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Insulin-like growth factor binding proteins inhibit oocyte maturation of zebrafish

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ABSTRACT

The function of insulin-like growth factor (Igf) system in ovary has attracted much attention, but the role of Igf binding proteins (Igfbps) in ovary is still largely unknown. In this study, the role of Igfbps in oocyte maturation was investigated in zebrafish. The expression of all eight identified Igfbps except Igfbp6b could be detected in the adult ovary and exhibited differential expression profiles during folliculogenesis. The expression of several Igfbps is dynamically changed during oocyte maturation induced by human chorionic gonadotropin (hCG). By treatment of an Igfbps inhibitor NBI-31772 *in vitro*, the oocyte maturation could be stimulated in a clear dose-, time- and stage-dependent manner. Such effects were also observed by administration of NBI-31772 *in vivo*. Igfbps are differentially expressed in both follicular cells and oocytes, but the effect of NBI-31772 could only be found in intact follicles and not in the denuded oocytes. Previous studies have demonstrated that Igf3 is the major Igf member in regulating oocyte maturation of zebrafish. Interestingly, NBI-31772 could increase the effect of Igf3 on oocyte maturation. Furthermore, we found the effect of NBI-31772 on oocyte maturation could be blocked by an Igf type 1 receptor inhibitor BMS-536924 *in vitro*, suggesting the Igfbps can inhibit the oocyte maturation via Igf/Igf1r pathway. Together, we provided the first evidence in fish that Igfbps inhibit oocyte maturation of zebrafish.

In most vertebrates, the oocyte remains arrested in prophase I (PI) stage during early stages of follicle growth. When the PI-arrested oocyte is fully grown, the oocytes receive a surge of luteinizing hormone (LH) from the pituitary to initiate meiotic resumption, inducing germinal vesicle breakdown (GVBD). After GVBD, the oocyte progresses through meiosis I and enters meiosis II and arrests again at metaphase II. This resumption of the meiotic cell cycle is called oocyte maturation (Jaffe and Egbert, 2017; Nagahama and Yamashita, 2008). Many paracrine factors have been identified to regulate oocyte maturation in vertebrates (Jaffe and Egbert, 2017; Sun et al., 2009; Van Der Kraak and Lister, 2011). In recent years, using zebrafish as a model, we have revealed the functions of several factors involved in the oocyte maturation including insulin-like growth factors (Igfs) (Li and Cheng, 2018; Li et al., 2015, 2019, 2011, 2018b,c).

The Igf system regulates a variety of fundamental processes including reproduction (Duan et al., 2010; Reinecke, 2010). In most vertebrates, the IGF signaling system is composed of two ligands (IGF1 and 2), two IGF receptors (IGF1r and IGF2r), and six IGF-binding proteins (IGFBP1-6). Systemic IGFs are mainly secreted by the liver, controlled by growth hormone (GH), but IGFs are also produced locally in many tissues. The significant role of IGF system in regulating oocyte maturation has attracted much attention in the past decades. In many vertebrates including fish, members of the IGF system are expressed in ovary tissue (Reinecke, 2010). IGF1 mutant female mice are infertile, due to an arrest of follicular development before the maturation stage and failure to ovulate even after administration of gonadotropins (Baker et al., 1996). The induction of oocyte maturation by either IGF1 and IGF2 has been reported in many species including mammals (Gomez et al., 1993; Grupen et al., 1997; Guler et al., 2000; Kiapekou et al., 2005) and several teleost species (Kagawa et al., 1994; Maestro et al., 1997; Paul et al., 2013; Weber and Sullivan, 2000). Recently, we and others have also demonstrated the importance of Igfs in zebrafish oocyte maturation. Most members of Igf system are expressed in the ovary of zebrafish (Li et al., 2015; Nelson and Van Der Kraak, 2010a; Nelson and Van Der Kraak, 2010b). The effects of different Igfs on oocyte maturation have been demonstrated in zebrafish (Aizen et al., 2018; Das et al., 2016; Xie et al., 2016). Several studies have demonstrated that Igf3, a gonad-specific Igf, could be regulated by LH signaling (Li et al., 2015, 2011, 2018a; Nelson and Van Der Kraak, 2010a; Zhou et al., 2016), its importance in mediating the LH action on oocyte maturation was recently demonstrated in zebrafish (Li et al., 2015, 2011).

The existence of multiple Igfbps can contribute to the fine-tuning of Igf signaling (Allard and Duan, 2018; Jones and Clemmons, 1995). Prior to Igf/Igfr interaction, Igfbps can bind Igf with equal or higher affinity than Igf1rs (Firth and Baxter, 2002). Therefore, Igfbps serve as the important modulator on the bioavailability of Igfs, thereby inhibiting or potentiating Igf actions (Duan and Xu, 2005). In contrast to

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many studies on the Igfs, limited attention has been paid to the role of Igfbps in ovary. Some studies have indicated the involvement of Igfbps in mammalian oocyte maturation. For example, the expression level of specific Igfbps are highly associated with the oocyte maturation in human (Kawano et al., 1997; Wang et al., 2006), and Igfbp3 can block the hCG-induced oocyte maturation in rabbit (Yoshimura et al., 1996). However, there are no other studies on Igfbps in other vertebrates. In the present study, using zebrafish as a model animal, we investigated the role of Igfbps in oocyte maturation. Herein, we provide the first *in vitro* and *in vivo* evidence on the inhibitory role of Igfbps in oocyte maturation of zebrafish.

1. Materials and methods

1.1. Animals

All zebrafish were purchased locally. Fish were maintained under 14-h light/10-h dark cycles, in circulating freshwater aquaria at 26–28 °C. The size of fish tank is around 1.5 m*0.8 m*0.8 m. Fish were fed twice daily with newly hatched brine shrimp (Brine Shrimp Direct, USA). Fish experiments were conducted in accordance to the regulations of the Animal Experimentation Ethics Committee of Northwest Normal University (Project number is NSFC [31601205 and 31560334]).

1.2. Chemicals

Analytical reagent grade chemicals and hCG were obtained from Sigma-Aldrich (St. Louis, Missouri, USA), culture media from Gibco (Grand Island, New York, USA), and enzymes from Promega (Madison, Wisconsin, USA). NBI-31772 was purchased from Cayman (Ann Arbor, Michigan, USA). BMS-536924 was purchased from MCE (Monmouth Junction, New Jersey, USA).

1.3. RNA isolation and RT-PCR

After anesthetization and decapitation, their body cavity of fish was opened along the belly, and the ovaries were dissected out by tweezers. Total RNA samples were isolated from ovarian follicles of zebrafish using TRIzol Reagent (Invitrogen, Carlsbad, California, USA). The amount and purity of the RNA were determined on a NanoDrop 2000C Spectrophotometer (Thermo, Waltham, Massachusetts, USA). For real-time PCR, elongation factor-1 alpha (*ef1a*) was used as the internal standard for target genes. Ct value of ef1α in folliculogenesis and different stages of oocyte maturation was summarized in the Supplementary Table 1. All primers used in this study are listed at Table 1. Real-time PCR was carried out as previously described in (Chu et al., 2014). The experimental system size is 20 μL which is consist of THUNDERBIRD® SYBR® qPCR Mix (10 μL) (Toyobo, Osaka, Japan), 10 μM Forward Primer (0.25 μL), 10 μM Reverse Primer (0.25 μL), the cDNA template (1 μL) and water (8.5 μL). The annealing temperature for PCR ranges from 55 to 65 °C, depending on the primer set used. For semiquantitative RT-PCR analysis, PCR was carried out on a Thermal Cycler 9600 (Eppendorf, GER).

