SUMMARY

Sertoli cells, the somatic cells of the seminiferous epithelium, play the key role in the proper course of spermatogenesis in mammals. One of the main regulators of Sertoli cells is pituitary follicle-stimulating hormone (FSH), which stimulates the production of paracrine factors and nutrients necessary for germ cell development. Previous studies indicate that also estrogens – hormones produced by androgen aromatization – affect Sertoli cell function, acting through both the nuclear estrogen receptors (ERα, ERβ) and the membrane estrogen receptor (GPER). Moreover, Sertoli cell activity is controlled by contact-dependent intercellular communication. One type of such communication is the Notch signaling pathway triggered by the interaction between transmembrane ligands and receptors located in the neighboring cells of the seminiferous epithelium.

The aim of the presented work was to explain the importance of FSH and estrogens in the regulation of the expression of Notch pathway ligands, receptor and effector genes and the activity of this pathway in rodent Sertoli cells.

To elucidate the role of FSH in the control of Notch signaling, *in vivo* experiments were performed using peripubertal male Wistar rats and sexually mature male bank voles after pharmacological and physiological inhibition of gonadotropin production, respectively, followed by FSH substitution. To demonstrate the role of estrogen signaling in the regulation of Notch pathway in the male gonad, rat testis explants (*ex vivo* model) incubated in the presence of 17β-estradiol and estrogen receptor antagonists (ICI 182,780; G15) were used. *In vitro* studies were conducted using primary rat Sertoli cells and the TM4 Sertoli cell line. To confirm the involvement of FSH and its receptor (FSHR) in the control of Notch pathway, Sertoli cells were either stimulated with FSH or silencing of FSHR expression was performed. To determine the role of estrogen receptors, Sertoli cells were incubated with 17β-estradiol and/or estrogen receptor antagonists (ICI 182,780; G15), or ERα, ERβ, and GPER silencing was carried out. Changes in the expression of Notch pathway components at the mRNA and protein level were demonstrated by RT-qPCR and western blot analyses, respectively. Immunohistochemical and immunofluorescence analyses were used to determine the localization of the studied proteins. Enzymatic immunoassays were carried out to assess FSH and cAMP levels. The activity of the RBP-J transcription factor and its binding to the promoters of Notch pathway effector genes were

determined using the luciferase reporter assay and chromatin immunoprecipitation, respectively.

RT-qPCR, western blot and immunohistochemistry analyses showed that in rats and bank voles after gonadotropin suppression, FSH administration caused an increase in the expression of the DLL1 ligand and the activity of the Notch1 receptor, and a decrease in the expression of *Hes1* and *Hey1* effector genes at mRNA and/or protein level. Similar changes in the expression of Notch pathway components in both species indicate a common mechanism of FSH action on Notch signaling in Sertoli cells during the initiation and recrudescence of spermatogenesis in rodents. *In vitro* studies demonstrated direct involvement of FSH-FSHR signaling in the regulation of Notch pathway components and activity in rodent Sertoli cells.

RT-qPCR and western blot analyses revealed that exposure to ICI 182,780 or G15 decreased mRNA and/or protein expression of DLL1, DLL4, Notch1, HES1 and increased HEY1 expression in rat testis explants. The results of *in vitro* experiments demonstrated that estrogens regulate the expression of DLL1 and JAG1 ligands, acting mainly through ERα, while ERβ and GPER are involved in the control of DLL4. It was also found that 17β-estradiol by binding to ERα reduces the expression of the effector gene *Hey1*, whereas 17β-estradiol action through ERβ increases the activation of the Notch1 receptor, the transcriptional activity of RPB-J and the expression of *Hes1*. The obtained data provide evidence for the role of estrogens in the control of Notch signaling in the male gonad and point out the interaction of ERα, ERβ and GPER receptors in the specific regulation of Notch pathway components in Sertoli cells.

In conclusion, the results of the presented studies indicate the involvement of FSH and estrogens in the regulation of Notch signaling pathway in Sertoli cells. The interplay between hormonal signaling and Notch pathway may therefore be a molecular mechanism controlling the function of Sertoli cells, and consequently, providing the proper course of spermatogenesis.