Introduction

Zebrafish sexual dimorphism is highly plastic during development, making this species an ideal model for investigation of the effects of endocrine disruption on gonadal development and function. However, the hormonal regulation of these processes in zebrafish is poorly understood. Here, we use androgen and glucocorticoid deficient fdx1b mutants to explore such processes.

Fdx1b is a co-factor to steroidogenic enzymes crucial for synthesis of cortisol and 11-ketotestosterone (11KT), the primary teleostean androgen.

Upon raising fdx1b mutant zebrafish to adulthood we observed that all of the adults exhibited female secondary sexual characteristics. However, dissection to expose the gonads led to the finding that despite their external female appearance, fdx1b mutants may possess either ovaries or testes.

Fdx1b mutant males exhibit feminised secondary sex characteristics and are infertile

- All adult fdx1b mutant males have female type pigmentation, especially in the dorsal and anal fins.
- Breeding behaviour is impaired in mutant males, but IVF was successful and sperm could be collected by manually.

Steroid deficiency due to mutation of fdx1b

Decreased concentrations of cortisol and 11KT measured by LC-MS/MS and decreased expression of cortisol responsive genes fkbp5 and pck1 and androgen responsive gene cyp2k22 measured by qPCR.

Disorganised seminiferous tubules and decreased sperm concentration in fdx1b mutants

- Fdx1b mutants have disorganised seminiferous tubules, fewer sperm and somatic cell hyperplasia.
- Decreased sperm concentration was confirmed by sperm counting.

Down-regulation of pro-male and spermatogenic genes

To further investigate the mechanism behind testicular disorganisation and decreased sperm concentration we analysed the expression of pro-male and spermatogenic genes using qPCR.

Sox9a is a transcription factor expressed in Sertoli cells with an important role in testis differentiation conserved in almost all vertebrates. In fish sox9a has also been linked to testicular tubule development. Interestingly, the expression of dmr1 and amh, genes also implicated in male development, was unaffected.

Igf3 and Ins3 are crucial for proliferation and differentiation of type A spermatogonia.

Inhibin A (Inha) exerts negative feedback on the hypothalamus-pituitary-gonadal (HPG) axis, reducing expression of fsh.

Disruption of spermatogenesis in Fdx1b mutants

- Expression of type A spermatogonia markers increased in Fdx1b mutants
- Indicates a blockade in spermatogenesis at the differentiation of type A→ type B spermatogonia

Summary and Conclusions

- Male fdx1b mutant zebrafish are androgen and cortisol deficient, exhibit feminised secondary sex characteristics, disorganised testicular structure and decreased sperm concentration.
- 11KT is not required, or only required at low levels, for testis differentiation but is required for correct development or maintenance of testis morphogenesis and function.
- Fdx1b promotes expression of sox9a, a transcription factor with a highly conserved role in male sex development. Sox9a has also been linked to testicular tubule development in another fish species.
- Fdx1b is required for expression of igf3 and ins3. Downregulation of these genes results in impaired spermatogenesis
- We anticipate that these insights will support further development of zebrafish mutants to study the interplay of genes and environmental factors in disorders of sex development.