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Seasonal changes and endocrine regulation of pejerrey (*Odontesthes bonariensis*) oogenesis in the wild

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ABSTRACT

The goal of this study was to evaluate the essential components controlling the brain–pituitary–gonad axis during pejerrey (*Odontesthes bonariensis*) oogenesis in the wild. Ovarian developmental stages from vitellogenesis up to ovulation were associated with increasing day length and water temperatures below 21 °C (winter and beginning of spring). Gonadal regression was observed when water temperature exceeded this value or when photoperiod decreased. Most females were arrested at primary growth stage during summer (high temperature) or at cortical alveoli stage between autumn and beginning of winter (short photoperiod). Plasma E_2 and transcript levels of *fshr*, *cyp19a1b* and *cyp19a1a* increased during vitellogenesis, while *fshb* remained high at all vitellogenic stages. A significant correlation between plasma sex steroids (T and E_2) and *cyp19a1b* as well as *lhcgr* transcript levels was observed during vitellogenesis, suggesting a steroid positive feedback. *Gnrh-I*, Gth subunits and *lhcgr* transcript levels increased significantly during late vitellogenesis and final maturation. Present results suggest that pejerrey vitellogenesis is controlled by Fsh/Fshr, stimulating gonadal aromatase and estradiol synthesis. Moreover, the increase of testosterone and estradiol during final vitellogenesis could induce coordinately the functioning of the Gnrh/Lh system (perhaps through brain P450 aromatase stimulation and brain estradiol increase) and the gonadal Lhcgr synthesis to promote the final maturation of oocytes. All these stimulation mechanisms of gonadal development would be possible only under permissive environmental conditions.

1. Introduction

In all vertebrates, female fish reproduction is regulated by different hormones and enzymes that jointly control the functioning of the brain–pituitary–gonad axis in order to stimulate gametogenesis and spawning (Lubzens et al., 2010). In temperate regions, photoperiod and temperature are the most important environmental cues that regulate fish reproductive axis and thereby promote the spawning seasonality (Pankhurst and Porter, 2003).

Gonadotropin releasing hormone (Gnrh) is an important neurohormone and neuromodulator implicated in fish reproductive control, which regulates the synthesis and release of the pituitary gonadotropins (Gths, Zohar et al., 2010). The Gths, follicle stimulating hormone (Fsh) and luteinizing hormone (Lh), and their respective receptors (Fshr and Lhcgr) are critical in the endocrine control of gametogenesis, promoting mainly the synthesis of sex steroids through the stimulation of specific enzymes (Nagahama, 1994; Kumar et al., 2000). In females, estradiol (E_2) is the main steroid that induces oocyte development, and the pathway that mediates the conversion of testosterone (T) to E_2 by means of gonadal P450 aromatase activity, represents a key step in the regulation of oogenesis (Lubzens et al., 2010).

Current knowledge about endocrine control of oogenesis suggests that, at least in synchronous spawner, Fsh through Fshr stimulates the P450 aromatase activity and E_2 production that promotes the vitellogenesis (Montserrat et al., 2004), while Gnrh, Lh and Lhcgr stimulate a shift in the steroidogenic pathway toward the synthesis of the maturing inductor steroid (MIS), which promotes the final maturation and spawning (Nagahama and Yamashita, 2008; Lubzens et al., 2010). However, these regulation mechanisms are not clear for multiple spawner fish, in which different results have been reported depending on the species, the methodologies used or because different oocyte stages coexist in the same ovary. For instance, different patterns of Gths at gene expression levels have been found during the reproductive cycle of different fish species, such as goldfish *Carassius auratus* (Sohn et al., 1999), red seabream *Pagrus major* (Gen et al., 2003), and stickleback *Gasterosteus aculeatus* (Hellqvist et al., 2006). At plasma level, two increments of both Fsh and Lh plasma levels were observed in *Oreochromis niloticus*, one during vitellogenesis and the other during spawning (Aizen et al., 2007).

