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# Current perspectives on the androgen 5 alpha-dihydrotestosterone (DHT) and 5 alpha-reductases in teleost fishes and amphibians

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ABSTRACT

The androgen 5 alpha-dihydrotestosterone (DHT) is a steroidogenic metabolite that has received little attention in non-mammalian species. DHT is produced by the reduction of the double-bond of testosterone by a group of enzymes called 5 alpha-reductases of which there can be multiple isoforms (i.e., *srd5a1*, *srd5a2*, and *srd5a3*). Data from amphibians suggest that the expression of the *srd5a* genes occurs in early development, and continues until adulthood; however insufficient data exist in fish species, where DHT is thought to be relatively biologically inactive. Here, we demonstrate that fathead minnow (FHM; *Pimephales promelas*) developing embryos and adults express *srd5a* enzyme isoforms. During FHM embryogenesis, both *srd5a1* and *srd5a3* mRNA levels were significantly correlated in expression levels while *srd5a2* showed a more unique pattern of expression. In adult FHMs, males had significantly higher levels of *srd5a2* in the liver and gonad compared to females. In the male and female liver, transcript levels for *srd5a2* were more abundant compared to *srd5a1* and *srd5a3*, suggesting a prominent role for *srd5a2* in this tissue. Interestingly, the ovary expressed higher mRNA levels of *srd5a3* than the testis. Thus, data suggest that *srd5a* isoforms can show sexually dimorphic expression patterns in fish. We also conducted a literature review of the biological effects observed in embryonic and adult fish and amphibians after treatments with DHT and DHT-related compounds. Treatments with DHT in teleost fishes and amphibians have resulted in unexpected biological responses that are characteristic of both androgens and anti-androgens. For example, in fish DHT can induce vitellogenin *in vitro* from male and female hepatocytes and can increase 17β-estradiol production from the teleost ovary. We propose, that to generate further understanding of the roles of DHT in non-mammals, studies are needed that (1) address how DHT is synthesized within tissues of fish and amphibians; (2) examine the full range of biological responses to endogenous DHT, and its interactions with other signaling pathways; and (3) investigate how DHT production varies with reproductive stage. Lastly, we suggest that the Srd5a enzymes can be targets of endocrine disruptors in fish and frogs, which may result in disruptions in the estrogen:androgen balance in aquatic organisms.



## 1. Introduction

The interest in the effects of endocrine disrupting chemicals (EDCs) in the aquatic environment continues to increase over the past decade, specifically those that act on steroidogenic pathways. EDCs that include synthetic androgenic and estrogenic compounds can severely affect endocrine systems in wildlife and exert significant impacts on their reproduction, development and sexual behavior (reviewed in Colborn et al., 1993; Söffker and Tyler, 2012). For example, the pharmaceutical androgen 17β-trenbolone is used as a growth promoter in beef cattle farming and it has been shown to masculinize female fish (Ankley et al., 2003). This occurs because it is found in the aquatic environment predominantly in runoff, feedlot surface soils and manure on animal farming facilities. Hence, characterizing the range and magnitude of effects of androgens on development and reproduction is crucial for understanding how EDCs perturb androgen receptor signaling in aquatic organisms.

### 1.1. Androgens in fishes: multiple biological effects in males and females

In many teleost fishes, androgens have been shown to be involved in a broad range of biological functions, including reproduction, development, and behavior. Furthermore, androgens have

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also been shown to be involved in growth and osmoregulation in several teleost species (Sparks et al. , 2003; Sangiao-Alvarellos et al., 2006). Recently, it has been suggested that androgens also have an important role in immune responses. For example, Àguila et al. (2013) discovered that two dominant androgens, testosterone and 11-ketotestosterone (11KT), are involved in regulating the response of professional phagocytes in gilthead seabream (*Sparus aurata*). In addition to modulation of the immune response, other androgen related functions based upon transcriptomics and proteomics data in fish include apoptosis, transport and oxidation of lipids, synthesis and transport of hormones, protein metabolism, and cell proliferation (Martyniuk and Denslow, 2012). Thus, androgens are involved in diverse functions in teleostean species.

### 1.2. Testosterone: roles and biosynthesis

The sex hormone testosterone is perhaps the most well studied androgen in vertebrate taxa and it has important functions in both male and female mammals (Konkle and McCarthy, 2011), birds (Goymann, 2009), amphibians (Eikenaar et al., 2012), reptiles (Eikenaar et al., 2012), and fish (Borg, 1994). Testosterone is a metabolic precursor for estrogens and other androgenic steroids such as 11KT, which has more potent androgenic characteristics in fish. Testosterone is not only involved in the development of male sexual characteristics but is also involved in the production of estrogens via aromatization, carbohydrate, fat and protein metabolism, and osmoregulation (Sangiao-Alvarellos et al., 2006; Kelly and Jones, 2013). Testosterone is synthesized from either androstenediol or androstenedione by 3β-HSD and 17β-HSD, respectively, and has several metabolic pathways involving a range of different enzymes as shown in Fig. 1.

