GONADAL EXPRESSION PATTERNS OF STEROIDOGENESIS-RELATED GENES DURING SEX DIFFERENTIATION IN ZEBRAFISH

Caulier M. (1) (2), Guérin A. (2), Cauty C. (2), Hinfray N. (1), Brion F. (1) Guiguen Y. (2)

(1) INERIS, Unité d'écotoxicologie in vitro et in vivo, Verneuil-en-Halatte, France, e-mail : morgane.caulier@rennes.inra.fr
(2) Institut National de la Recherche Agronomique, UR 1037, INRA-SCRIBE, IFR 140, Campus de Beaulieu, Rennes, France

Introduction:

Zebrafish is an important animal model recommended by the Organisation for Economic Co-operation and Development (OECD) for detecting and assessing the effects of pollutants. In fish, sex differentiation is often used to evaluate the effect of endocrine disrupting chemicals (EDCs) as many of them are known to alter gonadal differentiation. Steroidogenesis has been shown to be highly sensitive to hormonal perturbations and is also of importance for natural fish gonadal differentiation. In zebrafish, there is still however a lack of knowledge regarding the steroidogenic gene expression during gonadal differentiation leaving unexplored the precise mechanism of action of EDCs on this physiological process. To fulfill these gaps, we then investigated the normal expression profiles of some selected genes involved in steroidogenesis during early gonadal development in zebrafish. This information will serve as a basis to investigate the molecular mechanism of EDCs on steroidogenic gene expression during early gonadal development in zebrafish.

Methods:

Genes were selected for their well known implication in steroidogenesis including steroid enzymes *(cyp11b2* and *cyp17a1)*, and a cholesterol mitochondrial transfer protein (*star*). Expression patterns were characterized using whole mount *in situ* hybridization in males and females zebrafish sampled at different stages of development (20, 30, 40 and 60 dpf or days post-fertilization). Fish were fixed overnight in 4% paraformaldehyde in PBS and hybridizations were performed with DIG-labeled probes on 10 different fishes for each gene and developmental stage investigated.

Results and discussion:

At the later stages of development (40 and 60 dpf), *star* and *cyp11b2* were more expressed in male gonads whereas *cyp17a1* was more strongly expressed in female gonads. At earlier stages (20 and 30 dpf) the precise identification of the sex phenotype of the gonads was not possible but similar differences were suspected as labeling was only found in some gonads. Interestingly, *star* and *cyp11b2* displayed a rather similar expression pattern characterized by small internal clusters of positive cells in male gonads and scattered positive cells mostly located at the periphery of the female gonads. For *cyp17a1* a similar male expression pattern was recorded but in female gonads expression was localized in the cytoplasm of previtellogenetic oocytes.

Conclusion:

Our study confirm previous results showing that some genes involved in steroidogenesis are differentially expressed during early development between male and female gonads. We will now extend this analysis to other genes involved in steroidogenesis including for instance *amh*, *cyp19a1a*, *cyp11a1*, *hsd3b1*, *foxl2a*, *nr0b1*, and *nr5a1b*. These precise gene expression patterns will set grounds for future studies with an ecotoxicological prospective.