

Morphology, sex steroid level and gene expression analysis in gonadal sex reversal of triploid female (XXX) rainbow trout (*Oncorhynchus mykiss*)

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Abstract In non-mammalian vertebrates, estrogens and expressions of *cyp19a1* and *foxl2* play critical roles in maintaining ovary differentiation and development, while *dmrt1* and *sox9* are male-specific genes in testicular differentiation and are highly conserved. In order to deeply understand the morphological change, sex steroids level and molecular mechanism of triploid female gonadal reversal in rainbow trout, we studied the ovary morphology, tendency of estradiol-17 β (E2) and testosterone (T) levels and the relative expressions of *dmrt1*, *cyp19a1*, *sox9* and *foxl2* in juvenile and adult fish. Our results demonstrated that the development of triploid female gonads in rainbow trout went through arrested development,

oocytes dedifferentiation, ovary reconstruction and sex reversal finally. During early gonadal development (154–334 days post-fertilization), the expressions of *foxl2* and *cyp19a1* increased linearly, while expressions of *dmrt1* and *sox9* were extremely suppressed, and E2 level was higher, while T level was lower. During the mid-to-late period of triploid female gonadal development (574–964 days post-fertilization), the expressions of *dmrt1* and *sox9* remained high and were very close to the quantity of diploid male genes, and T levels were even reaching diploid male plasma concentrations, while expressions of *cyp19a1* and *foxl2* were decreased, leading to decrease in E2 level. We realized that the development model of rainbow trout triploid female gonads was extremely rare, and the regulatory mechanism was very special. Genes involved in gonadal development and endogenous estrogens are pivotal factors in fish natural sex reversal.

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Introduction

Most of triploid fish are sterile, so they can afford all energy to somatic growth, to improve feed utilization, in the juvenile and mature period; triploid fish generally grow faster than diploid. In salmonids, mature individuals often have a higher mortality rate

after breeding, known as the “mature death”; however, triploid fish infertility can avoid biological death, decrease production cost, form a large individual, and improve the economic benefits and experimental requirements. However, the mechanism about triploid rainbow trout (RBT) infertility has not been understood clearly.

According to the studies about all salmonid species so far, sex determination in the early period is strictly genetic, with the chromosomal XX/XY system (Nagahama et al. 2004). However, the genetic mechanism is easily altered by additional factors. For example, sex steroid could act as a pivotal role in these gonadal developmental pathways and cellular differentiations. Many research studies have been carried out on this issue. Maintenance of RBT normal ovarian differentiation is supported by sustained high estradiol-17 β (E2) level, and blockage of estrogen receptivity is bound to be the masculinization (Guiguen et al. 1999; Yao 2005). A recent work about male RBT chronically exposed to ethynyl estradiol (EE2) has been carried out (Depiereux et al. 2014), and in the morphological and physiological aspects, male RBT develop intersex gonads under exposure to high level of EE2. The male-to-female gonadal morphologies have a detailed analysis on different periods. The development of triploid female RBT ovarian differentiation has been explored. Triploid female RBT are sterile as they cannot produce primary oocytes (Thorgaard and Gall 1979; Thorgaard et al. 1992), and their ovaries are sting like, while diploid ovary is characterized by developing oocytes in the perinucleolar stage (Krisfalusi et al. 2000). After 17 α -methyl-dihydrotestosterone (MDHT) treatment, gonads of triploid female RBT are very similar to diploid male testis. Similarly, triploid male RBT gonads tend to be feminized and look identical to the ovary of diploid female under E2 treatment (Krisfalusi and Cloud 1999). In this paper, we found out gonadal sex reversal in triploid female (XXX) RBT under the natural condition. Without exogenous sex steroid treatment, triploid female RBT gonads had changed into the phenotypic male. Morphology of reversal gonads will be illustrated in detail.

