

**TITLE: Characterization and biological role of cysteine rich secretory protein (CRISP) in the male reproductive system of turkey (*Meleagris gallopavo*)**

**DESCRIPTION FOR THE GENERAL PUBLIC**

**State of the objective of the project.** Genomics, transcriptomics, proteomics and bioinformatics ('omics' technologies) will be introduced to studies of cysteine rich secretory protein (CRISP) of turkey seminal plasma. CRISP will be subjected of detailed physiological and functional studies. The specific objectives of the project are: (1) isolation of CRISP from turkey seminal plasma and characterization of its physicochemical properties, (2) an analysis of the turkey reproductive tract and egg transcriptome in relation to *CRISP* mRNA and (3) tissue distribution of CRISP in turkey male reproductive tract and comparative analysis of CRISP among avian species. Moreover, we will evaluate the biological function of CRISP in turkey reproduction: (4) the effect of CRISP on semen physiological characteristics and (5) its participation in sperm-egg interaction during fertilization.

**The research to be carried out.** Using hydrophobic interaction chromatography and gel filtration, CRISP will be isolated from turkey seminal plasma. Molecular weight, isoelectric point and glycoprotein structure and phosphorylation will be determined by electrophoretic and mass spectrometry analysis. RNA sequencing will be used to study the transcriptome, a set of all mRNA molecules in cell. Using RNA-seq method CRISP mRNA will be sequenced and an bioinformatic analysis of the turkey reproductive tract and egg transcriptome in relation to CRISP will be performed. Tissue distribution of CRISP in the male reproductive tract will be performed using immunohistochemistry and quantitative PCR analysis. Comparative analysis of CRISP among avian species will be analyzed using Western blot and anti-turkey CRISP antibodies. The biological role of turkey CRISP will be determined by ELISA test, computer-assisted sperm analysis (CASA) and flow cytometry what allow for correlation of CRISP concentration with semen quality parameters. Analysis of chemoattractant properties of turkey CRISP will be performed with  $\mu$ -Slide Chemotaxis and CASA. CRISP participation in sperm-egg interaction during fertilization in turkey will be performed by *in vivo* fertilization.

**Reasons for choosing the research topic.** The presence of CRISP in turkey seminal plasma has been demonstrated for the first time in our laboratory using proteomic approach. In contrast to mammals, where CRISP proteins play a role in sperm-zona pellucida interaction and gamete fusion, there is no information regarding CRISP function in avian reproduction. Due to the importance of CRISP in mammals reproduction, there is a urgent need to obtain information about CRISP in avian reproduction system. Characterization of physicochemical properties will be performed for the first time for avian CRISP and obtained information will allow for interspecific comparison of mammals and avian CRISP. Deciphering the nucleotide sequence of CRISP transcript from testis, epididymis and ductus deferens will allow to determine alternative gene spliced transcripts, post-transcriptional modifications, gene fusion, mutations and changes in gene expression can be predicted. Moreover, the involvement of CRISP in pathways taking place in reproductive tract. Tissue distribution analysis will determine the exact synthesis and secretion site. Experiments designated for understanding the biological role of avian CRISP will answer the questions i) does turkey CRISP modulate sperm motility and orientation during fertilization ? and ii) does turkey CRISP play a role in sperm-perivitelline layer interaction and gamete fusion ?