### 1.3. 11KT as the major androgen in male and female fishes

In teleost fishes, 11KT is considered to be the predominant androgen and has been shown to stimulate secondary sexual characteristics, spermatogenesis, reproductive behavior as well as the masculinization of female genotypes to a higher degree than testosterone (reviewed in Borg, 1994). Testosterone is converted to 11β-hydroxytestosterone by the enzyme cytochrome P450 11β-hydroxylase (P45011β) and then converted to 11KT by the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD). 11KT circulates in male teleost species at a higher plasma concentration than in their female counterparts and undergoes a seasonal cycle with the highest plasma concentration occurring during the pre-spawning season in some species (Borg, 1994). The abundance of 11KT in female plasma is generally not as high as testosterone, but in contrast to testosterone, the physiological actions of 11KT have not yet been fully described in female fishes (Lokman et al., 2002). Currently, most research with teleost fishes focuses on the biological functions of 11KT and testosterone; however other androgens with potentially important roles such as 5 alpha-dihydrotestosterone (DHT) have been understudied in fishes.

### 1.4. Dihydrotestosterone: roles and biosynthesis

In mammals, birds, reptiles, and amphibians the most potent androgen is considered to be DHT. Although 11KT is considered the major androgen in teleost fishes, it has been demonstrated by Asahina et al. (1985) that in urohaze goby (*Glossogobius olivaceus*), the main product of testosterone conversion was DHT and not 11KT, suggesting that some teleost species could show unexpected patterns in androgen biosynthesis. DHT is produced from testosterone by an enzyme class called the 5 alpha-reductases (*srd5a1*, *srd5a2*, and *srd5a3*; reviewed in Langlois et al., 2010a). These enzymes were previously found to be active in the teleostean central nervous system, indicating the potential for localized conversion of testosterone into DHT in the brain (Callard et al., 1980). Recent exposure of fathead minnows (*Pimephales promelas*; FHMs) to DHT revealed that both DHT and 11KT can induce androgenic responses in fish (Margiotta-Casaluci and Sumpter, 2011), suggesting that DHT may also have important physiological roles in male and female fishes similar to 11KT.

To better clarify some of the potential roles of DHT in non-mammalian species we (1) generated a developmental profile of 5 alpha-reductase transcripts during early embryogenesis in the FHM; (2) investigated the distribution of the three *srd5a* isoforms in both male and female brain, liver, ovary, and testis; (3) reviewed studies that investigate the effects of DHT during fish and amphibian development; and (4) synthesized evidence to suggest that DHT can be a biologically active androgen in fish and amphibians.

## 2. Dihydrotestosterone and 5 alpha-reductase in early development

Androgens are present in early vertebrate development and have been detected in oocytes and in unfertilized and fertilized eggs. Testosterone is present in Japanese rice fish (*Oryzias latipes*) female fully grown oocytes (4–5 pg), then decreases in concentration in unfertilized eggs (2–3 pg) until after ~1 d incubation (1–2 pg) (Iwamatsu et al., 2006a). After fertilization, testosterone concentration remains between 0.2 and 1.0 pg/animal (up to 10 dpf) which suggests roles for testosterone metabolites in early development. In frogs, both testosterone and DHT have been detected at levels ranging from 2000 to 4000 pg/g ww in Nieuwkoop-Faber stage 20 African clawed frog (*Xenopus laevis*) embryos which corresponds to approximately 22 h post fertilization (Bögi et al., 2002). Noteworthy is that high androgen levels decrease towards the limit of detection of radioimmunoassays at metamorphosis, implying that elevated androgen levels are essential for early frog development. In amphibians, the ontogeny of the enzymes involved in DHT production (i.e., *srd5a* isoforms) has been examined in detail during early development of the Western clawed frog (*Silurana tropicalis*; Langlois et al., 2010b). All three isoforms are maternally transferred as their transcripts are detected at the frog egg stage (Nieuwkoop-Faber stage 2) suggesting that the presence of DHT in frog embryos could also be produced *de novo* from the animal itself. At the Nieuwkoop-Faber stage 16, all three transcriptional profiles significantly increase until organogenesis, and *srd5a2* shows the larger surge of transcript being produced. Although *srd5a* isoform expression has been studied in amphibian development, there is no information as to the ontogeny of the *srd5a* isoforms in teleost fishes.

To address this knowledge gap, we collected FHM early life staged embryos and fry generated from six breeding pairs of one year old fish from a breeding colony at the University of New Brunswick (Saint John, NB, CAN). Embryos were collected at 1 day post fertilization (1 dpf), 3 dpf, 5 dpf (prior to hatch), one day post hatch (6 dpf), and 14 dpf. During these early stages of development, major landmarks in developing embryos include body patterning, development of the anterior dorsal section of the mouth, organogenesis, and central nervous system development (Kimmel et al., 1995; Liu and Chang, 2002). To measure the expression of the *srd5a* isoforms during early FHM development, we optimized real-time PCR bioassays and assessed expression levels over the first 2 weeks of life (Fig. 2). We point out that others have also recently identified *srd5a* isoforms in fish. For example, after screening the NCBI ESTs database, *srd5a1* and *srd5a2* were identified in FHMs by Margiotta-Casaluci et al. (2013b) and the zebrafish (*Danio rerio*) genome contains at least three isoforms for *srd5a* (NM_001076653, NM_001017703, NM_001044939).

The *srd5a* isoforms showed unique expression profiles in early FHM development (Fig. 2). Similar to amphibian embryos, there