More remarkably, the sex steroid effect on RBT gonadal development is determined mainly by genetic interaction, and genes in gonadal developmental system play important roles. The expressions of these genes between males and females are different to a large extent. These vital genes are always divided into

four categories: steroid enzymes, sex steroid hormone receptors (SSHR), transcription factors and growth factors (Baron et al. 2005c; Kobayashi et al. 2013). *Cyp19a1* is a main steroid enzyme, the terminal enzyme in the steroidogenic pathway, maintaining the balance between estrogen and testosterone (Lin et al. 2011). The rats lacking *cyp19a1* show that females failed to synthesize estrogens and develop testicular features (Huddleston et al. 2006). In fish, *cyp19a1* is also an important marker of ovarian differentiation (Patil and Gunasekera 2008). *Cyp19a1a* is an isoform of *cyp19a1* and is generally considered to be important in the early differentiated and adult gonads (mainly the ovary) (Cheshenko et al. 2008). *Foxl2* acts as a necessary transcription factor in maintaining female differentiation, and its expression in female RBT is reduced by testosterone treatment (Pailhoux et al. 2001). According to studies, *foxl2* and estrogens construct a positive feedback loop, and *foxl2* can regulate *cyp19a1* expression positively (Wang et al. 2007). *Dmrt1* is essential in male determination among different animals. In human, *dmrt1* haploinsufficiency leads to male-to-female sex reversal (Cheshenko et al. 2008). In KO mice lacking *dmrt1*, the testis differentiation has been severely destroyed (Fahrioglu et al. 2007). *Dmy* is a specialized form of *dmrt1* in medaka (*Oryzias latipes*), and it has been identified as the master sex-determining gene in medaka (Matsuda et al. 2007). Previous work has shown that *dmrt1* has an association with *cyp19a1*, and *dmrt1* high expression leads to reduction in *cyp19a1* (Atala 2012). *Sox9* is an important “male gene,” it is regulated by *sry* gene, and the mutation of *sox9* leads to sex reversal of male mammals. Up to now, there is still no normal research about these gonadal developmental genes in triploid female-to-male RBT. Based on these studies, we hypothesized sex reversal in triploid female RBT is due to the interaction between these genes. Therefore, we detected the relative expressions of *dmrt1*, *cyp19a1a*, *foxl2* and *sox9* (they all have been identified in rainbow trout) (Marchand et al. 2000; Baron et al. 2004; Alfaqih et al. 2009; Guiguen et al. 2010) and measured sex steroid levels in triploid female RBT and diploid RBT.

This paper focuses the development of triploid female RBT gonads tended towards testicular differentiation in normal condition. We described the morphological reversal in detail. Speculating the reversal was due to the interaction between gonadal

developmental genes, so we detected these gene expressions (*dmrt1*, *cyp19a1a*, *foxl2* and *sox9*). In addition, the sex steroid levels verified the sex reversal in triploid female RBT.

Materials and methods

Ethics statement

In this study, all experiments were performed according to the European Communities Council Directive (86/609/EEC). All fishes involved in this research were bred according to the guideline of Animal Husbandry Department of Heilongjiang, PRChina. All efforts were made to minimize suffering.

Fish and sampling

Genetically triploid all-female (XXX) and normal diploid individual (XX/XY) rainbow trout were obtained from experimental fish farms of Heilongjiang Fisheries Research Institute (Harbin, China). The triploid all-female eggs were obtained by the method of Espinosa et al. (2005). The embryos were incubated at 10 °C from fertilization until 24 days post-fertilization (dpf) and transferred to the experimental installations, which were 0.3 m³ tanks with a recirculating water system, at 10 ± 0.1 °C, under constant photoperiod (12L:12D), and 400 fish were divided into three batches after complete yolk resorption at 54 dpf, transferred to tanks at a constant temperature of 12 °C and fed daily with a commercial diet ad libitum. The groups were as follows: normal diploid all-male rainbow trout (XY), diploid all-female rainbow trout (XX) and genetically all-female (XXX) rainbow trout.

Histological analysis

In total, 54 fishes were analyzed. The samples were divided into six periods: 154, 274, 334, 574, 784 and 964 dpf. As each sample containing one fish with two gonads, six gonad samples for every group at each period were analyzed. The gonad samples were all at the same stage according to days post-hatching. Sampled fishes were anesthetized with MS222. The gonads were removed from Bouin's solution. Fish were fixed in 4 % buffered formalin. Gonadal samples were embedded in paraffin, and 5-µm-thick sections

were prepared and stained by hematoxylin–eosin (HE).

