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Blockage of progestin physiology disrupts ovarian differentiation in XX Nile tilapia (*Oreochromis niloticus*)

Linyan Zhou [a, 1], Feng Luo [a, 1], Xuelian Fang [a], Tapas Charkraborty [b], Limin Wu [a], Jing Wei [a], Deshou Wang [a, *]

[a] Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), Key Laboratory of Aquatic Science of Chongqing, School of Life Sciences, Southwest University, Chongqing, 400715, PR China
[b] South Ehime Fisheries Research Center, Ehime University, Ainan, 798-4206, Japan

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ABSTRACT

Previous studies indicated that maturation inducing hormone, 17α, 20β-Dihydroxy-4-pregnen-3-one (DHP), probably through nuclear progestin receptor (Pgr), might be involved in spermatogenesis and oogenesis in fish. To further elucidate DHP actions in teleostean ovarian differentiation, we analyzed the expression of *pgr* in the ovary of Nile tilapia (*Oreochromis niloticus*), and performed RU486 (a synthetic Pgr antagonist) treatment in XX fish from 5 days after hatching (dah) to 120dah. Tilapia Pgr was abundantly expressed in the follicular cells surrounding oocytes at 30 and 90dah. Continuous RU486 treatment led to the blockage of oogenesis and masculinization of somatic cells in XX fish. Termination of RU486 treatment and maintenance in normal condition resulted in testicular differentiation, and estrogen compensation in RU486-treated XX fish successfully restored oogenesis. In RU486-treated XX fish, transcript levels of female dominant genes were significantly reduced, while male-biased genes were evidently augmented. Meanwhile, both germ cell mitotic and meiotic markers were substantially reduced. Consistently, estrogen production levels were significantly declined in RU486-treated XX fish. Taken together, our data further proved that DHP, possibly through Pgr, might be essential in the ovarian differentiation and estrogen production in fish.

1. Introduction

In teleosts, 17α, 20β-dihydroxypregn-4-en-3-one (17α, 20β-DP, DHP) and 17, 20β, 21-trihydroxy-4-pregnen-3-one (17, 20β, 21-P), have been identified as two major biologically active progestins to induce oocytes maturation and ovulation in female [1,2], as well as final sperm maturation and spermiation in male [3,4]. Moreover, DHP, mediated through progestin receptors (Pgr), stimulates early stage of spermatogenesis in Japanese eel (*Anguilla japonica*) [5], Atlantic salmon (*Salmon salar*) [6], zebrafish (*Danio rerio*) [7], cod (*Gadus morhua*) [8] and tilapia (*Oreochromis niloticus*) [9]. However, the role of DHP in early stage of ovarian differentiation remains elusive. Previous report showed, by *in vitro* culture of ovarian epithelial, that a low concentration of DHP was sufficient to promote DNA synthesis, formation of Synaptonemal-Complexes (SC) oocytes, and Spo11 expression in Japanese huchen (*Hucho perryi*) and common carp (*Cyprinus carpio*) [10]. No further informations are available regarding the role of DHP in early stage of oogenesis in teleosts. Therefore, additional investigations are required to elucidate the mechanisms of DHP in germ cell meiotic initiation and ovarian differentiation.

Estrogen is regarded as a natural inducer of ovarian differentiation in fish during female sex determination/differentiation [11]. Gonadal aromatase (encoded by the *cyp19a1a* gene), an enzyme catalyzing the conversion of androgens to estrogens, increased its expression specifically in the ovary from 5 days after hatching (dah) [12]. The treatment of XX tilapia fry with fadrozole (an aromatase inhibitor) or tamoxifen (an estrogen receptor antagonist) causes their complete sex reversal to functional males [13,14]. In fadrozole-treated XX tilapia gonads, germ cells meiotic initiation was delayed, and expression profiles of *sycp3* were detected only

* Corresponding author. School of Life Sciences, Southwest University, 400715, Beibei, Chongqing, PR China
E-mail address: wdeshou@swu.edu.cn (D. Wang).
[1] The authors contribute to this work equally.

