Ontogeny of steroid hormone metabolism gene transcription during zebrafish embryonic development

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The zebrafish embryo has an impressive range of possible applications as a vertebrate model in both fundamental and applied research. Although the zebrafish model has many advantages for studying vertebrate development as well as impairment of normal development, many of the molecular mechanisms underlying the different developmental phases are still poorly understood. For example, surprisingly little is known about the role of steroid hormones in the early development of zebrafish. Although for some steroid hormones studies have shown that they have a role in various stages of development, available information is still limited.

Our study was designed to provide detailed baseline information about the transcriptional dynamics of key genes involved in the steroid hormone system during normal zebrafish embryonic development. We described the timing of the normal embryonic transcriptional activation of the hormone synthesis machinery and associated receptors during the early stages of development, which has never been done so far. We isolated RNA at 25 time points between 0 and 32 days post fertilisation of zebrafish development covering the most important events during the embryonic and larval stages. Here, we focus on early development starting from 1.5 hours post fertilisation (hpf) until 120hpf, since this is the age window in which zebrafish embryo experiments are currently considered alternative tests according to European regulation. We selected genes coding for the key enzymes involved in steroid hormones biosynthesis from the cytochrome P450 superfamily *(cyp11a1, cyp11a2, cyp17a1, cyp11c1, cyp19a1a, cyp19a1b)*, the hydroxysteroid beta dehydrogenases (*hsd17b3, hsd11b2* and *hsd17b1*), the estrogen and androgen receptors (*esr1, esr2a, esr2b, gper* and *ar*) as well as two enzymes involved in cholesterol biosynthesis *(hmgcrα, hmgcrβ)*, and vitellogenin (*vtg1*), which is widely used as a biomarker for toxicological experiments. Expression levels of these genes were measured using QPCR.

Our results show that some of the enzymes and receptors involved in the steroidogenesis pathway, like *hsd17b3*, *esr2a* and *ar*, are maternally transferred. This suggests an important role of steroid

hormones in programming the earliest stages of zebrafish development before the embryo's genome is activated around 3hpf. Further, we observed that the nuclear estrogen receptors have different transcriptional patterns during the development. Although *esr2a* is maternally transferred, *esr1* is abundantly transcribed by the embryo itself around the time of embryonic genome activation. Transcription of *esr2b* is low during the first 24 hours and gradually increases from that point on. Interestingly, transcription of *hsd17b3*, coding for an enzyme that is important to both estrogen and androgen synthesis, showed a high peak from the earliest stages, suggesting that *hsd17b3* plays an important role in steroid synthesis very early in development. After primary neurogenesis, *cyp19a1b, ar* and *hsd11b2* start to be transcribed, and increased transcription was observed during the pharyngula and organogenesis period.

These results will improve our fundamental understanding of the role of steroid hormones during early life stages of the zebrafish and will be used as a reference dataset for the development of new testing methods targeted at identifying various endocrine disrupting compounds which may act by altering these transcriptional patterns.