Quantitative real-time PCR

RNA extraction and RT

For quantitative real-time PCR, 54 samples were analyzed. Three samples for every group were analyzed at each period. Each sample contained six gonads from three fishes was extracted RNA. That is, nine fishes for every group at each period were analyzed. For this part, 162 fishes were sampled in total. Gonadal samples were stored in tank of liquid nitrogen. Total RNAs were extracted from gonads by Trizol reagent (Invitrogen). Only samples with a RIN (RNA integrity number) between 8 and 10 were kept for further analysis. Reverse transcription was used to generate cDNAs using PrimeScript RT Reagent Kit (TakaRa).

Quantitative real-time PCR

Real-time PCR was performed using SYBR Premix Ex Taq (TakaRa), and the 7500 Real-Time PCR System (Applied Biosystems) was handled with the following parameters: 95 °C for 10 min, followed by 40 two-step cycles: at 95 °C for 15 s and at 60 °C for 34 s. *β-actin* and *EF1α* were used as two reference genes. The primers designed for rainbow trout *dmrt1*, *cyp19a1a*, *foxl2* and *sox9* by Primer Premier Software 5.0 (PREMIER Biosoft International, USA) are given in Table 1. For each cDNA sample, both target and reference genes were always amplified independently in triplicate on the same plate and on the same experimental run. A melting curve analysis showed that all reactions were free of dimers or other non-specific PCR products. Ct values were measured by the Sequence Detection System software (ABI), and the value of target sequence normalized to reference sequence was calculated as $2^{-\Delta\Delta Ct}$.

Sex steroid

For blood samples, 75 fishes were analyzed (274, 334, 574, 784 and 964 dpf). Five blood samples of each group were analyzed at each point. Circulating

Table 1 Nucleotide sequences of real-time PCR primers

Gene	GenBank number	Forward sequence	Reverse sequence
<i>dmrt1</i>	AF209095	GGACACCTCCTACTACAACCTTCT	GTTCCGGCATCTGGTATTGTTGGT
<i>cyp19a1a</i>	BX083177	CTCTCCTCTCATACCTCAGGTT	AGAGGAACTGCTGAGTATGAAT
<i>sox9</i>	AB006448.1	ATGCAGGTGCCAAGGCTCA	CTCTGGCTGGGGCTCATATA
<i>foxl2</i>	CA341688	TGTGCTGGATTTGTTTTTGT	GTGTCGTGGACCATCAGGGCCA
<i>ef1a</i>	AF498320	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG
<i>β-actin</i>	AJ438158	ATCCTGACGGAGCGGTTACAG	TGCCATCTCCTGCTCAAAGTCCA

hormones were determined in blood samples. All plasma samples were radioimmunoassayed, for determining estradiol-17 β (E2) level according to Terqui and Thimonier (1974) and for determining testosterone (T) level according to Fostier and Jalabert (1986). Steroid levels determined by RIA were validated by checking that the curves for serial dilutions were parallel to the standard curves. Standards were determined in duplicate. The anti-E2 and anti-T were supplied by Sigma.

Statistical analysis

One-way ANOVA was used to assess differences between periods. Data are shown as mean \pm SD. Statistical analysis was performed using SPSS 13.0 for Microsoft Windows. $P < 0.05$ was considered as statistically significant.

Results

Gonadal morphology and structure of triploid female (XXX) rainbow trout

In triploid all-female rainbow trout, gonads in more than 90 % samples had appeared to have reversal after 784 dpf. The ovary development of triploid female RBT experienced stages as follows: oogonia, primary growth oocytes, dedifferentiated germ cells and spermatogenic-like cells within oogonial nests (Fig. 1a, c, e and d). In control group, ovary of diploid female samples developed integrality, containing normal perinucleolar oocytes with well-developed ovarian lamellae. Compared with diploid ovary, gonads of triploid female samples degenerated obviously and