1.4. Isolation and incubations of ovarian follicles

The staging system that we have adopted is based on the original definition of Selman (Selman et al., 1993). The ovaries were dissected out from 15 to 20 female zebrafish after anesthetization and decapitation, and placed in a 100-mm culture dish containing 60% Leibovitz L-15 medium. Follicles of different stages were manually isolated and grouped into the following stages: primary growth (PG, stage I; below 0.1 mm in diameter), previtellogenic (PV, stage II or cortical alveolus stage; about 0.30 mm in diameter), early vitellogenic (EV; about 0.40 mm in diameter), midvitellogenic (MV; about 0.50 mm in diameter), late vitellogenic (LV; about 0.60 mm in diameter) and full grown but immature (FG; about 0.65 mm in diameter). Follicles of different stages were incubated (around 30 follicles/well) in 24-well culture plates at 28 °C. After treatment, follicles that underwent GVBD were identified by their ooplasmic clearing (due to proteolytic cleavage of vitellogenin). Each group had four to five replicate wells and each experiment was repeated at least three times.

1.5. Separation of follicular cells and oocyte from ovarian follicles

For extraction of RNA, the follicular cell layer was carefully peeled off from the follicle with fine forceps as described (Li et al., 2018c). The isolated follicular cell layers and the denuded but intact oocytes from 10 to 20 follicles were pooled and subjected to RNA extraction. For treatment assay, the follicular cell layer was separated by pipetting up and down using a narrow glass tube (Iwaki, Japan). The glass tube was pulled to about 0.7 mm diameter using an alcohol burner. The denuded but intact oocytes were pooled, and the surviving denuded oocytes were collected for treatment assay. Two different marker genes including *gdf9* and *lhcgr* were used to confirm the clean separation of both follicular cell layer and oocyte by real-time PCR. *gdf9* is highly expressed in the oocyte but *lhcgr* is predominantly expressed in the follicular cell layer. The primers for both *gdf9* and *lhcgr* were listed in Table 1.

1.6. Intraperitoneal injection into adult zebrafish

The intraperitoneal injection procedure of Kinkel et al. (2010) was followed with minor modifications. Briefly, after fasting and anesthetization, zebrafish were quickly placed on an agar gel plate. Using a microinjection system (WPI, USA), 4 ul hCG (5 IU/ul) or 4 ul NBI-31772 (100 mM) (using DMSO as control) were carefully injected into the midline between the pelvic fins. After injection, the fish were immediately transferred back to the water tank for recovery.

1.7. Statistical analysis

All data were expressed as mean values ± SEM, $P < 0.05$ was considered statistically significant using one-way ANOVA, followed by Fisher's least significant difference test using the GraphPad InStat software (GraphPad Software, USA). Statistical comparisons of the *igfbps* expression levels between follicular cell and oocyte was conducted using an unpaired two-tailed Students's *t*-test.

2. Results

2.1. Eight Igfbps are expressed in the ovary of adult zebrafish

The existence of Igfbps in the mature zebrafish ovary was analyzed. Nine different Igfbps (*igfbp1a*, *igfbp1b*, *igfbp2a*, *igfbp2b*, *igfbp3*, *igfbp5a*, *igfbp5b*, *igfbp6a*, and *igfbp6b*) have been identified in zebrafish (Allard and Duan, 2018). Transcripts of all eight *igfbps* except *igfbp6b* could be detected (Fig. 1A). The results were confirmed by quantitative real-time PCR. The mRNA level of *igfbp3* elicited the highest expression level (Fig. 1B).

The expression profiles of *igfbps* in the follicles of different stages of development during folliculogenesis were further assessed using real-time PCR. The level of *igfbp1a, igfbp1b, igfbp2b, igfbp3* and *igfbp6a* increased from PG stage to PV stage and then decreased rapidly thereafter. The expression of *igfbp2a* started to increase from the PV stage, reaching its highest level in FG stage. The expression of *igfbp5a* plateaued during folliculogenesis, but *igfbp5b* was increased from PG stage and reached the highest level in LV stage (Fig. 1C). These data indicate that *igfbps* are dynamically expressed during folliculogenesis in zebrafish.

Table 1
Primers used in the present study.

Gene full name	Symbol	Sequence(5′ to 3′ direction)	Strand	Purpose	Product length
Insulin-like growth factor binding protein 1a	*igfbp1a*	AGTCAACGCGATACGCAAGA	S	Real-time PCR	141 bp
		TGTTTGTCGCAGTTTGGCAG	AS		
Insulin-like growth factor binding protein 1b	*igfbp1b*	CAGAGTCTCAGCAGGCGTTG	S	Real-time PCR	141 bp
		TCCAGGAGGACACACACCAG	AS		
Insulin-like growth factor binding protein 2a	*igfbp2a*	CCGCCAACAGCTGAAGTCTA	S	Real-time PCR	199 bp
		GTACTGCCCCCTCTTGTCAC	AS		
Insulin-like growth factor binding protein 2b	*igfbp2b*	ACGCGGTCTCCTCCTATGAA	S	Real-time PCR	160 bp
		CCAGCTCCTGTTGACACTGG	AS		
Insulin-like growth factor binding protein 3	*igfbp3*	ATCTCACGGACACGACAAGC	S	Real-time PCR	89 bp
		GATGGACCGTCTCAGCTTCC	AS		
Insulin-like growth factor binding protein 5a	*igfbp5a*	GGACCAGGAGCCATGTTGAC	S	Real-time PCR	124 bp
		GCCATCACTCTGGAAGTGTCC	AS		
Insulin-like growth factor binding protein 5b	*igfbp5b*	AAGGAAGCTGGACGGAATCA	S	Real-time PCR	157 bp
		GTCCACGCACCAGCAGATT	AS		
Insulin-like growth factor binding protein 6a	*igfbp6a*	TTCAGGAGGAAGCAGTGTCG	S	Real-time PCR	124 bp
		CGCTGCTACATGAGATTGATCC	AS		
Insulin-like growth factor binding protein 6b	*igfbp6b*	CTGTGACACTCGTGGCTTCT	S	Real-time PCR	97 bp
		GGTGTTGCCCAGTTCATCCA	AS		
Elongation factor 1a	*ef1a*	CTGGAGGCCAGCTCAAACAT	S	Real-time PCR	87 bp
		ATCAAGAAGAGTAGTACCGCTAGCATTAC	AS		
Growth differentiation factor 9	*gdf9*	CCGAACCAAACCTGACTCCA	S	Real-time PCR	105 bp
		GCGGTGTTGTACAGGTGAGA	AS		
Luteinizing hormone receptor	*lhcgr*	GAGCCTTCAGGAAAGACGCT	S	Real-time PCR	107 bp
		TCGGTTGATGTTCTCCGAGC	AS		

