Is the use of recombinant cGnRH may be a future alternative to control the fish spawning? Let us go with the goldfish example

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Keywords

Recombinant chicken GnRH, sex steroids, ovulation, spawning, carassius auratus.

Abstract

The use of recombinant gonadotropin-releasing hormone (rGnRH) has very rarely been tested in fish to promote spawning. This study evaluated the impact of recombinant chicken gonadotropin-releasing hormone (rcGnRH) with metoclopramide on the release of sex steroids and final maturation induction in goldfish (Carassius auratus) broodstock. For this purpose, goldfish broodstock was divided into four groups and treated with 0.9% NaCl with 20 mg/kg metoclopramide (Met) (C); 10 µg/kg body weight (BW) rcGnRH with 20 mg/ kg metoclopramide (rcGn10); 15 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/ kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). The capability of the rcGnRH for eliciting biological response was tested in vivo by evaluating the changes of 17β estradiol (E2), testosterone (T), and 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) and the induced spawning. Blood samples were obtained at 0 h, 12 h, and 24 h after injection. The rcGn10, rcGn15, and rcGn20 treatments induced lower E2 concentration, especially 24 h post-injection. T levels were significantly higher in rcGn10, rcGn15, and rcGn20 treatments 12 h post-injection than at 0 h and then decreased at 24 h post-injection. Furthermore, the rcGnRH tested significantly enhanced DHP secretion in rcGn10, rcGn15, and rcGn20 treatments 12 h post-injection before a decline at 24 h post-injection. No significant difference between the sampling times was found in the C treatment for the 3 sex steroids tested. The results also displayed that rcGnRH at 10–20 µg/kg of body weight can trigger spawning with the highest speed and efficiency of spawning at 20 µg/kg. The obtained results represent a possible strategy for enhancing the artificial reproduction and ovulation of broodstock fish by rGnRH and further support the use of recombinant hormones to promote reproduction in aquaculture.

1. Introduction

The development of aquaculture activity requires more sustainable strategies, including the artificial reproduction of broodstock (Mylonas and Zohar 2001). One of the prerequisites for successful artificial reproduction is controlling the reproductive process of fish in captivity and the obtention of high-quality eggs (sperm and eggs). Different hormones are used for the artificial reproduction of aquatic animals in breeding centers, notably the analogs of GnRH (Zohar and Mylonas 2001).

GnRH is a decapeptide neurohormone secreted by the hypothalamus (Okubo and Nagahama 2008). GnRH is composed of peptides that can be targets of endopeptidases that reduce its half-life and make GnRH available for only 5

min, and then, it can be degraded by peptidases (Gothilf and Zohar 1991). The synthesized GnRH analogs (GnRHa) were designed to resist circulating enzymatic degradation and have higher stability than natural GnRH against enzymatic degradation, with a half-life of 23 min compared to the one of original salmon GnRH, which lasts 5 min (Gothilf and Zohar 1991). Despite the higher stability of synthetic GnRHs than natural GnRH, a single injection is often not sufficient to trigger the induction of final oocyte maturation (FOM). Failure to respond to a single injection of GnRHa in the induction of final sexual development of fish is related to its short half-life in the blood circulation. Final oocyte maturation may take several days to be completed in fish, so high concentrations of GnRHa must be maintained in the circulatory system during this time to induce the required high concentration of LH. On the other hand, fish do not start FOM immediately after GnRHa injection, and plasma LH and steroid concentrations may increase several hours after hormone injection. Therefore, due to its short half-life in the circulatory system, it needs to be injected continuously (Mylonas and Zohar 2001). Numerous manipulations for GnRHa injection are problematic; they can be stressful and cause damage to fish and, in turn, may adversely affect their reproduction (Leather-land et al. 2010). The stressful farming conditions affect fish reproduction, making it necessary to find a hormonal treatment with a long half-life that can be effective with an optimal injection dose.

There are several chemical and genetic techniques to increase the half-life of peptides-based hormones. For the former ones, the addition of polyethylene glycol (PEG) and lipidation to the peptide is suggested to increase the half-life and reduce renal refinement rates (Kontermann 2005; Szlachcic et al. 2011; Che-kan et al. 2016). For the latter ones, one strategy consists of producing recombinant GnRH hormone dotted with gonadotropin-releasing hormone associated peptide (GAP) sequence that may further help to maintain the stability of the hormone produced. Although GAP was proposed to fix GnRH; however, it led to the inactivation of peptides activity (Andersen and Klungland 1993). Recombinant DNA results in forming active biologically polypeptides hormones in bacteria with reasonable cost compared to chemical synthesis (Xu et al. 2006). That technique has proved recent effectiveness in sterlet sturgeon (Mohammadzadeh et al. 2020a, b), but it is unknown if it applies to other teleost species.

Goldfish (Carassius auratus) belongs to the Cyprinidae family and originated from central Asia and China with a high marketable request in aquaculture, especially as an ornamental species (Wen et al. 2020). It has been recommended as a model fish species for reproductive, endocrinological, immunological, toxicological, and molecular related studies (Munakata and Kobayashi 2010; Ota and Abe 2016; Omori and Kon 2019). Therefore, it is a relevant model to further improve and understand the endocrine control of fish's ultimate maturity using promising hormonal therapies such as recombinant hormones.