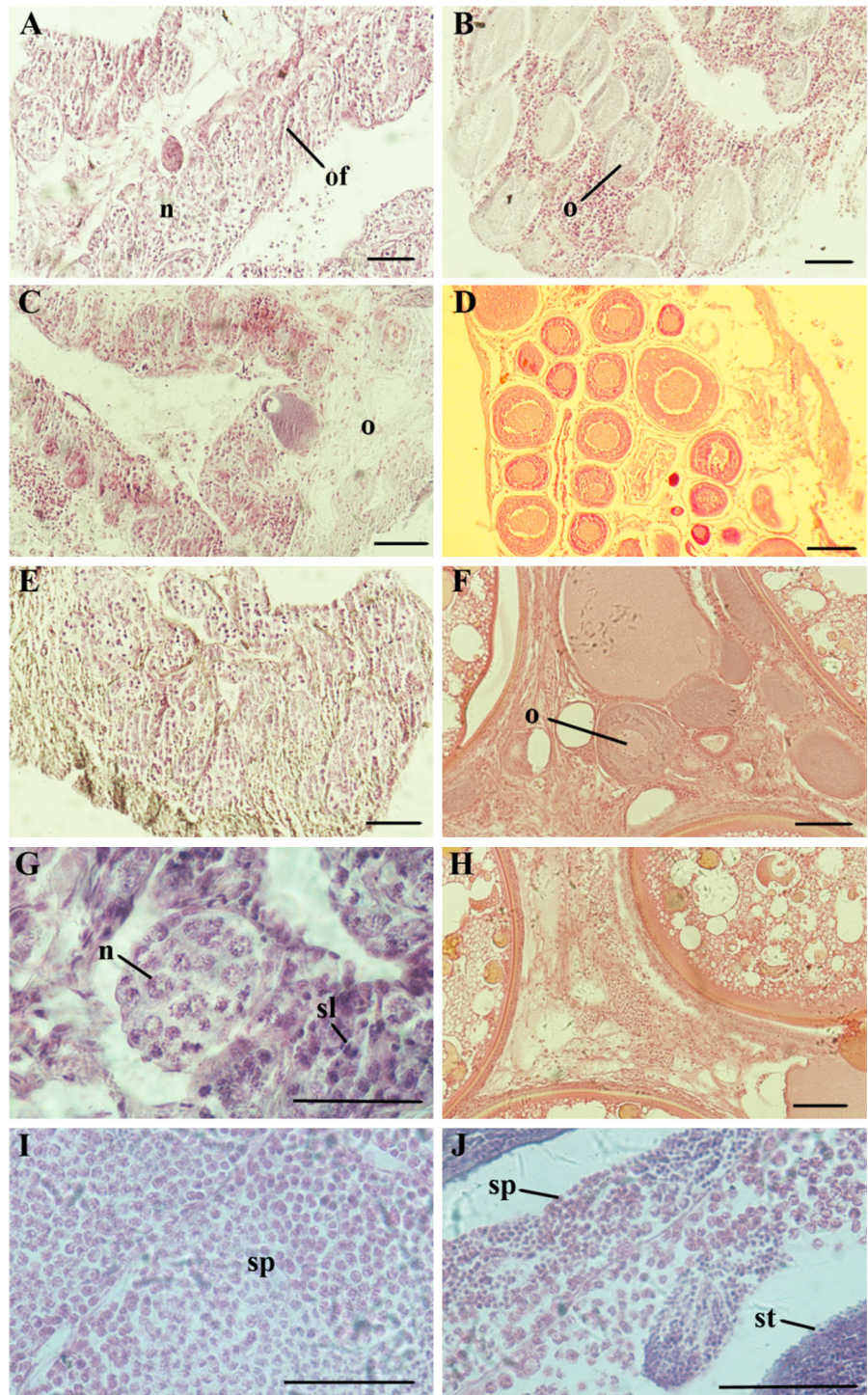
were full of undeveloped germ cells surrounded by substantial stromal tissue (Fig. 1e). At 784 dpf, a large number of germ cells in triploid female gonads tended towards dedifferentiation. And more notably, there was an accumulative, dark staining cell mass structure in gonads of 964 dpf (Fig. 1g). It was similar to the spermatogenic cysts in diploid testis at 574 dpf (Fig. 1i), occupying 30–50 % of the whole gonadal tissue.

The structure was more like spermatogonia or primary spermatocytes than typical oogonial nests. We named the cell mass as spermatogenic-like cells (Fig. 1g). Nevertheless, the situation was quite different from the morphology and structure of diploid male testis at 964 dpf (Fig. 1j). We considered that triploid female gonads had turned to dedifferentiate after 784 dpf and had the signs of gonadal reversal after 964 dpf.

