Sex-specific responses to growth hormone and luteinizing hormone in a model teleost, the Mozambique tilapia

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Highlights

- oGH and oLH acted synergistically to restore GSI and ovarian morphology in females
- *ghr2* expression was higher in ovary than in testis
- Gonadal *igf3* was increased by oGH and oLH in both sexes
- Ovarian *erβ* and *arβ* levels were restored by a combination of oGH and oLH
- Muscle and hepatic *ghr2* were more responsive to oGH in males versus females

Abstract

Across the vertebrate lineage, sexual dimorphism in body size is a common phenomenon that results from trade-offs between growth and reproduction. To address how key hormones that regulate growth and reproduction interact in teleost fishes, we studied Mozambique tilapia (*Oreochromis mossambicus*) to determine whether the activities of luteinizing hormone (Lh) are modulated by growth hormone (Gh), and conversely, whether targets of Gh are affected by the presence of Lh. In particular, we examined how gonadal morphology and specific gene transcripts responded to ovine GH (oGH) and/or LH (oLH) in hypophysectomized male and female tilapia. Hypophysectomized females exhibited a diminished gonadosomatic index (GSI) concomitant with ovarian follicular atresia. The combination of oGH and oLH restored GSI and ovarian morphology to conditions observed in sham-operated controls. A similar pattern was observed for GSI in males. In control fish, gonadal *gh receptor* (*ghr2*) and *estrogen receptor β* (*erβ*) expression was higher in females versus males. A combination of oGH and oLH restored *erβ* and *arβ* in females. In males, testicular *insulin-like growth factor 3* (*igf3*) expression was reduced following hypophysectomy and subsequently restored to control levels by either oGH or oLH. By contrast, the combination of both hormones was required to recover ovarian *igf3*

expression in females. In muscle, *ghr2* expression was more responsive to oGH in males versus females. In the liver of hypophysectomized males, *igf2* expression was diminished by both oGH and oLH; there was no effect of hypophysectomy, oGH, or oLH on *igf2* expression in females. Collectively, our results indicate that gene transcripts associated with growth and reproduction exhibit sex-specific responses to oGH and oLH. These responses reflect, at least in part, how hormones mediate trade-offs between growth and reproduction, and thus sexual dimorphism, in teleost fishes.

Keywords: gonadotropins, fish, growth, liver, muscle, receptors, reproduction, sexual dimorphism

1. Introduction

Sexual dimorphism with respect to body size is prevalent among animal taxa from crustaceans to mammals, including fishes. In bony fishes, this pattern is observed across a wide range of species, such as garfish (*Belone belone*; Samsun et al., 2006), mackerel (*Scomber scombrus*; Eltink, 1987), several groundfish (Hanson and Courtenay, 1997; Jiménez et al., 1998), and tilapia (Chervinski, 1965). Sexual dimorphism results from differences in sexual maturation, growth, and life span between sexes, in addition to other selective pressures (Fairbairn et al., 2007; Hüssy et al., 2012; Ralls and Mesnick, 2009; Stamps, 1993). Moreover, growth and reproductive processes interact in many vertebrates and evidence suggests that differences in the allocation of energy towards somatic growth versus reproductive capacity may underlie sex-specific growth rates (Cox, 2006; Rennie et al., 2008; Taylor and DeNardo, 2005).

Like many other teleost fishes, tilapia exhibit sexually dimorphic growth patterns, with males growing faster than females. In Mozambique tilapia (*Oreochromis mossambicus*), these differences in growth rate were previously attributed to the direct actions of androgens and estrogens as well as their modulation of the growth hormone (GH)/insulin-like growth factor 1 (Igf1) axis (Kuwaye et al., 1993; Riley et al., 2002; Seale et al., 2020; Sparks et al., 2003). Different growth rates between sexes may also result from the timing of gonadal development and sexual maturation (Bhatta et al., 2012b). In males, growth rates decreased only after complete sexual maturation, while in females, growth decreased prior to sexual maturation. Moreover, the removal of gonads retarded growth while ectopic transplantation of gonadal tissue restored normal body growth of Mozambique tilapia (Bhatta et al., 2012a), suggesting that gonadal hormones impact growth in both sexes. The endocrine intermediaries between growth and reproduction, however, require elucidation. In salmonids, somatic growth is reduced during sexual maturation when lipids are mobilized from visceral adipose tissue and muscle (Aussanasuwannakul et al., 2011; Manor et al., 2012; Nassour and Leger, 1989). During the sexual maturation of rainbow trout (*Oncorhynchus mykiss*), nutrition and energy are redirected away from somatic growth to support ovarian development by increasing muscle protein turnover (Cleveland and Weber, 2011, 2016; Weber et al., 2022). It remains unclear, however, how the interaction between growth and reproductive development is regulated by hormonal signals within males and females.

The endocrine system of fishes regulates a range of physiological processes, including growth and reproduction. Secreted by the pituitary gland, Gh regulates various physiological processes including somatic growth (Duan, 1997) through its receptors (Ghrs). Two *ghrs* subtypes were found in Mozambique tilapia, *ghr1* and *ghr2* (Kajimura et al., 2004; Pierce et al.,

2007), with *ghr2* encoding the primary Gh receptor (Kajimura et al., 2004; Pierce et al., 2007, 2012). Gh regulates growth and development directly through the Ghr2 and indirectly through the stimulation of Igfs (Butler and Le Roith, 2001; Duan, 1998; Duan et al., 2010; Le Roith et al., 2001; Reindl and Sheridan, 2012). Also secreted by the pituitary gland, follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh) regulate gametogenesis and the production of gonadal hormones in males and females (Levavi-Sivan et al., 2010; Schulz et al., 2001; Swanson et al., 2003). In teleosts, it was established that the two gonadotropins, Gth I and Gth II are orthologues of tetrapod Fsh and Lh, respectively (Li and Ford 1998; Quérat et al. 2000; Suzuki et al. 1988a, b, c). Lh and Fsh operate through their cognate receptors in Leydig cells and Sertoli cells in males, and follicular cells in females (Lubzens et al., 2017; Schulz et al., 2001), to stimulate the production of androgens and estrogens, respectively (Kagawa et al., 1998; Okada et al. 1994; Planas et al. 2000; Planas and Swanson, 1995; Swanson et al., 2003). Gonadal steroids further regulate gametogenesis and sexual maturation (Forsgren and Young, 2012; Miura and Miura, 2003; Monson et al., 2017; Nagahama, 1994; Nagahama et al., 1994; Schulz et al., 2001) with androgen and estrogen receptors (*ar* and *er*) mediating the gonadal responsiveness to sex steroid hormones (Gross and Yee, 2002; Park et al., 2007). Gh may also directly modulate reproduction by exerting both gonadotropin-dependent and -independent actions in both males and females (Hull and Harvey, 2014). Hence, it is necessary to examine the effects of hormones that regulate growth and reproduction on Ghr2, Igfs, and sex steroid receptors to understand the endocrine mechanisms that may underlie sexually dimorphic growth.

The combination of hypophysectomy with hormone replacement is a classic experimental approach to identify the endocrine effects of pituitary hormones. Through this approach, we previously described the effects of Gh on hepatic leptin levels (Douros et al., 2016), plasma Igfs

and hepatic *ghr2*, *igf1*, and *igf2* expression (Breves et al., 2014), and intestinal *ghr2* expression (Petro-Sakuma et al., 2020). Given their well-described patterns of sexually dimorphic growth, we used hypophysectomized Mozambique tilapia as a model to examine whether the activities of Lh are modulated by Gh, and conversely, whether targets of Gh are affected by the presence of Lh. We administered ovine GH and ovine LH alone or in combination before examining the morphology of gonads and gene expression patterns for *ghr2*, *igfs*, *ers*, and *ars* in gonad, muscle, and liver.

2. Materials and methods

2.1 Animals and rearing conditions

Male and female Mozambique tilapia (*O. mossambicus*) with mean (± S.E.M.) body weights (BW) of 92.6 ± 3.1 and 93.0 ± 3.0 g, respectively, were obtained from stocks maintained at the Hawai‘i Institute of Marine Biology. Given the sexually-dimorphic nature of this species, the mean BW was kept similar between males and females to control for any influence of BW on sex-specifc responses. Fish were maintained outdoors with a continuous flow of fresh water (FW; municipal water) under natural photoperiod and fed a commercial diet (Silver Cup Trout Chow, Nelson & Sons Inc., Murray, UT). Water temperatures were maintained between 24 and 26 °C. All housing and experimental procedures were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee of the University of Hawai‘i.

2.2 Hypophysectomy and hormone replacement

Hypophysectomy was performed by the transorbital technique developed by Nishioka (1994). Mozambique tilapia were anesthetized by immersion in buffered tricaine

methanesulfonate (100 mg/l, Argent Chemical Laboratories, Redmond, WA) and 2-phenoxyethanol (2-PE; 0.3 ml/l, Sigma-Aldrich, St Louis, MO) in FW. After removal of the right eye and underlying tissue, a hole was drilled through the neurocranium and the pituitary was aspirated with a modified Pasteur pipette. The orbit was then packed with microfibrillar collagen hemostat (Ethicon, Somerville, NJ) and fish were allowed to recover for 5 d in brackish water (12‰) composed of seawater diluted with FW. Following recovery, fish were transferred to re-circulating experimental aquaria containing aerated brackish water and treated with kanamycin sulfate (National Fish Pharmaceuticals, Tucson, AZ). Sham operations were carried out in the same manner but without aspiration of the pituitary.

To characterize the effects of GH and LH on gonadosomatic index (GSI; (gonad weight/BW)*100), and gonadal, muscle, and hepatic gene expression, hypophysectomized fish (n = 8) were administered ovine GH (oGH; 5 μg/g BW, National Hormone and Peptide Program) and ovine LH (oLH; 5 μg/g BW, National Hormone and Peptide Program) alone or in combination via intraperitoneal injections over the course of 5 d. oGH and oLH were delivered in saline vehicle (0.9% NaCl; 1.0 μl/g BW). Forty-eight hours after the initial injection, second and third injections were administered 48 h apart. Twenty-four hours after the third injection, fish were netted, lethally anesthetized in 2-PE, and weighed prior to the removal of the gonads for calculation of GSI. Muscle, liver, and gonad samples were collected, immediately snap-frozen in liquid nitrogen, and stored at −80 °C until RNA extraction. A portion of the gonads was fixed in 4% paraformaldehyde and processed for histological analyses. Two additional groups, hypophysectomized and sham-operated fish (control), were injected with saline vehicle only. Fish were not fed for the duration of the recovery and post-injection periods. At sampling, the

completeness of hypophysectomy was confirmed by post-mortem inspection of the hypothalamic region.

2.3 Histological analysis

Fixed ovary and testis fragments were dehydrated in a series of graded ethanol, cleared with xylene, and embedded in paraffin wax. The embedded gonadal fragments were cut into 5 μm sections and stained with hematoxylin-eosin for histological analysis. Gonadal sections were examined using a light microscope (Olympus BX43; Olympus Corp., Center Valley, PA) equipped with a digital camera (Infinity 3s; Lumenera, Ottawa, ON).

2.4 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from gonad, muscle, and liver using TRI Reagent (MRC, Cincinnati, OH) according to the manufacturer's protocols. The concentration and purity of extracted RNA were assessed using a microvolume spectrophotometer (NanoDrop One, Thermo Fisher Scientific, Waltham, MA). Total RNA (300-500 ng) was reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). The mRNA levels of reference and target genes were determined by the relative quantification method using a StepOnePlus real-time PCR system (Thermo Fisher Scientific). The qRT-PCR reaction mix (15 μl) contained Power SYBR Green PCR Master Mix (Thermo Fisher Scientific), 200 nM of forward and reverse primers, and 1 μl cDNA. Dilution of experimental cDNA from gonad, muscle, and liver ranged from 10- to 50-fold. PCR cycling parameters were: 2 minutes at 50 °C, 10 minutes at 95 °C followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 1 minute. All qRT-PCR primers have been previously described and are listed with PCR efficiencies in Table

1. The geometric mean ($\sqrt[3]{x_1 * x_2 * x_3}$; where x = quantity of each reference gene) of *elongation factor 1α* (*ef1α*), *ß-actin*, and *18s* levels was used to normalize target genes levels (Celino-Brady et al., 2019).