2. Material and Methods

2.1. Broodstock rearing condition and treatment

Sexually mature female goldfish with an average weight of 72.26 ± 6.21 g and age of 7 months were purchased from a local fish breeding center (Guilan, Iran) and transformed into a private wet lab in the local fish breeding center (Gorgan, Iran). The selection of female broodstock was made according to anatomical appearance (soft and protruding abdomen), which is a reliable way to determine spawning readiness during natural spawning and to assess the stage of oocyte development (central position of the nucleus) (Podhorec et al. 2016). The selected fish were randomly distributed into four treatments (n = 10), and was placed into a 250-L aquarium. After a 2-day adaptation period and a gradual increase in ambient temperature, the water conditions used were as follows: temperature 26.33 ± 0.3 °C, natural photoperiod (16 h light and 8 h dark), pH 8.3 ± 0.3, and dissolved oxygen 11.5 ± 0.8 mg/L. After two other days in these conditions, fish was injected a single intraperitoneal injection containing one of the following treatments with 0.9% NaCl as vehicle: 20 mg/kg of metoclopramide (C); 10 µg/kg body weight (BW) rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); 20 µg/kg BW rcGnRH with 20 mg/ kg metoclopramide (rcGn20). rcGnRH was produced base on our previous work (Mohammadzadeh et al. 2020a, b). Metoclopramide was incorporated in all treatments, like dopamine receptor antagonists, to optimize the ovulatory process in cyprinid fishes. Before each manipulation, fish were anesthetized with clove powder at a dose of 15 ppm. Breeders were tested for ovulation 8 h after the injection, and this manipulation was repeated at 2-h intervals up to 24 h post-injection. In response to gentle pressure, the spawning-ready fish were stripped to collect the eggs in a dry container. The latency period was recorded as the lapse of time between the hormone injection and spawning. Only spawned fish were thus taken into consideration for the calculation of the latency period.

2.2. Sampling and hormone analysis

First, fish were anesthetized with 15 ppm clove powder extract. Blood samples (6 fish into each tank) were collected from the behind of the anal fin with a 2-mL heparinized syringe at the injection time, 12 h post-injection, and 24 h post-injection (only in spawned fish). The samples were then transferred to the tubes, centrifuged (1600 g for 10 min) to separate plasma, and stored at − 20 °C for later analysis.

Enzyme-linked immunosorbent assay (ELISA) commercial kits were applied to measure sex steroid concentration (ng/mL) of testosterone (T, Cat. No: 3725–300, AccuBind ELISA Microwells, Monobind, Inc. Lake Forest, CA, USA), 17β estradiol (E2, Cat. No: 4925–300, AccuBind ELISA Microwells,

Monobind, Inc. Lake Forest, CA, USA), and 17α, 20β-dihydroxy-4-pregnen-3-one (DHP, Cat. No: CK-E91529, Eastbiopharm, USA). The intra- and inter-assay coefficients of variation were 5.8%, 9.8% (n = 10), 6.4%, 9.5% (n = 10), 10%, 12% (n = 8) for E2, T, and DHP, respectively.

2.3. Statistical analysis

SPSS software (version 16, Chicago, IL, USA) was used to analysis data. This research has been done using a completely randomized design with different treatments. Kolmogorov–Smirnov and Levene's tests were applied to check the data normality and homogeneity of variances, respectively. One-way analysis of variance (ANOVA) was used to analyze differences in the treatments' latency time. The effect of the treatments and the sampling times on the sex steroid levels was examined by a two-way analysis of variance (ANOVA). Differences between several treatments were determined by Tukey's post hoc tests. To investigate potential correlations between the sex steroid hormones, a Pearson correlation test was used.

3. Results

3.1. Spawning performance

The spawning productivity of goldfish broodstock injected with rcGnRH showed eight females injected with rcGn10, six females injected with rcGn15, and ten females injected with rcGn20 spawned, but no females spawned in the control group (Table 1). The ovulation rate of broodstock receiving rcGnRH was enhanced in females treated with 20 µg/kg of body weight. The groups of females treated with 20 µg/kg of rcGnRH exhibited a decreased latency period than the two other groups injected with rcGnRH (Table 1; $P < 0.05$).

3.2. Hormone levels

While the E2 level was similar among the groups at 0 h, it was impacted by the treatments at 12 h and 24 h post-injection (Fig. 1; $P < 0.05$). At 12 h, a significant decrease of E2 levels was observed in the rcGn15 treatment relative to the control group. However, all rcGn10, rcGn15, and rcGn20 treatments led to significantly lower E2 concentration 24 h post-injection than baseline measures (Fig. 1; $P < 0.05$).

After 12 h post-injection, the concentration of T was significantly higher in rcGn10, rcGn15, and rcGn20 than at 0h, and it was also higher than the concentration measured in the control group. Twenty-four hours post-injection, the T level returned to the initial level in the rcGn10 and rcGn20 groups while only the rcGn15 group showed a higher level than the control group (Fig. 2; $P < 0.05$). The control group did not show any significant difference among the different sampling times (Fig. 2; $P > 0.05$).

No difference in DHP levels was found among treatments at the injection time (Fig. 3; $P > 0.05$). Twenty-four hours post-injection, the level of DHP was increased in the rcGn10, rcGn15, and rcGn20 treatments, before decreasing 24 h post-injection (Fig. 3; $P < 0.05$). As for E2 and T, the DØ level remained steady along the control group's experimental time-course.

Negative correlation between plasma E2 and DHP was showed by a Pearson linear correlation test (Fig. 4; Pearson correlation = – 0.382; $P = 0.010$). There was a negative correlation between E2 and T (Fig. 5; Pearson correlation = – 0.263; $P = 0.026$). Finally, a significant positive correlation was also obtained between T and DHP levels (Fig. 6; Pearson correlation = 0.797; $P = 0.000$).

4. Discussion

In the artificial reproduction of fish, gametes' quality and quantity are essential factors that affect reproductive success. In some fish species, artificial reproduction is difficult to perform without using stimulation with relevant hormones (Brzuska 2001; Yaron et al. 2009). Besides, the stress resulting from the repeated manipulations of the injected breeders may affect the reproduction quality (Milla et al. 2009; Pourhosein Sarameh et al. 2012; Falahatkar and Poursaied 2013). Thus, it is essential to apply the hormonal stimulation using useful substances that minimize the stress with a few hormones and a limited number of injections.

