Research article

Insulin-like growth factors 1 and 2 regulate gene expression and enzymatic activity of cyp17a1 in ovarian follicles of the yellowtail, *Seriola quinqueradiata*

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1. Introduction

Insulin-like growth factors (IGFs: IGF-1 and IGF-2), primary mediators of somatic growth, play essential roles in gonadal development and maturation in a wide variety of vertebrates. In mammals, IGFs have been shown to affect gonadal steroidogenesis, differentiation and proliferation of somatic cells in the gonads, and be involved in oocyte maturation (Bondy et al., 2006). IGF-1 has also been shown to potentiate the stimulatory effects of gonadotropins on steroid production and expression of steroidogenic enzymes in mammalian theca cells (Bondy et al., 2006). In teleosts, as in mammals, IGF-1 stimulates steroid production in the ovarian follicles. Furthermore, both IGF-1 and IGF-2 induce maturational competence and final oocyte maturation (Reinecke, 2010). In general, fish IGFs are primarily produced in the liver and exert their actions in various tissues to induce growth, proliferation, and differentiation of peripheral tissues via the blood stream (Reindl and Sheridan, 2012). However, IGFs are also expressed in parenchymal cells of numerous extrahepatic sites where they are thought to act via autocrine/paracrine mechanisms (Reindl and Sheridan, 2012; Wood et al., 2005). In fact, IGFs as well as their receptors are expressed in the ovary of teleosts, such as the zebrafish (*Danio rerio*) (Zhou et al., 2016), coho salmon (*Oncorhynchus kisutch*) (Maestro et al., 1997), common carp (*Cyprinus carpio*)

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(Mukherjee et al., 2006), and red seabream (Pagrus major) (Kagawa et al., 1995). Moreover, these ovarian IGFs are likely to be directly controlled by GH in the same manner as hepatic IGFs (Gioacchini et al., 2005; Berishvili et al., 2010). Therefore, ovarian IGFs are likely to be autocrine/paracrine regulators and play essential roles in the reproductive physiology of fish.

Although evidence regarding the possible role of IGF-2 is still lacking, several studies in fish have revealed the species-specific effects of IGF-1 on ovarian steroidogenesis. In the common carp (Paul et al., 2010) and white perch (Morone americana) (Weber et al., 2007), IGF-1 stimulated both testosterone and 17β-estradiol (E2) synthesis in ovarian follicles. In the coho salmon, IGF-1 inhibited basal testosterone production by iso-ovarian follicles of the red seabream (Kagawa et al., 2003). Contrary to (Negatu et al., 1998) and in the zebra fish ovary, comparative studies in the mummichog (Fundulus heteroclitus) (Negatu et al., 1998) and in the zebrafish (Nelson and Van Der Kraak, 2010a).

The species-specific actions of IGFs in fish steroidogenesis are likely to correspond to their expression patterns in ovaries because the expression patterns of IGF genes in ovaries also varied among fish (Higuchi et al., 2016). For example, levels of igf-1 mRNA in the ovaries of adult tilapia (Oreochromis mossambicus) (Schmid et al., 1999) and shi drum (Umbrina cirrosa) (Patruno et al., 2006) were much higher than those of igf-2 mRNA, whereas igf-2 mRNA was present at higher levels than igf-1 in the rainbow trout (Oncorhynchus mykiss) (Lankford and Weber, 2010). In contrast, the expression of igf-1 gene was considerably low or not detected in gilthead seabream (Sparus aurata) (Perrot et al., 2000), zebrafish (Nelson and Van Der Kraak, 2010b), and cohso salmon ovaries (Yamamoto et al., 2011). Therefore, to understand the various steroidogenic actions of IGFs in the fish ovary, comparative studies regarding their gene expression and steroidogenic actions may be useful. In particular, autocrine/paracrine regulators are pivotal for the interactions between germ and somatic cells, as well as theca and granulosa cells in steroidogenesis. However, available information about the localization of IGFs in ovaries is still limited. Recently, they have shown that IGF-1 and IGF-2 in the yellowtail are produced in the theca and granulosa cells during the vitellogenic phase (Higuchi et al., 2016). In particular, the cellular localization of IGF-1 in the yellowtail (Seriola quinquergadiata) is distinct from that in other fish species studied so far, in which IGF-1 is mainly expressed in granulosa cells (Kagawa et al., 1995; Schmid et al., 1999; Perrot et al., 2000). The unique expression pattern of yellowtail IGF-1 may, thus, be indicative of a completely different function of IGF-1 in ovarian steroid production.

