Analysis of phosphoproteomes of turkey (*Meleagris gallopavo*) ejaculates with different biological quality

mgr inż. Katarzyna Teresa Rafalska

Summary

The professional turkey (*Meleagris gallopavo*) breeding relies entirely on artificial insemination due to the excessive sexual dimorphism between males and females of this species. Another problem is inability to use any kind of preserved semen, apart from fresh one, during mating. The optimal buffer composition which would allow for cryopreservation of turkey semen has still not been established. When birds are exposed to higher temperatures for too long, theirs thermoregulatory mechanisms can become overloaded. As a result, heat stress can occur, and the body's homeostasis can be disturbed. In the turkey population, approximately 10-16% of individuals are affected by yellow semen syndrome (YSS). It is mainly characterized by lower values of selected quantitative and qualitative parameters of the ejaculates as well as presence and/or absence of chosen proteins in semen. The usage of ejaculate affected by YSS in AI leads to reduced fertilization efficiency. The causes of yellow semen syndrome occurrence are still unknown.

The aim of the present study was to analyse the phosphoproteomes of seminal plasma and spermatozoa derived from normal and YSS ejaculates during the two distinguished periods i.e., spring-summer and autumn-winter season, which was preceded by in-depth analysis of chosen biological and biochemical parameters of semen.

In fresh semen, sperm concentration and motility were measured, and sperm viability was assessed, including cell membrane integrity in the acrosome region, mitochondrial functionality, occurrence of apoptotic changes and nitric oxide detection in sperm. The activity of selected antioxidant enzymes - SOD, GPx, CAT - and measurement of GSH and MDA content were determined both in seminal plasmas and spermatozoa. In addition, total protein content was determined in the plasmas and the activity of acid and alkaline phosphatase was measured. Furthermore, phosphorylated proteins were isolated from both semen components and separated by means of Native-PAGE, Tricine-PAGE, Urea-PAGE, SDS-PAGE and 2D-PAGE. Selected phosphoproteins, separated by the latter two techniques, were identified by Nano LC-MS/MS and/or MALDI-TOF/TOF.

The present study confirmed that turkey semen with YSS is characterized by reduced quality parameters as compared to white semen. For the first time, it was shown that the season affects selected biological and biochemical parameters of turkey semen. Statistically significantly higher levels of acid and alkaline phosphatase activities were observed in the plasmas of YSS semen and during the autumn-winter season. It was proven that the phosphorylation profiles of serine and tyrosine residues of proteins are affected by both the occurrence of yellow semen syndrome and the season. A total of 142 phosphoproteins were identified in turkey semen. Among them were the proteins encoded by the following genes: UBB, RPS27A, SPATA18, CDC42, ACTN, TBB, ACTA, ODF2, GAPDH, PGAM, PGK, TPI, ALDOC, LDH, SDHA, FASN, APOA, CKM, GOT, CA, CALM, TRFC, FTH, HSPA, HSP, GST, PRDX, QSOX, ASTL, ANXA, ACR, GSN, VDAC and PLCZ1. The identified phosphoproteins are involved in, among other things, interaction and binding with an oocyte, the fertilization process, cytoskeletal organization, sperm metabolism, intracellular transport, and determination of the proteome composition of individual cellular compartments. It has been shown that the degree of phosphorylation of mentioned proteins is affected by the type of semen as well as the ongoing season.

Keywords: turkey, seminal plasma, spermatozoa, white semen, YSS, season, biological and biochemical parameters of an ejaculate, semen phosphoproteome