2.5 Statistical analysis

Group comparisons were performed by two-way ANOVA followed by Fisher's protected LSD test when significant main or interaction effects were detected. In order to meet assumptions of normality (assessed by Shapiro-Wilk test), individual values were log-transformed, when necessary, prior to statistical analysis. Pearson correlation coefficients were used to describe the relationship between GSI and *igf3* levels. Statistical calculations were performed using Prism 9.0 (GraphPad, La Jolla, CA). Significance for all tests was set at $P < 0.05$.

3. Results

3.1 Effects of hypophysectomy and hormonal treatment on GSI and gonadal morphology

Overall, there was a strong effect of sex on GSI. GSI was higher in females than in males, and was reduced in females following hypophysectomy. While neither oGH nor oLH when administered alone could restore GSI, the combined administration of both hormones restored GSI to values similar to sham-operated controls. On the other hand, although not significant ($P = 0.4891$), there was a tendency for GSI in males to decrease following hypophysectomy and a tendency for the combined administration of oGH and oLH to restore GSI (Fig. 1).

Histological analysis indicated that sham-operated fish exhibited normal gonad morphology. Males displayed maturing testes with spermatozoa, and in females, ovaries with vitellogenic oocytes were visible (Figs. 2 and 3). Except for vacuolization, hypertrophy of

interstitial tissue, and death of a few germ cells, there were no profound histological changes between the testis from control and hypophysectomized groups (Figs. 2A and B). Testes morphology of fish treated with oGH, oLH, or both hormones in combination did not markedly differ from control fish (Fig. 2). In females, however, hypophysectomy was followed by extensive atresia of ovarian follicles in 57% of the fish (Figs. 3A and B**)**. The administration of oGH and oLH alone had no effect on hypophysectomy-induced atresia (Figs. 3C and D); the administration of oGH with oLH restored normal ovarian morphology in most of the fish (Fig. 3E).

3.2 Effects of hypophysectomy and hormonal treatment on gonadal ghr2 and igfs

In control groups, gonadal *ghr2* levels were higher in females than in males. Ovarian *ghr2* levels were reduced following hypophysectomy and restored by oLH or the combination of oLH and oGH; when administered alone, oGH only partially restored *ghr2* levels. By contrast, hypophysectomy did not affect *ghr2* levels in testis; administration of oLH alone or in combination with oGH reduced *ghr2* levels (Fig. 4A).

In controls, *igf1* levels were higher in the testis compared with ovary. Testicular *igf1* was reduced following hypophysectomy and restored by oGH alone or in combination with oLH. When administered alone, oLH had no effect on testicular *igf1* expression. In females, there were no effects of hypophysectomy or hormones on ovarian *igf1* levels (Fig. 4B). Ovarian *igf2* was reduced in hypophysectomized females but there were no clear effects of oGH or oLH administration. In males, while testicular *igf2* was unaffected by hypophysectomy, *igf2* expression was stimulated by oGH both alone or in combination with oLH (Fig. 4C). Gonadal *igf3* levels were higher in males than in females. Among gonadal *igfs*, *igf3* was markedly

reduced following hypophysectomy in both males and females. In testis, *igf3* levels were restored by both oGH and oLH alone or in combination. In females, *igf3* expression was only restored by the combination of both hormones (Fig. 4D). *igf3* expression in both males and females was significantly correlated with GSI (r^2=0.32 and r^2=0.29, respectively).

3.3 Effects of hypophysectomy and hormonal treatment on gonadal ar and er

The expression of gonadal *erα* was higher in males than females. Testicular *erα* levels were reduced following hypophysectomy; oGH and oLH alone, or in combination, restored or increased *erα* levels beyond controls. Ovarian *erα* was also reduced following hypophysectomy; neither oGH nor oLH had an effect on *erα* (Fig. 5A). Unlike *erα*, gonadal *erβ* expression was higher in females versus males. The co-administration of oGH and oLH stimulated ovarian *erβ* after hypophysectomy. There were no clear responses to hypophysectomy or hormone treatments in the testis (Fig. 5B).

There was no response of gonadal *arα* to any treatments in either males or females (Fig. 5C). On the other hand, *arβ* was higher in the testis compared with ovaries. Ovarian *arβ* decreased following hypophysectomy and was stimulated by oLH alone or in combination with oGH. The combined treatment of oLH with oGH increased *arβ* to levels observed in controls. By contrast, there were no effects of oGH or oLH on *arβ* in the testis (Fig. 5D).

3.4 Effects of hypophysectomy and hormonal treatment on muscle ghr2 and igfs

In both sexes, muscle *ghr2* expression decreased following hypophysectomy. The response of *ghr2* to hormonal treatments followed similar pattern in both sexes; oGH alone or combination with oLH stimulated *ghr2* levels. In males, however, *ghr2* was more responsive to

oGH (Fig. 6A). The responses of *igf1* to all treatments were similar between males and females. Hypophysectomy was followed by a reduction in *igf1* that was restored by oGH alone or combined with oLH. oLH did not affect *igf1* (Fig. 6B). In males, an increase in *igf2* expression occurred following hypophysectomy; *igf2* was further elevated by oGH alone or in combination with oLH. oLH alone, however, slightly diminished *igf2* in hypophysectomized fish. In females, *igf2* did not respond to hypophysectomy but was stimulated by oGH or oLH alone and in combination. Moreover, *igf2* was more responsive to oLh in females than in males (Fig. 6C).

3.5 Effects of hypophysectomy and hormonal treatment on hepatic ghr2 and igfs

In the liver, *ghr2* levels were higher in males than in females. In males, there were no changes in *ghr2* following hypophysectomy; oGH had the tendency to increase hepatic *ghr2* levels while treatment with oLH alone or in combination with oGH diminished *ghr2* when compared with fish treated with oGH alone. In females, *ghr2* expression was reduced following hypophysectomy; oGH and oLH could not fully restore *ghr2* expression to control levels (Fig. 7A). There were no changes in *igf1* expression in either sex following hypophysectomy. The administration of oLH alone or in combination with oGH reduced *igf1* in males. In females, oGH diminished *igf1* expression compared with saline-injected hypophysectomized fish (Fig. 7B). *igf2* expression was markedly increased following hypophysectomy in males; oGH and oLH alone or in combination diminished the *igf2* levels. There were no changes in *igf2* expression following hypophysectomy and hormone administration in females (Fig. 7C).

4. Discussion

While the manifestations of sexually dimorphic growth have been addressed in terms of energetic costs or reproductive strategy (Cox, 2006; Fairbairn et al., 2007; Henderson et al., 2003; Rennie et al., 2008; Taylor and DeNardo, 2005), the endocrine mechanisms underlying these phenomena have not been fully resolved. In this study, we identified sex-specific responses to oGH and oLH in hypophysectomized Mozambique tilapia by endocrine/paracrine regulators of growth and reproduction. We found that following hypophysectomy the combined administration of oGH and oLH restored GSI and ovarian morphology. Further examination of ovarian and testicular gene targets revealed sex-specific responses by *ghr2*, *igf*s, and steroid hormone receptors. Sex-specific responses of *ghr2* and *igf*s were also observed in muscle and liver.

The importance of Gh and Lh to gonadal function was underscored by the restoration of GSI following their combined administration to hypophysectomized females. By contrast, hypophysectomy had no effect on male GSI. These results were consistent with histological observations, where hypophysectomy resulted in atresia in ovarian follicles and vacuolization and hypertrophy of interstitial cells in the testis. The reduced GSI in hypophysectomized females and the tendency for GSI to decrease in males could be attributed to the morphological changes and degeneration of gonadal cells in both sexes. Ovarian atresia is a common phenomenon in teleosts under both natural and experimental conditions (Guraya, 1986; Saidapur, 1978;). Atretic ovarian follicles are frequently associated with changes in hormone levels (Nagahama, 1983, 1994; Nagahama et al., 1995). In fish, impairment of endocrine function through the reduction of pituitary Fsh, and plasma E_2 and Igf1, is associated with the induction of follicular atresia (Guraya, 1986; Yamamoto et al., 2011). Consistent with these studies, our results show that both

Gh and Lh are necessary for complete ovarian development. In males, like in females, gametogenesis is regulated by gonadotropins, Gth I and Gth II, orthologues of tetrapod Fsh and Lh (Levavi-Sivan et al., 2010; Schulz et al., 2001). Miura et al. (1991a, b) demonstrated the function of gonadotropins through the induction of complete spermatogenesis by human chorionic gonadotropin, an analog of Lh, *in vitro*.

In addition to its role in regulating growth, Gh also modulates reproduction. For example, Gh stimulated spermatogonial proliferation in Japanese eel (*Anguilla japonica*) (Miura et al., 2011). Despite only a tendency for hypophysectomy to reduce GSI and for hormonal administration to restore GSI, hypophysectomy elicited morphological changes in the testis. The more pronounced effect of hypophysectomy, oGH, and oLH in females may stem from the greater requirement for these hormones in ovarian development at later stages and the more energy that is allocated to gonadal development in females compared with males.

Gh plays an important role in the regulation of Ghr2 and Igfs in a variety of organs including gonads (Breves et al., 2014; Hull and Harvey, 2014; Petro-Sakuma et al., 2020; Pierce et al., 2011, 2012). Furthermore, Lh also regulates circulating and gonadal Igf1 (Bradley et al., 2006; Chandrashekar and Bartke, 2003). Our results indicate that oGH and oLH modulated gonadal *ghr2* and *igf* levels in both males and females. The Gh/Igf1 axis is not only central to vertebrate growth, but is also implicated in the control of reproduction (Duan, 1997; 1998). Inasmuch as sexual differentiation requires germ cell proliferation and gonadal tissue growth (Nakamura et al., 1998), growth factors are critical regulators of reproductive processes. In particular, Igfs exert actions on gonad development and differentiation (Duan et al., 1997; Lu et al., 2005; Wood et al., 2005).

Furthermore, gonadal steroid hormones modulate the Gh/Igf1 axis, a process that is important for the initiation of puberty and sexually dimorphic growth (Leung et al., 2004; Meinhardt and Ho, 2006). So far, there are limited studies comparing the expression of Gh/Igf axis genes between testis and ovary in fish. In Mozambique tilapia and fathead minnow (*Pimephales promelas*), *ghr2* expression is greater in ovary versus testis (Davis et al., 2008; Filby ad Tyler, 2007). These previous findings coincide with our results that show higher *ghr2* expression in control ovaries compared with testes. There were also differences in the sensitivity of gonadal *ghr2* to both hypophysectomy and hormonal treatments between sexes. In Nile tilapia (*O. niloticus*), *ghr2* transcripts were highest in the ovary during sexual maturation, while in the testis, *ghr2* expression was highest during sexual recrudescence and declined during maturation and regression (Ma et al., 2007). These findings concur with our results and explain the lower gonadal *ghr2* and its lack of response to oGH in males.

In contrast to *ghr2*, higher levels of *igf1* transcripts were found in the testis compared with the ovary. Furthermore, although Igf1 is implicated in both male and female gametogenesis, not much is known about the differences in the function of Igf1 in gonadal development between sexes. Igfs are responsive to Gh and direct follicular growth, lipid uptake, and steroid biosynthesis (Campbell et al., 2006; Kagawa et al., 2003; Lokman et al., 2007; Maestro et al., 1997; Paul et al., 2010; Weber and Sullivan, 2001). Igf1 is necessary for the action of the main teleost androgen, 11-ketotestosterone (11-KT), to initiate spermatogenesis. (Miura and Miura, 2001). Moreover, both systemic Igf1 and granulosa cell-derived Igf1 can affect ovarian function (Baroiller et al., 2014; Berishvili et al., 2006; Kagawa et al., 1995, Perrot et al., 2000; Reinecke, 2010). Thus, although both sexes might require Igf1 for normal gonadal development,

differences in expression levels could be attributed to the extent to which local Igf1 is required for steroid biosynthesis at each stage of gonadal maturation.

Unlike Igf1, relatively little is known about the physiological roles of Igf2 in fish reproduction. While Igf2 is capable of inducing germinal vesicle breakdown in red sea bream (*Pagrus major*), the effect of Igf1 is more pronounced (Kagawa et al., 1994). Interestingly, among gonadal *igfs*, the effects of hypophysectomy, oGH, and oLH were most pronounced on *igf3*, especially in males. Moreover, *igf3* responses to hypophysectomy and hormonal treatment in both males and females closely correlated with GSI. Li et al. (2012) reported that gonadal *igf3* expression was higher in male versus female Nile tilapia from 50 to 70 days after hatching, and varied with the age of individuals. Berishvili et al. (2010) also found higher *igf3* in the testis versus the ovary in adult Nile tilapia. The gonad-specific expression of Igf3 and its differing responses to oGH and oLH from Igf1 and Igf2, suggests distinct roles for Igf3 in gonad development (Wang et al., 2008). Our findings, therefore, are consistent with previous studies supporting sex-dependent patterns of *igf3* expression.

