

SUMMARY

CHANGES IN THE TRANSCRIPTOME OF GRANULOSA CELL LINE AVG-16 TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD)**mgr Agnieszka Sadowska**

Doctoral thesis was carried out in the Department of Animal Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn under the guidance of prof. dr hab. Renata E. Ciereszko (supervisor) and dr Anna Nynca (ancillary supervisor).

The doctoral thesis titled “Changes in the transcriptome of granulosa cell line AVG-16 treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)” comprises three papers published in 2015 (1 – *Folia Biologica (Praha)*, 61: 184-194) and 2017 (2 – *Journal of Genetics*, 96: 75-85, doi: 10.1007/s12041-017-0745-3; 3 – *Chemosphere*, 178: 368-377; doi: 10.1016/j.chemosphere.2017.03.055). The papers focus on aryl hydrocarbon receptor (AhR) gene and on the mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) action in porcine granulosa cells in the pig.

Aryl hydrocarbon receptor is a ligand-activated transcription factor belonging to the bHLH-PAS (ang. basic helix-loop-helix-Per-ARNT-Sim) family. AhR gene and protein was found in vertebrates and invertebrates. In mammals, AhR is expressed in a variety of tissues and organs, including the reproductive tract (ovary, oviduct and uterus). Although the first AhR studies were carried out in the 1970s, up to date the full AhR gene sequence was established only in few animal species. In the pig, AhR and ARNT (AhR nuclear translocator) genes have not yet been fully characterized. The AhR mediates the effects of some endocrine disruptors, such as polychlorinated dibenzo-*p*-dioxins (PCDDs, dioxins). Dioxins are by-products of human industrial activity. Among 75 congeners of PCDDs, seven of them which hold chlorine atoms in positions 2, 3, 7 and 8 of benzene rings are considered to be highly toxic. The most toxic dioxin congener is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The toxicity of TCDD depends on the animal species and strain, sex and age as well as exposure dose, target organs and cell types. An exposure of living organisms to TCDD is chronic and widespread. It results in broad spectrum of pathologies such as a neurological and immune system dysfunction. TCDD also affects male and female reproductive and endocrine systems. In females, many reproductive processes are determined by the proper development and functioning of ovarian follicles. Granulosa cells, *via* steroid hormone production, play a fundamental role in a proper growth, development and functioning of ovarian follicles. They also ensure an optimal environment for fertilization, implantation and embryo development.

The presence of AhR in granulosa cells and the documented impact of TCDD on steroidogenesis make granulosa cells suitable for studying molecular mechanism of TCDD action. Since primary granulosa cell cultures have some limitations, granulosa cell lines became an attractive alternative model for *in vitro* examination of follicular function. Presently, AVG-16 is the only porcine granulosa cell line commercially available.

The aims of the present study were: 1/ to examine the phenotypic, biochemical, and molecular features of AVG-16 cells for their suitability to investigate the effects of environmental factors on the granulosa cell functions; 2/ to determine the structure and chromosomal localization of AhR and ARNT genes in the pig and 3/ to examine the effects of TCDD on the transcriptome profile of AVG-16 cells. The studies enabled to fulfill the specified aims of the doctoral thesis and extended our knowledge on the mechanism of TCDD action in porcine granulosa cells. On the basis of the obtained results the following conclusions were drawn:

1. AVG-16 cells are similar to the granulosa cell lines and may serve as an excellent model for studying the effects of environmental factors on ovarian physiology.
2. The existence of AhR and ARNT cDNA variants may affect the specific cell response determined by e.g., the AhR ligand, its concentration or biochemical cell status.
3. TCDD may affect ovarian follicle fate by influencing granulosa cell cycle, proliferation and differentiation.
4. Short-term incubation with TCDD (3 hours) may induce defense processes in the cells including cell cycle arrest and DNA repair. In turn, long-term incubation with TCDD (24 hours) may lead to granulosa cell death and follicular atresia.
5. TCDD may affect the occurrence of atresia of porcine follicles by involving non-apoptotic mechanisms of cell death.