Gene expression

The gonadal reversal in triploid female (XXX) rainbow trout should have association with gene expressions, so we detected expressions of *dmrt1*, *cyp19a1a*, *sox9* and *foxl2*. These genes are main gonadal developmental factors. *Cyp19a1a* and *foxl2* are female-specific genes in ovary (Baron et al. 2005c, 2008), while *dmrt1* and *sox9* are male-specific genes in sertoli cells (Baron et al. 2007). Compared with diploid female samples, expressions of *cyp19a1a* and *foxl2* in triploid female ovary were weaker, and only during the period of 274–574 dpf, these expressions were relatively high, while the expressions of *dmrt1* and *sox9* in XXX ovary were continuously increasing and much higher than that in diploid female RBT (Fig. 2a, b). The expressions of *dmrt1* and *sox9* in gonads between triploid female and diploid male

Fig. 1 Gonadal morphology. **a** Rainbow trout ovary of triploid female (XXX) sample of 154 dpf. It is the typical ovarian structure with oogonia in ovigerous fold. *Scale bar A = 100 μ m*. **b** Rainbow trout normal diploid female (XX) ovary at 154 dpf, with a large amount of oogonia and oocytes. *Scale bar B = 100 μ m*. **c** Rainbow trout ovary of triploid female sample at 574 dpf, the primary growth oocyte in ovigerous fold is rarely seen and oogonia are dispersive. *Scale bar C = 100 μ m*. **d** Normal diploid female ovary at 574 dpf, including oocytes of different periods. *Scale bar D = 100 μ m*. **e** Rainbow trout XXX ovary at 784 dpf, spermatogenic-like cells start to appear within oogonial nests. *Scale bar E = 100 μ m*. **f** Normal XX ovary at 784 dpf. *Scale bar F = 100 μ m*. **g** Rainbow trout XXX ovary at 964 dpf, a large number of spermatogenic-like cells compose cell mass, the structure is similar with spermatogenic cysts. *Scale bar G = 400 μ m*. **h** Normal XX ovary at 964 dpf. *Scale bar H = 100 μ m*. **i**, **j** Rainbow trout normal diploid male (XY) testis at 334 and 784 dpf, with a large amount of spermatogonia and spermatid. *Scale bar I and J = 400 μ m*. *n* oogonial nests, *o* perinucleolar oocyte, *of* ovigerous fold, *sl* spermatogenic-like cells, *sp* spermatogonia, *st* spermatid



sample were distinguishing. They presented trend of increasing after 334 dpf in triploid female gonads, but in diploid male gonads their expressions were

fluctuating, and the highest expression of *dmrt1* and *sox9* were at 574 dpf (Fig. 2a, c). As for *cyp19a1a* and *foxl2*, their expressions in triploid female gonads were