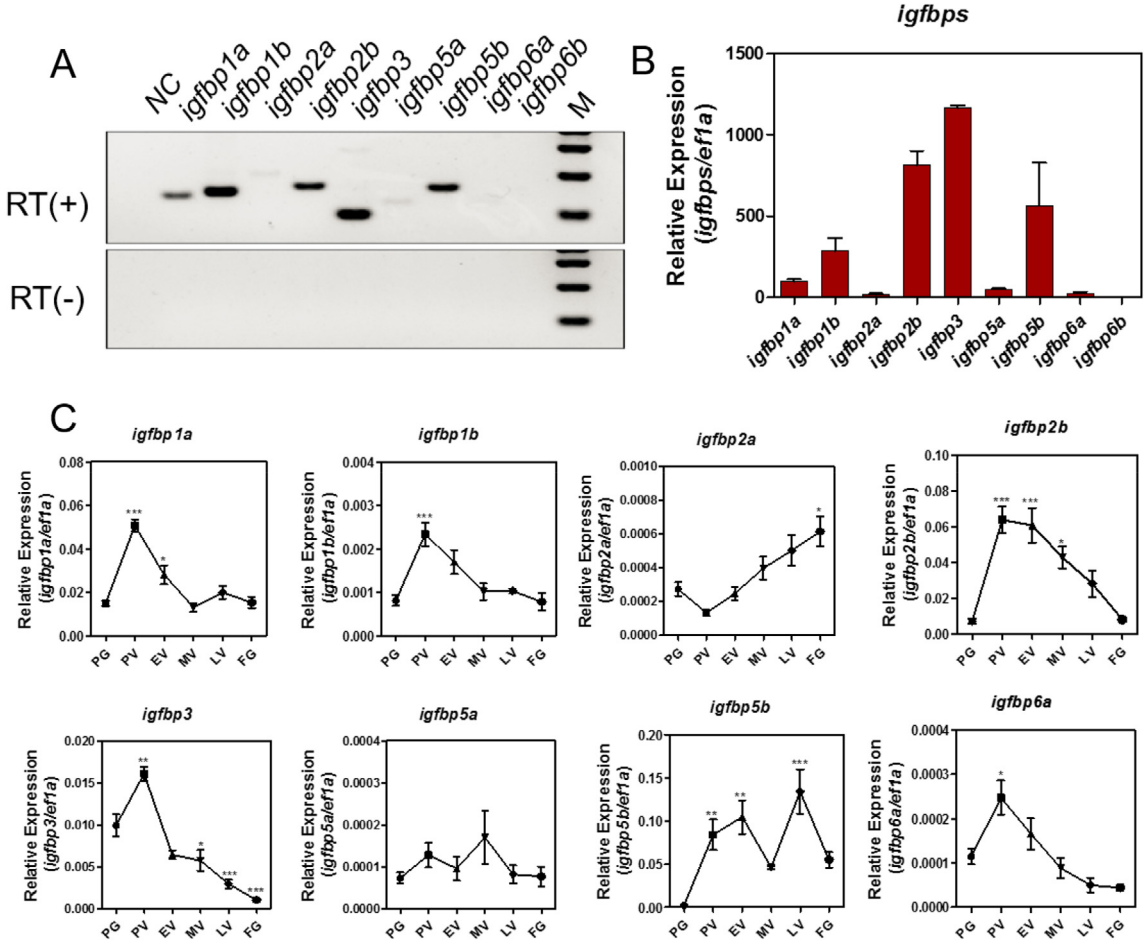

Fig. 1. Expression of *igfbps* during zebrafish folliculogenesis. (A) RT-PCR detection of *igfbp1a*, *igfbp1b*, *igfbp2a*, *igfbp2b*, *igfbp3*, *igfpb5a*, *igfpb5b*, *igfpb6a* and *igfbp6b* expression in the ovary of adult fish. RT(+): RNA with reverse transcription; RT(-): RNA without reverse transcription; M: marker. (B) Real-time PCR detection of *igfbp1a*, *igfbp1b*, *igfbp2a*, *igfbp2b*, *igfbp3*, *igfpb5a*, *igfpb5b* and *igfpb6a* expression in the ovary of adult fish. (C) Temporal expression of *igfbp1a*, *igfbp1b*, *igfbp2a*, *igfbp2b*, *igfbp3*, *igfpb5a*, *igfpb5b* and *igfpb6a* in the follicles of different stages isolated from the ovaries of adult fish. PG, primary growth; PV, previtellogenic stage; EV, early vitellogenic stage; MV, midvitellogenic stage; LV, late vitellogenic stage; FG, full grown stage. Each value represents the mean value ± SEM of quadruplicate assays ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. PG).

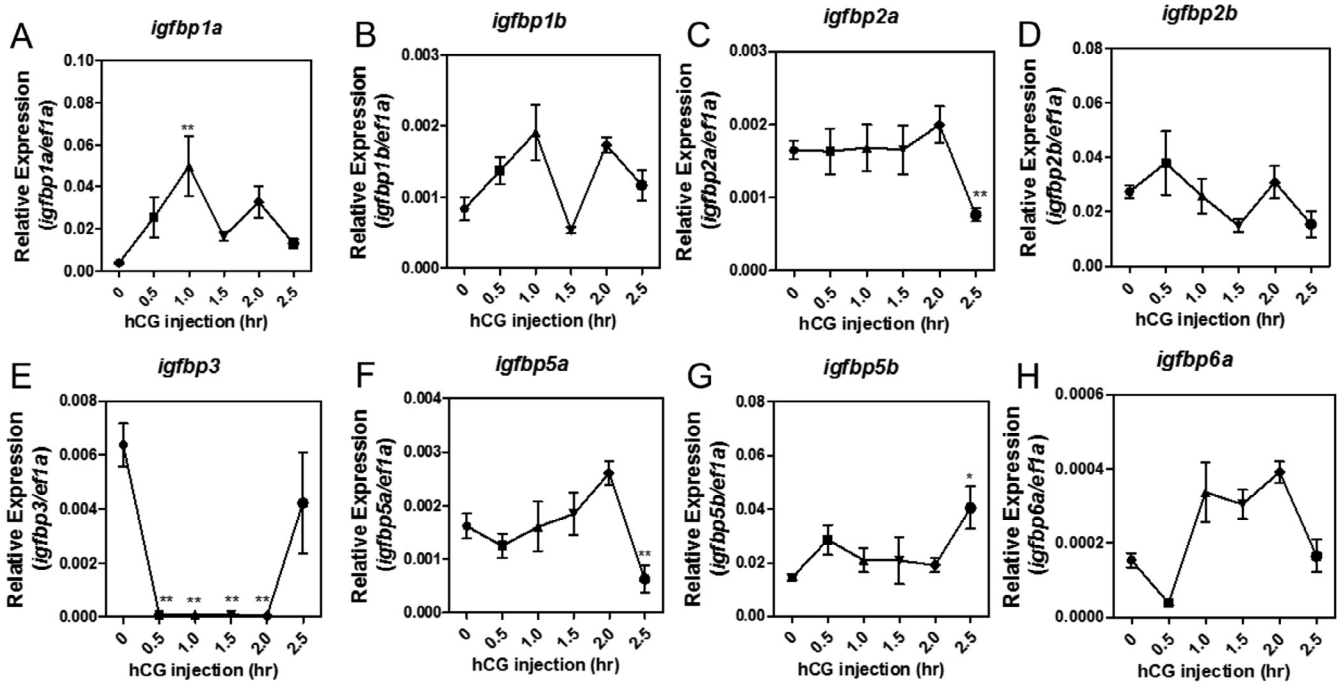

Fig. 2. The expression of *igfbps* during oocyte maturation induced by hCG in zebrafish. The relative expression of *igfbp1a*, *igfbp1b*, *igfbp2a*, *igfbp2b*, *igfbp3*, *igfpb5a*, *igfpb5b* and *igfpb6a* expression in preovulatory stage follicles after administration of hCG (20 IU/fish) assessed at different time points by real-time PCR. Each value represents the mean value ± SEM of quintuplicate assays ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control).