The present results showed that goldfish broodstocks injected with recombinant cGnRH showed a more efficient spawning rate than non-injected females. Concurrent with the present study, the injection with GnRHa resulted in improved spawning rate in tench (Tinca Tinca) broodstock (at 25 µg/kg mGnRHa) (Podhorec et al. 2016). In goldfish broodstock, the injection of GnRHa at 10 µg/kg resulted in enhanced final maturation (Zadmajid et al. 2009). The results confirmed that GnRH-type molecules could enhance final oocyte maturation and, thus, spawning rate (Drori et al. 1994; Targonska and Kucharczyk 2011). Synthetic hormones are excessively applied to induce final maturation in fish and have an adequate degree of success (Mahadevi et al. 2018).

But recombinant GnRH has been rarely used so far as it has only proved efficiency in Sterlet sturgeon (Mohammadzadeh et al. 2020a, b). With the present results, we suggest that recombinant GnRH can also promote the maturation of oocytes in the broodstock for sufficient spawning.

A thoroughly efficient and more rapid spawning was observed in the present study by injecting maturing goldfish females with 20 µg kĝ1 of rcGnRH (rcGn20) while some females failed to spawn with the doses of 10 µg/kg and 15 µg/kg. More recently, Mohammadzadeh et al. (2020a, b) investigated the possible induction of spawning in Sterlet sturgeon (Acipenser ruthenus) with recombinant GnRH and concluded that the injection of rGnRH at 10 µg/kg was the most effective dose to augment the maturation of broodstocks. Thus, the current study further supports that rcGnRH is recommended at a specific amount to induce an accelerated spawning fully effective. In this way, Drori et al. (1994) reported that the hormonal injection of GnRHa at low concentrations did not show high spawning effectiveness in common carp (Cyprinus carpio). Hence, the optimum level of GnRH-type molecules, including the recombinant GnRH, is one of the main factors that guarantee the efficacy of the hormonal injection in terms of efficiency and action speed.

The results showed that the level of E2 was markedly decreased after 12 h and/or 24 h post-injection with rcGnRH at 10, 15, and 20 µg/kg. Similarly, the concentration of E2 was decreased in the Sterlet sturgeon females injected with rGnRH (Mohammadza-deh et al. 2020a, b). Plasma E2 is generally increased during the vitellogenesis period, and then, it

decreases before the final stages (Mojazi Amiri et al. 1996; Barannikova 1999). The E2 reduction in the rGnRH treated groups is accompanied by completed oocyte maturation in goldfish broodstocks. In this regard, the estrogens are known to inhibit the meiotic maturation through the G-protein-coupled estrogen receptor (GPER) (Majumder et al. 2015). Altogether, data support that the action of rcGnRH on the ovulatory event is linked to the depression of the estrogen transduction pathway. However, in other species, the injection of GnRH analogs at the early FOM triggers either elevation of E2 with the induction of ovulation or no apparent effect on E2 (e.g., Podhorec et al. 2016; Zareski et al. 2019; Shokr 2020). Thus, further investigations are needed to understand if these discrepancies are due to species specificities or the type of GnRH-like molecules (GnRHa or rcGnRH).

In fish, the high levels of T before ovulation would participate in regulating the final stages, leading to the synchronicity of final oocyte maturation and ovulation (Bayunova et al. 2006). These high T levels are also associated with reproductive behavior initiation, allowing broodstock to reach the ovulation stage more quickly (Ceapa et al. 2002). In the present study, T levels showed values in the broodstock groups treated with rcGnRH after 12 h and 24 h postinjection higher than at the initial time or than the control groups. In agreement with the present study, Mohammadzadeh et al. (2020a, b) concluded that the level of T was increased by the hormonal injection of rGnRH at an equivalent dose (10 μg/kg) in Sterlet sturgeon. The high T levels after GnRH injection and the observed decline around ovulation reflect the influence of GnRH on the pituitary gland that releases the LH (Falahatkar et al. 2016; Podhorec et al. 2016) and, in turn, the ovarian T secretion. Our data further support that GnRH-like molecules are involved in the final stages of reproduction in fish.

In goldfish, DHP is the potential maturation-inducing steroid (MIS) (Nagahama et al. 1983). The present results show that DHP concentration had marked increases in broodstock treated with rcGnRH at 12 h post-injection compared to the control. In the same way, Mohammadzadeh et al. (2020a, b), Podhorec et al. (2016), and Aizen et al. (2017) showed that the injection of rcGnRH or GnRHa resulted in increased DHP concentration in sterlet sturgeon, tinca, and common carp, respectively. These data support that the effectiveness of GnRH-like molecules on fish spawning effectiveness passes through hypersecretion of the MIS. The present results also show concomitant enhanced production of DHP levels and reduced E2 levels in goldfish broodstock treated with rcGnRH, which is in line with Mohammadzadeh et al. (2020a, b), who reported similar observation for progesterone and E2 in Persian sturgeon. These results suggest that enhanced progestagens and lowered E2 are associated with the shift in the steroidization pathway. This result may stem from decreased 17–20 lyase activity and increased 20-β-hydroxysteroid dehydrogenase activity (Aizen et al. 2012, 2017). In many teleosts, two genes were identified encoding 17, α-hydroxylase (P450c17); one of which is P450c17-I, and its expression indicates lyase activity producing 19-carbon steroids that may be precursors of estrogen and this variant of P450c17 is expressed in ovarian granulosa cells during vitellogenesis (Zhou et al. 2007). Another variant is P450c17-П, which encodes 17-α, a hydroxylase that lacks lyase activity and is expressed only in oocytes at the maturation stage (Nagahama and Yamashita 2008). Therefore, increased DHP level and decreased E2 level in rGn10, rGn15, and rGn20 treated broodstock may indicate that the females reached the ovulation stage by increased DHP levels, potentially through stimulation of P450c17-П and inhibition of P450c17-I. To link them with goldfish ovulation, further investigations would be needed to analyze the kinetics of these enzymes' activity and E2/DHP blood plasma levels along the process of final oocyte maturation in goldfish.

