ELSEVIER

Tissue and Cell 40 (2008) 299–306

Tissue&Cell

www.elsevier.com/locate/tice

Histological evaluation of gonadal differentiation in fathead minnows (*Pimephales promelas*)

Cevdet Uguz *

Department of Medical Biology and Genetics, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03200 Afyonkarahisar, Turkey

Received 16 November 2007; received in revised form 5 February 2008; accepted 20 February 2008
Available online 3 April 2008

Abstract

The timing of sex determination and the pattern of sex differentiation have not been studied in fathead minnow even though this species of fish are commonly used as a research model for toxicological studies. In this study, the developmental histology of gonadal development was investigated. Fish were cultured in the laboratory conditions and spawning obtained at a photoperiod of 16 h-light and 8 h-dark. Samples were collected from day 7 fish post-spawning (day 7 fps) to day 150 fps and their gonads were processed for histological examination. Developmental histology was assessed by using a light microscopy. The results showed that ovarian differentiation normally occurs at around day 13 fps, while testicular differentiation normally occurs at around day 22 fps.

Keywords: Fathead minnow; Gonads; Developmental histology; Sex differentiation

1. Introduction

There is a growing concern about the adverse effects of man-made chemicals that have been introduced into the environment within the last 50 years (Curtis and Skaar, 2002). A scientific term, environmental endocrine disrupter, coined to define chemicals when they exogenously administered to an organism. They usually change the normal function(s) of the endocrine system in the target organism (Damstra et al., 2002). Therefore, many researchers and some environmental organization have been emphasizing to develop a testing program to determine the adverse effects of these man-made chemicals on reproduction and development in animals and human (USEPA, 1998; OECD, 1992a,b).

Fishes have commonly been utilized as animal model in toxicological studies since the late XIX Century (Ankley and Villeneuve, 2006). To date, small fresh water fishes such as fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*) and zebra fish (*Danio rerio*) are widely used as toxicological research models to test the effects of toxic chemicals in a testing programs (Ankley and Johnson, 2004).

It has been shown that steroids are natural approximate factors in sex determination and differentiation in fishes (Yamamoto, 1969) and amphibians (Adkins-Regan, 1987; reviewed in Uguz et al., 2003). It has been widely shown that the exogenous steroids can alter sex ratio when administered to fishes in a certain critical time period during embryonal or larval development (reviewed in Uguz et al., 2003). Estrogens or androgens induce sex reversal in non-mammalian vertebrates including fishes (Schreck, 1974; Hunter and Donaldson, 1983). Man-made environmental endocrine disrupters called xenobiotics or xenoestrogens have also been reported to alter sex ratio in fishes since they act either estrogen agonist or androgen antagonist in fish (Drastichova et al., 2005; Whitehead, 2006; Filby et al., 2007).

To determine the effects of xenobiotics on sex differentiation as well as on reproduction, it is necessary to determine the developmental gonadal histology of the target animals used in a testing program.

Fathead minnow is commonly used organism in many EPA mandated toxicological studies. Therefore, this study was design to determine the timing of sex differentiation and the pattern of developmental sex differentiation.

* Correspondence address: Afyon Kocatepe Üniversitesi, Veteriner Fakültesi, A.N. Sezer Kampüsü, 03100 Afyon, Turkey.
Tel.: +90 272 228 13 12x133; fax: +90 272 228 1349.
E-mail address: cuguz@aku.edu.tr.

0040-8166/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tice.2008.02.003

2. Materials and methods

2.1. Fish

Laboratory-bred fathead minnow brood stocks were used in this study. One male and four females were maintained under a photoperiod of 16 h-light and 8 h-dark at 24 °C in 35 L semi static glass aquarium system. Polyvinyl-coated (PVC) pipes (11 cm in diameter and 20 cm long) were cut in half through length-wise and were used as spawning substrates. To obtain accurate estimates of spawning time and to maximize spawn size, substrates were removed from the tanks for 1 or 2 days and then placed back into tanks to induce maximum spawning. Substrates containing eggs were collected from the 10 brood stock aquaria and were placed in a 10 L plastic bucket containing dechlorinated tap water. Air-stone were placed under neat each bridge-like concave substrates to vigorously aerate the eggs, which were attached on the concave surface of the substrates. As soon as the hatching occurs larvae were removed from buckets.