2.2. Igfbps in follicles are differentially regulated during oocyte maturation induced by hCG

To analyze the expression of *igfbps* during oocyte maturation, the oocyte maturation was artificially induced by hCG (20 IU/fish). The oocyte maturation and ovulation could be induced by hCG at around 2 h and 3 h, respectively. The gene expression of the eight *igfbps* during oocyte maturation was assessed using real-time PCR. The level of *igfbp1a and igfbp5b* significantly increased, but the levels of *igfbp2a*, *igfbp3* and *igfbp5a* significantly decreased. The expression of *igfbp1b*, *igfbp2b* and *igfbp6a* remained constant (Fig. 2).

2.3. Inhibition of Igfbps by NBI-31772 could stimulate oocyte maturation in vitro and in vivo

In order to analyze the biological functions of Igfbps in oocyte maturation, an Igfbps inhibitor NBI-31772 was employed to block the binding of Igfbps to Igfs. After treatment of FG stage follicles with this inhibitor, oocyte maturation could be significantly stimulated in a clear dose-, time- and stage-dependent manner (Fig. 3A, B and C). To further test the *in vivo* effects of NBI-31772 on oocyte maturation, we injected this inhibitor into adult zebrafish. GVBD could be induced in the ovaries of zebrafish injected with the NBI-31772 (Fig. 3D). The oocyte maturation ratio after administration of NBI-31772 was also calculated (Fig. 3E). Taken together, these data indicate that blocking the binding of Igfbps to Igfs by NBI-31772 could induce oocyte maturation *in vitro* and *in vivo*.

2.4. Igfbps regulate oocyte maturation through the follicular cells

To investigate whether the Igfbps exert their action directly on oocytes or through the follicular cells, the expression of Igfbps in ovarian follicles was examined by real-time PCR. This was done by separating the somatic follicular cell layer from the full grown follicles and analyzing the expression of *igfbps* in the two compartments. Clean separation was confirmed by two marker genes *viz. gdf9* and *lhcgr*, the former being oocyte specific and the other being follicular cell specific (Fig. 4A). The results showed that the expression of eight different Igfbps could be detected in both oocyte and follicular cells. *igfbp1b*, *igfbp2b*, *igfbp3*, *igfbp5a* and *igfbp6a* are highly expressed in the oocyte, but *igfbp1a, igfbp2a* and *igfbp5b* are predominantly expressed in the follicular cells (Fig. 4B). In order to know whether Igfbps exert their effects through follicular cell or oocyte on oocyte maturation, the effects of NBI-31771 on the oocyte maturation was analyzed in the denuded oocytes. We found that treatment of the denuded oocytes by NBI-31772 could not significantly affect spontaneous oocyte maturation (Fig. 4C). These results suggest that Igfbps might regulate oocyte maturation through follicular cells.

2.5. Igfbps regulate oocyte maturation via the Igf/Igf1r pathway

In order to understand the mechanism on Igfbps-regulated oocyte maturation, we investigated whether Igfbps can regulate oocyte maturation via modulation of Igfs. Previously Igf3 has been demonstrated as the major form of Igfs in regulating oocyte maturation of zebrafish. We asked if a low concentration of Igf3 not eliciting effects by itself, does stimulate oocyte maturation when NBI-31772 was present as well. Indeed, oocyte maturation could be significantly stimulated by treatment with both Igf3 (100 nM) and NBI-31772 *in vitro* (Fig. 5A). Next, we examined whether Igfbps regulate oocyte maturation via Igf1r. An Igf1r inhibitor (BMS-536924) was employed to block Igf1r action. We found the effect of NBI-31772 on oocyte maturation was completely abolished by treatment with BMS-536924 (Fig. 5B). All these data indicate that Igfbps regulate oocyte maturation via the Igf/Igf1r pathway. Furthermore, we found that the effect of NBI-31772 on oocyte maturation was dramatically suppressed by a translation inhibitor (cycloheximide) but not by a transcription inhibitor (actinomycin) (Fig. 5C), indicating that translational event is indispensable for the action of Igfbps on oocyte maturation.

3. Discussion

It has been well demonstrated that the Igf system plays an important role in the oocyte maturation of vertebrates (Reinecke, 2010; Silva

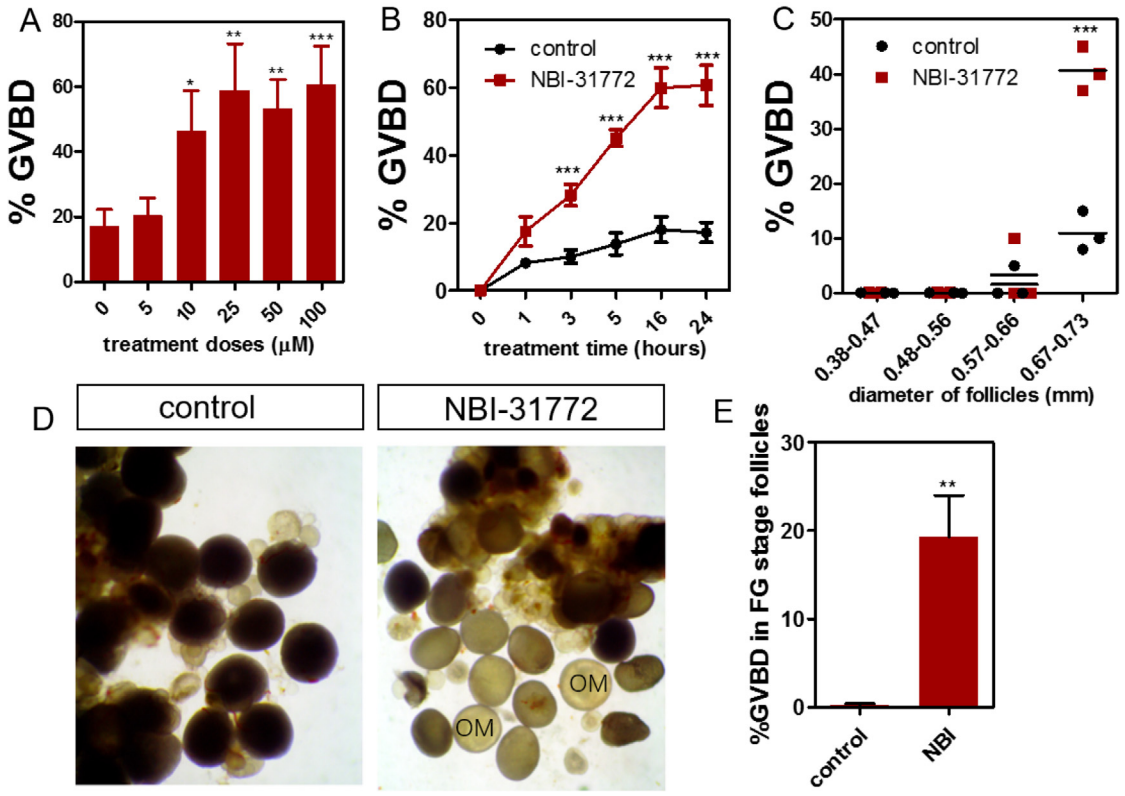

Fig. 3. Oocyte maturation could be induced by NBI-31772 *in vitro* and *in vivo*. (A) Dose dependence of NBI-31772 effect (16 h treatment) on the rate of oocyte maturation. For each dose point, four wells each containing 30 follicles were used. Each value represents the mean value ± SEM. (B) Time dependence of NBI-31772 (100 μM) on the rate of oocyte maturation. For each time point, four wells each containing 30 follicles were used. (C) Stage dependence of NBI-31772 effects (100 μM, 16 h treatment) on the rate of oocyte maturation. For each stage, three wells each containing 30 follicles were used. The solid lines show the mean. (D) Gross morphology of ovaries dissected from adult zebrafish after administration of NBI-31772 (100 mM, 4 μL/fish) for 4 h. FG, full grown stage. OM, oocyte maturation stage. (F) Quantitative assessment of oocyte maturation induction by administration of NBI-31772 (100 mM, 4 μL/fish) after 4 h. Five fish in each group was used. Each value in the above experiments represents the mean value ± SEM of quadruplicate assays ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control).

