Streszczenie w języku angielskim

The ovarian follicle is the basic structural and functional unit of the mammalian ovary. In many mammalian species, the process of follicle development, known as folliculogenesis, begins in fetal life. Once formed, primordial follicles determine the ovarian reserve, which represents the total number of oocytes potentially available to ovulate during the reproductive lifetime. The female reproductive potential depends on the size of this pool and the rate of recruitment of primordial follicles for further development. The mechanisms that regulate the recruitment, growth, and development of follicles are under the control of hormones and growth factors. At various stages of development, most follicles undergo atresia and only some of them have the possibility of reaching the preovulatory stage. After ovulation, the corpus luteum (CL) is formed from the follicle wall. Its main function is to secrete progesterone, which is essential to prepare the uterus for implantation. In a nonfertile cycle, the CL regresses in the process known as luteolysis. There is increasing evidence showing that the disruption of hormonal balance during the prenatal and neonatal period, which are critical windows of development, may cause long-term effects on the reproductive system in adult life. The hormonal homeostasis may be disrupted by endocrine-active chemicals (EACs), that are present in the environment and may mimic or block the action of endogenous hormones. These compounds derive from various sources, including chemical, agricultural and pharmaceutical industries. It is presumed that long-term effects observed after exposure to EACs are caused by epigenetic changes such as DNA methylation and microRNA (miRNA) expression profile.

Therefore, the aim of this doctoral dissertation was to determine the effects of neonatal exposure to EACs on the CL function and the factors involved in the regulation of folliculogenesis in sexually mature pigs. To achieve this goal, newborn piglets were randomly allocated into five experimental groups (n = 5 per each group) and subcutaneously injected between postnatal days 1 and 10 with testosterone propionate (TP, androgen, 20 mg/kg body weight [bw]), flutamide (FLU, antiandrogen; 50 mg/kg bw), 4-*tert*-octylphenol (OP, a compound with estrogenic activity; 100 mg/kg bw), ICI 182,780 (ICI, antiestrogen; 400 µg/kg bw), or methoxychlor (MXC, a compound with estrogenic, antiestrogenic and antiandrogenic activity, 20 µg/kg bw). Piglets in the control group (n = 5) were given a vehicle only (corn oil). Animals were maintained until sexual maturity and following two estrous cycles, between days 8 and 11 of the next estrous cycle, gilts were slaughtered at a local abattoir. Blood samples and CLs were collected. In addition, preantral (primary, primary, early secondary) and small antral

follicles were isolated from the ovaries of the control and MXC-exposed animals. The collected tissues were snap-frozen in liquid nitrogen for RNA, DNA, and protein isolation or fixed in Bouin's solution for routine H&E staining and immunohistochemistry. In the current study, the following assays were performed: (1) hematoxylin and eosin staining to analyze the CL histology as well as Oil Red O and Sudan III staining to detect lipid droplets in luteal cells, (2) colorimetric assays to determine plasma level of triglycerides, cholesterol, high and low-density lipoproteins, (3) radioimmunological assays to determine the concentration of steroid hormones in plasma and luteal tissue, (4) enzyme-linked immunosorbent assays to investigate total DNA methylation and prostaglandin E_2 and $F_{2\alpha}$ concentration in luteal tissue, as well as, plasma FSH and AMH levels, (5) Western blot analysis to examine the abundance of proteins involved in DNA methylation and miRNA biosynthesis and function in luteal tissue. In addition, in luteal tissue of the MXC-treated group, the Next Generation Sequencing (NGS) was performed to determine the expression profile of mRNA and miRNA transcripts, along with bioinformatics data analysis and validation of results using real-time PCR. Moreover, in ovarian follicles obtained from MXC-treated gilts, the mRNA expression and protein abundance of selected members of the transforming growth factor type β family and their receptors, as well as FSH receptor, were examined.

Results showed that neonatal exposure to EACs led to changes in plasma steroid and lipid concentrations in sexually mature pigs. Additionally, hypertrophy and vacuolization of luteal cells were also observed as a result of neonatal exposure to all examined EACs. Moreover, a predominant abundance of lipid droplets was found in luteal cells of TP-, FLU- and ICI-treated animals. Neonatal exposure to ICI and MXC resulted in increased global DNA methylation, as well as higher DNMT1 protein abundance. Changes in the abundance of proteins involved in miRNA biogenesis were also detected. OP treatment led to a lower DROSHA protein abundance, while ICI treatment resulted in a greater DROSHA protein abundance. On the other hand, both FLU and ICI increased DICER1 protein abundance in the luteal tissue. Moreover, neonatal exposure to MXC caused changes in the intra-luteal concentration of androstenedione, estrone, and prostaglandin E_2. Transcriptomic analysis revealed 53 differentially expressed miRNAs and 359 differentially expressed genes in luteal tissue following the neonatal MXC treatment. MXC affected the expression of genes involved in lipogenesis, steroidogenesis, membrane transport, immune response, cell signalling, and adhesion. Furthermore, in preantral follicles, MXC exposure increased *GDF9*, *BMPR1B*, *TGFBR1*, and *BMPR2* mRNA levels, while decreasing *AMH*, and *BMP15* mRNAs. Additionally, a lower protein abundance of BMP15 and BMPR1B was observed. In small antral

follicles, exposure to MXC increased the mRNA levels for *BMPR1B*, *BMPR2*, and *AMHR2*, while decreased for *AMH*, *BMPR1A*, and *FSHR*. Moreover, neonatal exposure to MXC led to a decrease in protein abundance of AMH and all examined receptors.

In conclusion, the results of the current study suggest that neonatal exposure to EACs has a long-term impact affecting CL function and morphology. It may derive from altered plasma lipid profile as well as epigenetic changes in luteal tissue, including impaired DNA methylation and miRNA biogenesis. Furthermore, neonatal exposure to MXC affects intra-luteal prostaglandin and steroids concentration, as well as the expression of genes related to the maintenance of CL function suggesting an earlier onset of structural luteolysis in pigs. The expression of specific miRNAs was also disturbed by MXC, which indicates that these miRNAs are potential mediators of the long-term MXC effect on the CL function. Moreover, neonatal exposure to MXC may accelerate initial recruitment and impair the cyclic recruitment of follicles in gilts. Therefore, all these results confirm a crucial role of steroid milieu during neonatal development which is critical for the proper ovarian function in adult animals. The disturbance of the hormonal homeostasis during this time caused by environmental toxicants such as endocrine-active chemicals may have long-term consequences, which may lead to abnormalities in folliculogenesis and the corpus luteum function.