Er and Ar play major roles in mediating the responsiveness of target tissues to estrogens and androgens (Park et al., 2007). The neuroendocrine control of reproductive and metabolic homeostasis is affected by changes in receptor expression and/or function (Goksoyr, 2006; Tabb and Blumberg, 2006; Vijayan et al., 2005). Here, we observed sex- and subtype-specific modulation of gonadal steroid hormone receptors in male and female tilapia.

The steroid hormone E_2 functions in both males and females to regulate reproductive processes (Esterhuyse et al., 2010) by binding specific receptors, Erα and Erβ (Gross and Yee, 2002; Park et al., 2007). *erα* levels were higher in the testis than in the ovary, while *erβ* levels were higher in the ovary than in the testis. Previous studies indicate that patterns of *er* subtype

366 expression vary according to age, sex, reproductive stage, and species (Chakraborty et al., 2011;
367 Davis et al., 2008; Nagasawa et al., 2014). Transcripts of testicular and ovarian *erα* were both
368 diminished following hypophysectomy. While all hormonal treatments strongly upregulated
369 testicular *erα* levels, only oLH or its co-administration with oGH, stimulated ovarian *erα* levels.
370 In contrast, *erβ* seems to be less susceptible to the effects of hypophysectomy, oGH, and oLH.
371 Hence, our data indicate both sex-dependent and receptor sub-type specific responses by *ers* to
372 hypophysectomy, oGH, and oLH. In a breeding stock of Mozambique tilapia, no sex-specific
373 expression patterns of gonadal *erα* and *erβ* were detected (Esterhuyse et al., 2010). In another
374 study, however, both *ers* were higher in ovaries of sexually mature fish (Davis et al., 2008). In
375 all-male or all-female Nile tilapia fry produced by artificial fertilization, there were no
376 differences in *erα* levels between ovary and testis until 70 days after hatch when *erα* transcripts
377 were higher in males (Ijiri et al., 2008). These differences between studies may indicate a
378 diversity of E_2 signaling requirements of germ cells among tilapia species. The differential
379 responsiveness of *erα* and *erβ* to hypophysectomy, oGH and oLH, as well as the higher level of
380 testicular *erα*, indicates an important role for *erα* in spermatogenesis. E_2 induced spermatogonial
381 stem cell renewal and spermatogonial proliferation in Japanese eel and threespot wrasse
382 (*Halichoeres trimaculatus*) (Kobayashi et al., 2011; Miura et al., 1999, 2003), thus as mediators
383 of E_2 effects, Ers play a role in normal testicular development.

384 As observed with *ers*, *ars* also exhibited sex- and sub-type dependent patterns of
385 expression, where *arβ* was higher in testis than in ovary. In males, 11-KT appears to be the major
386 sex steroid that influences male reproduction (Borg, 1994; Kobayashi et al., 1999; Nagahama et
387 al., 1994; cf Turcu et al., 2018). Moreover, 11-KT can induce all stages of spermatogenesis in *A.*
388 *japonica* (Miura et al., 1991a, b). In *Astatotilapia burtoni*, Arβ2 is preferentially activated by 11-

KT compared with testosterone (Olsson et al., 2005), which is consistent with the elevated levels of *arβ* found in the testis of *O. mossambicus*. Although *arβ* levels in the ovary were much lower than that of testis, *arβ* respond to hypophysectomy; and the co-administration of oLH and oGH strongly induced ovarian *arβ* levels in hypophysectomized females. Androgens have been implicated in female sexual development of several teleost species (Lokman et al., 1998). For example, in cod (*Gadus morhua*), androgen treatment promoted primary follicle development (Kortner et al., 2008, 2009), while in coho salmon (*Oncorhynchus kisutch*), 11-KT induced follicle growth and the appearance of cortical alveoli in the cytoplasm (Forsgren and Young, 2012). Thus, the stimulation of *arβ* by oLH and oGH in female tilapia supports pituitary control of ovarian androgenic responsiveness. The importance of *arβ* signaling during follicle development is further supported by observations in which levels of this isoform, but not *arα*, varied with follicular stages (García-López et al., 2011).

In muscle, Ghr2 and Igfs are stimulated by Gh, which in turn induce a variety of growth-promoting effects (Butler and Le Roith, 2001; Duan et al., 2010; Le Roith, 2003; Mommsen, 2001; Wood et al., 2005). Consistent with these patterns, *ghr2* and *igf1* transcript levels were diminished following hypophysectomy and subsequently stimulated by oGH. The magnitude of muscle *ghr2* induction, however, was greater in males. These results not only reinforce the essential role of Gh in regulating its receptor and Igf1, but also imply that sensitivity of this signaling pathway varies between male and females. A greater sensitivity of the Gh/Igf axis in males is consistent with males outgrowing females in this species. Specifically, our previous studies in Mozambique tilapia showed that somatic growth is positively correlated with pituitary *gh* and muscle *ghr2* expression (Moorman et al., 2016) and that pituitary *gh* expression is greater in males than in females (Seale et al., 2020). Thus, the current findings are consistent with sex-

specific modulation of *ghr2* in a fashion that supports enhanced somatic growth in males. Both Igf1 and -2 have direct effects on muscle growth in fish (Fuentes et al., 2013; Garikipati and Rodgers, 2012a, 2012b; Montserrat et al., 2012;). Specific aspects underlying the control of muscle growth, however, may be differentially controlled by the Igf isoforms. Igf2, for example, had stronger effects than Igf1 on myocyte proliferation in sea bream (*Sparus aurata*) (Rius-Francino et al., 2011). Because *igf2* was relatively unresponsive to pituitary control, the underlying differences between *igf1* and *-2* regulation are not clear.

Consistent with a previous experiment in Mozambique tilapia, our results showed higher hepatic *ghr2* expression in males compared with females (Davis et al., 2008). Other than the tendency of hypophysectomy to decrease *ghr2*, and a tendency of oGH to restore *ghr2*, there were no marked effects of hypophysectomy on *ghr2* and *igf1* expression. Previous studies showed that oGH stimulated plasma Igf1 and hepatic *ghr2* levels, and restored *ghr2* levels in anterior and middle intestine in hypophysectomized fish (Breves et al., 2014; Petro-Sakuma et al., 2020). These previous experiments, however, employed only a single intraperitoneal injection of oGH, while in the present study, three intraperitoneal injections of oGH were administered. There was no effect of sham operation on somatotropic axis genes, indicating that handling stress may not be directly linked to the lack of response of *igf1* to oGH treatment. While there was an increase in hepatic *ghr2*, this lack of response of hepatic *igf1* to oGH was also observed by Breves et al. (2014) in hypophysectomized tilapia. In contrast, Pierce et al. (2011) found that oGH injection resulted to increase in hepatic *igf1* levels. This experiment, however, did not use hypophysectomized fish. Hence, differences in responses could be attributed to differences in experimental design or frequency of injections.

Hypophysectomy resulted in increased *igf2*; oGH and oLH suppressed the hypophysectomy-induced rise in hepatic *igf2*. These effects, however, were not seen in females. Hypophysectomy-induced stimulation of *igf2* and its strong suppression by oGH could be explained by a feedback effect of hepatic *igf2* brought about by the loss of Gh. The removal of Gh may trigger the Gh/Igf axis to compensate by elevating *igf2*.

In summary, the current study identified regulators of growth and reproduction in tilapia that exhibit sex-specific regulation by oGH and oLH. The high sensitivity of ovarian transcripts to a lack of Lh and Gh compared with males implies important synergistic functions of these hormones on ovarian development. The distinct responses of *igf3* when compared with other *igf* isoforms indicate that its translated product plays key roles in gonadal development. The higher expression of hepatic *ghr2* and the strong response of *ghr2* to oGH in muscle in males suggests enhanced Gh signaling in support of somatic and systemic growth promotion, whereas the higher *ghr2* levels in ovary compared to testis indicate a shift towards gonadal development in females. Taken collectively, our data provide new insight into how hormones underlie sexual dimorphism in tilapia by resolving the interactions between somatotropic and gonadotropic endocrine axes.

CRediT authorship contribution statement

Fritzie T. Celino-Brady: Conceptualization, Methodology, Investigation, Formal Analysis, Validation, Writing - original draft, Writing- review & editing, Visualization. **Jason P. Breves:** Conceptualization, Methodology, Investigation, Formal Analysis, Writing - review & editing, Funding acquisition. **Andre P. Seale:** Conceptualization, Methodology, Investigation, Formal Analysis, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Aussanasuwannakul, A., Kenney, P.B., Weber, G.M., Yao, J., Slider, S.D., Manor, M.L., Salem, M., 2011. Effect of sexual maturation on growth, fillet composition, and texture of female rainbow trout (*Oncorhynchus mykiss*) on a high nutritional plane. Aquaculture 317 (1), 79–88. https://doi.org/10.1016/j.aquaculture.2011.04.015.

Baroiller, J., D'Cotta, H., Shved, N., Berishvili, G., Toguyeni, A., Fostier, A., Eppler, E., Reinecke, M., 2014. Oestrogen and insulin-like growth factors during the reproduction and

480 growth of the tilapia *Oreochromis niloticus* and their interactions. Gen. Comp. Endocrinol.
481 205, 142–150. https://doi.org/10.1016/j.ygcen.2014.07.011.
482 Berishvili, G., D'Cotta, H., Baroiller, J.F., Segner, H., Reinecke, M., 2006. Differential
483 expression of IGF-I mRNA and peptide in the male and female gonad during early
484 development of a bony fish, the tilapia *Oreochromis niloticus*. Gen. Comp. Endocrinol. 146,
485 204–210. https://doi.org/10.1016/j.ygcen.2005.11.008.
486 Berishvili G, Baroiller JF, Eppler E, Reinecke M., 2010. Insulin-like growthfactor-3 (IGF-3) in
487 male and female gonads of the tilapia: development and regulation of gene expression by
488 growth hormone (GH) and 17alphaethinylestradiol (EE2). Gen. Comp. Endocrinol. 167,
489 128–134. https://doi.org/10.1016/j.ygcen.2010.01.023.
490 Bhatta, S., Iwai, T., Miura, C., Higuchi, M., Shimizu-Yamaguchi, S., Fukada, H.,Miura, T.,
491 2012a. Gonads directly regulate growth in teleosts. Proceedings of the National Academy of
492 Sciences of the United States of America, 109, 11408–11412 2012.
493 Bhatta, S., Iwai, T., Miura, T., Higuchi, M., Maugars, G., Miura, C., 2012b. Differences between
494 male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). J.
495 Sci. Eng. Technol. 8 (2), 57–65. https://doi.org/10.3126/kuset.v8i2.7326
496 Borg, B., 1994. Androgens in teleost fishes. Comp. Biochem. Physiol. C Toxicol. Pharmacol.
497 109 (3), 219–245. https://doi.org/10.1016/0742-8413(94)00063-G.Breves, J.P., Tipsmark, C.
498 K., Stough, B. A., Seale, A. P., Flack, B. R., Moorman, B. P., Lerner, D. T., Grau, E.G.,
499 2014. Nutritional status and growth hormone regulate insulin-like growth factor binding
500 protein (igfbp) transcripts in Mozambique tilapia. Gen. Comp. Endocrinol. 207, 66–73.
501 https://doi.org/10.1016/j.ygcen.2014.04.034.

Butler, A.A., LeRoith, D., 2001. Minireview: tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology 142 (5), 1685-1688. https://doi.org/10.1210/en.142.5.1685.

Campbell, B., Dickey J., Beckman B, Young G, Pierce A, Fukada H, Swanson P., 2006. Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor-1, estradiol- 17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. Biol. Reprod. 75, 34–44. https://doi.org/10.1095/biolreprod.105.049494.

Celino-Brady FT, Petro-Sakuma CK, Breves JP, Lerner DT, Seale AP. Earlylifeexposure to 17beta-estradiol and 4-nonylphenol impacts the growth hormone/insulin-like growth-factor system and estrogen receptors in Mozambique tilapia, Oreochromis mossambicus. Aquat Toxicol (2019) 217:105336. doi: 10.1016/j.aquatox.2019.105336.

