ISSN: 0975-833X

Available online at http://www.journalajst.com

International Journal of Current Research
Vol. 5, Issue, 06, pp.1565-1567, June, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

OOGONIAL PROLIFERATION, OOGENESIS, FOLLICULOGENESIS AND VITELLOGENESIS IN THE OVARY OF ZEBRAFISH (*Danio rerio*): A HISTOLOGICAL AND HISTOCHEMICAL ANALYSIS

Prasad A. Deshpande and *Katti Pancharatna

Department of Zoology, Karnatak University, Dharwad-580 003, India

ARTICLE INFO

Article History:
Received 31st March, 2013
Received in revised form
03rd April, 2013
Accepted 21st May, 2013
Published online 15th June, 2013

Key words:
Zebrafish,
Oogenesis,
Folliculogenesis,
Vitellogenesis,
Histochemistry.

ABSTRACT

Histological and histo chemical analysis of zebrafish ovary with reference to oogonial proliferation, oogenesis, folliculogenesis and vitellogenesis were studied. Ovaries from adult females were processed for paraffin embedding and sections (3µm) thick were cut and stained with hematoxylin-eosin and for histochemical detection of proteins, carbohydrates, mucopolysaccharides, lipids, neutral lipids and acidic lipids. Sections of ovaries exhibited oogonia and oocytes (previtellogenic and vitellogenic). Oogonia were spherical cells found in nests on the ovarian epithelium and occasionally dividing; they ranged from 9-11 µm in size. Newly transformed oocytes ranged from 20-40 µm and primary oocytes measured 40-140 µm in their size. In larger primary oocytes (>170 µm) yolk vesicles appeared beneath plasma membrane marking beginning of the vitellogenesis. Ooplasm of previtellogenic oocytes stained to mercuric bromophenol blue and alcian blue. Nuclear extrusions (nuage) stained positive to Sudan black B and Oil Red O. Extraneous yolk granules stained positive to nile blue, alcian blue, bromophenol blue and PAS. The findings reveal that oogonial proliferation and their differentiation into oocytes (oogenesis) are prevalent in adult ovary; yolk contains nuclear extrusions (RNA?) and yolk granules deposited from the periphery of the oocyte.

INTRODUCTION

Zebrafish (*Danio rerio*) has emerged in recent years as a powerful and versatile model to study the vertebrate development and organogenesis. As a model system, this fish has several advantages such as (i) small size (ii) short generation time (iii) optical transparency of embryo and (iv) *ex utero* development etc. In addition, the genome of this fish has been sequenced completely and is found to have strong synteny with that of the human genome that has made this fish a close vertebrate model to study human diseases and is intensely employed in drug/pharmaceutical discovery research. Owing to its growing importance it is essential to understand the reproductive processes of this fish in detail. This fish is a non-seasonal/acyclic breeder with asynchronous ovarian follicular development. The morphology and classification of stages of oocyte development (Selman *et al.*, 1993), ultra structure of the ovary (Nazan Deniz Koc *et al*., 2008) are studied in this fish. Reports on hormonal control of reproduction, development of gonads and primordial germ cells are also available (Maack, 1964; Van Ree, 1977; Garg, 1998; Weber *et al*., 2002; Orn *et al*., 2003; Fenske and Segner, 2004; Raz, 2003; Nagahama and Yamashita, 2008). The present study was aimed to elucidate the processes that determine the fecundity of female such as, oogonial proliferation, oogenesis, folliculogenesis, development and growth of follicle with special reference to histochemical analysis and distribution of yolk in the adult ovary of zebrafish.

MATERIALS AND METHODS

Animals

Adult (body length: 3.0–4.0 cm) *Danio rerio* (wild-type) were obtained from commercial suppliers (Aquastar Aquarists, Chennai, India). They were housed in aquaria connected to water re-circulating system generated indigenously. Fishes were maintained in the laboratory under natural temperature (27 ± 1°C) and photoperiod (14L: 10D) using conditioned water (pH 7.0 - 7.2; hardness, 117 – 120 mg L^{-1}) and were fed on commercial pellets *ad libitum* twice a day (Rajapurohit and Pancharatna, 2007; David and Pancharatna, 2009ab). Permission to work on zebrafish was obtained from CPCSEA, India under Institutional registration.

***Corresponding author:** Katti Pancharatna
Department of Zoology, Karnatak University, Dharwad-580 003, India

Histology

Ovaries were removed from freshly euthanized females and immediately fixed in Bouin's fluid for 24 hours and processed for paraffin embedding. Sections of 3µm thick were cut on a semi-automated microtome (Leica-RM 2255) and stained with hematoxylin-eosin. The sections were observed under microscope (Olympus BX51TRF). The size of oogonia and follicles at various stages of development were measured (*n = 100*) using an ocular micrometer.

Histochemistry

Parallel paraffin sections were simultaneously processed for histochemical detection of proteins (mercury bromophenol blue), glycoproteins (periodic acid Schiff's - PAS), mucopolysaccharides (alcian blue), lipids (Sudan black B), neutral lipids (oil red O) and acidic lipids (Nile blue) (Table 1). Stained sections were observed and photographed with a digital camera (Progress Capture, Pro 2.5, Jenoptik, Germany).

