

A Computational Model of Male Fathead Minnow Reproductive System Regulation: Linking changes in gene expression with reproductive effects

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In animals, including humans, the endocrine system regulates processes vital for reproduction, growth, and metabolism through an array of biochemical and biomolecular signals that facilitate both positive and negative feedback control. Within the endocrine system, the hypothalamic-pituitary-gonadal (HPG) axis is viewed as the primary subsystem regulating processes important for reproduction. Fish exposed to wastewater treatment plant effluents in the U.S. have been found with gross reproductive abnormalities (e.g., intersex), altered steroid hormone levels, and altered male:female sex ratios in the populations which have been attributed to mixtures of low levels of endocrine disruption compounds (EDCs) in the water. Thus, our research goal is to understand how the fathead minnow (FHM, *Pimephales promelas*) HPG axis responds to selected EDCs, such as 17 α -ethinylestradiol (EE₂, a synthetic estrogen used in birth control pills).

We investigated FHM responses using a systems toxicology approach, which is a combination of traditional toxicology, computational modeling, and cutting-edge molecular-level assays. In our study, male FHMs were exposed to EE₂ for 48 hours. Reproductive endpoints such as concentrations of steroid hormones (i.e., 17 β -estradiol (E₂), testosterone (T), and 11-ketotestosterone (11-KT)) and vitellogenin (Vtg, a precursor to the egg yolk protein) in plasma were measured using traditional methods. Brain, gonad, and liver tissues were collected and gene expression changes were evaluated using 44 K FHM-specific oligonucleotide microarrays. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to verify microarray results of selected genes relevant to the HPG axis.

A physiologically based computational model (Fig. 1) was developed to simulate the HPG axis and reproductive endpoints in unexposed male FHMs and male FHMs exposed to EE₂. Mass balances were used to formulate a set of differential equations

describing steroid hormone and Vtg concentrations. The model was calibrated with measurements of E₂, T, 11-KT and Vtg concentrations in 154 unexposed male FHMs which were controls in different experiments (1-9), and measurements of EE₂, Vtg and T concentrations in four EE₂-exposed FHMs.

Within 48 hours, we observed a measurable, treatment-specific effect on gene expression and on steroid hormone concentrations in the fish treated with EE₂. These data suggest that short-term exposures may be a useful approach to examine the early estrogen receptor-mediated effects on the regulation of reproduction in fishes. The computational model successfully predicted E₂, T, 11-KT, Vtg, and EE₂ concentrations in both unexposed and EE₂-exposed FHMs, but all the predictions had variances lower than the measured data.

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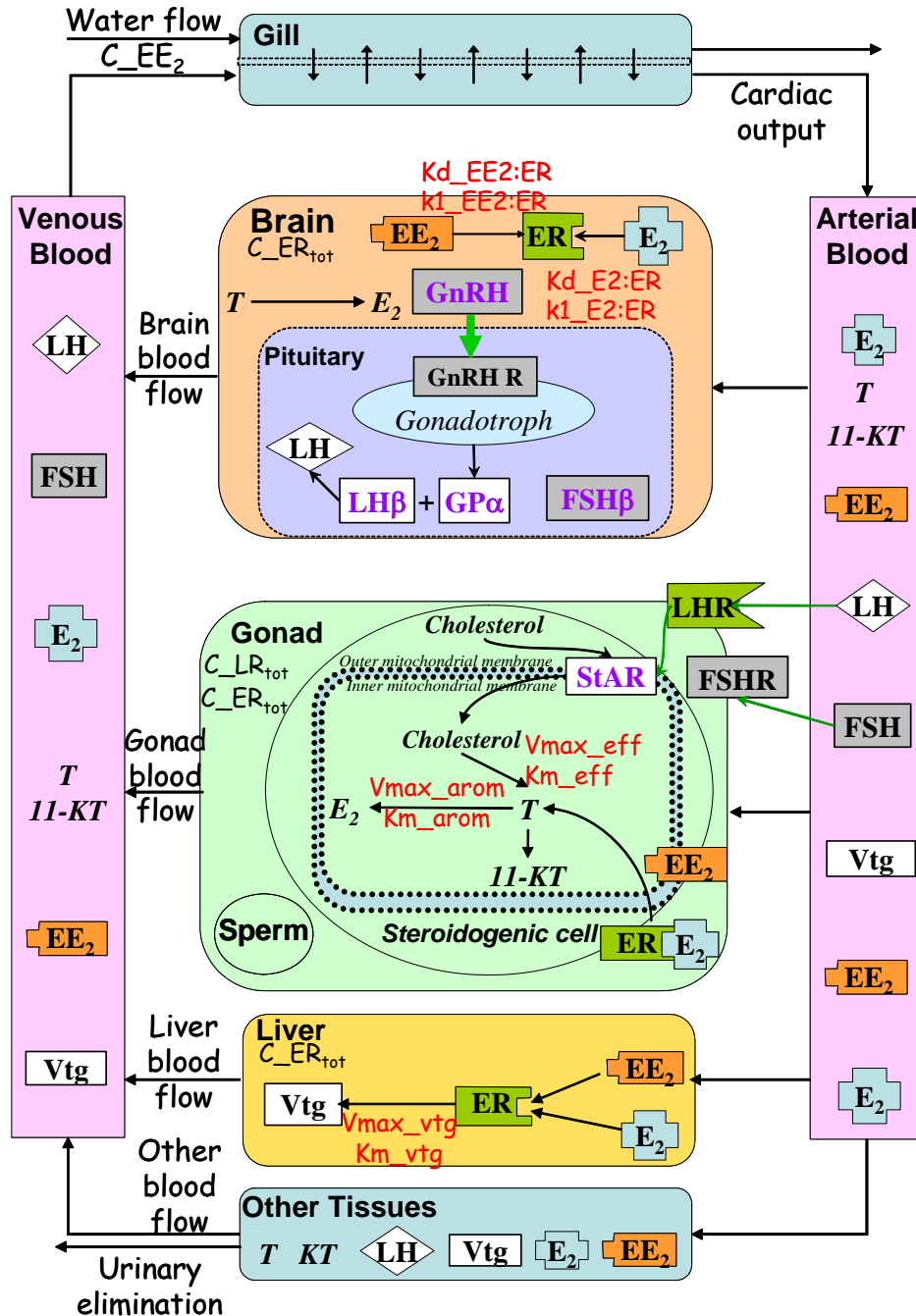


Fig. 1: Physiologically based model of the HPG axis in male FHM. Gray boxes represent features that are not currently formulated in the model. GnRH = gonadotropin releasing hormone; FSH β = follicle stimulating hormone-like subunit β ; LH β = luteinizing hormone-like

subunit β ; GP α = glycoprotein hormone α subunit; LH = luteinizing hormone, consists of GP α and LH β subunits; FSH = follicle stimulating hormone, consists of GP α and FSH β subunits; StAR = steroidogenic acute regulatory protein; ER = estrogen receptor; LHR = luteinizing hormone receptor; Vtg = vitellogenin; E₂ = 17 β -estradiol; T = testosterone; & 11-KT = 11-ketotestosterone. Red text indicates a model parameter. Based upon Villeneuve et al (10).