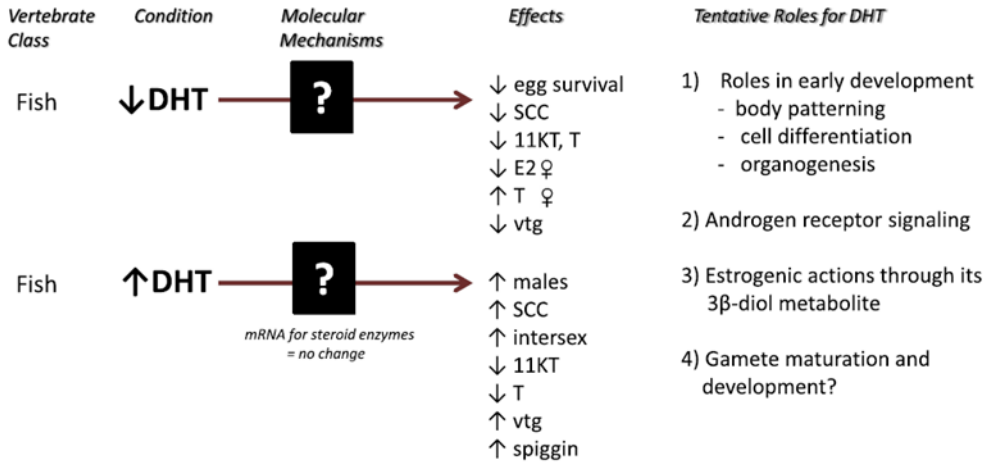

Effects of DHT in fish. The mechanisms associated with the biological responses (Martyniuk, Bissegger et al. 2013)

Relationships between two key events

Title	Relationship	Adjacency	Evidence	Quantitative understanding
5α-reductase, inhibition leads to Decrease, DHT level	MIE-KE1	Adjacent	High	High
Decrease, DHT level leads to Reduction, Plasma 17beta-estradiol concentrations	KE1-KE2	Adjacent	Low	Low
Reduction, Plasma 17beta-estradiol concentrations leads to Reduction, Plasma vitellogenin concentrations	KE2-KE3	Adjacent	High	High
Reduction, Plasma vitellogenin concentration leads to Reduction, plasma vitellogenin concentration	KE3-AO	Adjacent	High	High

DHT, dihydrotestosterone; MIE, molecular initiation event; KE, key event; AO, adverse outcome

WP 1: Development of metabolomics platform for evaluation of endocrine metabolism and disruption

Molecular initiating events (MIEs) and key events (KEs) will be identified for depicted Dutasteride and finesteride on steroidogenesis. This will allow us to build up an AOP for these substances. After exposure of organisms to EDCs in non-lethal concentrations, a metabolomics study using high resolution mass spectrometry will help to identification MIEs and the KEs on mitochondrial or endoplasmic reticulum CYP450, hydroxysteroid dehydrogenase, short-chain dehydrogenase/reductase. Protein and mRNA expression level will be measure for verification and identification of pathway.

- Development of analytic methods using mass spectrometry for cholesterol metabolism including steroids under endocrine disruption chemicals treatment. Selection of target enzymes in steroidogenesis and prediction of biological effects on knock-out and knock-in cell models.

  *Potential target enzymes: Steroid 5α-reductase, CYP11A1, CYP17, CYP19, CYP21, CYP11B1, CYP11B2, 3β-Hydroxysteroid dehydrogenase, and 17β-Hydroxysteroid dehydrogenases, aldoketo reductase, membrane-associated progesterone receptors

WP 2: Quantitative evaluation of endocrine disruption in developed platform

Effects of depicted quantitative metabolomics will be tested in certain *in vitro* cell models experiments using knockout/knock-in system. These experiments will be clarified possible effects on toxicity pathway.

- Evaluation of endocrine disruption effects using chemical inhibition on *in vitro* cell models
- Development of stable 5α reductase knockout/knock-in system using CRISPR/Cas9 system (dCas9/CRISPR-SAM)
- Identification of pathway using knockout/knock-in models

WP 3: Development of the quantitative adverse outcome pathway

Dose-response based qAOPs can be developed by systems toxicology approach

- Quantification of endocrine disruption effects using *in vivo* models which are invertebrates (water flea of brine shrimp) and vertebrates. To integrate the quantitative relationships generated by CYP450 expression or *in vivo* testing, quantitative AOPs (qAOPs) providing dose-time response are more critical than AOPs. We will compare previous approaches to qAOP building
- Identification of interspecies comparison of new target toxicity pathway in human, rodents, fish cell line and vertebrates model.
- Quantitative comparison of key event relationship