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It is known that sex steroids play a pivotal role in the communication (steroid feedback) between the gonads, the brain and the pituitary in order to regulate gonad development, although this mechanism is not fully understood (Zohar et al., 2010). In relation to steroid feedback, it is important to note the presence of brain aromatase in the brain as well as in the pituitary of fish (Pasmanik and Callard, 1985; Strobl-Mazzulla et al., 2008). Although brain aromatase has mainly been implicated in neurogenesis process, itsrole has also been suggested on fish reproductive control (Diotel et al., 2010; Strobl-Mazzulla et al., 2010). It must be noted that the activity and/or gene expression of brain aromatase fluctuate during the reproductive cycle in some teleost fish (Kazeto et al., 2003; Ezagouri et al., 2008; Rasheeda et al., 2010; Geraudie et al., 2011; Trubiroha et al., 2012).

Our fish model, the pejerrey (*Odontesthes bonariensis*), is a multiple spawner fish that inhabits lagoons of the Pampas region of Argentina. This species has a marked seasonal reproductive cycle, possessing a major spawning period during spring and a minor one in autumn (Calvo and Morriconi, 1972; Strüssmann, 1989). It has also been reported in captivity that both photoperiod and water temperature conditions determine jointly the pejerrey spawning season, the optimal temperatures being between 18 and 20 °C and a photoperiod of 14 h of light (Strüssmann, 1989; Miranda et al., 2006; Miranda et al., 2009).

As already mentioned, and notably for multiple spawner fish, the mechanisms associated with the coordinated functioning of the entire reproductive axis, and its interaction with the environmental cues, are far to be completely understood. In this context, the aim of the present study was to evaluate the essential components controlling the brain–pituitary–gonad axis during pejerrey oogenesis in order to obtain an overview of the natural functioning of the entire reproductive axis at each gonadal stage. Specifically, the expression of brain aromatase (*cyp19a1b*), brain Gnrh variants (*gnrh-I*, *gnrh-II* and *gnrh-III*), pituitary Gths subunits (*gphα*, *fshb* and *lhb*), gonadal Gth receptors (*fshr* and *lhcgr*), gonadal aromatase (*cyp19a1a*), and E_2 and T plasma levels were studied in the different gonad stages of pejerrey females from Chascomús Lagoon (Buenos Aires, Argentina). In addition, the seasonal occurrence of each gonadal stage was assessed throughout a whole year (May 2010–April 2011) in relation to the natural variations of photoperiod and water temperature recorded in the lagoon.

2. Materials and methods

2.1. Animal sampling

Adult pejerrey females were sampled monthly in Chascomús Lagoon (35°36′S 58°02′W) from May, 2010 to April, 2011 using a towing net, at 100 m far from the lagoon coast and approximately 1.2 m in depth. Fish caught were immediately taken to the Instituto de Investigaciones Biotecnológicas/Instituto Tecnológico de Chascomús laboratory. Every month 5 females were chosen (standard length, SL: 16.00 ± 0.29 cm; total weight, TW: 44.61 ± 2.74 g), euthanized with 2-phenoxyethanol and dissected. The size of all selected females was above the length of the first maturation reported for this species in Chascomús Lagoon (Calvo and Morriconi, 1972). Previously, blood samples were taken from the caudal vessels using heparinized syringes, and plasma samples were obtained by centrifugation at 4 °C and stored at −80 °C. Brains, pituitaries, gonads and livers were excised immediately after bleeding. Gonads and livers were weighed (GW and LW ± 0.1 g, respectively) for gonadosomatic index (GSI = 100GW/TW) and hepatosomatic index (HSI = 100LW/TW) calculations. A portion of each gonad, brain and pituitary were stored in TRIzol Reagent (Invitrogen, Germany) at 4 °C for approximately 24 h until processed for RNA extraction. The brains were sectioned, and only telencephalon, diencephalons and mesencephalon were used. A section of each gonad was fixed in Bouin's fixative and processed by routine methods for embedding in Paraplast Plus and histological analysis. All fish were handled and sacrificed in accordance with the UFAW Use and Care Committee Handbook on the Care and Management of Laboratory Animals (http://www.ufaw.org.uk/pubs.htm#Lab) and local regulations.