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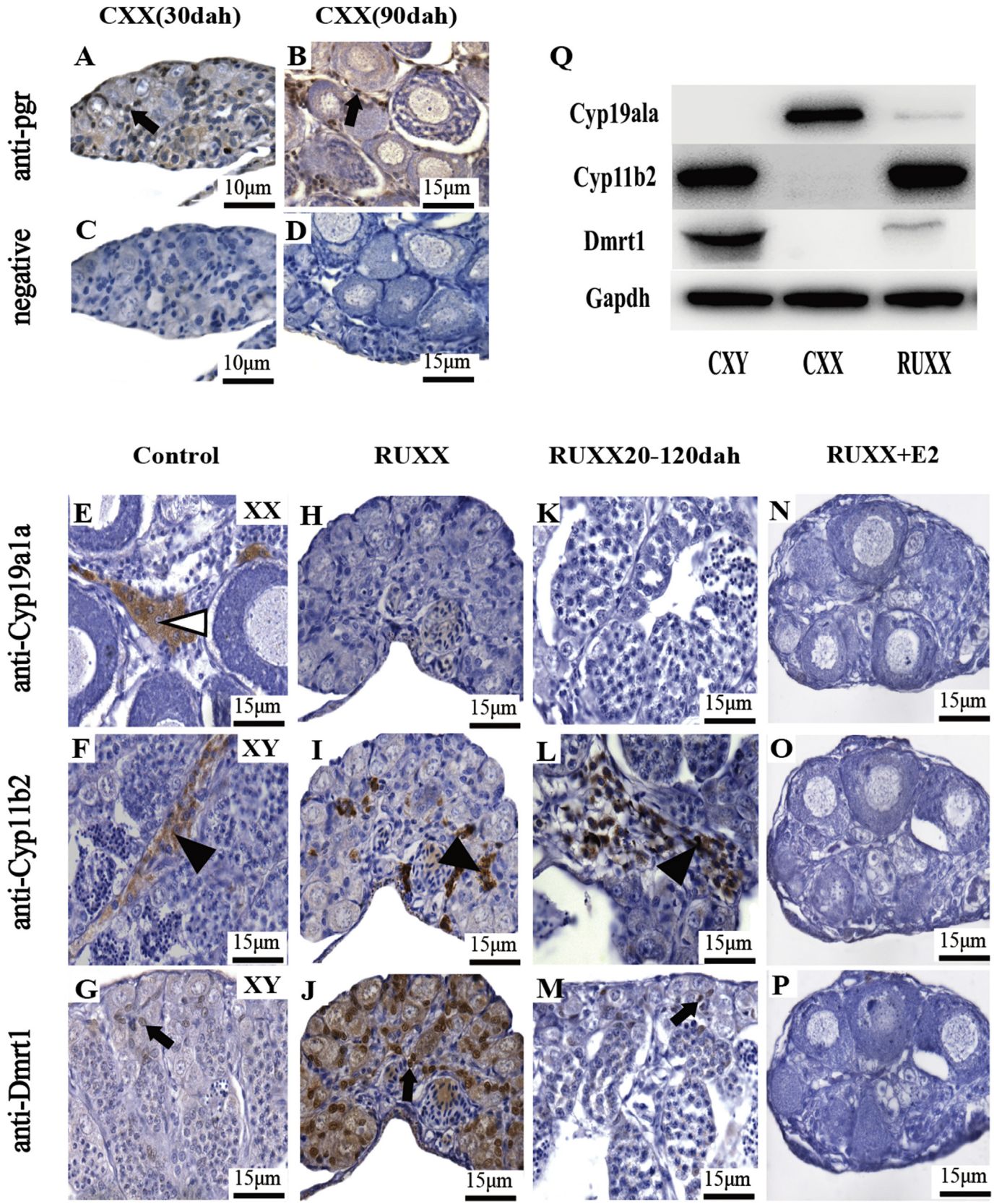

Fig. 1. Pgr expression and effects of RU486/RU486 + E2 treatment on ovarian differentiation. **(A–D)** Immunohistochemistry of Pgr in the ovary of 30 and 90dah. Positive staining was detected in the interstitial cells of the ovarian tissue at 30dah (A). The expression of Pgr was also detected in the follicular cells surrounding oocytes at 90dah (B). No immunostaining was detected in the sections incubated with normal rabbit serum as the first antibody (C, D). **(E–P)** Results of immunohistochemistry of three genes (Cyp19a1a, Cyp11b2, Dmrt1) expression in the gonad of different treatments and control fish at 120dah. Abundant expression of Cyp19ala was detected in interstitial cells of the gonads in XX control fish (E), while no positive signal of Cyp19ala expression was detected in the gonads of RU486-treated XX fish (H, K). Cyp11b2 and Dmrt1, which were strongly expressed in