To gain a better understanding of the various physiological roles of IGFs in fish reproduction, we examined the regulatory roles of IGF-1 and IGF-2 for the gene expression and activity of steroidogenic enzymes in ovaries of our model fish species, the yellowtail, which is known as a multiple-spawner with an asynchronous-type ovary (Matsuyama et al., 1996). We also investigated the possible regulation of its steroidogenic enzymes through IGF receptors at different ovarian developmental stages.

2. Materials and methods

2.1. Fish and sampling procedures

All experiments were performed in accordance with the Guidelines for the care and use of live fish, Seikai National Fisheries Research Institute (SNFRI), Japan Fisheries Research and Education Agency (FRA). Yellowtail juveniles were captured from the wild and maintained for 2 years under natural water temperature and photoperiod conditions in square sea cages (5 m side, 5 m depth) at Goto station, SNFRI, FRA (Nagasaki, Japan). Fish were fed a commercial pellet diet (Hamachi special 15, Marubeni Nissin Feed, Tokyo, Japan) to satiation by hand each morning, 3 days/week.

For in vitro culture, ovaries at various developmental stages were sampled from yellowtail aged >2 years [approximately 6 kg in body weight (BW)] between January and April. The females were netted from cages, killed by decapitation, and BW was measured. Ovarian tissues were removed and weighed to determine the gonadosomatic index (GSI = gonad weight (g) × 100/BW (g)). A piece of ovary was fixed with Bouin’s fixative for histological examination of the ovarian developmental stage, and other samples were placed in chilled Leibovitz’s L-15 culture medium (Thermo Fisher Scientific Inc., Waltham, MA) supplemented with 0.1% bovine serum albumin (BSA, Sigma-Aldrich Inc., St. Louis, MO), 100 μg/ml streptomycin, 100 U/ml penicillin (Thermo Fisher Scientific Inc.), and 10 mM HEPES adjusted to pH 7.4 for ovarian culture. Developmental stages of ovaries were classified into the perinucleolar stage (Pn), yolk vesicle stage (Yv), primary yolk stage (Py), secondary yolk stage (Sy), and tertiary yolk stage (Ty), according to the most advanced types of oocytes found, as described previously (Higuchi et al., 2016).

To investigate the distribution of IGF receptor gene expression among tissues, four females of age >2 years were sampled on January 2014 (Higuchi et al., 2016). The brain, pituitary, gill, heart, liver, kidney, stomach, spleen, muscles and ovary were collected, immediately placed in RNAlater (Ambion, Austin, TX), and stored at -30 °C until analysis. To investigate changes in cyp17a1 gene expression associated with ovarian development, we sampled 88 females aged >2 years at various stages of ovary development between July 2012 and May 2013 (Higuchi et al., 2016). The ovary samples were placed in RNAlater, and stored at -30 °C.

2.2. Ovarian culture

Human recombinant IGF-1 and IGF-2 were purchased from Bachem AG (Bubendorf, Switzerland) and Sigma-Aldrich, respectively. IGFs were dissolved at 50 μM in phosphate-buffered saline (PBS) with 0.1% BSA. Wortmannin (Wort) was purchased from Cayman Chemical (Ann Arbor, MI), and dissolved at 10 mM in DMSO. Progesterone (P) and 17α-hydroxyprogesterone (17P) were purchased from Sigma-Aldrich. The steroids were first dissolved in absolute ethanol at 1 mg/ml, then diluted in L-15 medium to 10 μg/ml. All stock solutions of chemicals were dissolved directly in the culture medium at less than 0.1% (v/v) vehicle. Ovarian tissue culture was performed by the method widely used in fish species (Kagawa et al., 2003; Weber et al., 2007; Luckenbach et al., 2011; Yamamoto et al., 2011; Yang et al., 2015) with some modifications. Briefly, ovaries were cut into approximately 40 mg pieces. One piece was transferred into a well of a 48-well polystyrene culture plate containing 0.5 ml of L-15 medium. After 1 h of pre-incubation in L-15 medium without any additives at 20 °C, the medium was removed and replaced with either fresh medium alone (control) or medium containing IGF-1 (1, 10 and 100 nM) or IGF-2 (1, 10 and 100 nM) and incubated over 8–48 h. Wort (1 and 10 μM), P (100 ng/ml) or 17P (100 ng/ml) were also tested in combination with IGFs. After incubation, the sampled ovarian fragments were stored in RNAlater at -80 °C for RNA isolation. The culture medium was collected and frozen at -80 °C for steroid assays. All incubations were performed in triplicate wells per treatment. In addition, each experiment was repeated using 2–4 different ovaries to confirm the reproducibility.