et al., 2009), but the role of Igfbps in this process remains to be fully elucidated. In this study, we have demonstrated for the first time in fish that Igfbps inhibit oocyte maturation using zebrafish as a model.

The expression of Igfbp genes in ovary has been reported in different species especially in mammals. All Igfbps except Igfbp1 have been detected in mammalian ovaries (Mazerbourg and Monget, 2018). Similarly, in rainbow trout, the expression of all Igfbps except Igfbp1 has been also detected in ovary (Kamangar et al., 2006). In zebrafish, nine different Igfbps have been identified. Different from mammals and some teleost species, Igfbp4 was not found in zebrafish (Allard and Duan, 2018). In the present study, all Igfbps except Igfbp6b are expressed in adult ovary. All these results suggest the involvement of Igfbps in ovarian development of vertebrates. In mammals, the intrafollicular Igfbps expression varies in different species, which shows the high species specificities (Mazerbourg and Monget, 2018). For example, *igfbp2* mRNA was strongly decreased during follicular growth in ovine (Besnard et al., 1996), porcine (Liu et al., 2000), bovine (Armstrong et al., 1998) species, but increased in monkey and human follicles (Arraztoa et al., 2002; Kwon et al., 2010). In fish, the information on the expression of Igfbps during follicle growth is very limited. In this study, we have analyzed the expression pattern of different *igfbps* during folliculogenesis. Comparing the available data on the expression of Igfbps in the ovary of rainbow trout (Kamangar et al., 2006), the expression of most *igfbps* during folliculogenesis is different between rainbow trout and zebrafish.

In this study, we found *igfbp2a*, *igfbp3* and *igfbp5a* is significantly decreased, but *igfbp1a* and *igfbp1b* is significantly increased during oocyte maturation induced by hCG, indicating these *igfbps* could be regulated by LH signaling. Since oocyte maturation could be induced by injection of hCG at around 2 h, only *igfbp1a* and *igfbp3* could be regulated before 2 h. Thus, both Igfbp1a and Igfbp3 might be involved in regulating oocyte maturation. The modulation of *igfbps* by gonadotropins in ovary has been reported in several different species (Mazerbourg and Monget, 2018). In sheep, inhibiting gonadotropins by gonadotrophin-releasing hormone antagonist (GnRHa) injection leads to a transient increase in Igfbp5 but decreased Igfbp4 expression (Hastie and Haresign, 2010). In primate ovary *igfbp4* mRNA expression was increased by hCG treatment *in vivo* (Zhou et al., 2003), but a more recent study reported a reduction of *igfbp4* mRNA levels in monkey granulosa cells after injection of hCG (Brogan et al., 2010). In rainbow trout, a down-regulation of *igfbp2b*, *-4*, and *-5* occurs in the oocyte in response to gonadotropins, whereas an up-regulation of *igfbp2a* and *-6* occurs in follicular layers in response to gonadotropic stimulation (Kamangar et al., 2006; Rodgers et al., 2008). The regulation of Igfs by gonadotropin has been analyzed in the ovary of different species including zebrafish (Kwintkiewicz and Giudice, 2009; Li et al., 2015). The modulation of Igfbps by gonadotropins might further fine-tune the biological activity of Igfs in ovary.

To investigate the action of Igfbps and the interaction between Igfs and Igfbps in oocyte maturation, we employed the Igfbps inhibitor NBI-31772. This inhibitor can displace Igfs from the Igfs-Igfbps complex, increasing the levels of free bioactive Igfs (Liu et al., 2001). Several studies have used this inhibitor to study the function of Igfbps in different species (Malberg et al., 2007; Schertzer et al., 2007). In zebrafish, NBI-31772 was successfully used to study the role of Igfbps in spermatogonial development and embryogenesis (Choi et al., 2013; Safian et al., 2016; Safian et al., 2017). Interestingly, we found in the present study that NBI-31772 could induce oocyte maturation of zebrafish *in*