5. Conclusion

In conclusion, the obtained results illustrated that the hormonal injection with rcGnRH in goldfish broodstock resulted in achieving ovulation in relation to modulation of the significant sex steroids. Recombinant GnRH seems to be a practical strategy applied in the artificial reproduction of fish to increase seed production and allow their availability throughout the year.

Table 1. Reproduction performance in goldfish (Caras-sius auratus) broodstock after injection with 0.9% NaCl with 20 mg/kg metoclopramide (C); 10 µg/kg body weight (BW) rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD

Treatment	No. of injected fish	No. of spawned fish	Spawning success (%)[1]	Latency period (h)[2]
0.9% NaCl	10	0	-	-
rcGn10	10	8	80	22.6 ± 0.5[a]
rcGn15	10	6	60	21.5 ± 0.5[a]
rcGn20	10	10	100	17.5 ± 0.5[b]

Different letters designate significant differences as determined by Tukey's post hoc tests
1 Spawning success (%): the number of females that ovulated after injection divided by the total number of injected females
2 Latency period (h): time between the hormone injection and ovulation

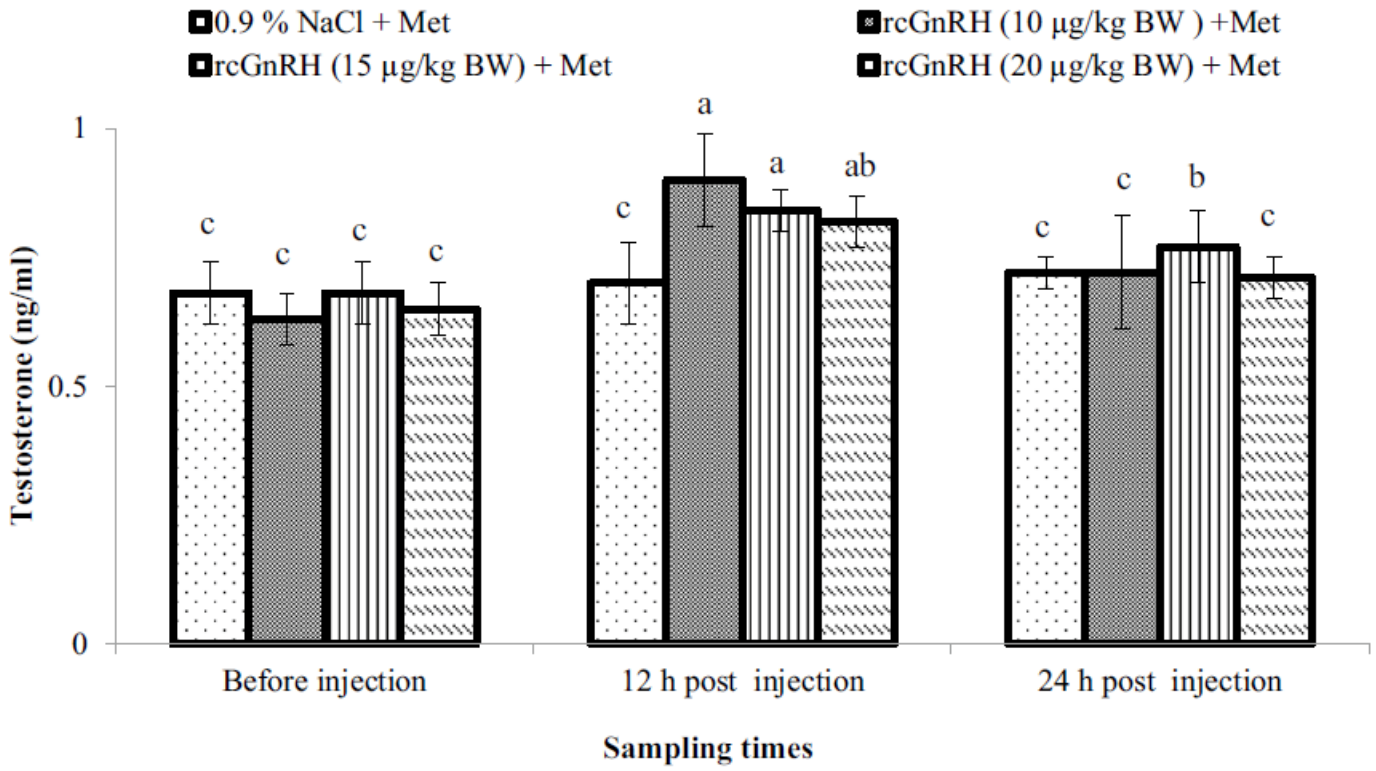

Figure 1.
Plasma 17ß-estradiol levels in goldfish (Carassius auratus) broodstock after injection with 0.9% NaCl with 20 mg/kg metoclopramide (C); 10 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD; n = 6 for each tank. Different letters designate significant differences as determined by Tukey's post hoc tests.

