Comparative Biochemistry and Physiology - Part D 27 (2018) 23–29

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology - Part D

journal homepage: www.elsevier.com/locate/cbpd

Comparison of proteomic profiles in the ovary of Sterlet sturgeon (*Acipenser ruthenus*) during vitellogenic stages

Mohammad Youneszadeh-Fashalami[a,b], Amir Parviz Salati[a,*], Saeed Keyvanshokooh[a]

[a] Department of Fisheries, Faculty of marine Natural Resources, Khorramshahr University of Marine Science and Technology, Iran
[b] South Iranian Aquaculture Research Center, Ahwaz, Iran

ARTICLE INFO

Keywords:
Vitellogenesis
Proteome
Sex steroids
Acipenser ruthenus

ABSTRACT

One of the challenges of sturgeon aquaculture is that sturgeon takes an extended amount of time to reach sexual maturity. The pattern of the protein expression in relation to the late maturity of sturgeon can help to better understand changes in sexual maturity. 17β-estradiol (E2), testosterone (T) and vitellogenin (Vtg) levels were examined at all stages of sexual maturation in Sterlet sturgeon (*Acipenser ruthenus*). Two-dimensional gel electrophoresis and mass spectrometry analysis were used to show the pattern of the ovarian proteins. The T levels increased from the previtellogenic to the postvitellogenic stages ($P < 0.05$) and Vtg showed a decremental pattern in pre- and postvitellogenic, and atresia (not significantly). The analysis showed 900 protein spots, 19 of which were successfully identified and had significant differences between the previtellogenic and the vitellogenic groups ($P < 0.05$). Among the identified proteins, 40% involved in cell defense (heat shock protein, Glutathione peroxidase, natural killer enhancing factor, peroxiredoxin-2), 30% in transcription and translation (constitutive photomorphogenesis 9 and Ybx2), 20% in metabolism and energy production (triose-phosphate isomerase (TPI)) and 10% in transport (glycolipid transfer protein). In the vitellogenic stage, the proteins were related to metabolism and energy production (TPI, ES1, creatin kinase, enolase, nucleoside diphosphate kinase, 50%), cell defense (thioredoxin and dislophid isomerase, 20%) and transport (fatty acid binding protein, 10%). Our findings show changes in protein expression pattern from cell defense to metabolism during egg development.

1. Introduction

One of the challenges in rearing sturgeon is the late age at which they reach maturity. Maturation of females is stopped in the previtellogenic stage, and the primary reproductive cells enter a stagnant phase. The length of the previtellogenic stage is different in sturgeons, but a long time is needed from the previtellogenic to shift to the vitellogenic stage (Moberg et al., 1991).

Today, scientific knowledge and new technologies are used to obtain the best breeding methods for production as well as sustainable development. One of the most important emerging technologies is proteomics, useful in biology and aquaculture (Pandey and Mann, 2000). Compared to genomics, proteomics not only examines information at the mechanistic level, but also changes in proteins after translation (Rodrigues et al., 2012). One of the applications of proteomics is the study of oogenesis. Proteomic analysis has been successfully used in determining the profile of proteins in mammal and fish oocytes, and the changes in their expression during oogenesis (Ziv et al., 2008; Keyvanshokooh and Vaziri, 2008; Sirard et al., 2003). The proteomic study of oocytes provides an insight into biochemical processes that involve maturation processes.

Among sturgeon fishes, Sterlet sturgeon (*Acipenser ruthenus* Linnaeus, 1758), are considered as a suitable model for studying reproductive physiology of sturgeon (Holcik, 1989), due to quicker sexual maturation, small size and lower maintenance costs compared to the other sturgeons. The molecular changes in oocytes during the oogenesis from the early oogonia to mature oocytes are well characterized in teleosts (Bidwell and Carlson, 1995). Unlike bony fish, little information is available on sturgeon species. Following these molecular events and comparing them among various species, a framework will be provided for understanding the mechanisms involved in the oocyte maturation in sturgeon (Bidwell and Carlson, 1995). Therefore, studying the ovarian proteome and measuring the blood value of sex steroids and vitellogenin in Sterlet, as a sturgeon, in different stages of sexual maturation can answer many of the questions about the reproductive physiology female sturgeons.

* Corresponding author at: Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran.
E-mail address: apsalati@kmsu.ac.ir (A.P. Salati).

https://doi.org/10.1016/j.cbd.2018.04.006
Received 24 January 2018; Received in revised form 22 April 2018; Accepted 27 April 2018
Available online 02 May 2018
1744-117X/

2. Material and methods

The Sterlet stocks were imported from Hungary in 2004 and kept at the Shahid Dr. Beheshti Sturgeon Fish Propagating and Rearing Complex, Iran. The fish sampled in this research were obtained from the Hungarian broodstock, which was kept in special flow-through rectangular concrete tanks (25 × 3 × 0.6 m) in natural photoperiod and temperature (10–26.5 °C). All fish used in this study were taken from this reservoir.

Twelve fish aged < 3 years and in the early stage of sexual maturation with average weight 178.22 ± 24.28 g and total length 33.32 ± 22.76 cm were selected. Blood from the venous vein was captured using a 5-mm heparin syringe, centrifuged (Eppendorf, Germany) at 1600*g* for 10 min, and obtained plasma was isolated and stored at −80 °C. Due to the small size of the fish, they were euthanized and gonad tissue samples were taken. Part of the sample was fixed in Bouin solution for histological analysis and the other was directly transmitted into nitrogen tank and then stored at −80 °C until proteomic analysis.

The fish aged 5 years ($n = 40$) with mean weight of 768.33 ± 40.45 g and total length of 56.9 ± 2.8 cm were used for sampling in the later stages of maturation. The biopsy of gonads was done according to Chebanov and Galich (2011) by a special steel probe (diameter 3–4 mm groove length 3–6 cm).