2.2. Histological analysis

Ovarian sections of 6 μm thick were stained with hematoxylin and eosin for observation of histological characteristics and estimation of the reproductive status of each animal. The proportion of oocytes at different developmental stages was analyzed at random in an area of 7.5 mm^2 in three different histological sections (separated by 1 mm each other) in the middle of the right ovary (see Elisio et al., 2012). The quantifications were performed on micrographs taken with a light microscope Nikon Eclipse E600, equipped with a digital photomicrographic system (Nikon Digital Sight DS-Fi1). Gonad stages were defined according to the proportion of different oocyte developmental stages present in the ovary following the guidelines proposed by Strüssmann (1989). Primary growth (PG): ovaries with all oocytes in primary growth stage (oocyte without evidence of cortical alveoli, lipids or yolk droplets); cortical alveoli (CA): ovaries with a clutch of oocytes in cortical alveoli stage (oocytes filled with cortical alveoli and lipid droplets) and without vitellogenic or final maturation oocytes; initial vitellogenesis (VtgA): ovaries with a leading clutch of oocytes at initial vitellogenesis stage (oocytes with small yolk droplets, cortical alveoli and lipid droplets that appear in the periphery of oocyte) and without final maturation oocytes; advanced vitellogenesis (VtgB): ovaries with a leading clutch of oocytes at advanced vitellogenesis stage (oocytes filled with large yolk droplets) and without final maturation oocytes; final maturation (FM): ovaries with a leading clutch of oocytes at final maturation stage (oocytes with fused yolk droplets and the germinal vesicle migrating or ex-centric); atretic (AT): ovaries with more than 10% of atretic oocytes (oocytes with chorionic filaments and follicle investments irregular in shape and shriveled, and with signs of nuclear and cytoplasmic disintegration); ovulated (OV): ovaries of ovulated or recently spawned females (shrunken follicle with its follicular layer folded). The histological features of the different gonad stages of pejerrey females are shown in Fig. 1.

2.3. Gene expression measurements

The relative transcript levels of brain Gnrh variants (*gnrh-I*, *gnrh-II* and *gnrh-III*), brain aromatase (*cyp19a1b*), pituitary Gth subunits (*fshb*, *lhb*, *gphα*), gonadal Gth receptors (*fshr*, *lhcgr*) and gonadal aromatase (*cyp19a1a*) genes were determined in each female using real-time RT-PCR with the standard curve method following the procedure published by Applied Biosystems (1997). The gene expression data were normalized using β-actin mRNA levels. For this purpose, total RNA was extracted for each sample using TRIzol Reagent following the manufacturer's instructions. Briefly, RNA samples were treated with DNase I (Invitrogen) and reverse transcribed using SuperScript III RNase H (Invitrogen) and oligo$(dT)_{12–18}$. Gene-specific primers for real-time PCR analysis were designed to generate amplicons no longer than 155 bp (Table 1) using the Primer Express software (Applied Biosystems, Foster City, CA, USA). The PCR mix consisted of 1 μL of diluted cDNA (ca. 100 ng), 1 pmol of each primer and 7.5 μL of FastStart Universal SYBR Green Master (ROX, Roche Applied Science, Mannheim, Germany) in a final volume of 15 μL. The reactions were performed in an M×3005P® QPCR System (Stratagene, Agilent Technology Company, Santa Clara, CA, USA). Amplification of the target genes was done simultaneously with β-actin in separate tubes and the results were analyzed with the Stratagene M×3005P® QPCR System software version 4.01. The efficiencies of RT-qPCR ranged between 80% and 100%. Dissociation-curves analyses were run after each real-time experiment to ensure that there was only one product. A reverse-transcriptase negative control was run for each template and primer pair.

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