2.3. RNA isolation and reverse transcription

Total RNA was extracted from the cultured ovaries using ISOGEN II (NIPPON GENE, Toyama, Japan), and treated using TURBO DNase (Ambion) according to the manufacturer’s protocol. One μg of total RNA, quantified using a NanoDrop (ND-1000, Thermo Scientific Inc.), was
reverse-transcribed using the Omniscript RT kit (QIAGEN GmbH, Düsseldorf, Germany), after priming with random hexamers (QIAGEN GmbH). For the across-stage comparisons of transcript levels, mRNA was further isolated from total RNA samples to mitigate issues associated with comparing ovarian follicles during different stages of oogenesis, which may be dramatically different in size and RNA composition (Yamamoto et al., 2011). mRNA was isolated from 60 μg of total RNA/sample using the Poly(A) Purist MAG kit (Ambion), and 50 ng of mRNA was reverse-transcribed as described above. As in vitro culture experiments were done with ovaries at the same stage, total RNA was used for cDNA synthesis.

2.4. RT-PCR and real-time quantitative PCR

RT-PCR was used to study tissue distribution of IGF receptors (igf1ra, igf1rb, igf2r) mRNA in yellowtail females, and the PCR products were then electrophoresed and visualized in 2.0% agarose gel containing ethidium bromide. The primers for RT-PCR were designed and synthesized by Greiner Bio One International GmbH (Kremsmunster, Austria) (Table 1).

| Targeted gene | Primer/probe sequence (5’-3’) | Product size (bp) |
|---------------|-------------------------------|-------------------|
| igf1ra        | Forward: AGGGCAATCTGGGACATCAAC | 300           |
|               | Reverse: ACATGCGAGTTTGGGTTTC  |                |
| igf1rb        | Forward: GCATATCAACATCGGCGAGG | 445           |
|               | Reverse: TGGCAGGTCAGTTGTACC  |                |
| igf2r         | Forward: ATGCCGACCCACCGCGAGG  | 432           |
|               | Reverse: CACGTGACCTCGCTGGTA   |                |
| actinb        | Forward: GATCCAAGGCCAGAGAGAG  | 382           |
|               | Reverse: GAGGAGTACGCGACGCTTG  |                |

Data are presented as the mean ± standard error of the mean (SEM). Time course data from ovarian culture experiments were analyzed using two-way analysis of variance (ANOVA) with treatment and time as independent variables, and where significant differences were observed, Tukey’s multiple comparison tests were conducted. The across-stage gene expression data and IGF concentration-response data (with or without Wort, P and 17-P) were subjected to one-way ANOVA followed by Tukey’s multiple comparison tests. Significant differences in cyp17a1 mRNA levels between IGF-treated and control samples in ovaries at different stages were analyzed using Student’s t-test. Statistical analyses were performed using Prism 6.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. Effects of IGF-1 and IGF-2 on expression of ovarian steroidogenesis-related genes

To examine effects of IGF-1 and IGF-2 on expression of ovarian steroidogenesis-related genes, IGFS time-course experiments were conducted using yellowtail ovary at the TY stage. The transcript levels of steroidogenesis-related genes showed clear responses to time-course treatment with IGF-1 and IGF-2 (Figure 1). Transcript abundance for cyp17a1 was significantly increased in ovarian fragments treated with both IGFS by 8 h, and remained elevated above controls at all time points (P < 0.05). IGF-1 and IGF-2 had no effect on mRNA levels of other steroidogenesis-related genes over the time course investigated (P > 0.05).
Transcription of cyp17a1 that exhibited significant upregulation at 8 h in the time-course experiments was further assessed with different concentration of IGFs using ovary tissue at the TY stage (Figure 2). Transcript abundance for the cyp17a1 gene increased in a concentration-dependent manner, reaching approximately 2-fold maximum elevation when treated with 100 nM IGFs ($P < 0.05$).