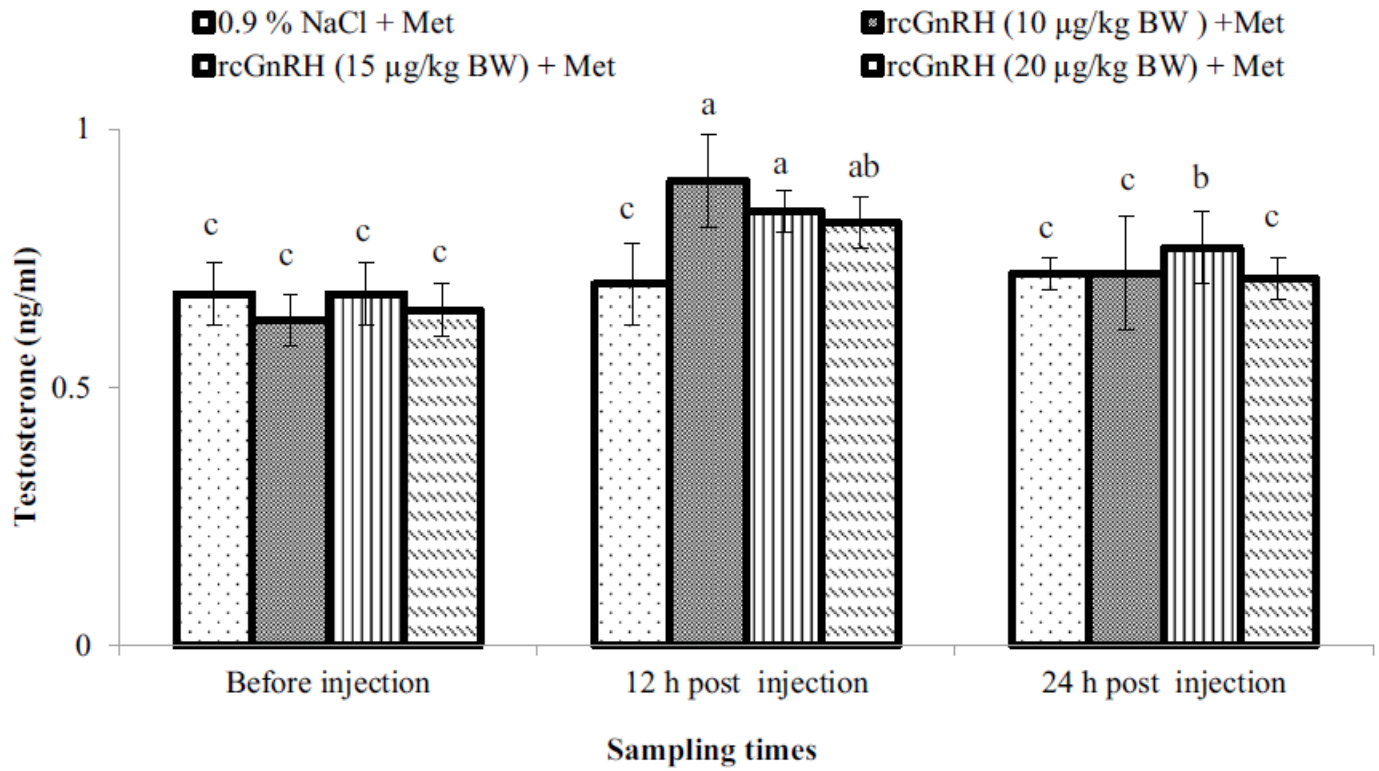

Figure 2.
Plasma testosterone levels in goldfish (Carassius auratus) broodstock after injection with 0.9% NaCl with 20 mg/kg metoclopramide (C); 10 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD; n = 6 for each tank. Different letters designate significant differences as determined by Tukey's post hoc tests

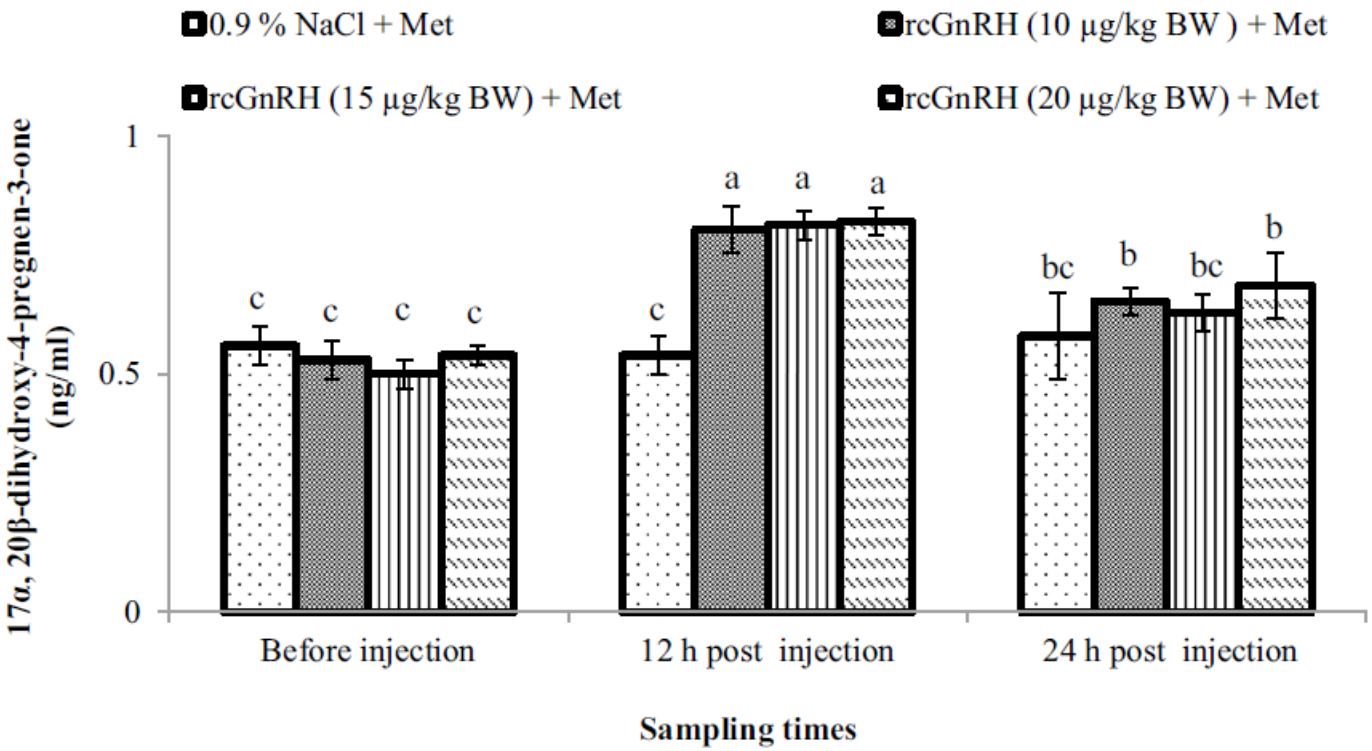

Figure 3.
Plasma 17α, 20β-dihydroxy-4-pregnen-3-one levels in goldfish (Carassius auratus) broodstock after injection with 0.9% NaCl with 20 mg/kg metoclopramide (C); 10 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/ kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD; n = 6 for each tank. Different letters designate significant differences as determined by Tukey's post hoc tests