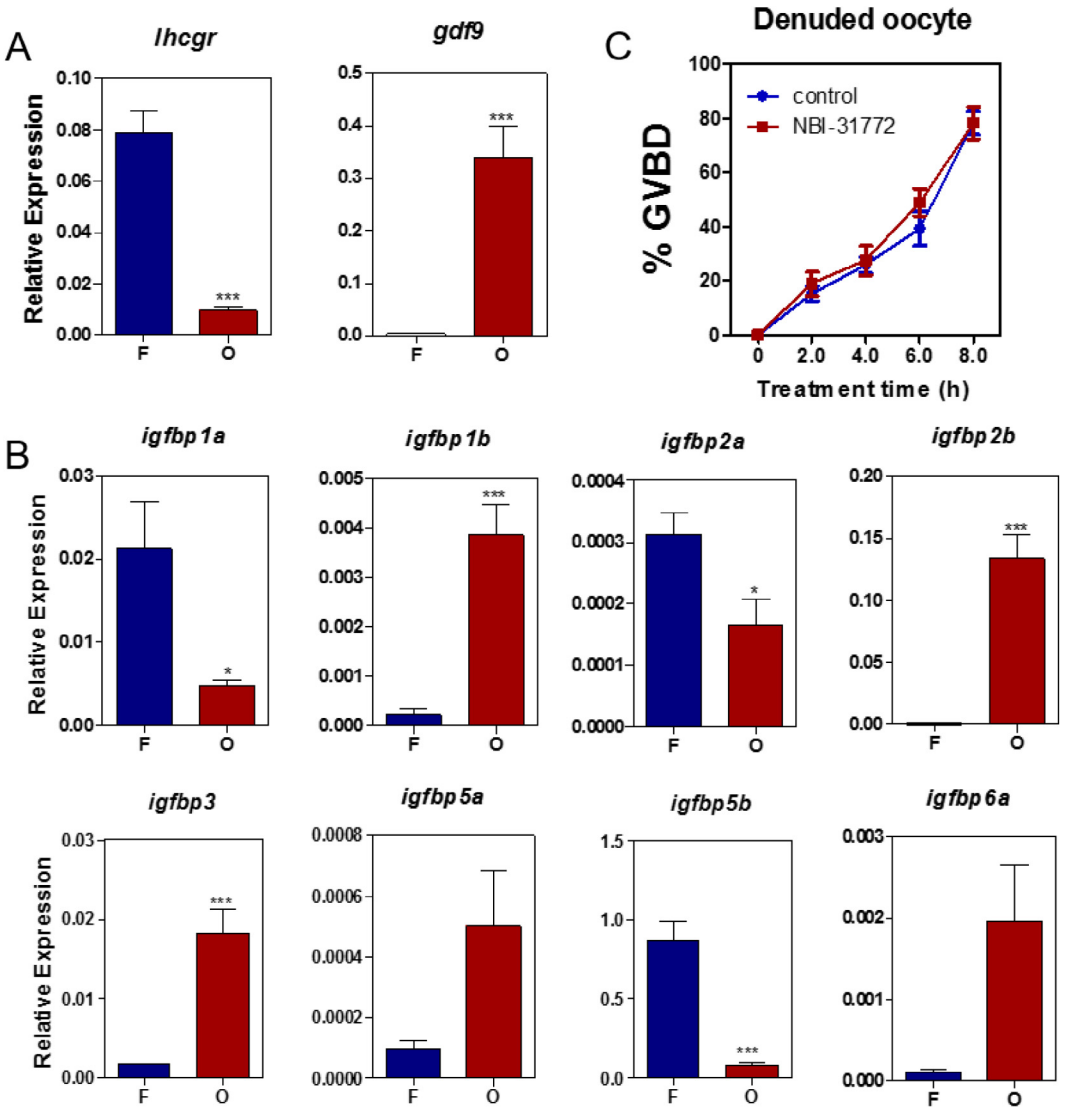

Fig. 4. The expression of *igfpbs* in the ovarian follicles and effect of NBI-31772 on denuded oocytes in zebrafish. (A) Real time PCR of *lhcgr* and *gdf9* expression in full grown zebrafish follicles indicate the clean separation of both follicular cell layers and denuded oocytes compartments. (B) Real time PCR results for the expression of *igfbp1a*, *igfbp1b*, *igfbp2a*, *igfbp2b*, *igfbp3*, *igfpb5a*, *igfpb5b* and *igfpb6a* in isolated follicular cell layers and denuded oocytes. Each value represents the mean value ± SEM of quintuplicate assays ($^{*}P < 0.05$ and $^{***}P < 0.001$ vs. follicular cell). (C) Effects on the rate of spontaneous oocyte maturation of denuded oocytes by treatment with NBI-31772 at different doses at different time points. For each time point, four wells each containing 30 follicles were used. Each value represents the mean value ± SEM of quadruplicate assays.

vitro and *in vivo*, indicating that Igfbps are involved in oocyte maturation and also suggest that occupancy of Igfbps with Igf family members is high in the zebrafish ovary. Previously we have demonstrated Igf3 as one of major Igf members in regulating oocyte maturation (Li et al., 2015, 2011, 2018a). In this study, we found NBI-31772 could increase the biological activity of a sub-threshold dose of Igf3 on oocyte maturation, suggesting that Igfbps might inhibit oocyte maturation through binding Igf3. We also found NBI-31772 could not stimulate oocyte maturation in denuded oocytes, indicating that Igfbps exert their inhibitory effect through follicular cells. This finding is consistent with our previous findings that Igf3 is mainly expressed in follicular cells of zebrafish (Li et al., 2015, 2011), which suggest that Igfbps might regulate oocyte maturation through Igf3 in follicular cells. Such speculation was further confirmed by our finding that an Igf1r inhibitor (BMS-536924) could totally block the effect of NBI-31772 on oocyte maturation. Consistently, same as the action of Igfs on oocyte maturation in which translational events are involved in (Li et al., 2011), the effect of NBI-31772 on oocyte maturation could only be blocked by a translational inhibitor but not a transcriptional inhibitor. So far, we still do not know which Igfbp is the major member involved in regulating this process. Igfbp3 is a potential candidate. As mentioned above, the expression of Igfbp3 is the highest one among all Igfbps in adult ovary. Its expression is decreased from the PV stage to the FG stage. Igfbp3 can be dramatically and rapidly downregulated in zebrafish oocyte maturation induced by hCG. In rabbit, Igfbp3 has been demonstrated as an inhibitory factor in oocyte maturation induced by hCG (Yoshimura et al., 1996). Based on analysis on the regulation of Igfbps during oocyte maturation, Igfbp1a can be another candidate for regulating oocyte maturation. As mentioned in the Introduction, Igfbps can either activate or inhibit Igfs action. Therefore, it is possible that Igfbp1a can activate but Igfbp3 can block the action of Igf3 on oocyte maturation. However, this possibility warrants studies in the future.

In conclusion, we have demonstrated Igfbps could inhibit zebrafish oocyte maturation by sequestering Igf signaling. Combining with our previous findings on the role of Igfs in zebrafish oocyte maturation (Li et al., 2015, 2011), we can propose a new working model on the regulations and functions of the Igf system in oocyte maturation in zebrafish (Fig. 5D). LH uses two ways to increase Igf3 activity. One way is

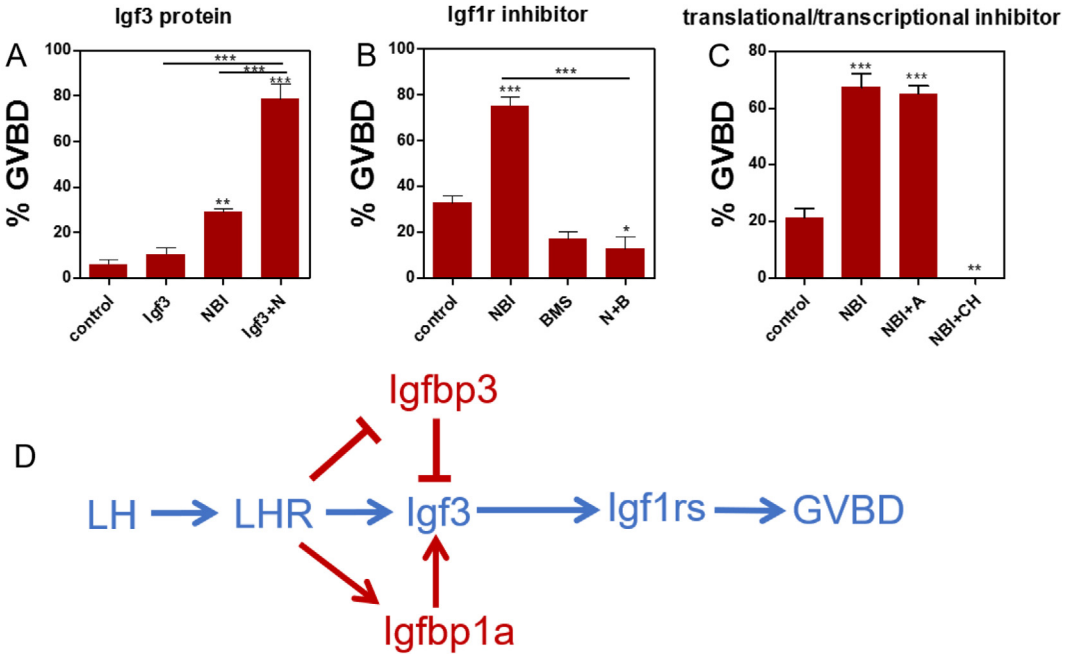

Fig. 5. Igf signaling is involved in the NBI-31772-induced oocyte maturation in zebrafish. (A) The effects on oocyte maturation treatment with Igf3 protein (100 nM) with or without NBI-31772 (100 μM) after 8 h. (B) The effects on oocyte maturation treatment with an Igf1r inhibitor BMS-536924 (10 μM) with or without NBI-31772 (100 μM) after 16 h. (C) The effects on oocyte maturation treatment with actinomycin (transcriptional inhibitor) or cycloheximide (translational inhibitor) with NBI-31772 (100 μM) after 16 h. For each control or treatment group, four wells each containing 30 follicles were used. Each value represents the mean value ± SEM of quadruplicate assays ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control). (D) A hypothetic model for the role of Igfbps in regulation of oocyte maturation in zebrafish. After the LH surge, Igf3 expression is increased, while the expression of Igfbps including Igfbp1a and Igfbp3 is regulated to release active Igf3, and the increased Igf3 can stimulate the oocyte maturation by activating Igf1rs signaling.

to increase the expression of Igfs especially the Igf3 in follicular cell directly, while regulating Igfbps expression including Igfbp3 and Igfbp1a to fine-tune the release of active Igf3. These increased Igfs including Igf3 can bind to Igf1rs in oocyte membrane to stimulate oocyte maturation in zebrafish.