3.2. Stage-dependent effects of IGF-1 and IGF-2 on ovarian cyp17a1 gene expression and developmental changes in ovarian cyp17a1 mRNA levels

To examine reproductive stage-dependent effects of IGF-1 and IGF-2 on cyp17a1 gene expression, ovarian fragments at various developmental stages (PN, YV, PY, SY, or TY stages) were incubated for 8 h with 100 nM of IGF-1 or IGF-2. The cultures were conducted using 3 or 4 different females at each developmental stage (GSI, 0.55 ± 0.03 at PN stage; 0.61 ± 0.05 at YV stage; 0.98 ± 0.08 at PY stage; 1.88 ± 0.24 at SY stage; 2.81 ± 0.10 at TY stage). Transcript abundance for cyp17a1 were significantly increased by IGF-1 relative to control in SY and TY stage ovaries ($P < 0.05$), but not in PN, YV and PY stage ovaries (Figure 3A). IGF-2 significantly stimulated cyp17a1 mRNA levels only in TY stage ovaries ($P < 0.05$, Figure 3A).

Table 2. Quantitative PCR primer and probe sequences for targeted genes, PCR product sizes, and mean cycle threshold (Ct) values for all cDNA samples measured.

| Targeted gene | Primer/probe sequence (5'→3') | Product size (bp) | Mean Ct |
|---------------|--------------------------------|-------------------|---------|
| cyp17a2       | Forward: GGGAGGACTGTTGACATTAC  | 85                | 31.3    |
|               | Probe: FAM-CTTGGATGTACCTCTTTAACAAGTCTCTGA-IBFQ |
|               | Reverse: TCTGACAGTGATACCTCTCCCCAGTTT |
| actinb        | Forward: ACCCTGTCGTCCTCAGAGG  | 137               | 20.5    |
|               | Probe: FAM-AGATGACCCGATCGTCTGGA-IBFQ |
|               | Reverse: ACCAGGCGATACGGGACA |

Figure 1. Effects of IGF-1 and IGF-2 on mRNA levels of steroidogenesis-related genes, star (A), cyp11a1 (B), hsd3b (C), cyp17a1 (D), cyp17a2 (E) and cyp19a1 (F), and reference gene, actinb (G) in yellowtail ovaries at the tertiary yolk stage. Ovarian fragments were incubated in 0.5 ml culture medium containing IGF-1 or IGF-2 (100 nM) for 8–48 h at 20 °C. Quantitative PCR data for genes of interest were normalized to actinb mRNA levels, whereas actinb data were not normalized to another gene. The data represent mean ± SEM ($n = 3$). Significant differences between IGF treatment and control groups at each time point are indicated by different letters ($P < 0.05$, two-way ANOVA followed by Tukey’s multiple comparison test). The figure shows a representative result of replicates from at least three experiments.
The expression of igf1ra, igf1rb and igf2r was detected in all tissues investigated, including the ovary (Figure 4A).

3.4. Effects of PI3 kinase inhibitor on IGF-induced cyp17a1 gene expression

To examine whether PI3 kinase activation was necessary for IGF-induced cyp17a1 gene expression, TY stage ovary fragments were pre-incubated for 2-h with increasing concentrations of Wort (0, 1 or 10 μM), followed by incubation with IGF-1 or IGF-2 (100 nM) for a further 8-h. Wort, at its increasing concentrations, significantly inhibited both IGF-1 and IGF-2-stimulated cyp17a1 gene expression almost in a dose-dependent manner (P < 0.05, Figure 4B).

3.5. Effects of IGF-1 and IGF-2 on 17α-hydroxylase and C17,20 lyase activities

To examine whether IGF-1 and IGF-2 stimulate 17α-hydroxylase and C17,20 lyase activities, TY stage ovary fragments were incubated with IGFs in the absence or presence of P or 17-P as precursor substrate for 24-h, and then androstenedione content in the medium was measured. IGF-1 and IGF-2 at 100 nM in the absence of P or 17-P did not affect androstenedione production (Figures 5 and 6). Androstenedione production, however, increased significantly after high doses of IGF-1 (100 nM) and IGF-2 (10 and 100 nM) in the presence of P in the medium (P < 0.05, Figure 5). IGF-1 had stimulatory effects on androstenedione production in a concentration-dependent manner when 17-P was present in the incubation medium (P < 0.05, Figure 6). Moreover, IGF-2 significantly elevated androstenedione production with addition of 17-P to the medium (P < 0.05), but the highest dose of IGF-2 (100 nM) did not promote such production (Figure 6).