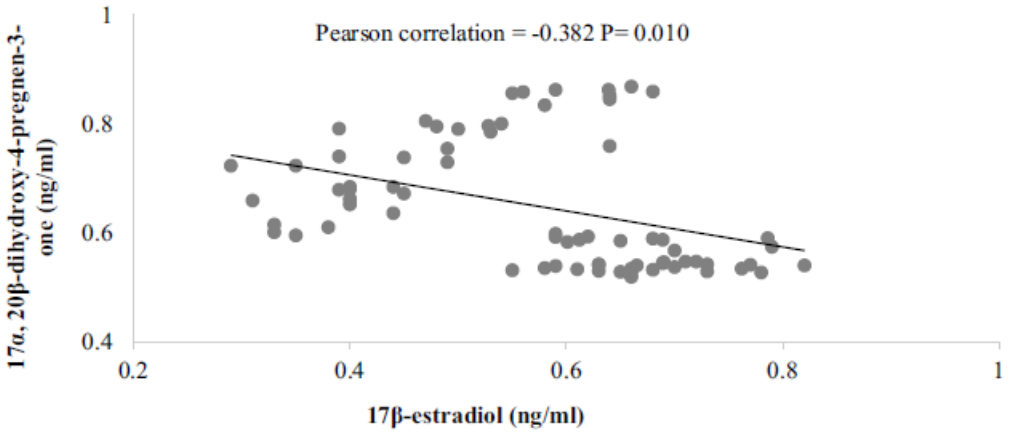

Figure 4.

Negative correlation between plasma concentrations of 17α, 20β-dihydroxy-4-pregnen-3-one, and 17β-estradiol in goldfish (Carassius auratus) broodstock after injection with 0.9% NaCl with 20 mg/kg metoclopramide (C); 10 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/ kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD; n = 6 for each tank

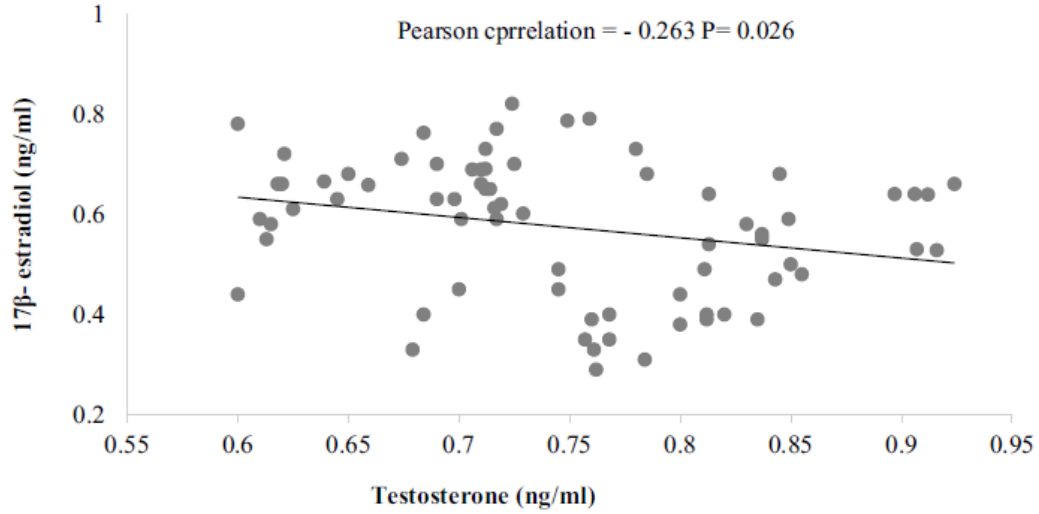

Figure 5.

Negative correlation between plasma concentrations of 17ß-estradiol and testosterone in goldfish (Carassius auratus) broodstock after injection with 0.9% NaCl with 20 mg/ kg metoclopramide (C); 10 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/kg BW rcGnRH with 20 mg/ kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD; n = 6 for each tank

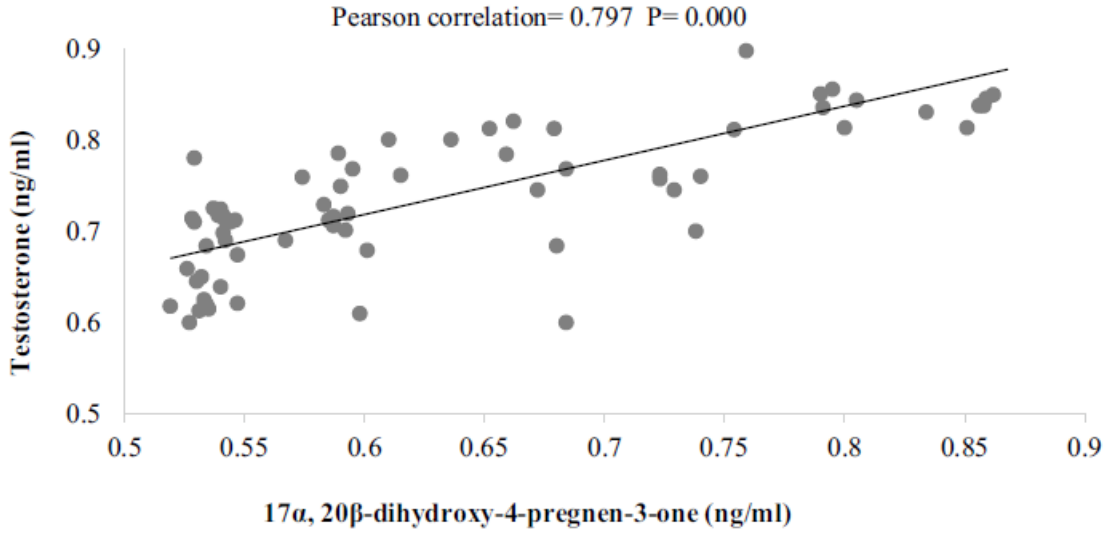

Figure 6.