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Disclosure statement

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.06.002.

References

Aizen, J., Pang, Y., Harris, C., Converse, A., Zhu, Y., Aguirre, M.A., Thomas, P., 2018. Roles of progesterone receptor membrane component 1 and membrane progestin receptor alpha in regulation of zebrafish oocyte maturation. General Comparative Endocrinol. 263, 51–61.
Allard, J.B., Duan, C., 2018. IGF-binding proteins: why do they exist and why are there so many? Front. Endocrinol. 9, 117.
Armstrong, D.G., Baxter, G., Gutierrez, C.G., Hogg, C.O., Glazyrin, A.L., Campbell, B.K., Bramley, T.A., Webb, R., 1998. Insulin-like growth factor binding protein -2 and -4 messenger ribonucleic acid expression in bovine ovarian follicles: effect of gonadotropins and developmental status. Endocrinology 139, 2146–2154.
Arraztoa, J.A., Monget, P., Bondy, C., Zhou, J., 2002. Expression patterns of insulin-like growth factor-binding proteins 1, 2, 3, 5, and 6 in the mid-cycle monkey ovary. J. Clin. Endocrinol. Metabolism 87, 5220–5228.
Baker, J., Hardy, M.P., Zhou, J., Bondy, C., Lupu, F., Bellve, A.R., Efstratiadis, A., 1996. Effects of an Igf1 gene null mutation on mouse reproduction. Mol. Endocrinol. (Baltimore Md) 10, 903–918.
Besnard, N., Pisselet, C., Monniaux, D., Locatelli, A., Benne, F., Gasser, F., Hatey, F., Monget, P., 1996. Expression of messenger ribonucleic acids of insulin-like growth factor binding protein-2, -4, and -5 in the ovine ovary: localization and changes during growth and atresia of antral follicles. Biol. Reprod. 55, 1356–1367.
Brogan, R.S., Mix, S., Puttabyatappa, M., VandeVoort, C.A., Chaffin, C.L., 2010. Expression of the insulin-like growth factor and insulin systems in the luteinizing macaque ovarian follicle. Fertil. Steril. 93, 1421–1429.
Choi, W.Y., Gemberling, M., Wang, J., Holdway, J.E., Shen, M.C., Karlstrom, R.O., Poss, K.D., 2013. In vivo monitoring of cardiomyocyte proliferation to identify chemical modifiers of heart regeneration. Development 140, 660–666.
Chu, L., Li, J., Liu, Y., Hu, W., Cheng, C.H.K., 2014. Targeted gene disruption in zebrafish reveals noncanonical functions of LH signaling in reproduction. Mol. Endocrinol. 28, 1785–1795.
Das, D., Pal, S., Maitra, S., 2016. Releasing prophase arrest in zebrafish oocyte: synergism between maturational steroid and Igf1. Reproduction 151, 59–72.
Duan, C., Ren, H., Gao, S., 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. Gen. Comp. Endocrinol. 167, 344–351.
Duan, C., Xu, Q., 2005. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen. Comp. Endocrinol. 142, 44–52.
Firth, S.M., Baxter, R.C., 2002. Cellular actions of the insulin-like growth factor binding proteins. Endocr. Rev. 23, 824–854.
Gomez, E., Tarin, J.J., Pellicer, A., 1993. Oocyte maturation in humans: the role of gonadotropins and growth factors. Fertil. Steril. 60, 40–46.
Grupen, C.G., Nagashima, H., Nottle, M.B., 1997. Role of epidermal growth factor and insulin-like growth factor-I on porcine oocyte maturation and embryonic