4. Discussion

In the present study, we demonstrated that IGF-1 and IGF-2 could stimulate only cyp17a1 gene expression in the yellowtail ovary in vitro. However, although information is limited regarding the effects of IGF-2 on ovarian steroidogenesis, the steroidogenic actions of ovarian IGF-1 detected in this study differ from those of other reports involving other fish species. For example, in the common carp, IGF-1 enhanced cyp19a1 gene expression in isolated granulosa cells, although the administration of IGF-1 stimulated basal testosterone and E2 production in vitellogenic follicles (Paul et al., 2010). Moreover, the release of E2 from the ovaries was elevated through an increase in cyp19a1 mRNA levels after IGF-1 treatment in several Salmonidae species, whereas testosterone production is inhibited (Maestro et al., 1997; Nakamura et al., 2016). Furthermore, in red seabream ovary, IGF-1 promoted only the conversion of testosterone to E2 by stimulating aromatase activity and cyp19a1 gene expression (Kagawa et al., 2003). Interestingly, red seabream IGF-1 proteins were found only in the granulosa cell layers at different developmental stages (Kagawa et al., 1995). This cellular localization of IGF-1 in the ovary is strongly correlated with the steroidogenic action, because the theca cells (potential sites expressing cyp17a1 gene) supply testosterone to the granulosa cells that express cyp19a1 and produce E2 in vitellogenic fish (Lubzens et al., 2010). In contrast, yellowtail IGF-1 was mainly expressed in the theca cells (Higuchi et al., 2016), and then stimulated gene expression of cyp17a1 and enzymatic activities, i.e., 17α-hydroxylase/C17,20 lyase. In addition, IGF-1 had no effect on cyp19a1 gene expression and aromatase activity in yellowtail ovaries (data not shown). Therefore, the steroidogenic actions of IGF-1 are, in part, likely to be derived from differences in IGF expression among fish species. Meanwhile, autocrine/paracrine regulators including IGFs are secreted proteins that can diffuse and interact between the different gonadal cell types involved in steroid production. This fact suggests that cellular localization of IGF receptors may be also correlated with the different steroidogenic actions of fish IGFs. In future, further studies are needed to examine the localization of IGF receptors in ovaries of the yellowtail as well as other fish species.

The transcript levels of steroidogenesis-related genes except for cyp17a2 showed a slight decline between 24 and 48 h, regardless of the presence of IGFs. Luckenbach et al. (2011) identified that expression of genes associated with cell survival were decreased over time in culture of coho salmon ovary. Although we did not determine the expression levels of cell survival-related genes in the cultured yellowtail ovaries, transcript levels of actinb was tended to be decreased from 24 h onward, suggesting the possibility of a decline in at least cell activity of the ovarian follicles. Therefore, these results could probably explain the decreasing expression levels observed for steroidogenesis-related genes.
In teleosts, as in mammals, there are two types of IGF receptor; type 1 (IGF-1R) and type 2 (IGF-2R) (Caruso and Sheridan, 2011). IGFs binding to IGF-1R leads to activation of tyrosine kinase, resulting in phosphorylation of insulin receptor substrate (IRS) which subsequently activates downstream signaling molecules including the PI3 kinase and MAP kinase signaling cascades (Backer et al., 1992a, 1992b; Chuang et al., 1993). Although it is not certain how IRS associates with PI3 kinase in inducing ovarian steroidogenesis in fish, PI3 kinase existed in carp