Positive correlation between plasma concentrations of testosterone and 17α, 20ß-dihydroxy-4-pregnen-3-one in goldfish (Carassius auratus) broodstock after injection with 0.9% NaCl with 20 mg/kg metoclopramide (C); 10 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn10); 15 µg/ kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn15); and 20 µg/kg BW rcGnRH with 20 mg/kg metoclopramide (rcGn20). Mean ± SD; n = 6 for each tank

6. References

Aizen J, Hollander-Cohen L, Shpilman M, Levavi-Sivan B (2017) Biologically active recombinant carp LH as a spawning-inducing agent for carp. J Endocrinol 232:391– 402. https://doi.org/10.1530/JOE-16-0435

Aizen J, Kobayashi M, Selicharova I, Sohn YC, Yoshizaki G, Levavi-Sivan B (2012) Steroidogenic response of carp ovaries to piscine FSH and LH depends on the reproductive phase. Gen Comp Endocronol 178: 28-36 https://doi.org/10.1016/j.ygcen.2012.04.002

Andersen O, Klungland H (1993) The salmon GnRH encoding gene in teleost fish. Int Rev Cytol 147:165–191. https:// doi.org/10.1016/S0074-7696(08)60768-8

Barannikova IA (1999) Sex steroids in the serum of Caspian sturgeons and their specific cytosol binding in brain and gonads during the migratory cycle. J Appl Ichthyol 15:193–195 .https://doi.org/10.1111/j.1439-0426.1999.tb00232.x

Bayunova L, Canario AV, Semenkova T, Dyubin V, Sverdlova O, Trenkler I, Barannikova I (2006) Sex steroids and cortisol levels in the blood of stellate sturgeon (Acipenser stellatus Pallas) during final maturation induced by LH- RH-analogue. J Appl Ichthyol 22:334–339

Brzuska E (2001) Artificial spawning of herbivorous fish: use of an LHRH-a to induce ovulation in grass carp Ctenopharyngodon idella (Valenciennes) and silver carp Hypophthalmichthys molitrix (Valenciennes). Aquac Res 30:849–856. https://doi.org/10.1046/j.1365-2109.1999.00413.x

Ceapa C, Williot P, MennF Le, Cuisset D (2002) Plasma sex steroids and vitellogenin levels in stellate sturgeon (Aci-penser stellatus Pallas) during spawning migration in the Danube River. J Appl Ichthyol 18:391–396. https://doi.org/10.1046/j1439-0426.2002.00370.x

Chekan JR, Koos JD, Zong C, Maksimov MO, Link AJ, Nair SK (2016) Structure of the lasso peptide Iso peptidase identifies a topology for processing threaded substrates. J Am Chem Soc 138:16452–16458. https://doi.org/10.1021/jacs.6b10389

Drori S, Ofir M, Levavi-Sivan B, Yaron Z (1994) Spawning induction in common carp, Cyprinuscarpio, using pituitary extract or GnRHsuperactive anagoge combined with metoclopramide: analysis of profile, progress of oocyte maturation and dependence on temperature. Aquaculture 119:393–407. https://doi.org/10.1016/0044-8486(94)90303-4

Falahatkar B, Barzafshan H, Asadi M (2016) Effects of LHRH-A2 on sex steroids levels, stress indices, and some plasma biochemical parameters in female Sterlet sturgeon, Aci-penser ruthenus, broodstock. Iran J Fish Sci 3:121–136 ((In Persian))

Falahatkar B, Poursaied S (2013) Effects of hormonal manipulation on stress responses in male and female broodstocks of pikeperch Sander lucioperca. Aquacultint 22:235–244. https://doi.org/10.1007/s10499-013-9678-x

Gothilf Y, Zohar Y (1991) Clearance of different forms of GnRH from the circulation of the gilthead seabream, Sparus aurata, in relation to their degradation and bioactivities. In: Scott, A.P., Sumpter, J.P., Kime, D.E. and Rolfe, M.S. (eds.), Reproductive Physiology of Fish. Fish Symposium 91, Sheffield, pp. 35–37

Kontermann RE (2005) Recombinant bispecific antibodies for cancer therapy. Acta Pharmacol Sin 26:1–9. https://doi.org/10.1111/j.1745-7254.2005.00008.x

Leatherland JF, Li M, Barkataki S (2010) Stressors, glucocorticoids and ovarian function in teleosts. J Fish Biol 76:86– 111. https://doi.org/10.1111/j.1095-8649.2009.02514.x

Mahadevi SF, Cheryl A, Bhosale MM, Gopalakannan A, Gna-navel K (2018) Induced breeding of telescopic gold fish (Carassius auratus auratus) using synthetic hormone (WOVA-FH). J Entomol Zool Stu 6:1368–1373

Majumder S, Das S, Moulik SR, Mallick B, PalP MD (2015) G-protein coupled estrogen receptor (GPER) inhibits final oocyte maturation in common carp, Cyprinus carpio. Gen Comp Endocrinol 211:28–38. https://doi.org/10.1016/j.ygcen. 2014.11.011

Milla S, Wang N, Kestemont MSNM, P, (2009) Corticosteroids: friends or foes of teleost fish reproduction? Comp Biochem Physiol 153A:242251. https://doi.org/10.1016/j.cbpa. 2009.02.027

Mohammadzadeh S, Moradian F, Yeganeh S, Falahatkar B, Milla S (2020) Design, production and purification of a novel recombinant gonadotropin-releasing hormone associated peptide as a spawning inducing agent for fish. Protein Expr purif 166 (In press). https://doi.org/10.1016/j.pep. 2019.105510

