Sexual reproduction requires the formation of haploid gametes – sperm and egg cells – during a complex developmental process called gametogenesis. These cells must recognize and fuse to form a diploid zygote that develops into an embryo, and then into a new organism. Animals produce sperm directly through meiosis in male gonads (testis) that are successively transported to the **epididymis**. The adult epididymis in mammals is structurally and functionally divided into several unique anatomical segments that create distinct luminal environments to promote the functional transformation of spermatozoa and their subsequent storage in viable state in readiness for ejaculation. The initial segment (above the caput) is a loosely coiled tubule with a wide diameter and a low concentration of spermatozoa. Highly specialized **epithelial cells** in this segment are elongated and possess high microvilli. The caput segment is characterized by a narrow luminal diameter, while both the luminal diameter and the sperm concentration increase distally within the corpus and cauda epididymis. Differing epithelial cell types within these segments are responsible for the creation of a specialized luminal microenvironment that promotes the sequential maturation of spermatozoa (in the caput and corpus) and their subsequent storage (in the cauda). The establishment of the unique microenvironment in the particular segments of the epididymis features the varied endocytic and exocytic contributions of the specialized epididymal epithelial cells: the principal, basal, narrow, and clear cells.

Despite recognition of the importance of the bidirectional epithelial transport (exo- and endocytosis) in regulating of the epididymal luminal microenvironment, little is currently known about the molecular machinery that controls these complementary pathways. There is long recognized that the **actin cytoskeleton** together with actin-binding proteins (ABPs) play important roles in cells and tissues specialization and functioning. For example, actin filaments are important for the cell-cell communications that mediate tissue organization and integrity. Cell shape and development of specialized features, such as microvilli or stereocilia on the apical domain of polarized epithelial cells require actin polymerization as well. One of the ABPs involved in actin organization and dynamics in the polarized epithelia in mammals is **myosin VI** (**MYO6**). This unique motor protein is essential for normal stereocilia architecture of the inner ear sensory hair cells in mouse and human. Mutation in the *Myo6* gene in Snell's waltzer mice (*sv/sv* mutants) leads to deafness as a result of neurosensory epithelia degeneration. These mice display also several other defects, such as aberrations in Golgi morphology, reduced secretion, defective endocytosis, and impaired morphology of brush border enterocytes and hippocampal neurons. To date, no data are available on the involvement of MYO6 in functional organization of the polarized epididymal epithelium. However, we have recently revealed that depletion of MYO6 in Snell's waltzer mice causes structural disruptions during spermiogenesis and spermiation. The structural defects observed in MYO6-deficient developing spermatids result in reduced number of sperm in the epididymis and reduced male fertility. **I hypothesize** that reduced fertility of Snell's waltzer male mice may also be a result of impaired maturation of sperm in the epididymis.

Therefore, the **main objective** of the proposal is to determine the probable role of MYO6 in functional organization of the epididymal epithelium in mouse. During comparative studies in control mice and Snell's waltzer mutants we intend to answer to the following main questions: Do the expression and localization patterns of MYO6 differ in different segments of the epididymis? How does lack of MYO6 impair the structure and molecular composition of the epididymal epithelium? What is the main function/s of MYO6 during maturation and storage of spermatozoa in the epididymis? We postulate that MYO6 may be involved in functional organization of the epididymal epithelium in different ways. First, it may be required for the polarized delivery of cargo from the Golgi complex to the lumen of the epididymis or maturing sperm. Second, it can play very specific roles during endocytosis from the apical domain of polarized epithelial cells. In addition to the transporting role, MYO6 may also anchor the apical membranes of the epithelial cells to the actin framework, thereby supporting the organization and function of the highly polarized epididymal epithelium. Thus, MYO6 would be a structural element of the specific protein complex/es responsible for maintaining the unique architecture of the epididymal epithelium.

The project includes technically advanced experimental work on tissue, cellular and molecular levels with the use of the epididymis dissected from euthanatized animals (*sv/+* and *sv/sv* males) and sperm isolated form the epididymis. We will use a wide spectrum of histo/cytochemical, immunohisto/cytochemical, biochemical, and molecular biology techniques, such as fluorescence *in situ* hybridization (FISH), immunofluorescence labeling combined with F-actin staining, confocal microscopy of semi-thin and cryo-sections, a high-resolution stimulated emission depletion (STED) microscopy, single/double post-embedding immunogold labeling using transmission electron microscopy (TEM), and comparative ultrastructural analyses. The fluorescent and confocal microscopy, alternatively the STED microscopy or TEM, will be used to perform co-localization of the particular protein complexes in different epithelial segments and cells. The proposed project is **innovative** and will provide **new data** on the function of the unique motor protein MYO6 in polarized mammalian epithelia. Moreover, the project may help to understanding of male fertility regulation with implications for contraceptive intervention and infertility diagnostics.