# Precocious steroidogenesis in the gonads of Oreochromis niloticus, during and after sexual differentiation

Jean-François Baroiller, Alexis Fostier, Bernard Jalabert

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# Precocious steroidogenesis in the gonads of *Oreochromis niloticus*, during and after sexual differentiation

J.F. BAROILLER*, A. FOSTIER and B. JALABERT

*INRA, Laboratoire de Physiologie des Poissons,*
*Campus de Beaulieu, 35042 Rennes Cedex, France*
** CTFT, 45bis Avenue de la Belle Gabrielle, 94130 Nogent-sur-Marne, France*

*In Oreochromis niloticus, histological features showing ovarian differentiation (i.e. meiotic prophase and oocyte auxocytosis) were observed between 28 and 35 days P.F. at 27°C.*

*Steroidogenic potentials of the fry gonads were analyzed in vitro from day 35 P.F. to day 90 P.F. The capacity to metabolize tritiated steroids could be demonstrated as early as 35 days P.F. An initial analysis (TLC) of the incubation medium extract revealed differences in radiochromatogram patterns between ovaries at 35 and 42 days P.F. A preliminary scheme of ovarian steroidogenesis at 90 days P.F. is proposed based on further purified metabolites of P5.*

One of the most commonly used techniques for sex control in Tilapia sp. is steroid treatment of juvenile fish (Hunter and Donaldson, 1983). Very little is known at present about steroidogenesis during gonad differentiation in Tilapia. The modification of sexual differentiation by some exogenous steroids may be due to a pharmacological action (Reinboth, 1970).

In order to improve sex control methodologies in Oreochromis niloticus, morphological and endocrinological studies have been carried out. This parallel approach was taken so as to better understand the timing of gonadal sex differentiation, with respect to rearing temperature, and to collect information on the physiological role of sex steroids in gonadal differentiation.

Some of the results, obtained on ovarian differentiation are presented.

Five days after hatching and 9 days postfertilization (P.F.), broods of Oreochromis niloticus (a mouth brooder species) were taken from

the mouth of incubating females and each brood was reared separately at 27°C with food ad libitum.

Gonadal ontogenesis was followed until day 90 P.F. by histological examination of 4 µm serial sections stained with Regaud's hematoxylin, Orange G and Aniline blue. The paired indifferent gonads could be detected by the ninth day P.F. They appeared to be suspended by a mesogonium on the dorsal peritoneal wall in the coelomic cavity.

During the first 20 days P.F. morphological changes were minimal with the gonads mainly composed of somatic cells, although a few germ cells were scattered in the stroma.

At day 25 P.F., about half of the fish exhibited gonads with numerous germ cells. The first unequivocal signs of ovarian differentiation, observed between days 28 and 35 P.F., were the different stages of meiotic prophase, mainly leptotene and zygotene, and the ovarian cavity (fig. 1). Soon after, diplotene stages occurred in gonads of all females (fig. 2 and table 1).

Fig. 1 : Longitudinal section of day 30 P.F. fry gonad with meiotic prophase stages and ovarian cavity.

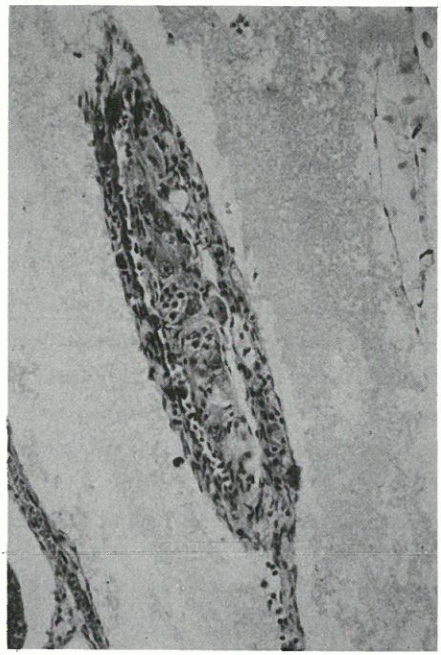


Fig. 2 : Longitudinal section of day 39 P.F. fry gonad with diplotene stages.

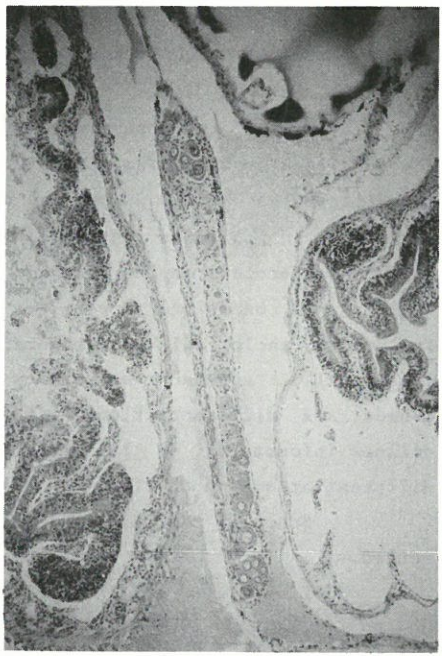


Table 1 : Ovarian differentiation chronology in Oreochromis niloticus reared at 27°C

| Age days postfertilization | Histological events |
|---|---|
| 9 ----- 20 | A few scattered germ cells in somatic tissue |
| 20 ----- 28 | Active germ cell proliferation |
| 28 ----- 35 | Meiotic prophase |
| | ------auxocytosis-------- |
| 35 ----- 90 | previtellogenesis |

