

Yellow semen syndrome (YSS) in domestic male turkeys (*Meleagris gallopavo*) was detected over thirty years ago by identifying seminal plasma having both a yellow color and a high protein concentration. More recently, spermatozoa motility characteristics, demonstrated by a computer-assisted sperm analysis has been reported as disturbed in YSS turkeys. Although semen quality parameters have been described in detail, the cause of YSS still remains unclear. Studies of mammalian species revealed that metabolism of androgen to estrogens, due to the enzyme aromatase that catalyzes the transformation of androgens into estrogens, is crucial for the male reproduction. Recently, the role of estrogens in testes of domestic goose during annual reproductive cycle has been reported. Since the authors demonstrated that plasma steroids' concentrations do not correspond to steroid hormone profiles within the tissue and the results of our preliminary studies revealed altered aromatase gene expression within the reproductive tissues of YSS turkey, it seems of special importance to measure the steroid hormone levels *in situ*, in the testis, epididymis, and ductus deferens of the YSS. Moreover, it is well established that in addition to spermatogenesis being regulated by FSH and testosterone, intercellular junctions between adjacent cells within the testis and epididymis play an important role in the control of spermatogenesis. Of note, cell-cell interactions *via* specialized cell junctions are required for normal functions of testis and epididymis, and are of vital interest in adult male reproduction. Several rodent models have recently been proposed to illustrate an important role of testosterone signaling in the regulation of Sertoli cell junctions dynamics, however the nature of testosterone effects after its aromatization and the underlying molecular and cellular mechanisms remain poorly understood. It should be added that the general organization of the turkey testis is similar to that of mammals, i.e. basal and adluminal compartments of the seminiferous tubule are present, due to several different types of cell-cell junctions. Moreover, the structural features of avian Sertoli cells indicate that they produce steroidal and peptide hormones like mammalian Sertoli cells. Compared with extensive studies on mammals, there has been very little work on the intercellular junctions in birds. In our opinion, the implication of testosterone/estradiol imbalance in the regulation of junction proteins' gene expression cannot be rejected in the turkey.

Thus, in the research project molecular and cellular approaches will be included to gain new insight into steroid hormone levels, cell-cell junction proteins' gene expression and the protein subcellular localization in the testis, epididymis, and ductus deferens of YSS turkeys. Several specific objectives are planned: (1) to confirm and widen the initial results, i.e. to localize aromatase in reproductive tissue cells and to measure testosterone and estradiol levels within the tissues, (2) to assess a potential effect of the steroid hormones' imbalance on selected junction proteins' gene expression in the testis, epididymis, and ductus deferens, and (3) to demonstrate precise distribution of the junction proteins within the tissues' epithelia. A particular emphasis will be placed on understanding a connection between the testosterone:estradiol ratio and junctional communication within the reproductive tissue cells as well as the lower quality of sperm occurring in YSS turkeys. In the second part of the project, it will be important to show (4) whether sperm cells are a putative source of estrogen and a target for both testosterone and estradiol, and finally, (5) to investigate whether impaired steroids' ratio may lead to alterations in sperm ultrastructure.

This is likely that the local balance in action between androgens and estrogens, rather than their absolute levels, may be crucial in having an impact on the cell junction proteins' expression. Our research hypothesis (based on our preliminary study) assumes that augmented expression of aromatase (at mRNA and protein level) within the testis, epididymis, and ductus deferens and, in consequence, loss of steroid hormones' balance are related to the appearance of the YSS, through its impact on the cell junction proteins' gene expression.

To verify the hormonal control of the junction protein expression within the tissues' epithelia, and that of sperm functions specific objectives will be realized using modern cell and molecular biology techniques. In all the experiments turkeys with white normal semen (WNS) will serve as a control.

The results of the proposed project will provide new information on the role of hormonal milieu within the tissues and intercellular communication within the seminiferous tubule epithelium, epididymal epithelium, and ductus deferens epithelium of domestic turkey with the YSS. Moreover, the results gathered in the frame of this project may lead to understanding mechanisms involved in the action of estrogens in the male gonad of turkey. It seems likely that in birds as in mammals excess estrogen may affect sperm function. The novelty of the proposed project, lies mainly in elucidation of the link between hormonal and structural alterations within the reproductive tissues of YSS turkey that could have an impact on the lower semen quality occurring in YSS turkeys. The obtained results may widen our knowledge at the cellular and molecular level of domestic turkey reproductive tissues' functions. The results will be important in the field of reproductive biology and may have a relevance in applied sciences including domestic avian reproduction. The proposed studies will open up new perspectives for further studies on the turkey reproductive tissues' functions at cellular and molecular levels.