Chakraborty, Shibata, Y., Zhou, L.-Y., Katsu, Y., Iguchi, T., & Nagahama, Y., 2011. Differential expression of three estrogen receptor subtype mRNAs in gonads and liver from embryos to adults of the medaka, *Oryzias latipes*. Mol. Cell. Endocrinol. 333 (1), 47–54. https://doi.org/10.1016/j.mce.2010.12.002..

Chandrashekar, V., Bartke, A., 2003. The role of insulin-like growth factor-I in neuroendocrine function and the consequent effects on sexual maturation: inferences from animal models. Reprod. Biol. 3 (1), 7–28.

Chervinski, J., 1965. Sexual dimorphism in tilapia. Nature 208:703.

523 Cleveland, B.M., Weber, G.M., 2011. Effects of sex steroids on indices of protein turnover in
524 rainbow trout (*Oncorhynchus mykiss*) white muscle. Gen. Comp. Endocrinol. 174, 132–142.
525 https://doi.org/10.1016/j.ygcen.2011.08.011.
526 Cleveland, B.M., Weber, G.M., 2016. Effects of steroid treatment on growth, nutrient
527 partitioning, and expression of genes related to growth and nutrient metabolism in adult
528 triploid rainbow trout (*Oncorhynchus mykiss*). Domest. Anim. Endocrinol. 56, 1-12.
529 https://doi.org/10.1016/j.domaniend.2016.01.001.
530 Cox, R. M., 2006. A Test of the Reproductive Cost Hypothesis for Sexual Size Dimorphism in
531 Yarrow's Spiny Lizard *Sceloporus jarrovi*i. J. Anim. Ecol. 75 (6), 1361–1369.
532 https://doi.org/10.1111/j.1365-2656.2006.01160.x.
533 Davis, L.K., Pierce, A.L., Hiramatsu, N., Sullivan, C.V., Hirano, T., Grau, E.G., 2008. Gender-
534 specific expression of multiple estrogen receptors, growth hormone receptors, insulin-like
535 growth factors and vitellogenins, and effects of 17 beta-estradiol in the male tilapia
536 (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 156, 544-551.
537 https://doi.org/10.1016/j.ygcen.2008.03.002.
538 Douros, J.D., Baltzegar, D. A., Mankiewicz, J., Taylor, J., Yamaguchi, Y., Lerner, D. T., Seale,
539 A. P., Gordon Grau, E., Breves, J. P., Borski, R. J., 2016. Control of leptin by metabolic
540 state and its regulatory interactions with pituitary growth hormone and hepatic growth
541 hormone receptors and insulin like growth factors in the tilapia (*Oreochromis*
542 *mossambicus*). Gen. Comp. Endocrinol. 240, 227–237.
543 https://doi.org/10.1016/j.ygcen.2016.07.017.
544 Duan, C., 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. J.
545 Nutr. 128, 306S-314S. https://doi.org/10.1093/jn/128.2.306S.

546 Duan, C., 1997. The insulin-like growth factor system and its biological actions in fish. Am
547 Zool. 37, 491–503. https://doi.org/10.1093/icb/37.6.491.
548 Duan, C., Ren, H., Gao, S., 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-
549 binding proteins: roles in skeletal muscle growth and differentiation. Gen. Comp.
550 Endocrinol. 167, 344–351. https://doi.org/10.1016/j.ygcen.2010.04.009.
551 Eltink, A. T. G. W. 1987. Changes in age- and size distribution and sex ratio during spawning
552 and migration of Western mackerel (*Scomber scombrus L.*). J. Cons. Int. Explor. Mer., 44:
553 10–22.
554 Esterhuyse, Helbing, C., van Wyk, J., 2010. Isolation and characterization of three estrogen
555 receptor transcripts in *Oreochromis mossambicus* (Peters). J. Steroid Biochem. Mol. Biol.
556 119 (1), 26–34. https://doi.org/10.1016/j.jsbmb.2009.12.002.
557 Fairbairn, D. J., Blanckenhorn, W. U., Székely, T., 2007. Sex, size and gender roles:
558 Evolutionary studies of sexual size dimorphism. Oxford University Press.
559 https://doi.org/10.1093/acprof:oso/9780199208784.001.0001.
560 Filby, A.L., Tyler, C.R., 2007. Cloning and characterization of cDNAs for hormones and/or
561 receptors of growth hormone, insulin-like growth factor-I, thyroid hormone, and
562 corticosteroid and the gender-, tissue-, and developmentalspecific expression of their mRNA
563 transcripts in fathead minnow (*Pimephales promelas*). Gen. Comp. Endocrinol. 150, 151–
564 163. 151–163. https://doi.org/10.1016/j.ygcen.2006.07.014.
565 Forsgren, K.L., Young, G., 2012. Stage-specific effects of androgens and estradiol-17beta on the
566 development of late primary and early secondary ovarian follicles of coho salmon
567 (*Oncorhynchus kisutch*) in vitro. Biol. Reprod. 87, 64.
568 https://doi.org/10.1095/biolreprod.111.098772.

569 Fuentes, E.N., Valdés, J. A., Molina, A., Björnsson, B. T., 2013. Regulation of skeletal muscle
570 growth in fish by the growth hormone – Insulin-like growth factor system. Gen. Comp.
571 Endocrinol. 192, 136–148. https://doi.org/10.1016/j.ygcen.2013.06.009.
572 García-López, A., Sánchez-Amaya, M. I., Prat, F., 2011. Targeted gene expression profiling in
573 European sea bass (*Dicentrarchus labrax, L.*) follicles from primary growth to late
574 vitellogenesis. Comp. Biochem. Physiol. A Mol Integr. Physiol. 160 (3), 374–380.
575 https://doi.org/10.1016/j.cbpa.2011.07.006.
576 Garikipati, D.K., Rodgers, B.D., 2012a. Myostatin stimulates myosatellite cell differentiation in
577 a novel model system: evidence for gene subfunctionalization. Am. J. Physiol. Regul. Integr.
578 Comp. Physiol. 302, R1059–1066. https://doi.org/10.1152/ajpregu.00523.2011.
579 Garikipati, D.K., Rodgers, B.D., 2012b. Myostatin inhibits myosatellite cell proliferation and
580 consequently activates differentiation: evidence for endocrine-regulated transcript
581 processing. J. Endocrinol. 215, 177–187. 215(1), 177–187. https://doi.org/10.1530/JOE-12-
582 0260..
583 Goksoyr, A., 2006. Endocrine disruptors in the marine environment: mechanisms of toxicity and
584 their influence on reproductive processes in fish. J. Toxicol. Environ. Health A. 69, 175–
585 184. https://doi.org/10.1080/15287390500259483.
586 Gross, J.M., Yee, D., 2002. How does the estrogen receptor work? Breast Cancer Res. 4, 62-64.
587 https://doi.org/10.1186/bcr424.
588 Guraya, S.S., 1986. The cell and molecular biology of fish oogenesis, in: Sauer, H.W. (ed.),
589 Monographs in Developmental Biology, Karger, Basel, pp.169-180.

590 Hanson, J. M., Courtenay, S. C., 1997. Seasonal distribution, maturity, condition, and feeding of
591 smooth flounder (*Pleuronectes putnami*) in the Miramichi estuary, southern Gulf of St.
592 Lawrence. Can. J. Zool. 75: 1226–1240. https://doi.org/10.1139/z97-145.
593 Henderson, B., Collins, N.C., E Morgan, G., Vaillancourt, A., 2003. Sexual size dimorphism of
594 walleye (*Stizostedion vitreum vitreum*). Can. J. Fish. Aquat. Sci. 60, 1345–1352.
595 https://doi.org/10.1139/f03-115.
596 Hull, K.L., Harvey, S., 2014. Growth Hormone and Reproduction: A Review of Endocrine and
597 Autocrine/Paracrine Interactions. Int. J. Endocrinol. 2014, 234014–234024.
598 https://doi.org/10.1155/2014/234014.
599 Hüssy, K. Coad, J. O., Farrell, E. D., Clausen, L. W., Clarke, M. W, 2012. Sexual Dimorphism
600 in Size, Age, Maturation, and Growth Characteristics of Boarfish (*Capros Aper*) in the
601 Northeast Atlantic. ICES J. Mar. Sci 69 (10), 1729–1735.
602 https://doi.org/10.1093/icesjms/fss156
603 Ijiri, S., Kaneko, H., Kobayashi, T., Wang, D.-S., Sakai, F., Paul-Prasanth, B., Nakamura, M.,
604 Nagahama, Y., 2008. Sexual Dimorphic Expression of Genes in Gonads During Early
605 Differentiation of a Teleost Fish, the Nile Tilapia *Oreochromis niloticus*. Biol. Reprod. 78
606 (2), 333–341.
607 Jiménez, M. P., Sobrino, I., and Ramos, F., 1998. Distribution pattern, reproductive biology, and
608 fishery of the wedge sole *Dicologlossa cuneata* in the Gulf of Cadiz, south-west Spain. Mar.
609 Biol. 131 (1), 173–187. https://doi.org/10.1007/s002270050308.
610 Kagawa, H., Kobayashi, M,. Hasegawa, Y., Aida K., 1994; Insulin and insulinlike growth
611 factors I and II induce final maturation of oocytes of red seabream, *Pagrus major*, in vitro.
612 Gen Comp Endocrinol 95: 293–300. https://doi.org/10.1006/gcen.1994.1126.

613 Kagawa, H., Gen, K., Okuzawa, K., Tanaka, H., 2003. Effects of Luteinizing Hormone and
614 Follicle-Stimulating Hormone and Insulin-Like Growth Factor-I on Aromatase Activity and
615 P450 Aromatase Gene Expression in the Ovarian Follicles of Red Seabream, *Pagrus major*.
616 Biol. Reprod. 68 (5), 1562–1568. https://doi.org/10.1095/biolreprod.102.008219.
617 Kagawa, H., Moriyama, S., Kawauchi, H., 1995. Immunocytochemical localization of IGF-I in
618 the ovary of red seabream, *Pagrus major*. Gen. Comp. Endocrinol. 95, 293–300.
619 https://doi.org/10.1006/gcen.1995.1114.
620 Kagawa, H., Tanaka, H., Okuzawa, K., Kobayashi, M., 1998. GTH II but Not GTH I Induces
621 Final Maturation and the Development of Maturational Competence of Oocytes of Red
622 Seabream in Vitro. Gen. Comp. Endocrinol. 112 (1), 80–88.
623 Kajimura, S., Kawaguchi, N., Kaneko, T., Kawazoe, I., Hirano, T., Visitacion, N., Grau, E.G.,
624 Aida, K., 2004. Identification of the growth hormone receptor in an advanced teleost, the
625 tilapia (*Oreochromis mossambicus*) with special reference to its distinct expression pattern
626 in the ovary. J. Endocrinol. 181, 65–76. https://doi.org/10.1677/joe.0.1810065.
627 Kobayashi M, Nakanishi T., 1999. 11-ketotestosterone induces male-type sexual behavior and
628 gonadotropin secretion in gynogenetic crucian carp, *Carassius auratus langsdorfii*. Gen.
629 Comp. Endocrinol. 115 (2), 178–87. https://doi.org/10.1006/gcen.1999.7314.
630 Kobayashi, Y., Nozu, R., Nakamura, M., 2011. Role of Estrogen in Spermatogenesis in Initial
631 Phase Males of the Three-Spot Wrasse (*Halichoeres trimaculatus*): Effect of aromatase
632 inhibitor on the testis. Dev. Dyn. 240, 116–121. https://doi.org/10.1002/dvdy.22507.
633 Kortner, T.M., Rocha, E., Arukwe, A., 2009. Androgenic modulation of early growth of Atlantic
634 cod (*Gadus morhua L*.) previtellogenic oocytes and zona radiata-related genes. J. Toxicol.
635 Environ. Health. A 72:184–195. https://doi.org/10.1080/15287390802539020.