![Figure 3. Stage-dependent effects of IGF-1 and IGF-2 on cyp17a1 mRNA levels in yellowtail ovaries. Developmental stages of ovaries were classified into the perinucleolar stage (Pn), yolk vesicle stage (Yv), primary yolk stage (Py), secondary yolk stage (Sy), and tertiary yolk stage (Ty), according to the most advanced types of oocytes found. (A) Effects of IGF-1 and IGF-2 on cyp17a1 mRNA levels in ovaries at different developmental stages in female yellowtail fish. Ovarian fragments at different developmental stages were incubated in 0.5 ml culture medium containing IGF-1 or IGF-2 (100 nM) for 8-h at 20 °C. The data represent mean ± SEM of replicates from 3 or 4 different ovaries (n = 3 or 4). Asterisks denote significant differences between IGF-treated and control groups (P < 0.05, Student’s t-test). (B) Changes in levels of transcripts of cyp17a1 gene during ovarian development. The gene expression was absolutely quantified using the standard curve constructed from serial dilution of a plasmid containing a partial cDNA sequence of cyp17a1 gene. Quantitative PCR data for cyp17a1 gene were not normalized using a reference gene. The data represent means ± SEM (n = 48, Pn; n = 40, Yv; n = 3, Py; n = 8, Sy; n = 12, Ty). Different letters indicate statistically significant differences at different stages of ovarian development (P < 0.05, one-way ANOVA followed by Tukey’s multiple comparison test).]
ovarian follicle cells and can be activated by IGF-1 for steroid production (Paul et al., 2013). In the present study, ovarian gene expression of IGF-1R (igf1ra and igf1rb) and IGF-2R was demonstrated and, furthermore, a PI3 kinase inhibitor, Wort, blocked IGF-1-and IGF-2-induced cyp17a1 expression in yellowtail ovarian follicles. These findings suggest that PI3 kinase plays an essential role in IGF-mediated steroid production, and the actions of both IGF-1 and IGF-2 may be mediated through activation of IGF-1R but not IGF-2R in yellowtail ovarian follicles. In general, the affinity of IGF-1R for IGF-1 is typically greater than that for IGF-2, and the affinity of IGF-2R for IGF-2 is higher than that for IGF-1 (Jones and Clemmons, 1995; Méndez et al., 2001; Hawkes and Kar, 2004). However, steroidogenic actions of mammalian IGF-1 and IGF-2 in granulosa, theca, and luteal cells are mediated via IGF-1R but not IGF-2R (Adashi et al., 1990; Blakesley et al., 1996; Willis et al., 1998). Moreover, ligand binding assays performed with zebrafish cells indicated that IGF-1 and IGF-2 bound to IGF-1R with similar affinities (Pozios et al., 2001). In future studies, it would be useful to examine the affinity of IGF-1 and IGF-2 for yellowtail IGF receptors for improved understanding of regulatory mechanisms involving IGF-1 and IGF-2 in ovarian steroid production.

In the present study, both IGFs significantly promoted the conversion of P and 17-P to androstenedione, suggesting that IGFs stimulate both 17α-hydroxylase and C17-20 lyase activities. In teleosts, unlike other vertebrates, two cytochrome P450c17 enzymes (P450c17-I and -II encoded by cyp17a1 and cyp17a2 genes, respectively) have been isolated and characterized (Zhou et al., 2007; Su et al., 2015). It has been demonstrated that P450c17-I exhibits both 17α-hydroxylase and C17,20 lyase activities, whereas P450c17-II is responsible for only 17α-hydroxylase activity. Therefore, the enhancement of both 17α-hydroxylase and C17,20 lyase activities by IGFs in the yellowtail ovary seems to be consistent with IGF-induced gene expression of cyp17a1. In addition, our previous study showed that both IGFs mRNA levels in yellowtail ovaries were elevated during the secondary oocyte growth phase, and then IGF-1 and IGF-2 proteins were produced in the follicle cell layers (Higuchi et al., 2016). Taken together, these findings suggest that ovarian IGF-1 and IGF-2 are potential autocrine/paracrine regulators in yellowtail ovaries, and act directly on follicle cells to stimulate steroid production through an increase in gene expression and enzymatic activity of cyp17a1. On the other hand, a high dose of IGF-2, but not IGF-1, did not affect the conversion of 17-P to androstenedione in the TY stage ovary of the yellowtail. The underlying mechanisms involving IGF-2, at this point, remain unclear. Further studies are needed to clarify the detailed mechanisms.

