Recent epidemiological reports reveal significant increase in the number of tumors of spermatogenic cells as well as steroidogenic cells (Leydig cells, LC) of the testis interstitial tissue. Leydigioma is diagnosed in adult men but also in children. Under the development of these diseases lies among others endocrine and genetic factors as well as environmental factors (exposure to chemicals). Diagnosis of the causes of male infertility is often difficult, due to incomplete knowledge about hormonal and genetic control of gonad function.

The main role of LC is the production of steroid hormones: androgens and estrogens. In LC sex hormone receptors exhibit high expression, indicating on the critical role of sex steroids in the control of steroidogenesis and spermatogenesis. Androgen/estrogen ratio is essential for normal development and male reproductive function. Knockout of genes for steroid receptors or enzymes or inhibition of sex steroid signaling in laboratory animals results in sterility and/or morphological and functional defects in the reproductive system including hypertrophy (enlarged size) and hyperplasia (increased number) of LC. After birth, LC are mitotically inactive. However, in many disorders of testis function e. g. azoospermia or Klinefelter's syndrome restoration of LC proliferation due to imbalance of endogenous steroids and other regulatory molecules was described. In animal models exposed to environmental hormone-like chemicals LC hyperplasia and hypertrophy was reported. These compounds may act *via* steroid hormone receptors (exerting estrogenic, antiandrogenic effect) as well as *via* activation of membrane G-coupled estrogen receptor (GPER) and non hormonal receptors peroxisome proliferator-activated receptor (PPAR α, β and γ, respectively). GPER and PPAR also bind sex hormones. It is not known whether and which hormonal mechanisms induce LC hyperplasia and its transformation into tumor. In tumor cells, disturbed intercellular communication leads to disruption of growth, cell division but also altered cell behavior and function including lipid homeostasis.

The main objective of the project is to determine the role of GPER and PPAR in LC physiology and tumorigenesis based on the recent data indicating overlapping of both signaling in the control of cell proliferation, migration, angiogenesis and lipid metabolism. Genes regulated by GPER and PPAR as well as activity of these receptors after endogenous steroid hormones and environmental hormone-like chemicals and growth factors treatment will be studied. In addition, GPER and PPAR interactions with signaling pathways and signaling molecules in regulation of LC cellular processes and function will be analyzed to elucidate involvement of GPER and PPAR in LC hypertrophy/hyperplasia and/or tumorigenesis. A comprehensive study will be conducted in both *in vitro* and *ex vivo* (tissue culture) systems in (i) tumor mouse LC (MA-10), (ii) LC/interstitial tissue of transgenic mice AROM+ (with estrogen synthase overexpression), and (iii) human Leydigioma. These complex approaches will enable to find universal GPER and PPAR signaling mechanisms in LC. The research will be carried out after earlier blockage or activation of these receptors (gene silencing techniques and pharmacologically) in mouse LC *in vitro.* Next the results will be verified in AROM + mice and human Leydigioma as well as in mouse LC/interstitial tissue exposed to sex steroids, mixtures of environmental chemicals (anti-androgens and xenoestrogens, respectively) or selected growth factors. In addition, hypoxia conditions (oxygen limitation) and excess of chloride ions for hyperplasia induction in LC will be performed. We will study the processes of cell migration, adhesion as well as tissue angiogenesis. Analysis of the mRNA, proteins and steroids will be performed using qRT-PCR, microarrays, immunohistochemical techniques, immunoenzematic and radioimmunological assays. Also, special emphasis will be put on signaling proteins e.g. kinases MAP/ERK, transcription factor DAX-1, cyclooxygenase 2, metalloproteinases, insulin-like peptide 3, steroidogenic factors as their role in control of LC was strongly confirmed.

Elucidation of GPER and PPAR mechanisms signaling in the control of physiological and pathological processes in LC can be a starting point to identify the molecular causes of LC hypertrophy/hyperplasia and Leydigioma development. The results will be an important contribution to the reproductive endocrinology and andrology. Undertaken research in terms of endocrine, genetic and cytophysiological aspects requires a deep knowledge of the experimental andrology, biochemical endocrinology, cell biology and molecular genetics of reproduction together with professional experience in imaging techniques. We believe that our multifactorial approaches and passion will lead to development of novel solution and discovery of unknown biological events and research areas.