636 Kortner, T.M., Rocha, E., Silva, P., Castro, L.F., Arukwe, A., 2008. Genomic approach in
637 evaluating the role of androgens on the growth of Atlantic cod (*Gadus morhua*)
638 previtellogenic oocytes. Comp. Biochem. Physiol. Part D Genomics Proteomics 3, 205–218.
639 https://doi.org/10.1016/j.cbd.2008.04.001.
640 Kuwaye, T.T., Okimoto, D.K., Shimoda, S.K., Howerton, R.D., Lin, H.R., Pang, P.K.T., Grau,
641 E.G., 1993. Effect of 17 alpha -methyltestosterone on the growth of the euryhaline tilapia,
642 *Oreochromis mossambicus*, in fresh water and in sea water. Aquaculture. 113, 137–152.
643 https://doi.org/10.1016/0044-8486(93)90347-2.
644 Le Roith, D., 2003. The insulin-like growth factor system. Exp. Diabesity Res. 4, 205–212.
645 https://doi.org/10.1155/EDR.2003.205.
646 Le Roith, D., Scavo, L., Butler, A., 2001. What is the role of circulating IGF-I? Trends
647 Endocrinol. Metab. 12, 48-52. https://doi.org/10.1016/S1043-2760(00)00349-0.
648 Leung, K.C., Johannsson, G., Leong, G.M., Ho, K.K.Y., 2004. Estrogen regulation of growth
649 hormone action. Endocr. Rev. 25, 693–721. https://doi.org/10.1210/er.2003-0035.
650 Levavi-Sivan, B., Bogerd, J., Mañanós, E.L., Gómez, A., Lareyre, J.J., 2010. Perspectives on
651 Fish Gonadotropins and Their Receptors. Gen. Comp. Endocrinol. 165 (3), 412–437.
652 https://doi.org/10.1016/j.ygcen.2009.07.019.
653 Li, M.D., Ford, J.J., 1998. A comprehensive evolutionary analysis based on nucleotide and
654 amino acid sequences of the α- and β-subunits of glycoprotein hormone gene family. J.
655 Endocrinol. 156: 529–542. https://doi.org/10.1677/joe.0.1560529.
656 Li, M., Wu, F., Gu, Y., Wang, T., Wang, H., Yang, S., Sun, Y., Zhou, L., Huang, X., Jiao, B.,
657 Cheng, C.H., Wang, D., 2012. Insulin-like growth factor 3 regulates expression of genes encoding

steroidogenic enzymes and key transcription factors in the Nile tilapia gonad. Biol. Reprod. 86 (5), 1–10. https://doi.org/10.1095/biolreprod.111.096248.

Lokman, P.M., George, K.A., Divers, S.L., Algie, M., Young, G., 2007. 11-Ketotestosterone and IGF-I increase the size of previtellogenic oocytes from shortfinned eel, *Anguilla australis*, in vitro. Reproduction 133, 955–967. https://doi.org/10.1530/REP-06-0229.

Lu, C., Lam, H.N., Menon, R.K., 2005. New members of the insulin family: regulators of metabolism, growth and now...reproduction. Pediar. Res.57, 70R–73R. https://doi.org/10.1203/01.PDR.0000159573.55187.CA.

Lubzens, E., Bobe, J., Young, G., Sullivan, C.V., 2017. Maternal investment in fish oocytes and eggs: The molecular cargo and its contributions to fertility and early development. Aquaculture, 472(SI), 107–143. https://doi.org/10.1016/j.aquaculture.2016.10.029.

Ma, X., Liu, X., Zhang, Y., Zhu, P., Ye, W., Lin, H., 2007. Two growth hormone receptors in Nile tilapia (*Oreochromis niloticus*): molecular characterization, tissue distribution and expression profiles in the gonad during the reproductive cycle. Comp. Biochem. Physiol. B 147, 325–339. https://doi.org/10.1016/j.cbpb.2007.01.021.

Maestro, M.A., Planas, J.V., Moriyama, S., Gutierrez, J,, Planas, J., Swanson, P., 1997. Ovarian receptors for insulin and insulin-like growth factor I (IGF-I) and effects of IGF-I on steroid production by isolated follicular layers of the preovulatory coho salmon ovarian follicle. Gen. Comp. Endocrinol. 106:189–201. https://doi.org/10.1006/gcen.1996.6863.

Manor, M.L., Weber, G.M., Salem, M., Yao, J.B., Aussanasuwannakul, A., Kenney, P.B., 2012. Effect of sexual maturation and triploidy on chemical composition and fatty acid content of energy stores in female rainbow trout, *Oncorhynchus mykiss*. Aquaculture 364, 312–321. https://doi.org/10.1016/j.aquaculture.2012.08.012.

Magdeldin, S., Uchida, K., Hirano, T., Grau, E.G., Abdelfattah, A., Nozaki, M., 2007. Effects of environmental salinity on somatic growth and growth hormone/insulin-like growth factor-I axis in juvenile tilapia, *Oreochromis mossambicus*. Fish. Sci. 73, 1023–1032. https://doi.org/10.1111/j.1444-2906.2007.01432.x.

Meinhardt, U.J., Ho, K.K.Y., 2006. Modulation of growth hormone action by sex steroids. Clin. Endocrinol. 65, 413–422. https://doi.org/10.1111/j.1365-2265.2006.02676.x.

Miura,T., Miura, C., Ohta,T., Nader, M.R.,Todo, T.,Yamauchi, K., 1999. Estradiol-17β stimulates the renewal of spermatogonial stem cells in males. Biochemical and Biophysical Research Communications 264, 230–234.

Miura, T., Miura, C., 2001. Japanese eel: a model for analysis of spermatogenesis. Zoological Science 18 1055–1063.

Miura, T., Ohta, T., Miura, C., Yamauchi, K., 2003. Complementary deoxyribonucleic acid cloning of spermatogonial stem cell renewal factor. Endocrinology 144, 5504–5510.

Miura, C., Shimizu, Y., Uehara, M., Ozaki, Y., Young, G., Miura, T., 2011. Gh is produced by the testis of Japanese eel and stimulates proliferation of spermatogonia. Reproduction 142 (6), 869–877.

Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1991a. Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). PNAS 88 5774–5778.

Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1991b. Induction of spermatogenesis in male Japanese eel, *Anguilla japonica*, by a single injection of human chorionic gonadotropin. Zoological Science 8 63–73.

703 Mommsen, T.P., 2001.Paradigms of Growth in Fish.Comparative Biochemistry and Physiology,
704 Part B 129.(2-3), 207–219.
705 Montserrat, N., Capilla, E., Navarro, I., Gutiérrez, J., 2012. Metabolic effects of insulin and IGFs
706 on Gilthead sea bream (Sparus aurata) muscle cells. Front. Endocrinol. 3, 55.
707 Monson, C., Forsgren, K., Goetz, G., Harding, L., Swanson, P., Young, G., 2017. A teleost
708 androgen promotes development of primary ovarian follicles in coho salmon and rapidly
709 alters the ovarian transcriptome. Biology of Reproduction, 97(5), 731–745.
710 Moorman, B.P.1., Yamaguchi, Y., Lerner, D.T., Grau, E.G., Seale, A.P., 2016. Rearing
711 Mozambique tilapia in tidally-changing salinities: Effects on growth and the growth
712 hormone/insulin-like growth factor I axis. Comparative Biochemistry and Physiology. Part
713 A, Molecular and Integrative Physiology 198, 8-14. doi: 10.1016/j.cbpa.2016.03.014.
714 Nagahama, Y., 1983. The Functional Morphology of Teleost Gonads. In Fish Physiology
715 (Chapter 6, Vol. 9, pp. 223–275). Elsevier Science & Technology.
716 Nagahama, Y., 1994. Endocrine regulation of gametogenesis in fish. The International Journal of
717 Developmental Biology, 38(2), 217–229.
718 Nagahama, Y., Miura, T., Kobayashi, T,. 1994. The onset of spermatogenesis in fish. Ciba
719 Found Symp.182: 255–67.
720 Nagahama, Y., Yamashita, M., Tokumoto, T., Katsu, Y., 1995. Regulation of oocyte growth and
721 maturation in fish. Current topics in Developmental Biology 30: 30, 103–145.
722 Nagasawa, K., Presslauer, C., Kirtiklis, L., Babiak, I., & Fernandes, J. M. O., 2014. Sexually
723 dimorphic transcription of estrogen receptors in cod gonads throughout a reproductive cycle.
724 Journal of Molecular Endocrinology, 52 (3), 357–371.

Nakamura, M., Kobayashi, T., Chang, X.T., Nagahama, Y., 1998. Gonadal sex differentiation in fish. J Exp Zool 281:362–372.

Nassour, I., Leger, C.L., 1989. Deposition and mobilisation of body fat during sexual maturation in female trout (*Salmo gairdneri* Richardson). Aquat. Living Resour. 2, 153–159.

Okada, T., Kawazoe, I., Kimura, S., Sasamoto, Y., Aida, K., Kawauchi, H., 1994. Purification and characterization of gonadotropin I and II from pituitary glands of tuna (T*hunnus obesus*). Int. J. Pept. Protein Res. 43, 69–80.

Olsson, P.E., Berg, A.H., von Hofsten, J., Grahn, B., Hellqvist, A., Larsson, A., Karlsson, J., Modig, C., Borg, B., Thomas, P., 2005. Molecular cloning and characterization of a nuclear androgen receptor activated by 11-ketotestosterone. Reprod Biol Endocrinol 3:37.

Park, C.B., Takemura, A., Aluru, N., Park, Y.J., Kim, B.H., Lee, C.H., Lee, Y.D., Moon, T.W., Vijayan, M.M., 2007. Tissue-specific suppression of estrogen, androgen and glucocorticoid receptor gene expression in feral vitellogenic male Mozambique tilapia. Chemosphere 69, 32-40.

Paul, S., Pramanick, K., Kundu, S., Kumar, D., Mukherjee, D., 2010. Regulation of ovarian steroidogenesis in vitro by IGF-I and insulin in common carp, *Cyprinus carpio*: stimulation of aromatase activity and P450arom gene expression, Mol. Cell. Endocrinol. 315: 95–103.

Perrot, V., Moiseeva, E.B., Gozes, Y., Chan, S.J.,, 2000. Funkenstein B. Insulin-like growth factor receptors and their ligands in gonads of a hermaphroditic species, the gilthead seabream (*Sparus aurata*): expression and cellular localization. Biol Reprod; 6:229–241.

Petro-Sakuma, C.K., Celino-Brady, F. T., Breves, J. P., Seale, A.P., 2020. Growth hormone regulates intestinal gene expression of nutrient transporters in tilapia (*Oreochromis mossambicus*). General and Comparative Endocrinology, 292, 113464–113464.

748 Pierce, A.L., Breves, J.P., Moriyama, S., Hirano, T., Grau, E.G., 2011. Differential regulation of
749 Igf1 and Igf2 mRNA levels in tilapia hepatocytes: effects of insulin and cortisol on GH
750 sensitivity. Journal of Endocrinology 211, 201–210.
751 Pierce, A;L., Breves, J., Moriyama, S., Uchida, K., Grau, E., 2012. Regulation of growth
752 hormone (GH) receptor (GHR1 and GHR2) mRNA level by GH and metabolic hormones in
753 primary cultured tilapia hepatocytes. General and Comparative Endocrinology, 179:1, 22–
754 29.
755 Pierce, A.L., Fox, B.K., Davis, L.K., Visitacion, N., Kitahashi, T., Hirano, T., Grau, E.G., 2007.
756 Prolactin receptor, growth hormone receptor, and putative somatolactin receptor in
757 Mozambique tilapia: tissue specific expression and differential regulation by salinity and
758 fasting. General and Comparative Endocrinology 154, 31-40.
759 Planas, J.V., Athos, J., Goetz, F.W., Swanson, P., 2000. Regulation of Ovarian Steroidogenesis
760 In Vitro by Follicle-Stimulating Hormone and Luteinizing Hormone During Sexual
761 Maturation in Salmonid Fish1. Biology of Reproduction, 62(5), 1262–1269.
762 Planas, J.V., Swanson, P., 1995. Maturation-associated changes in the response of the salmon
763 testis to the steroidogenic actions of gonadotropins (GtH-I and GtH-II) in vitro. Biol.
764 Reprod. 52, 697–704.
765 Quérat, B., Sellouk, A. and Salmon, C.. 2000. Phylogenetic analysis of the vertebrate
766 glycoprotein hormone family including new sequences of sturgeon (*Acipenser baeri*)
767 subunits of two gonadotropins and the thyroid-stimulating hormone. Biol. Reprod. 63: 222–
768 228.