development in vitro. Reprod. Fertil. Dev. 9, 571–575.
Guler, A., Poulin, N., Mermillod, P., Terqui, M., Cognie, Y., 2000. Effect of growth factors, EGF and IGF-I, and estradiol on in vitro maturation of sheep oocytes. Theriogenology 54, 209–218.
Hastie, P.M., Haresign, W., 2010. Modulating peripheral gonadotrophin levels affects follicular expression of mRNAs encoding insulin-like growth factor binding proteins in sheep. Animal Reprod. Sci. 119, 198–204.
Jaffe, L.A., Egbert, J.R., 2017. Regulation of mammalian oocyte meiosis by intercellular communication within the ovarian follicle. Annu. Rev. Physiol. 79, 237–260.
Jones, J.I., Clemmons, D.R., 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev. 16, 3–34.
Kagawa, H., Kobayashi, M., Hasegawa, Y., Aida, K., 1994. Insulin and insulin-like growth factors I and II induce final maturation of oocytes of red seabream, Pagrus major, in vitro. Gen. Comp. Endocrinol. 95, 293–300.
Kamangar, B.B., Gabillard, J.C., Bobe, J., 2006. Insulin-like growth factor-binding protein (IGFBP)-1, -2, -3, -4, -5, and -6 and IGFBP-related protein 1 during rainbow trout postvitellogenesis and oocyte maturation: molecular characterization, expression profiles, and hormonal regulation. Endocrinology 147, 2399–2410.
Kawano, Y., Narahara, H., Matsui, N., Nasu, K., Miyamura, K., Miyakawa, I., 1997. Insulin-like growth factor-binding protein-1 in human follicular fluid: a marker for oocyte maturation. Gynecol. Obstet. Invest. 44, 145–148.
Kiapekou, E., Loutradis, D., Drakakis, P., Zapanti, E., Mastorakos, G., Antsaklis, A., 2005. Effects of GH and IGF-I on the in vitro maturation of mouse oocytes. Hormones (Athens, Greece) 4, 155–160.
Kinkel, M.D., Eames, S.C., Philipson, L.H., Prince, V.E., 2010. Intraperitoneal injection into adult zebrafish. J. Vis. Exp.
Kwintkiewicz, J., Giudice, L.C., 2009. The interplay of insulin-like growth factors, gonadotropins, and endocrine disruptors in ovarian follicular development and function. Seminars Reprod. Med. 27, 43–51.
Kwon, H., Choi, D.H., Bae, J.H., Kim, J.H., Kim, Y.S., 2010. mRNA expression pattern of insulin-like growth factor components of granulosa cells and cumulus cells in women with and without polycystic ovary syndrome according to oocyte maturity. Fertil. Steril. 94, 2417–2420.
Li, J., Cheng, C.H.K., 2018. Evolution of gonadotropin signaling on gonad development: insights from gene knockout studies in zebrafish. Biol. Reprod. 99, 686–694.
Li, J., Chu, L., Sun, X., Liu, Y., Cheng, C.H.K., 2015. IGFs mediate the action of LH on oocyte maturation in zebrafish. Mol. Endocrinol. 29, 373–383.
Li, J., Huang, D., Sun, X., Li, X.H., Cheng, H.K., 2019. Zinc mediates the action of androgen in acting as a downstream effector of luteinizing hormone on oocyte maturation in zebrafish. Biol. Reprod. 100, 468–478.
Li, J., Liu, Z., Wang, D., Cheng, C.H.K., 2011. Insulin-like growth factor 3 is involved in oocyte maturation in zebrafish. Biol. Reprod. 84, 476–486.
Li, J., Niu, C., Cheng, C.H.K., 2018a. Igf3 serves as a mediator of luteinizing hormone in zebrafish ovulation. Biol. Reprod. 99, 1235–1243.
Li, J., Wang, Y., Zhou, W., Li, X., Chen, H., 2018b. The role of PKG in oocyte maturation of zebrafish. Biochem. Biophys. Res. Commun. 505, 530–535.
Li, J., Zhou, W., Wang, Y., Niu, C., 2018c. The dual role of cGMP in oocyte maturation of zebrafish. Biochem. Biophys. Res. Commun. 499, 998–1003.
Liu, J., Koenigsfeld, A.T., Cantley, T.C., Boyd, C.K., Kobayashi, Y., Lucy, M.C., 2000. Growth and the initiation of steroidogenesis in porcine follicles are associated with unique patterns of gene expression for individual componentsof the ovarian insulin-like growth factor system. Biol. Reprod. 63, 942–952.
Liu, X.J., Xie, Q., Zhu, Y.F., Chen, C., Ling, N., 2001. Identification of a nonpeptide ligand that releases bioactive insulin-like growth factor-I from its binding protein complex. J. Biol. Chem. 276, 32419–32422.
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.
Malberg, J.E., Platt, B., Rizzo, S.J., Ring, R.H., Lucki, I., Schechter, L.E., Rosenzweig-Lipson, S., 2007. Increasing the levels of insulin-like growth factor-I by an IGF binding protein inhibitor produces anxiolytic and antidepressant-like effects. Neuropsychopharmacology: Official Publ. Am. College Neuropsychopharmacology 32, 2360–2368.
Mazerbourg, S., Monget, P., 2018. Insulin-like growth factor binding proteins and IGFBP proteases: a dynamic system regulating the ovarian folliculogenesis. Front. Endocrinol. 9, 134.
Nagahama, Y., Yamashita, M., 2008. Regulation of oocyte maturation in fish. Dev. Growth Differ. 50 (Suppl 1), S195–219.
Nelson, S.N., Van Der Kraak, G., 2010a. Characterization and regulation of the insulin-like growth factor (IGF) system in the zebrafish (Danio rerio) ovary. Gen. Comp. Endocrinol. 168, 111–120.
Nelson, S.N., Van Der Kraak, G., 2010b. The role of the insulin-like growth factor (IGF) system in zebrafish (Danio rerio) ovarian development. Gen. Comp. Endocrinol. 168, 103–110.
Paul, S., Pramanick, K., Kundu, S., Roy Moulik, S., Pal, P., Mukherjee, D., 2013. Involvement of PI3 kinase and MAP kinase in IGF-I and insulin-induced ovarian steroidogenesis in common carp Cyprinus carpio. Gen. Comp. Endocrinol. 181, 98–106.
Reinecke, M., 2010. Insulin-like growth factors and fish reproduction. Biol. Reprod. 82, 656–661.
Rodgers, B.D., Roalson, E.H., Thompson, C., 2008. Phylogenetic analysis of the insulin-like growth factor binding protein (IGFBP) and IGFBP-related protein gene families. Gen. Comp. Endocrinol. 155, 201–207.
Safian, D., Morais, R.D., Bogerd, J., Schulz, R.W., 2016. Igf binding proteins protect undifferentiated spermatogonia in the Zebrafish testis against excessive differentiation. Endocrinology 157, 4423–4433.
Safian, D., van der Kant, H.J.G., Crespo, D., Bogerd, J., Schulz, R.W., 2017. Follicle-stimulating hormone regulates igfbp gene expression directly or via downstream effectors to modulate Igf3 effects on zebrafish spermatogenesis. Front. Endocrinol. 8, 328.
Schertzer, J.D., Gehrig, S.M., Ryall, J.G., Lynch, G.S., 2007. Modulation of insulin-like growth factor (IGF)-I and IGF-binding protein interactions enhances skeletal muscle regeneration and ameliorates the dystrophic pathology in mdx mice. Am. J. Pathology 171, 1180–1188.
Selman, K., Wallace, R.A., Sarka, A., Qi, X., 1993. Stages of oocyte development in the zebrafish, *Brachydanio rerio*. J. Morphol. 218, 22.
Silva, J.R., Figueiredo, J.R., van den Hurk, R., 2009. Involvement of growth hormone (GH) and insulin-like growth factor (IGF) system in ovarian folliculogenesis. Theriogenology 71, 1193–1208.
Sun, Q.Y., Miao, Y.L., Schatten, H., 2009. Towards a new understanding on the regulation of mammalian oocyte meiosis resumption. Cell Cycle 8, 2741–2747.
Van Der Kraak, G., Lister, A.L., 2011. The inhibitory control of oocyte maturation in the zebrafish (Danio rerio): the role of the G protein-coupled estrogen receptor and epidermal growth factor. Biol. Reprod. 85, 6–8.
Wang, T.H., Chang, C.L., Wu, H.M., Chiu, Y.M., Chen, C.K., Wang, H.S., 2006. Insulin-like growth factor-II (IGF-II), IGF-binding protein-3 (IGFBP-3), and IGFBP-4 in follicular fluid are associated with oocyte maturation and embryo development. Fertil. Steril. 86, 1392–1401.
Weber, G.M., Sullivan, C.V., 2000. Effects of insulin-like growth factor-I on in vitro final oocyte maturation and ovarian steroidogenesis in striped bass, Morone saxatilis. Biol. Reprod. 63, 1049–1057.
Xie, L., Tang, Q., Yang, L., Chen, L., 2016. Insulin-like growth factor I promotes oocyte maturation through increasing the expression and phosphorylation of epidermal growth factor receptor in the zebrafish ovary. Mol. Cell. Endocrinol. 419, 198–207.
Yoshimura, Y., Nagamatsu, S., Ando, M., Iwashita, M., Oda, T., Katsumata, Y., Shiokawa, S., Nakamura, Y., 1996. Insulin-like growth factor binding protein-3 inhibits gonadotropin-induced ovulation, oocyte maturation, and steroidogenesis in rabbit ovary. Endocrinology 137, 438–446.
Zhou, J., Wang, J., Penny, D., Monget, P., Arraztoa, J.A., Fogelson, L.J., Bondy, C.A., 2003. Insulin-like growth factor binding protein 4 expression parallels luteinizing hormone receptor expression and follicular luteinization in the primate ovary. Biol. Reprod. 69, 22–29.
Zhou, R., Yu, S.M., Ge, W., 2016. Expression and functional characterization of intrafollicular GH-IGF system in the zebrafish ovary. Gen. Comp. Endocrinol. 232, 32–42.