Mohammadzadeh S, Yeganeh S, Moradian F, Milla S, Falahatkar B (2020) Spawning induction in Sterlet sturgeon (Acipenser ruthenus) with recombinant GnRH: analysis of hormone profiles and spawning indices. Aquaculture 533 (in press). https:// doi.org/10.1016/j.aquaculture.2020.736108

MojaziAmiri B, Maebayashi M, Hara A, Adachi S, Yamauchi K (1996) Ovarian development and serum sex steroid and vitellogenin profiles in the female cultured sturgeon hybrid, the bester. J Fish Biol 48:1164–1178. https://doi.org/10.1111/j.1095-8649.1996.tb01812.x

Munakata A, Kobayashi M (2010) Endocrine control of sex behavior in teleost fish. Gen Com Endocrinol 165:456– 468. https://doi.org/10.1016/j.ygcen.2009.04.011

Mylonas C, Zohar Y (2001) Use of GnRHa-delivery systems for the control of reproduction in fish. Rev Fish Biol Fisher 10:463–491

Nagahama Y, Yamashita M (2008) Regulation of oocyte maturation in fish. Development, Growth & Differentiation 50:S195–S219

Nagahama Y, Hirose K, Suzuki K, Tamaokis B-I (1983) Relative in Vitro Effectiveness of 17a,20/3-Dihydroxy-4-pregnen-3-one and Other Pregnene Derivatives on Germinal Vesicle Breakdown in Oocytes of Ayu (Plecoglos-sus altivelis), Amago Salmon (Oncorhynchus rhodurus), Rainbow Trout (Salmo gairdneri), and Goldfish (Carassius auratus). Gen Comp Endocrinol 51:15–23 https:// doi.org/10.1016/0016-6480(83)90092-8

Okubo K, Nagahama Y (2008) Structural and functional evolution of gonadotropinreleasing hormone in vertebrates. Acta Physiolo 193:3–15. https://doi.org/10.1111/j.1748-1716.2008.01832.x

Omori Y, Kon T (2019) Goldfish: an old and new model system to study vertebrate development, evolution and human disease. J Biochem 165:209–18. https://doi.org/10.1093/jb/mvy076

Ota KG, Abe G (2016) Goldfish morphology as a model for evolutionary developmental biology. Wiley Interdiscip Rev Dev Biol 5:272–95. https://doi.org/10.1002/wdev.224

Podhorec P, Socha M, Amma BL, Sokolowska M, Brzuska E, Milla S, Gosiewski G, Stejskal V, Simko M, Kouril J (2016) The effects of GnRHa with and without dopamine antagonist on reproductive hormone levels and ovum viability in tench Tinca tinca. Aquaculture 465:158–163. https://doi.org/10.1016/j.aquaculture.2016.09.012

PourhoseinSarameh S, Falahatkar B, AzariTakami G, Efat-panah I (2012) Effects of different photoperiods and handling stress on spawning and reproductive performance of pikeperch Sander lucioperca. Anim Reprod Sci 132:213– 222. https://doi.org/10.1016/j.anireprosci.2012.05.011

Shokr EAM (2020) Effect of gonadotropin releasing hormone injection on physiological changes and reproductive hormones in Clarias gariepinus. Egypt J Aquat Biol Fish 24:119–129. https://doi.org/10.21608/ejabf.2020.69364

Szlachcic A, Zakrzewska M, Otlewski J (2011) Longer action means better drug: tuning up protein therapeutics. Bio-technol Adv 29:436–441. https://doi.org/10.1016/j.biotechadv.2011.03.005

Targonska K, Kucharczyk K (2011) The application of hCG, CPH and Ovopel in successful artificial reproduction of goldfish (Carassius auratus auratus) under controlled conditions. Reprod Domestic Anim 46:651–655. https:// doi.org/10.1111/j.1439-0531.2010.01723.x

Wen M, Feron R, Pan Q, Guguin J, Jouanno E, Herpin A, Klopp C, Cbau C, Zahm M, Parrinello H, Journot L, Burgess SM, Omori Y, Postlethwait JH, Schartl M, Guiguen Y (2020) Sex chromosome and sex locus characterization in gold fish, Carassius auratus (Linnaeus, 1758). BMC Genomics 21:552 https://doi.org/10.1186/s12864-020-06959-3

Xu J, Zhu Z, Duan P, Li W, Zhang Y, Wu J, Hu Z, Liu RSR, J, (2006) Cloning, expression, and purification of a highly immunogenic recombinant gonadotropin-releasing hormone (GnRH) chimeric peptide. Protein Expr Purify 50:163–170. https://doi.org/10.1016/j.pep.2006.08.016

Yaron Z, Bogomolnaya A, Drori S, Biton I, Aizen J, Levavi-Sivan KZ, B, (2009) Spawning induction in the carp: past experience and future prospects - review. Israeli J Aquac-ult 61:5–26

Zadmajid V, Imanpour MR, Soudagar M, Shabani A (2009) The effect of GnRHa, HCG and pituitary extract on biochemical parameters of seminal plasma in godfish (Carassius auratus). Iranian J Anim Res 22:333–342 (In Persian)

Zareski D, Fontaine P, Alix M, Blecha M, Broquard C, Milla KJ, S, (2019) Time of response to hormonal treatment but not the type of a spawning agent affects the reproductive effectiveness in domesticated pikeperch, Sander lucioperca. Aquaculture 503:527–536. https://doi.org/10.1016/j.aquaculture

Zhou LY, Wang DS, Kobayashi T, Yano A, Paul-Prasanth B, Suzuki A, Nagahama SF, Y, (2007) A novel type of P450c17 lacking the lyase activity is responsible for C21-steroid biosynthesis in the fish ovary and head kidney. Endocrinol 148:4282–4291. https://doi.org/10.1210/en.2007-0487

Zohar Y, Mylonas CC (2001) Endocrine manipulation of spawning in cultured fish: from hormones to genes. Aquaculture 197:99–136. https://doi.org/10.1016/S0044-8486(01)005841

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