine epididymal spermatozoa. Additionally, sonication was used for the separation of dog epididymal sperm morphological elements (heads, tails and acrosomes). The results revealed the presence of DAPs in both sperm protein fractions significant for sperm function and fertilizing ability. Additionally, the age-dependent variations were detected in the sperm proteome composition and were related to important metabolic pathways, which might suggest that some proteins implicated in sperm maturation could be potential aging biomarkers.