One hundred-ten larvae were put into each 5 L stainless steel tanks and were fed with brine shrimp alone for 10 days, and brine shrimp and tetra fin flakes for the next 20 days. Fish older than day 30 dps were transferred in to the 75 L glass aquaria and fed with ground trout chow until the end of the experiment.

2.2. Sampling schedule

Samples were collected from day 7 fish post-spawning (fps) through day 150 fps. Samples (6–10 fish) were collected every 3 days during the first month, every 8 days during the second month, and every 15 days during the third month. Samples were also collected once at 4 and 5 months. Sampling was discontinued when spawning was first detected in the rearing tanks (5 months).

2.3. Histology

The tissue samples (larvae or fish anesthetized with MS-222) were fixed in Bouin's fixative overnight. Bouin's fixative was washed from the samples with running tap water for several hours. Samples were then soaked in 40% ethanol for another several hours and stored in 70% ethanol until further processing. After measuring fork length, fish larger than 30 mm in length were trimmed by cutting the head just in front of the operculum, and the tail just behind the anal fin. Trimmed fish were dehydrated in 95 and 100% ethanol for 2 h each. Ethanol was removed from the tissues with xylene. Paraffin infiltration was done in a vacuum oven for 1 h (with a change after 30 min). Paraffin-infiltrated tissues were then embedded in paraffin blocks and 5 μm thick sections were taken from the tissues by using a rotary microtome.

All fish smaller than 20 mm in length for each treatment were sectioned from head to tail, whereas only one fish (larger than 20 mm in length) from each treatment group was decapitated and sectioned from head to tail to determine the exact location of gonad in their body. After the detection of gonads location, five fish were sectioned from each group and five slides were prepared from each fish for analysis.

Sections were mounted on albumin-coated glass slides and stained with Harris's hematoxylin regressive method and counterstained with eosin as described by Humasan (1962). Gonadal development was evaluated by using a light microscope.

The width and length of the largest gonad were measured and 15 germ cell nuclear diameters (width and length) in each of these gonads were also measured. Mea-

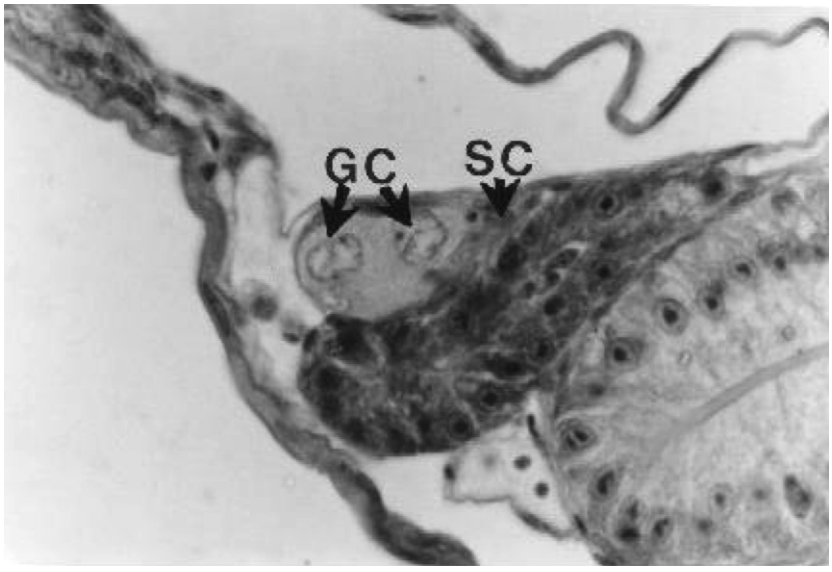

Fig. 1. Micrographic representation of gonad at day 7 fps: GC, germ cells; SC, somatic cells; 825×.