769 Ralls, K., Mesnick, S., 2009. Sexual dimorphism. In W. F. Perrin, B. Würsig, & J. G. M.
770 Thewissen (Eds.). Encyclopedia of marine mammals (pp. 1005–1011). (2nd ed.). London:
771 Academic Press.
772 Reindl, K.M., Sheridan, M.A., 2012. Peripheral regulation of the growth hormone-insulinlike
773 growth factor system in fish and other vertebrates. Comparative Biochemistry and
774 Physiology. Part A, Molecular and Integrative Physiology 163, 231–245.
775 Reinecke, M., 2010. Insulin-like Growth Factors and Fish Reproduction. Biology of
776 Reproduction, 82:4, 656–661.
777 Rennie, M.D., Purchase, C.F., Lester, N., Collins, N.C., Shuter, B.J., Abrams, P.A., 2008. Lazy
778 males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual
779 size dimorphism in percids. J. Anim. Ecol. 77, 916–926.
780 Riley, L.G., Richman, N.H., Hirano, T., Grau, E.G., 2002. Activation of the growth
781 hormone/insulin-like growth factor axis by treatment with 17alphamethyltestosterone and
782 seawater rearing in the tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol. 127,
783 285–292..
784 Rius-Francino, Acerete, L., Jiménez-Amilburu, V., Capilla, E., Navarro, I., Gutiérrez, J., 2011.
785 Differential effects on proliferation of GH and IGFs in sea bream (Sparus aurata) cultured
786 myocytes. General and Comparative Endocrinology, 172(1), 44–49
787 Saidapur, S.K., 1978. Follicular atresia in the ovaries of nonmammalianvertebrates. Int Rev
788 Cytol, 54:225-244.
789 Samsun, O., Samsun, N., Bilgin, S., and Kalayci, F. 2006. Population biology and status of
790 exploitation of introduced garfish *Belone belone euxini* (Gu¨nther, 1866) in the Black Sea.
791 Journal of Applied Ichthyology, 22: 353–356.

792 Schulz, R.W., Vischer, H., Cavaco, J. E., Santos, E., Tyler, C., Goos, H.J.T., Bogerd, J., 2001.
793 Gonadotropins, their receptors, and the regulation of testicular functions in fish.
794 Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,
795 129(2-3), 407–417.
796 Seale, A.P., Pavlosky, K.K., Celino-Brady, F.T., Lerner, D.T., 2020. Sex, salinity and sampling
797 period dependent patterns of growth hormone mRNA expression in Mozambique tilapia
798 Aquaculture 519, 734766.
799 Sparks, R.T., Shepherd, B.S., Ron, B., Richman, N.H., Riley, L.G., Iwama, G.K., Hirano, T.,
800 Grau, E.G., 2003. Effects of environmental salinity and 17 alpha-methyltestosterone on
801 growth and oxygen consumption in the tilapia, *Oreochromis mossambicus*. Comp. Biochem.
802 Physiol. 136, 657–665.
803 Stamps, J. A. 1993. Sexual size dimorphism in species with asymptotic growth after maturity.
804 Biological Journal of the Linnean Society, 50: 123–145.
805 Suzuki, K., Kawauchi, H. and Nagahama, Y., 1988a. Isolation and characterization of two
806 distinct gonadotropins from chum salmon pituitary glands. Gen Comp. Endocrinol. 71: 292–
807 301.
808 Suzuki, K., Kawauchi, H. and Nagahama, Y., 1988b. Isolation and characterization of subunits
809 of two distinct gonadotropins from chum salmon pituitary glands. Gen. Comp. Endocrinol.
810 71: 302–306.
811 Suzuki, K., Nagahama, Y. and Kawauchi, H., 1988c. Steroidogenic activities of two distinct
812 salmon gonadotropins. Gen. Comp. Endocrinol. 71: 452–458.
813 Swanson, P., Dickey, J. T., Campbell, B., 2003. Biochemistry and physiology of fish
814 gonadotropins. Fish Physiology and Biochemistry, 28(1-4), 53–59.

Tabb, M.M., Blumberg, B., 2006. New modes of action for endocrinedisrupting chemicals. Mol. Endocrinol. 20, 475–482.

Taylor, E.N., DeNardo, D. F., 2005. Sexual size dimorphism and growth plasticity in snakes: an experiment on the Western Diamond-backed Rattlesnake (*Crotalus atrox*). Journal of Experimental Zoology Part A: Comparative Experimental Biology, 303A(7), 598–607.

Tipsmark, C.K., Breves, J.P., Seale, A.P., Lerner, D.T., Hirano, T., Grau, E.G., 2011. Switching of Na+, K+-ATPase isoforms by salinity and prolactin in the gill of a cichlid fish. Journal of Endocrinology 209, 237–244.

Turcu, A.F., Nanba, A.T., Auchus, R.J., 2018. The Rise, Fall, and Resurrection of 11-Oxygenated Androgens in Human Physiology and Disease. Hormone research in paediatrics 89 (5), 284–291.

Vijayan, M.M., Prunet, P., Boone, A.N., 2005. Xenobiotic impact on corticosteroid signaling. In: Mommsen, T.P., Moon, T.W. (Eds.), Biochemistry and Molecular Biology of Fishes, vol. 5. Elsevier, New York, pp. 365–394.

Wang, D.S., Jiao, B., Hu, C., Huang, X., Liu, Z,, Cheng, C,H., 2008. Discovery of a gonad-specific IGF subtype in teleost. Biochem Biophys Res Commun. 367:336–341.

Weber, G.M., Ma, H., Birkett, J., Cleveland, B.M., 2022. Effects of Feeding Level and Sexual Maturation on Expression of Genes Regulating Growth Mechanisms in Rainbow Trout (Oncorhynchus Mykiss). Aquaculture 551, 737917.

Weber, G.M., Sullivan, C., 2001. In vitro hormone induction of final oocyte maturation in striped bass (*Morone saxatilis*) follicles is inhibited by blockers of phosphatidylinositol 3-kinase activity. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 129(2-3), 467–473.

Wood, A.W., Duan, C., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. Int. Rev. Cytol. 243, 215–285.

Yamamoto, Y., Luckenbach, J.A., Goetz, F.W., Young, G., Swanson, P., 2011. Disruption of the salmon reproductive endocrine axis through prolonged nutritional stress: changes in circulating hormone levels and transcripts for ovarian genes involved in steroidogenesis and apoptosis. Gen. Comp. Endocrinol. 172, 331–343.

Table 1. List of primers used in qPCR assays.

Gene	Primer Sequence (5'–3')	R^2	Eff. %	Amplicon size (bp)	Accession no.	Reference
18s	F: GCTACCACATCCAAGGAAGGC	0.989	87.4	69	AF497908	Magdeldin et al., 2007
	R: TTCGTCACTACCTCCCCGAGT					
ef1α	F: AGCAAGTACTACGTGACCATCATTG	0.999	96.7	85	AB075952	Breves et al., 2010
	R: AGTCAGCCTGGGAGGTACCA					
β-actin	F: CTCTTCCAGCCTTCCTTCCT	0.992	81.1	100	FN673689	Tipsmark et al., 2011
	R: ACAGGTCCTTACGGATGTCG					
ghr2	F: CACACCTCGATCTGGACATATTACA	0.995	94.4	102	EF452496	Pierce et al., 2007
	R: CGGTTGGACAATGTCATTAACAA					
igf1	F: CTGCTTCCAAAGCTGTGAGCT	0.991	85.9	75	AF033796	Kajimura et al., 2004
	R: GATCGAGAAATCTTGGGAGTCTTG					
igf2	F: GCTTTTATTTCAGTAGGCCAACCA	0.990	119.6	90	AH006117*	Davis et al., 2008
	R: CACAGCTACAGAAAAGACACTCCTCTA					
igf3	F: CAGACACTCCAGGTGCTGTGTG	0.993	82.2	168	NM_001279636.1	Li et al., 2012
	R: CAAGCCTTTACGTAAATAGATTCC					
erα	F: GGCTCAGCAGCAGTCAAGAA	0.989	77.6	302	AM284390	Park et al., 2007
	R: TGCCTTGAGGTCCTGAACTG					
erβ	F: ACCTTCCGGCAGCAGTACAC	0.994	95.6	149	AM284391	Park et al., 2007
	R: TCCAACATCTCCAGCAACAG					
arα	F: GTCCCTGCTCAGCATCCTAC	0.976	92.83	221	AB045211	Park et al., 2007
	R: TCACTCCCATCCATGACAGC					
arβ	F: CAGCCTCAATGAATTGGGAGA	0.997	97.5	146	AB045212	Park et al., 2007
	R: ATCCCAAGGCAAACACCATC					

* Primer sequences were designed from the nucleotide sequences with the provided accession number. This exact accession number, however, is not available anymore in NCBI, and the revised versions do not contain the forward primer. Hence, XM_025908435.1 was used to deduce the amplicon size.

870 **Figure captions**

871 **Fig. 1.** Effects of hypophysectomy (Hx), and ovine growth hormone (oGH) and ovine luteinizing
872 hormone (oLH) or their combination (oLH + oGH) on gonadosomatic index (GSI) in male and
873 female Mozambique tilapia. Following Hx, Mozambique tilapia received three intraperitoneal
874 injections of oGH alone, oLH alone (5 μg/g body weight), or their combination over 5 days.
875 Sham-operated and Hx fish received saline injections. Values represent means ± S.E.M. (n = 7-
876 9). Data were analyzed by two-way ANOVA followed by Fisher's protected LSD test when
877 significant main or interaction effects were detected (**P < 0.01, ***P < 0.001). Means not
878 sharing the same letter are statistically significant at P < 0.05. Male and female treatment means
879 not sharing the same uppercase and lowercase letters, respectively, are significantly different.
880 ††† indicates significant difference between sexes at P < 0.001.

881

882 **Fig. 2.** Representative micrographs of testis from sham-operated control (A),
883 hypophysectomized (Hx) males (B), or Hx males injected with ovine growth hormone (oGH)
884 (C), luteinizing hormone (oLH) (D), or combination of oLH and oGH (E). Following Hx,
885 Mozambique tilapia received three intraperitoneal injections of oGH alone, oLH alone (5 μg/g
886 body weight), or their combination over 5 days. Sham-operated and Hx fish received saline
887 injections. SG, spermatogonia; SC, spermatocyte; ST, spermatid; SZ, spermatozoa; dG, dead
888 germ SG; VC, vacuole; asterisk, enlarged interstitial tissue. Scale bar = 50 μm.

889

890 **Fig. 3.** Representative micrographs of ovary from sham-operated control (A),
891 hypophysectomized (Hx) females (B), or Hx females injected with ovine growth hormone (oGH)
892 (C), luteinizing hormone (oLH) (D), or combination of oLH and oGH (E). Following Hx,

Mozambique tilapia received three intraperitoneal injections of oGH alone, oLH alone (5 μg/g body weight), or their combination over 5 days. Sham-operated and Hx fish received saline injections. PV, previtellogenic follicle; VO, vitellogenic follicle; AT, atretic follicle. Scale bar = 200 μm.

Fig. 4. Effects of hypophysectomy (Hx), and injections of ovine growth hormone (oGH) and ovine luteinizing hormone (oLH) or their combination (oLH + oGH) following Hx on gonadal *ghr2* (A), *igf1* (B), *igf2* (C), and *igf3* (D) mRNA levels in male and female Mozambique tilapia. Values represent means ± S.E.M. (n = 4-9). Data were analyzed by two-way ANOVA followed by Fisher's protected LSD test when significant main or interaction effects were detected (*P < 0.05, **P < 0.01, ***P < 0.001). Means not sharing the same letter are statistically significant at P < 0.05. Male and female treatment means not sharing the same uppercase and lowercase letters, respectively, are significantly different. †, ††† indicates significant difference between sexes at P < 0.05, and P < 0.001, respectively.