In conclusion, we have shown that IGF-1 and IGF-2 stimulate cyp17a1 gene expression and the conversion of P to androstenedione (17α-
expression of IGF-1 acted synergistically with luteinizing hormone to increase follicle maturation in the zebra fish (Wang et al., 2008; Song et al., 2016) and amphibians expression of a novel IGF subtype, IGF-3, has been discovered in teleosts (Young and McNeilly, 2010). These results suggest that IGFs play different biological roles in ovarian steroid production (Adashi, E.Y., Resnick, C.E., Rosenfeld, R.G., 1990. Insulin-like growth factor-I (IGF-I) and IGF-II hormonal action in cultured rat granulosa cell: mediation via type 1 but not type II IGF receptors. Endocrinology 126, 216–222. Adashi, K., Kanbeaw, A., Higata, T., 1995. Development of a microtiter plate enzyme-linked immunosorbent assay for 17α, 20β, 3β-trihydroxy-5-pregnen-3-one, a teleost gonadal steroid. Fish. Sci. 61, 491–494. Backer, J.M., Myers, M.G., Shoelston, S.E., Chin, D.J., Sun, X.J., Miralpeix, M., Hu, P., Margolis, B., Sokolik, Y., Schlesinger, J., White, M.F., 1992b. Phosphatidylinositol 3-kinase is activated by association with IRS-1 during insulin stimulation. EMBO J. 11, 3469–3479. Backer, J.M., Schroeder, G.C., Kahn, C.R., Myers, M.G., Wilden, P.A., Cahill, D.A., White, M.F., 1992b. Insulin stimulation of phosphatidylinositol 3-kinase activity maps to insulin receptor regions for endogenous substrate phosphorylation. J. Biol. Chem. 267, 1367–1374. Berishvili, G., Baroiller, J.F., Eppler, E., Reinecke, M., 2010. Insulin-like growth factor-three (IGF-3) in men and female gonads of the tilapia: development and regulation of gene expression by growth hormone (GH) and 17α-ethinylestradiol (EE2). Gen. Comp. Endocrinol. 167, 128–134. Blakesley, V.A., Siringourou, A., Espistoir, D., Le Roth, D., 1996. Signaling via the insulin-like growth factor-I receptor: does it differ from insulin receptor signaling? Cytokine Growth Factor Rev. 7, 153–159. Bondy, C.A., Zhou, J., Arzoffa, J.A., 2006. Growth hormone, insulin-like growth factors, and the ovary. In: Neill, J.D. (Ed.), Knobil and Neill's Physiology of Reproduction. Academic press, Cambridge, pp. 527–546. Caruso, M.A., Sheridan, M.A., 2011. New insights into the signaling system and function of insulin in fish. Gen. Comp. Endocrinol. 173, 227–247. Chiang, L.M., Myers, M.G., Backer, J.M., Shoelston, S.E., White, M.F., Birnbaum, M.J., Kahn, C.R., 1993. Insulin-stimulated oocyte maturation requires insulin receptor substrate 1 and interaction with the SH2 domains of phosphatidylinositol 3-kinase. Mol. Cell Biol. 13, 6653–6660. Gioacchini, G., Cardinali, M., Maradonna, F., Funkenstein, B., Mosconi, G., Carnavalli, O., 2005. Hormonal control of the IGF system in the sea bream ovary. Ann. N. Y. Acad. Sci. 1040, 320–322. Hawkess, C., Kar, S., 2004. The insulin-like growth factor-1/insulin-like growth factor-I receptor: structure, distribution and function in the central nervous system. Brain Res. Rev. 44, 117–140. Higuchi, K., Gen, K., Izumida, D., Kazeto, Y., Hotta, T., Aono, H., Soyano, K., 2016. Changes in gene expression and cellular localization of insulin-like growth factors 1 and 2 in the ovaries during ovary development of the yellowtail, Seriola quinqueradiata. Aquaculture 479, 609–615. Jones, J.J., Clemmons, D.R., 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev. 16, 3–34. Kagawa, H., Moriyama, S., Kawauchi, K., 1995. Immunocytochemical localization of IGF-1 in the ovary of the red seabream, Pampus major. Gen. Comp. Endocrinol. 99, 307–315. Kagawa, H., Gen, K., Okuzawa, K., Tanaka, H., 2003. Effects of luteinizing hormone and follicle-stimulating hormone and insulin-like growth factor-I on aromatase activity and P450 aromatase gene expression in the ovarian follicles of red seabream, Pampus major. Biol. Reprod. 68, 1562–1568. Lankford, S.E., Weber, G.M., 2010. Temporal mRNA expression of transforming growth factor-beta superfamily members and inhibitors in the developing rainbow trout ovary. Gen. Comp. Endocrinol. 166, 250–258. Li, J., Chu, L., Sun, X., Liu, Y., Cheng, C.H., 2015. IGFs mediate the action of LH on oocyte maturation in zebrafish. Mol. Endocrinol. 29, 373–383. Li, M., Wu, F., Gu, Y., Wang, T., Wang, H., Yang, S., Sun, Y., Zhou, L., Huang, X., Jiao, B., Cheng, C.H., Wang, D., 2012. Insulin-like growth factor 3 regulates expression of...
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