

TITLE: Soluble scavenger receptor cysteine-rich domain-containing protein - SSc5D, a new player of innate immunity in turkey (*Meleagris gallopavo*) semen involved in yellow semen syndrome

DESCRIPTION FOR THE GENERAL PUBLIC

Objective. The objective of this project is to obtain new knowledge about turkey semen SSc5D protein, belonging to scavenger receptor cysteine rich family member (SRCR). SRCR were reported to play a role in the regulation of innate immune responses, they belong to pathogen recognition receptors. In mammals, the SRCR localization was confirmed in immune cells - macrophages as well as in male reproductive tract. At present no information on specific function of SSc5D is available in avian reproduction. In our opinion, SSc5D present in turkey semen is a new important player in avian innate immune response. Therefore, the proposed project has been designed to provide detailed information on SSc5D structure, tissue localization and exact role in antibacterial protection of semen. The analysis of SSc5D function will be expanded to study the yellow semen syndrome (YSS), an endemic disease within domestic turkey population. Low quality of YSS semen leads to reduction of fertility and hatchability. The yellow semen is characterized by an increased number of abnormal spermatozoa, spermatids, and spermiphages (semen macrophages) what indicates the inflammation within reproductive tract. Therefore, using high throughput sequencing methods, we would like to expand study of YSS to immune system molecules, including all members SRCR superfamily in YSS and to examine microbial communities of semen. Because scavenger receptors have been implicated in the pathogenesis of a number of diseases, it is of interest to determine SSc5D protein may be involved in YSS pathogenesis. Increasing the knowledge on this subject may be important to improve the reproduction in turkey, an important agricultural species in Poland.

The research hypothesis assumes that (i) SSc5D present in turkey seminal plasma is anti-microbial agent of turkey semen; (ii) SSc5D is secreted by reproductive tissue and/or by activated spermiphages; (iii) expression profile of SSc5D within reproductive tract is affected by YSS; (iv) the presence of spermiphages in semen reflects sperm quality; and (v) semen microbiota composition and immune system response, including SRCR superfamily can differentiate white, normal semen from YSS.

Description of the research. Using ion exchange chromatography, gel filtration and reverse phase chromatography SSc5D will be isolated from turkey seminal plasma. Molecular weight, isoelectric point and post-translational modifications (glycosylation and phosphorylation) will be determined by electrophoretic and mass spectrometry analysis. Antibodies against SSc5D protein will be produced by rabbit immunization and will be used for immunohistochemical detection of SSc5D and developing of ELISA test. The antibacterial properties of turkey SSc5D will be determined by the colony-forming unit (CFU) method and antimicrobial susceptibility testing. Tissue distribution of SSc5D in the male reproductive tract (including YSS) will be performed using immunohistochemistry and quantitative PCR analysis. Moreover, using the same techniques semen immune cells - spermiphages will be examined as possible source of SSc5D in white semen of good quality and YSS semen. Then, the effect of spermiphages on semen quality will be determined by flow cytometry, ELISA, and computer-assisted sperm analysis. Using proteomic tools (2DE-DIGE and MALDI TOF/TOF) proteomic profile of activated spermiphages from white and YSS semen will be compared. RNAseq and 16S rRNA sequencing will be used to study differentially expressed genes and microbiome in relation to YSS.

Justification. The presence of SRCR member, known as SSc5D has been demonstrated for the turkey seminal plasma. In contrast to mammals, where SSc5D was reported to play a role in the regulation of innate immune responses, there is no information regarding SSc5D function in avian reproduction. Therefore, it is of utmost importance to obtain solid information on SSc5D, both regarding its structure and tissue localization and exact role in bacterial infection in semen. The gaps in the knowledge regarding turkey immune system response, including SRCR superfamily and semen microbiota will be fill in in relation to YSS. It is unknown at present if SSc5D can be implicated in the pathogenesis of YSS. Increasing the knowledge on this subject may be important to improve the turkey reproduction.

Expected effects. We believe that our project will provide a truly important contribution to knowledge regarding avian reproductive immunity by describing unknown mechanism of antimicrobial immune defence in semen. In this context our results should be important for better understanding the possible role of SSc5D in coping with bacterial diseases in avian semen. The knowledge regarding molecular mechanism of YSS disease will be expanded.