Fig. 5. Effects of hypophysectomy (Hx), and injections of ovine growth hormone (oGH) and ovine luteinizing hormone (oLH) or their combination (oLH + oGH) following Hx on gonadal *erα* (A), *erβ* (B), *arα* (C), and *arβ* (D) mRNA levels in male and female Mozambique tilapia. Values represent means ± S.E.M. (n = 4-9). Data were analyzed by two-way ANOVA followed by Fisher's protected LSD test when significant main or interaction effects were detected (**P < 0.01, ***P < 0.001). Means not sharing the same letter are statistically significant at P < 0.05. Male and female treatment means not sharing the same uppercase and lowercase letters,

915 respectively, are significantly different. †, ††, ††† indicates significant difference between sexes
916 at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
917
918 **Fig. 6.** Effects of hypophysectomy (Hx), and injections of ovine growth hormone (oGH) and
919 ovine luteinizing hormone (oLH) or their combination (oLH + oGH) following Hx on muscle
920 *ghr2* (A), *igf1* (B), and *igf2* (C) mRNA levels in male and female Mozambique tilapia. Values
921 represent means ± S.E.M. (n = 4-9). Data were analyzed by two-way ANOVA followed by
922 Fisher's protected LSD test when significant main or interaction effects were detected (*$P <$
923 0.05, ***$P < 0.001$). Means not sharing the same letter are statistically significant at $P < 0.05$.
924 Male and female treatment means not sharing the same uppercase and lowercase letters,
925 respectively, are significantly different. ††, ††† indicates significant difference between sexes at
926 $P < 0.01$, and $P < 0.001$, respectively.
927
928 **Fig. 7.** Effects of hypophysectomy (Hx), and injections of ovine growth hormone (oGH) and
929 ovine luteinizing hormone (oLH) or their combination (oLH + oGH) following Hx on hepatic
930 *ghr2* (A), *igf1* (B), and *igf2* (C) mRNA levels in male and female Mozambique tilapia. Values
931 represent means ± S.E.M. (n = 4-9). Data were analyzed by two-way ANOVA followed by
932 Fisher's protected LSD test when significant main or interaction effects were detected (*$P <$
933 0.05, **$P < 0.01$, ***$P < 0.001$). Means not sharing the same letter are statistically significant at
934 $P < 0.05$. Male and female treatment means not sharing the same uppercase and lowercase
935 letters, respectively, are significantly different. †, †† indicates significant difference between
936 sexes at $P < 0.05$, and $P < 0.01$, respectively.

Figure 1

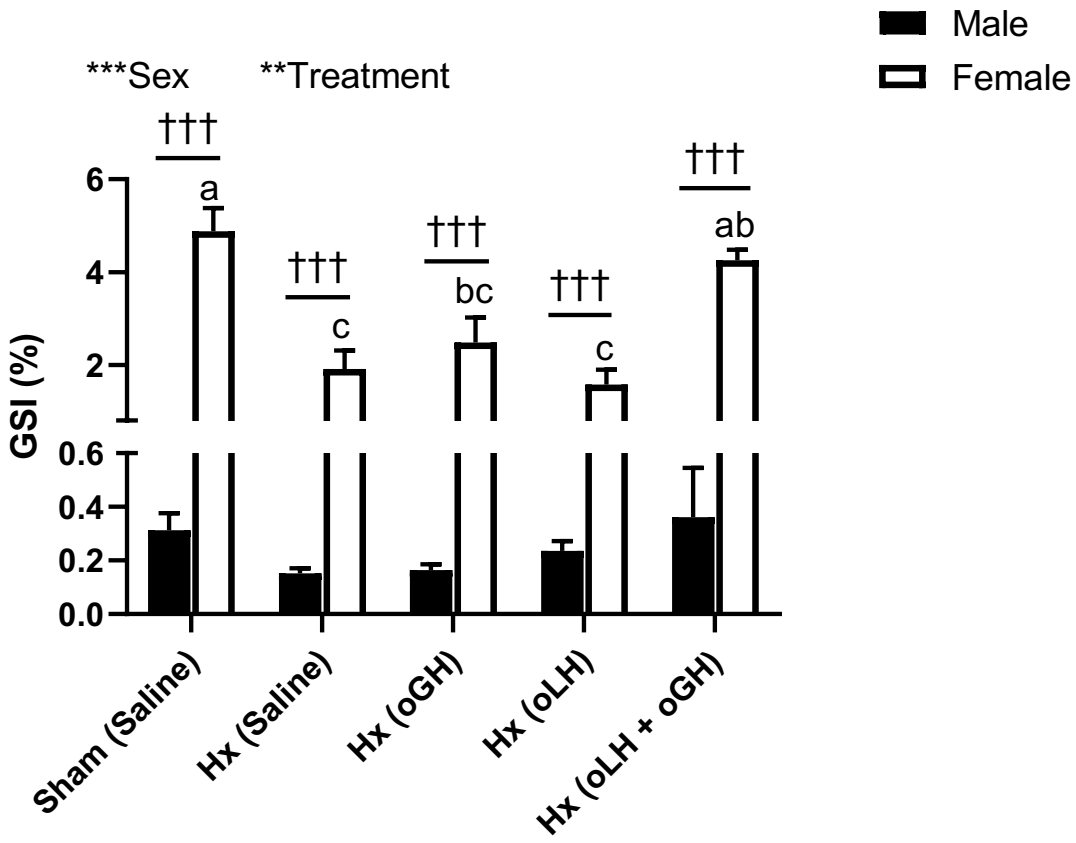
Male
Female
***Sex
**Treatment
†††
a
†††
c
†††
bc
†††
c
†††
ab
GSI (%)
6
4
2
0.6
0.4
0.2
0.0
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH + oGH)

Figure 2

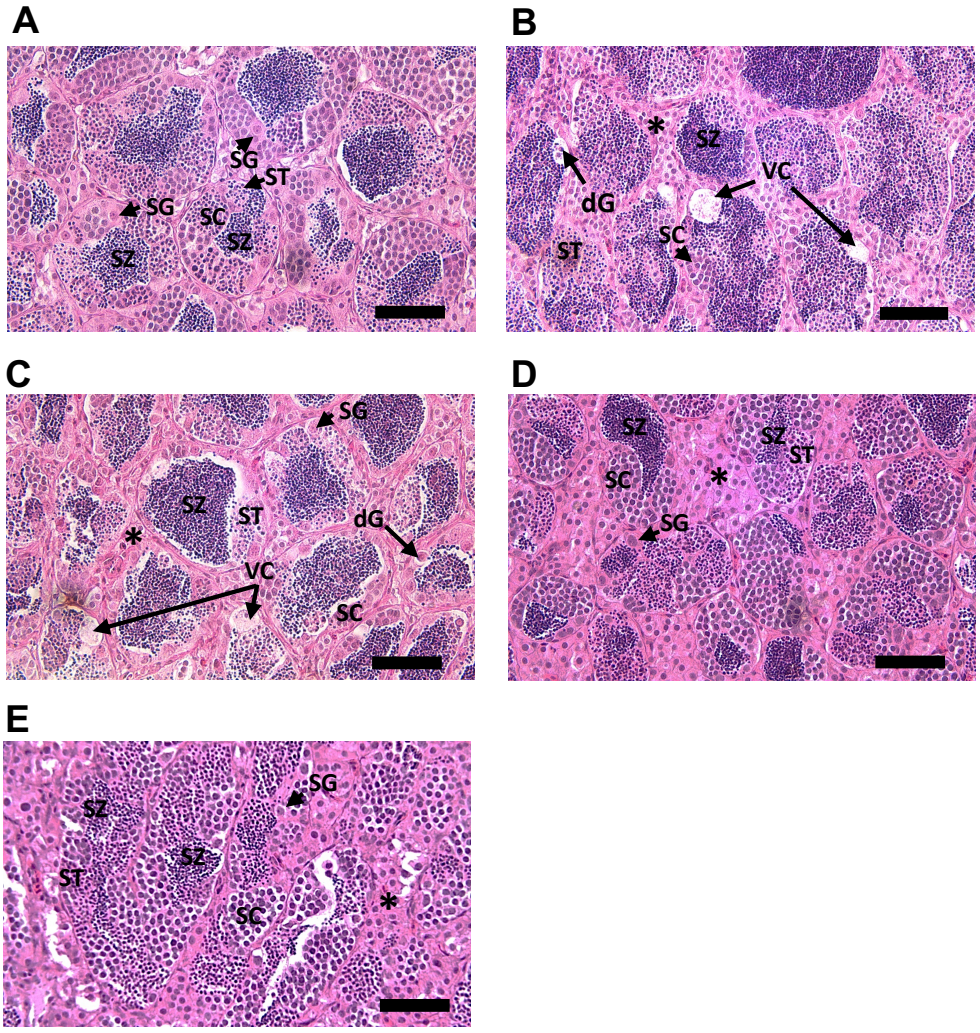
A
SG
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Figure 3