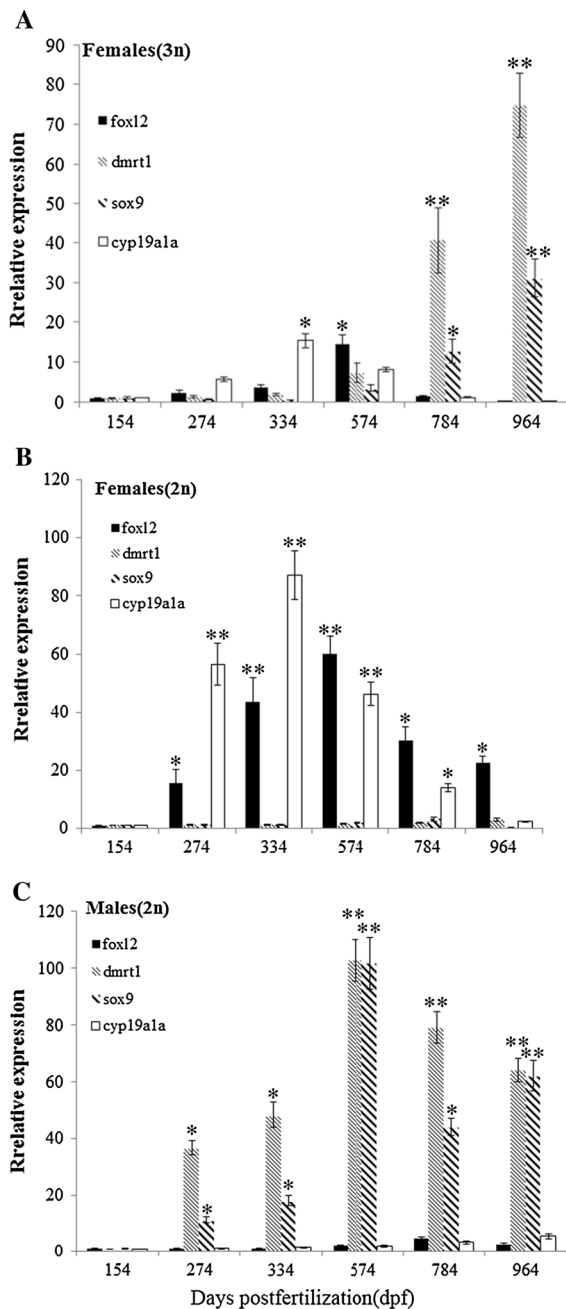


Fig. 2 Gene expression profiles in gonads. **a** The expression of genes in rainbow trout triploid female (XXX) gonads at different days post-fertilization. **b** The expression of genes in rainbow trout diploid female (XX) ovary at different stages. **c** The expression of genes in rainbow trout diploid male (XY) testis at different stages. Error bars are mean + SD from three replicates measured independently. (* $P < 0.05$ vs 154 dpf, ** $P < 0.01$ vs 154 dpf)

overexpressed than that in diploid male testis during the period of 154–574 dpf, but after that, the status was opposite during 574–964 dpf. Therefore, we got the phenomenon that in the early period of triploid female gonadal differentiation (154–574 dpf), *cyp19a1a* and *foxl2* may inhibit the expression of “male genes,” while during the late triploid female gonadal dedifferentiation (574–964 dpf), expressions of *dmrt1* and *sox9* increased continuously, almost to that in diploid male testis. So at this stage, *dmrt1* and *sox9* exerted their inhibitive effect on *cyp19a1a* and *foxl2* expressions. At 964 dpf, the inhibition reached the maximum. The “male genes” expressions almost reached to that of diploid male testis at the same period, and this could explain the phenomenon that a large amount of spermatogenic-like cells began to appear after 784 dpf. It is worthy of attention.

Sex steroid levels

During the gonadal development period (334–964 dpf), estradiol-17 β (E2) levels in diploid female and male plasma were both gradually increasing (Fig. 3a, b), while E2 level in triploid female plasma presented a trend of increase first and then decrease (Fig. 3c), reaching the peak value at 574 dpf. Compared to E2 level in diploid female plasma, the level in triploid female plasma had no significant differences during 334–574 dpf, but it was significantly lower than that in diploid female plasma after this period (Fig. 3b). Compared to diploid male samples, E2 level of triploid female plasma was significantly higher during 334 and 784 dpf, but after that until 964 dpf, E2 level was significantly lower than diploid male plasma (Fig. 3a).

As for testosterone (T) level, we found that in both control group (XY) and triploid female samples, T levels increased linearly with the days post-fertilization (Fig. 3a, c). In the period of 334–964 dpf, T level of triploid female gonads was significantly lower than that in diploid male sample. However, T level was far higher than E2 level after 784 dpf in triploid female plasma. Therefore, the reversed gonads in triploid female samples could be due to the imbalance between “male genes” and “female genes,” leading to continuously increasing level of testosterone and the reduction in E2 level.