We were able to collect ovaries as early as 35 days P.F. for the study of the metabolism of pregenolone (P5) in vitro. Labelled steroids were extracted from the incubation medium and tissues and separated by thin layer chromatography (TLC) on Silica Gel with the following eluant systems : Benzene-Acetone (8:2) and Cyclohexane-Ethylacetate (1:1).

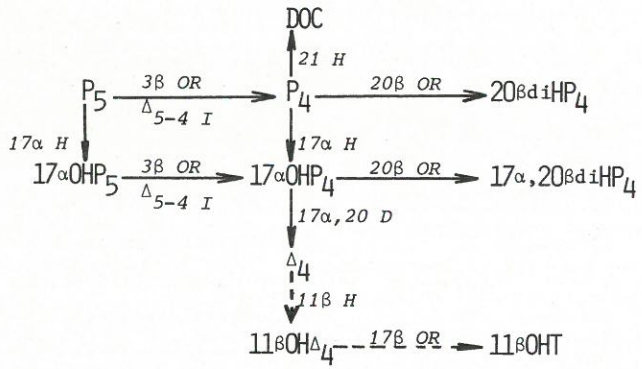
Radiochromatogram records revealed that tritiated P5 was actively metabolized at all ages studied (35, 42, 56, 69 and 90 days P.F.) showing that in Oreochromis niloticus, ovaries possess steroidogenic enzymes, as early as 35 days P.F.

Although absent later on, important modifications in radiochromatogram patterns during ovarian ontogenesis could be observed between 35 and 42 days P.F. , especially in polar and apolar steroids areas. Some of the metabolites from ovarian incubations at 90 days P.F. were identified. Metabolites such as 17α-hydroxypregnenolone (17αOHP5), progesterone (P4), 17α-hydroxyprogesterone (17αOHP4) and androstenedione (Δ4) were identified after purification by high pressure liquid chromatography (HPLC) and recrystallisation to constant specific activity (17αOHP5, P4,17αOHP4) or constant isotope ratio 3H/14C (Δ4). Some radioactivity was still associated with deoxycorticosterone (DOC) and 20β-dihydroprogesterone (20βdiHP4) carriers after 2 to 4 TLC runs in various systems and one run in HPLC. There was also some radioactivity associated with 17α-hydroxy, 20βdihydroprogesterone (17α, 20β diHP4), 11β-hydroxyandrostenedione (11βOHΔ4) and 11β-

hydroxytestosterone (11βOHT) carriers after 2 to 3 TLC runs.

These preliminary results suggest a scheme of ovarian biosynthesis at 90 days P.F. associating the $\Delta 5$ and $\Delta 4$ pathways (fig. 3)

Fig 3 : Steroid metabolic pathways in ovaries 90 days P.F.




*Enzyme abbreviations = OR: Oxydoreductase*

*H : Hydroxylase*

*D : Desmolase*

These in vitro data demonstrate the precocious capacities of the ovaries of Oreochromis niloticus fry to metabolize P5, at least concomitantly with gonadal sex differentiation. At this time (i.e. appearence of premeiotic oocytes) a gradual increase of the steroid producing cells (identified a few days before) takes place in the same species (Nakamura and Nagahama, 1985).

Based on radiochromatogram patterns, changes in these metabolic activities appear during ovarian ontogenesis. These in vitro qualitative or quantitative variations seem to occur mainly between 35 and 42 days P.F. when meiotic oocytes enter into previtellogenesis.

It is hoped that further studies on these precocious metabolic variations will lead to the elaboration of a scheme of steroid metabolism during some of the most important stages of the differentiation.

*Résumé* :

*Chez Oreochromis niloticus, les caractéristiques histologiques de la différentiation ovarienne (premières prophases méiotiques, auxocytose ovocytaire) apparaissent entre 28 et 35 jours P.F. à 27°C. Les potentialités stéroïdogènes des gonades ont été analysées in vitro, du 35è au 90è jour P.F. : La capacité de métabolisation de stéroïdes tritiés est démontrée dès 35 jours P.F. Après une première étape analytique (TLC) des extraits du milieu d'incubation, des différences dans les profils des radiochromatogrammes sont observées entre les ovaires de 35 et 42 jours P.F. La purification pour identification de métabolites tritiés de la P5 nous a permis de proposer un premier schéma de stéroïdogenèse ovarienne.*

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