In mammalian ovary, the oocyte is surrounded by a glycoprotein zona pellucida layer (ZP). In human zona pellucida contains four glycoproteins ZP1, ZP2, ZP3, ZP4. The primary role of the zona pellucida is participation in fertilization, prevention of polyspermy and involvement in embryos pre-implantation development. Zona pellucida is characterized by immunogenicity and it has been successfully used in animal studies as target antigen for active animal immunization. ZP3 expression in cancer tissues have made opportunity to use it in active or passive immunization. Surprisingly, we demonstrated ZP3 expression in male testis. Hence, ZP3 immunotherapy could cause changes in the testis and result contraceptive effect or bring side effects like testicle damage. Therefore, it is necessary to explain the role of ZP3 in the testis. The ZP3 expression investigation could shed the light on the ZP3 role in testis. The main aim of this project is to make functional characterization of the ZP3 expression during the testicular ontogeny in mice. Specific goals include: (1) to characterize the ZP3 expression profile in male mouse testis tissue during ontogenesis; (2) to characterize the mechanisms regulating ZP3 expression in the testis and it's function during ontogenesis; (3) to identify the potential co-regulators of ZP3 action in the testis.

In this planned research study, we will use commercially available mouse cell line GC-2spd(ts) and mouse male testis tissues preserved in 4% PFA. Due to significant changes for androgen and gonadotropins levels in various stages of male maturation, we will analyze the gene expression profile in mouse testis during ontogenesis. Then, we will check the ZP3 transcript localization using RNAscope *in situ* hybridization, ZP3 protein level by Western Blot and assess protein localization by immunohistochemistry. Until now, there exist a little data about potential hormones or factors that may regulate the ZP3 expression and function. To analyze factors which could regulate the ZP3 action we will treat mouse spermatocyte cells with selected hormones and gonadotropins. Subsequently, we will assess ZP3 expression at the molecular level and ZP3 cellular localization using immunofluorescence assay. We will also make a knockout of ZP3 gene using the CRISPR/Cas9 gene editing method in order to discover functional changes after deleting ZP3 gene in mouse spermatocyte cells. We will check the role of ZP3 in cells proliferation, survival and the putative activation of cell death pathways. Additionally, we check expression of genes involved in the steroidogenic pathway, hormones receptors and chosen potentially co-working with ZP3 proteins.

We expect that findings from this science research project would further help to understand the ZP3 function in the male ontogeny process, which might be a milestone for the next proof of principal ZP3 male contraception immunization study. All the results obtained in this project will be presented during scientific congresses and published in scientific journals.