After the histological studies (Fig. 1), fish were divided into 4 groups ($n = 12$): previtellogenic, vitellogenic, postvitellogenic and atresia (Amiri et al., 1996). Six blood samples were taken at each reproductive stage.

2.1. Blood analysis

Plasma levels of 17β-estradiol and testosterone were measured by radioimmunoassay using the Immunotech Kit (Marseille, France) and with a gamma counter (LKB model, Finland) and expressed as nanogram per milliliter (ng/ml). Vitellogenin measurements were performed by ELISA with polyclonal fish vitellogenin (VTG) ELISA kit (east-abiopharm, China) as described company.

2.2. Proteomic analysis

Three biological replicates (with consideration of individual genetic diversity) were prepared for each group for proteomics analysis. The gonads were homogenized in the cold lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 50 mM Tris, 0.2% carrier ampholyte, 0.25% RNase, 1% DNase, and 0.2 ml protease inhibitors Cocktail (Complete Mini, Roche). The homogenates incubated for 1 h at room temperature, centrifuged at 15000*g* for 20 min at 4 °C. The supernatant was collected and its protein concentration was assayed by Bradford (1976) with bovine serum albumin (BSA) as standard.

Two-dimensional gel electrophoresis (2DE) was used to separate the proteins extracted from Sterlet sturgeon ovaries. Proteins (2 mg) for each sample were diluted in a buffer containing 8 M urea, 4% CHAPS, 50 mM DTT, and 0.2% ampholytes at pH 3–10, and then loaded into IPG strips (17 cm, pH 3–10; Bio-Rad) by passive rehydration for 16 h at room temperature. Isoelectric focusing (IEF) was carried out using Protean IEF Cell (Bio-Rad, USA). Low voltage (250 V) was applied for 20 min, and then, the voltage was gradually increased to 10,000 V over 2.5 h and hold at that level until a total of 50,000 Vh. Prior to the second step of the 2DE method, the proteins separated in the IPG strips were equilibrated for 20 min in a solution of 2% DTT for protein reduction and for another 20 min in a solution of 2.5% iodoacetamide for alkylation. Both reagents were dissolved in the equilibration buffer containing 50 mM Tris–HCl pH 8.8, 6 M urea, 20% glycerol, 2% SDS, and 0.01% bromophenol blue. The equilibrated strips were then placed

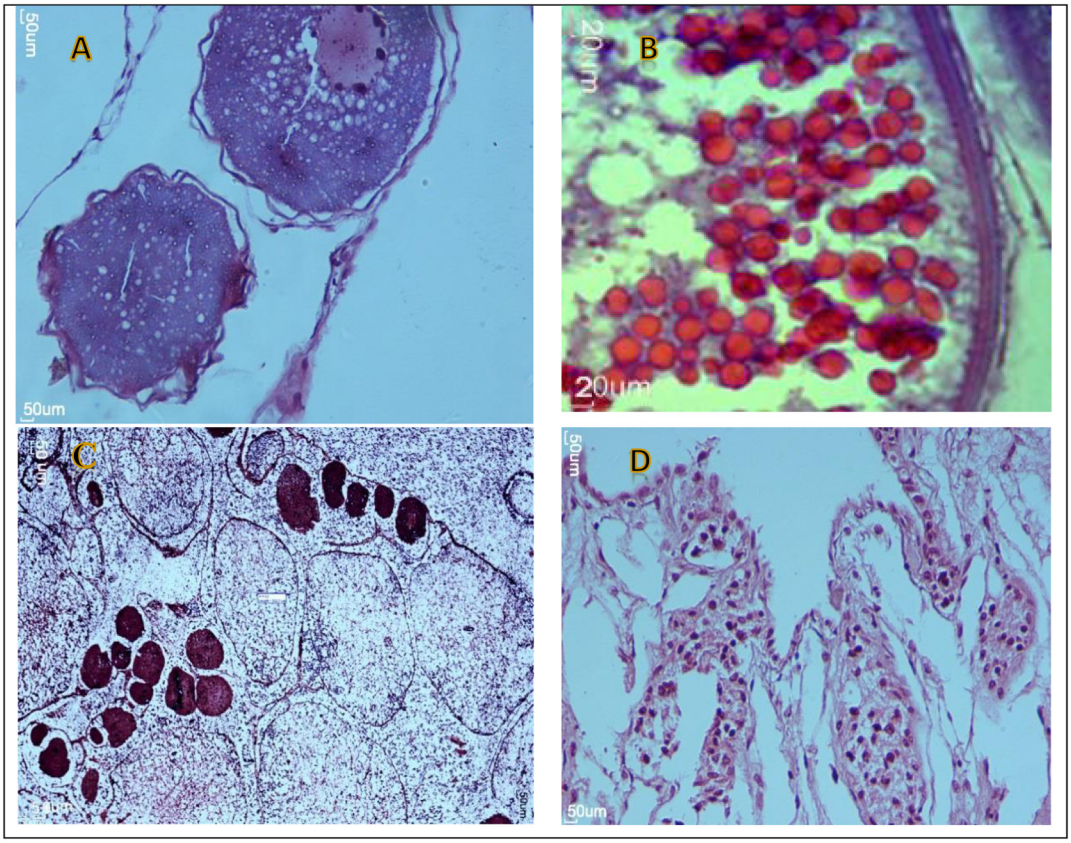

Fig. 1. Different developmental stages of Sterlet sturgeon, *A. ruthenus* ovary. Slides stained with hematoxylin & eosin. Previtellogenic (a), Vitellogenic (b), Postvitellogenic (c) and Atresia (d).