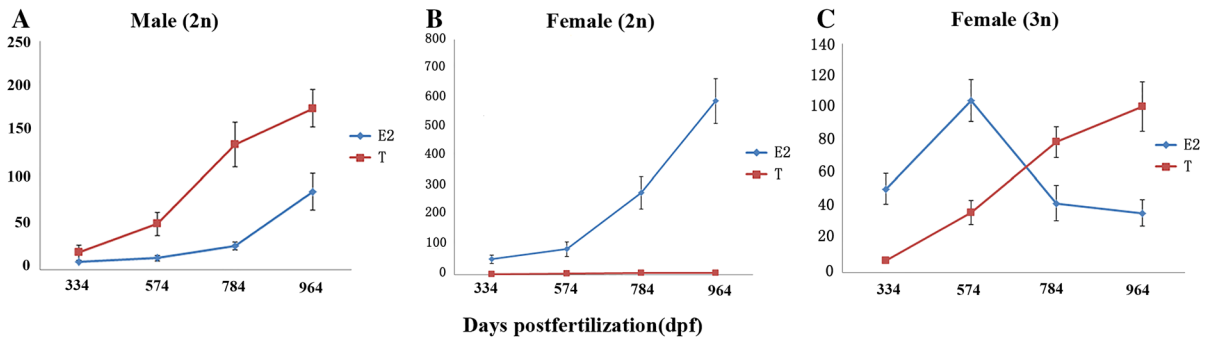


Fig. 3 Sex steroid levels. This graph shows the relationship between sex steroid levels and days post-fertilization (dpf). **a** Estradiol-17 β (E2) and testosterone (T) levels at different days post-fertilization of diploid male (XY) testis. **b** E2 and T levels

at different stages of triploid female (XXX) gonads. **c** E2 and T levels at different stages of diploid female (XX) ovaries. For each point, data represented the mean + SD from 3 replicates. (* $P < 0.05$ vs 334 dpf, ** $P < 0.01$ vs 334 dpf)

Discussion

The important distinction of gonadal differentiation between the mammals and teleosts is that mammalian gonads have decided to ovaries or testis after fertilization, while teleosts still have the tendency of sex reversal after sex determination (Vizziano et al. 2008). In triploid rainbow trout, three sets of chromosomes lead to functional sterility; however, the gonadal developments of different genders are quite different (Thorgaard and Gall 1979; Krisfalusi and Cloud 1999). Under normal circumstances, the morphology of triploid fish testis is similar with diploid males, and sex steroids are at the same level, and germ cells in triploid fish gonads can enter into the meiosis stage and product integral sperm (Krisfalusi and Nagler 2000). On the contrary, when diploid female ovary is full of oocytes, triploid female fish possesses only string-like ovary at the same period, with the arrest of ovary development and a tiny amount of oocytes (Krisfalusi et al. 2000). Carrasco firstly reported that there were spermatogonia in triploid female ovaries of rainbow trout. They found male germ cells in triploid female ovaries at 15 months of age, and the proportion increased with development process. They ascribed the phenomenon to the inhibitive effect of oogonia nests on the interaction between somatic cells and germ cells, so that somatic cells cannot differentiate normally, leading ovary recombination to spermatogenic tissue (Carrasco et al. 1998). In our study, there were a tiny amount of oocytes in triploid female ovaries, and the ovary morphology tended to be string-like. During the period of gonadal development

(154–964 dpf), partial female cells tended towards dedifferentiation. At 964 dpf, structure of triploid female ovary begun to reconstruct, spermatogenic-like cells appeared in more than 90 % gonads of our triploid female samples, and these cells were slightly smaller than oogonia, scattered in oogonia nests, as if they were generated from oogonial redifferentiation. In addition, there were no recognizable follicular granulosa cells in triploid female ovaries at 154 dpf, and the number of oogonia nests in early differentiation period was rather small, so it did not inhibit the interaction between somatic cells and germ cells. Based on the observations, we speculated that the special sex reversal in triploid female gonads was largely due to continuous high expression of certain main male specific genes.

In the researches about rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*) (Ijiri et al. 2008), crucian carp (*Gobiocypris rarus*) (Cao et al. 2012) and Atlantic cod (*Gadus morhua*) (Haugen et al. 2012), the expressions of many genes are prior to the first differentiation of phenotypic gonads on histological analysis, and most genetic expressions have significant sexual dimorphism. There are some female-specific genes *cyp19a1*, *foxl2a*, *foxl2b* and *bmp4* in the early gonadal development. Their expressions will be suppressed when female rainbow trout are treated with androgen (Baron et al. 2005b). *Foxl2a* needs more attention, because it is highly conserved in the mammals (Baron et al. 2005a), the birds (Hudson et al. 2005) and the fish (Nakamoto et al. 2006). Numerous studies have demonstrated estrogen can induce *foxl2* expression intensely in fish,