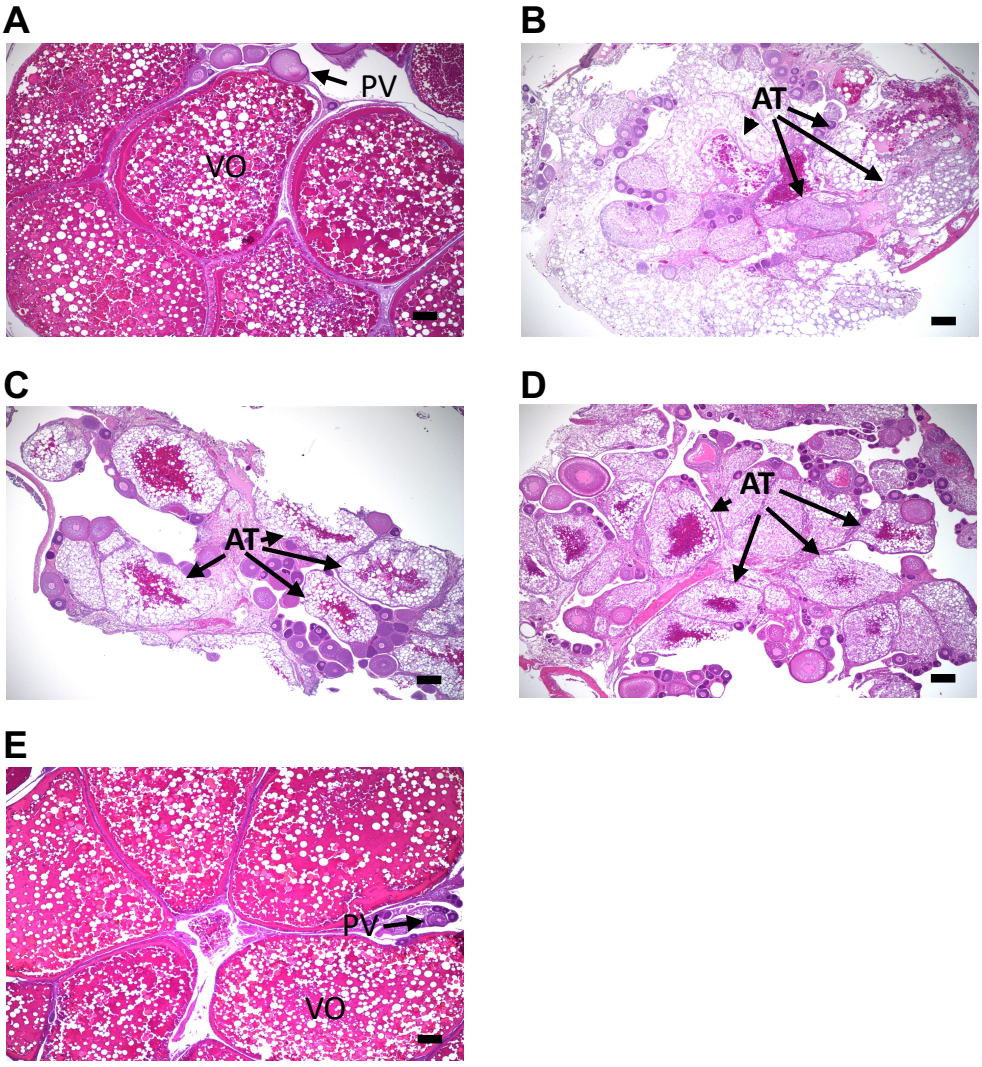


Figure 4

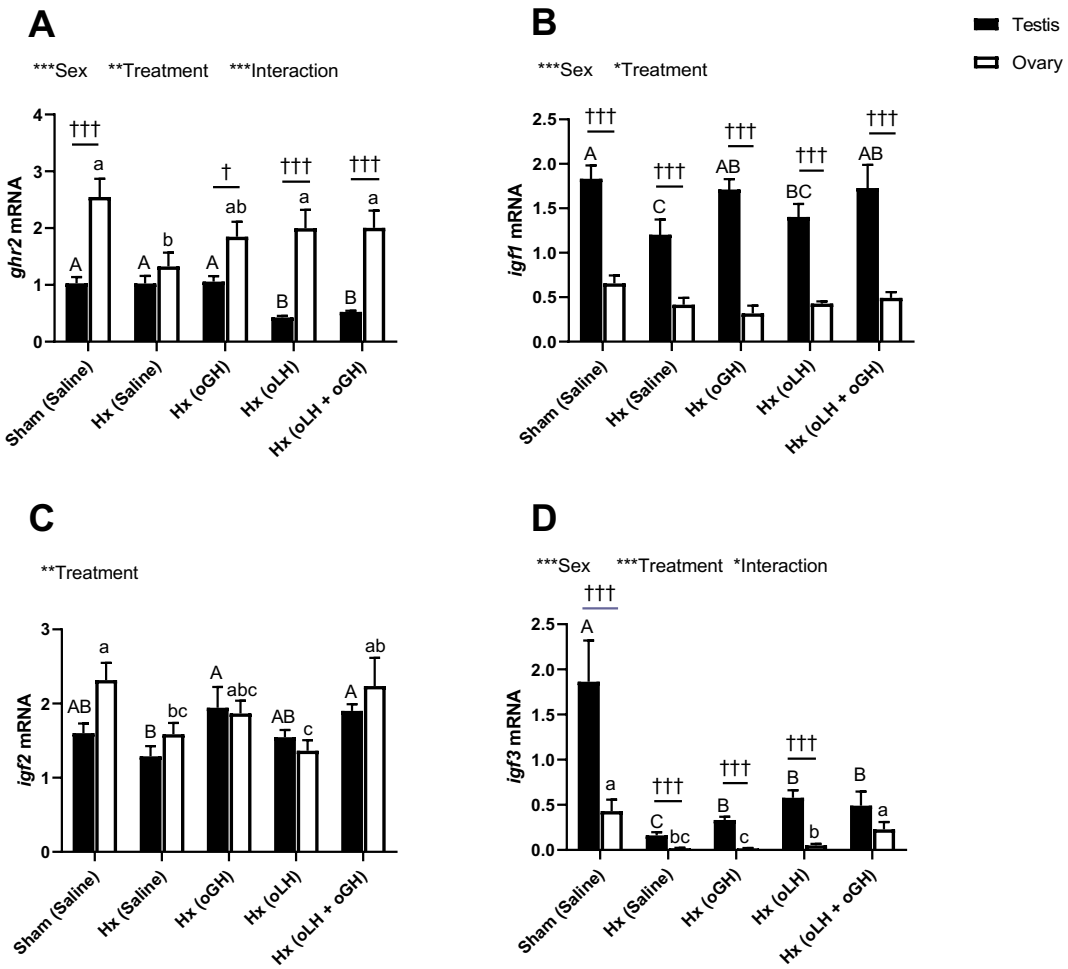

A
***Sex **Treatment ***Interaction
ghr2 mRNA
B
***Sex *Treatment
igf1 mRNA
Testis
Ovary
C
**Treatment
igf2 mRNA
D
***Sex ***Treatment *Interaction
igf3 mRNA
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH + oGH)

Figure 5

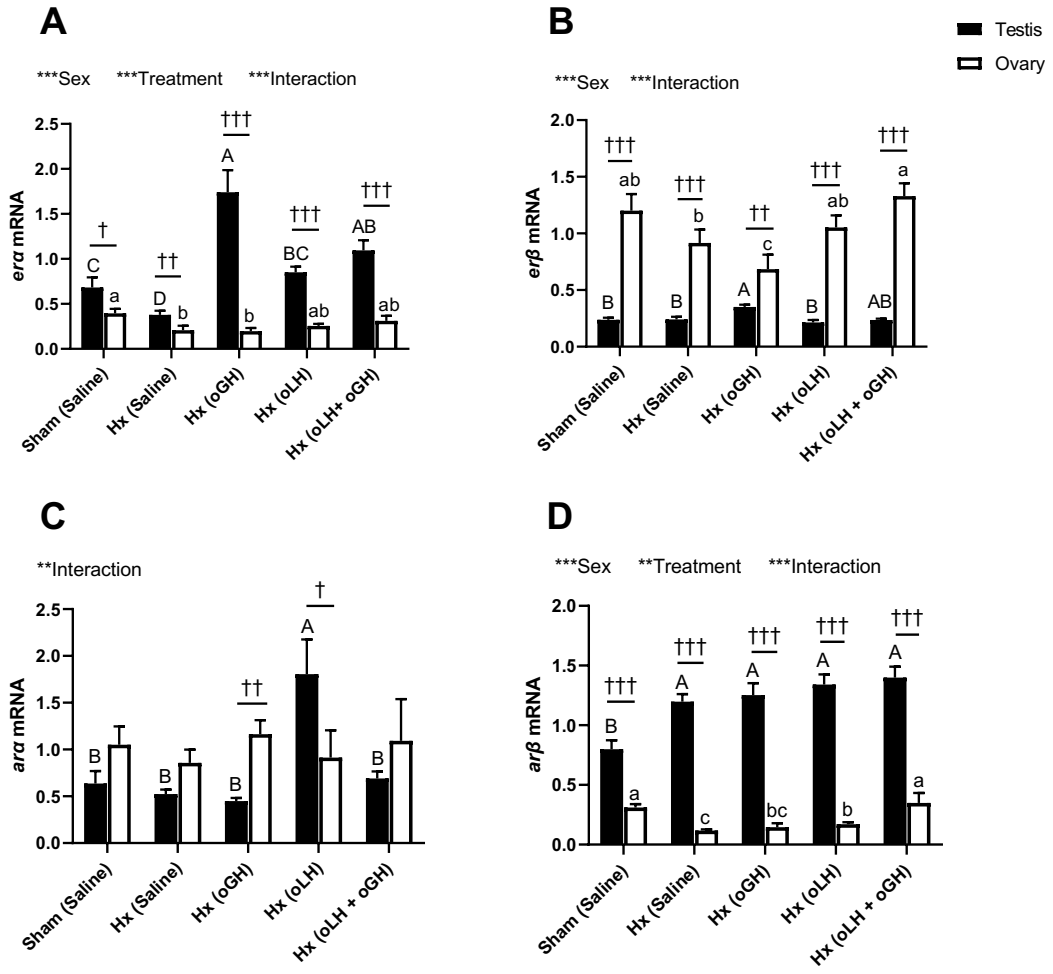

A
***Sex ***Treatment ***Interaction
erα mRNA
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH+ oGH)
B
***Sex ***Interaction
erβ mRNA
Testis
Ovary
C
**Interaction
arα mRNA
Hx (oLH + oGH)
D
***Sex **Treatment ***Interaction
arβ mRNA

Figure 6

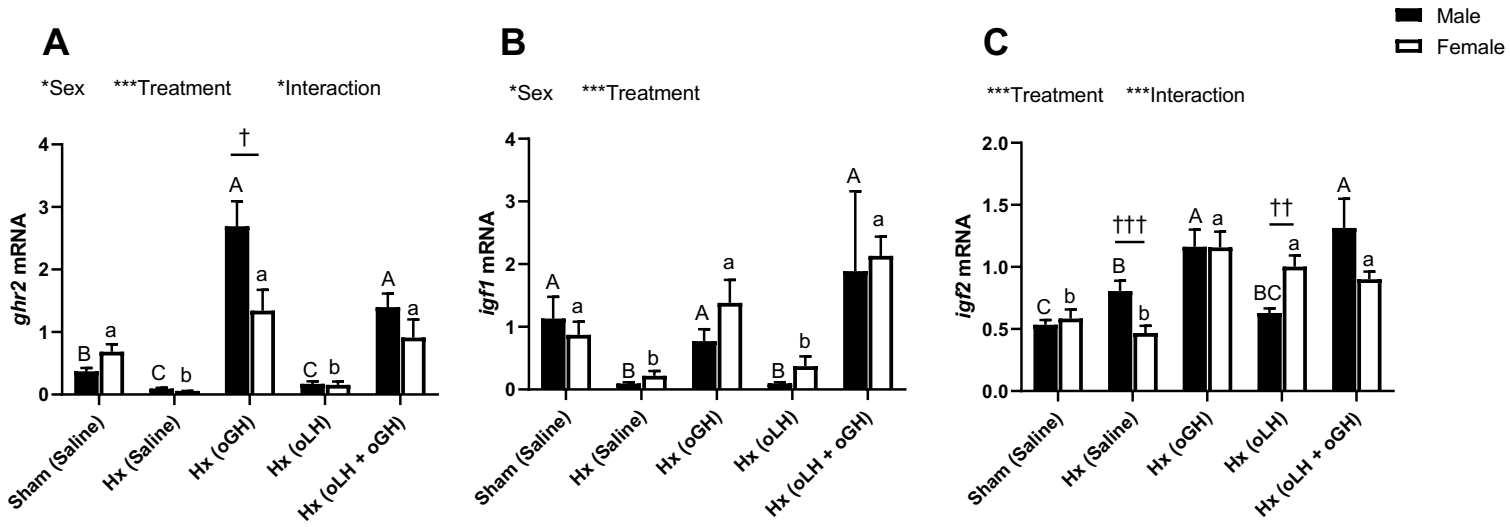
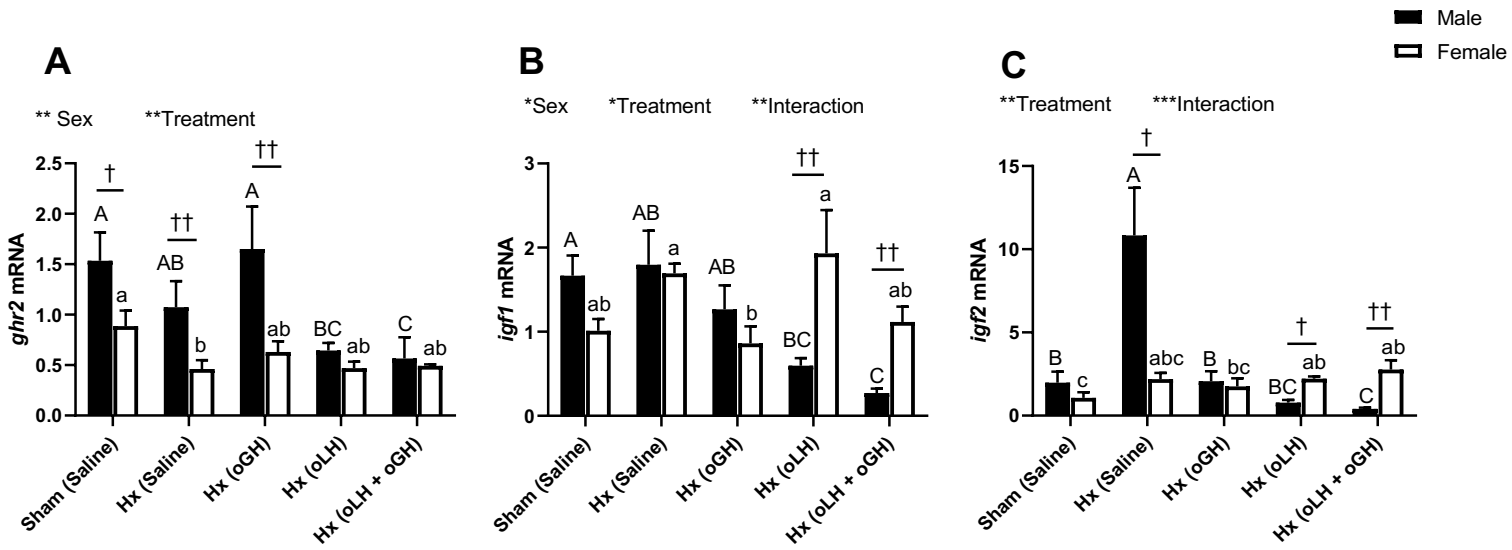

A
*Sex ***Treatment *Interaction
ghr2 mRNA
B
*Sex ***Treatment
igf1 mRNA
C
***Treatment ***Interaction
igf2 mRNA
Male
Female
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH + oGH)

Figure 7


Male
Female
A
** Sex
**Treatment
ghr2 mRNA
2.5
2.0
1.5
1.0
0.5
0.0
†
††
A
AB
A
BC
C
a
b
ab
ab
ab
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH + oGH)
B
*Sex
*Treatment
**Interaction
igf1 mRNA
3
2
1
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††
A
AB
AB
BC
C
a
a
b
a
ab
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH + oGH)
C
**Treatment
***Interaction
igf2 mRNA
15
10
5
0
†
†
††
B
A
B
BC
C
c
abc
bc
ab
ab
Sham (Saline)
Hx (Saline)
Hx (oGH)
Hx (oLH)
Hx (oLH + oGH)