and *foxl2* regulates the expression of *cyp19a1* positively, and there is a positive feedback loop between these two genes. Our results showed that triploid female plasma was the highest, and *foxl2* and *cyp19a1* were overexpressed at the same time. In addition, our data demonstrated that although triploid female E2 was maintaining relatively high level, it could not induce *foxl2* overexpression consistently after entering into adulthood (574 dpf) with the expression of *cyp19a1* also becoming extremely low, it explained the estrogenic function and *foxl2* expression could not compete the inhibitive effect male-specific genes exerted on *cyp19a1* expression. In line with previous studies, expressions of some female-specific genes (*foxl2a*, *foxl2b*, *cyp19a1*, *fst*, *inha*) are suppressed intensely when treated with androgen. We demonstrated the assumption that *foxl2* expression and estrogenic level can regulate the expression of *cyp19a1* positively, and there is a short feedback loop, and when entered into adulthood, the E2 level decreased, and expressions of *foxl2* and *cyp19a1* were suppressed drastically.

Dmrt1 and *Sox9* are considered as main male-specific genes of teleosts and play vital roles in growth development and sex determination (Koopman and Loffler 2003; Raghuvver and Senthilkumar 2009; Herpin and Schartl 2011). Previous studies have shown *amh*, *sox9a* and *dmrt1* of sertoli cells are induced to increase at early testicular differentiation, while female-specific genes, such as *cyp19a1*, are suppressed obviously. The inhibitory effect on *cyp19a1* may be due to the high expression of *dmrt1* associated with other male-marked genes (*Nr0b1*, *sox9* and *amh*) (Guiguen et al. 2010). In our results, we found that the expressions of *dmrt1* and *sox9* in rainbow trout adult gonads have similar sexual dimorphism, and the testicular (XY) expressions are significantly higher than ovary (XX) expressions. However, the expressions of *dmrt1* and *sox9* in triploid female (XXX) gonads changed periodically, their expressions were extremely low at juvenile period (154–334 dpf), the expressions increased with age increment (574–964 dpf) linearly and the expressions almost reached ones of diploid male adulthood. This phenomenon can ascribe to the inhibition of estrogen and *cyp19a1* at juvenile fish, as well as the decrease in plasma estrogen and the drastic suppression of *cyp19a1* during adulthood. As

for sex steroid level studies, T level of adult triploid female (574–964 dpf) increased gradually, and *dmrt1* and *sox9* were increasing continuously at the same period. The statue is similar with female-to-male sex reversal treated with androgen in diploid female rainbow trout (Guan et al. 2000). Therefore, according to our results, gonadal arrested development and the dedifferentiation of oocytes during triploid female juvenile period lead to the reconstruction of adult gonads, even the female-to-male reversal. The development model is consistent with the sex reversal treated with androgen; that is, androgen inhibits significantly the expression of *cyp19a1* and leads to lacking of estrogen, then the reversal of female-to-male gonads. These results demonstrated sex steroids play vital roles in gonadal differentiation and development of triploid female rainbow trout, and they affected normal genetic expressions and physiological processes, leading to sex reversal.

In summary, triploid female gonads of rainbow trout went through arrested development, oocytes dedifferentiation, ovary construction and sex reversal finally. During the early gonadal development, the expressions of *foxl2* and *cyp19a1* increased linearly, while expressions of *dmrt1* and *sox9* were extremely suppressed, and E2 level was higher, while T level was lower. In the mid-to-late period of triploid female gonadal development, expressions of *dmrt1* and *sox9* overexpressed continuously almost to diploid male genes, and T levels were even reaching diploid male plasma concentrations, while expressions of “female genes” were reduced, leading to decrease in E2 level. These results illustrate a question that the development model of rainbow trout triploid female gonads is extremely rare and the regulatory mechanism is very special. The experimental results indicate that there is a negative feedback loop between oocytes dedifferentiation and female-specific gene expressions in rainbow trout triploid female gonads. Suppression of “female genes” and low E2 level enhance the dedifferentiation of oocytes and generation of spermatogenic-like cells. Therefore, we can realize that high estrogen level acts as a critical role in maintaining ovary normal differentiation and development, and the suppression of female-specific genes by male genes and testosterone can lead to female-to-male sex reversal.

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