RESULTS

Histological details of adult D. rerio ovary

Sections of adult ovaries exhibited ovarian epithelium with oogonia, previtellogenic and vitellogenic oocytes at various stages of development (Fig. 1A-C). Oogonia were spherical cells with prominent nucleus, always found in nests on the ovarian epithelium and occasionally dividing; they ranged from 9-11 µm in size (Fig. 1A). Newly transformed oocytes ranged from 20-40 µm. Primary oocytes ranged from 40 to 140 µm in their size; they consisted a large spherical nucleus with one or two prominent nucleoli and relatively less cytoplasm. With the growth of the primary oocytes, yolk nucleus

appeared in ooplasm (Fig. 1B). In the larger primary oocytes yolk vesicles appeared at the periphery of the oocytes marking the beginning of the yolk accumulation and vitellogenesis (Fig. 1B). Vitellogenesis began when the follicles reached a size of 170 µm. With the progress of vitellogenesis, the rate of accumulation of yolk was enhanced (Fig. 1C). Vitellogenesis resulted in the exponential increase in the size of the oocytes and they attained a size of 550 to 690 µm at the end of this process.

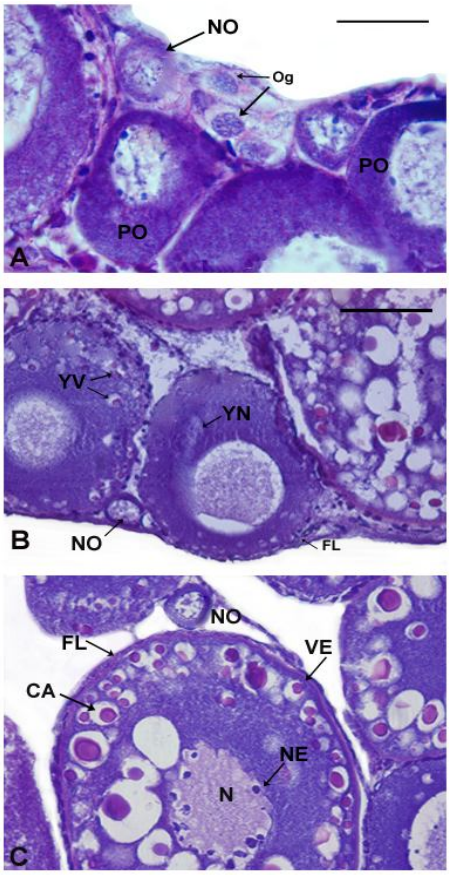

Fig. 1. Cross section of zebrafish ovary (Haematoxylin-eosin) showing oocytes at different stages of development.

A: Oogonia (Og), newly formed oocytes (NO) and primary oocytes (PO). Scale bar: 50 µm, B: Newly formed oocytes (NO), primary oocyte with single layered follicular epithelium, yolk vesicles (YV) and yolk nucleus (YN). Scale bar: 50 µm, C: Cortical alveolar oocyte with cortical alveoli (CA), follicular epithelium (FL), vitelline envelope (VE), nuclear extrusions (NE) and nucleus (N). Scale bar: 25µm.

Histochemistry of developing and vitellogenic oocytes

Processing of the parallel sections to various histochemical staining techniques (listed in Table. 1) indicated that previtellogenic oocytes stained positive but very faintly to mercuric bromophenol blue and alcian blue, and negative to PAS, oil Red O, nile blue and Sudan black B (Fig. 2A-F). In vitellogenic follicles yolk granules stained positive to all above mentioned stains indicating the protein, carbohydrate and lipid composition of yolk granules (Fig. 2A-F).

Table 1. Histochemical techniques used

S. No.	Detection of	Method used
1.	Proteins	Bromophenol blue
2.	Glycogen & Polysaccharides	Periodic Acid Schiff's
3.	Acid mucopolysaccharide	Alcian blue
4.	Neutral fats	Oil Red O
5.	Lipids	Sudan black B
6.	Phospholipids	Nile blue

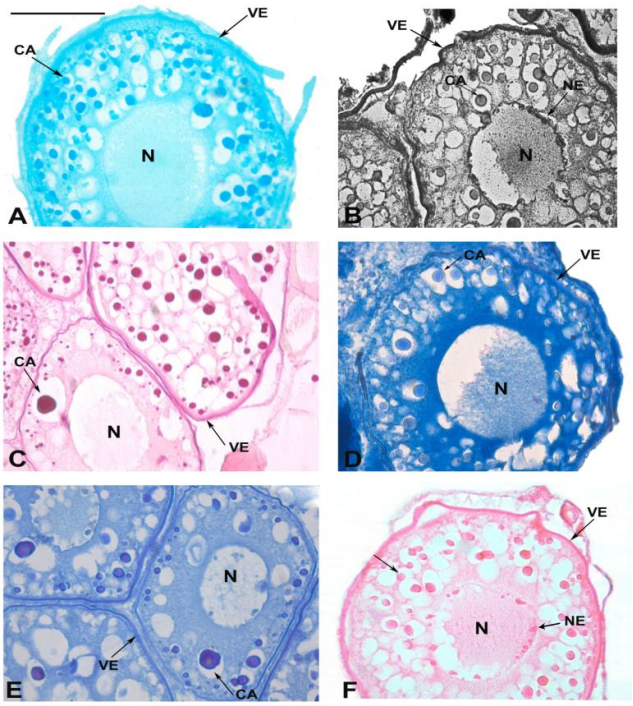

Fig. 2. Cortical alveolar and vitellogenic oocytes with different histochemical methods

A: Alcian blue (acid mucopolysaccharides), B: Sudan black B (lipids), C: Periodic Acid Schiff's (PAS) (Glycogen and polysaccharides), D: Nile blue (Phospholipids), E: Bromophenol blue (proteins) and F: Oil red O (Neutral fats). CA: cortical alveoli, VE: vitelline envelope, NE: nuclear extrusions, N: nucleus. Scale bar: 25µm.

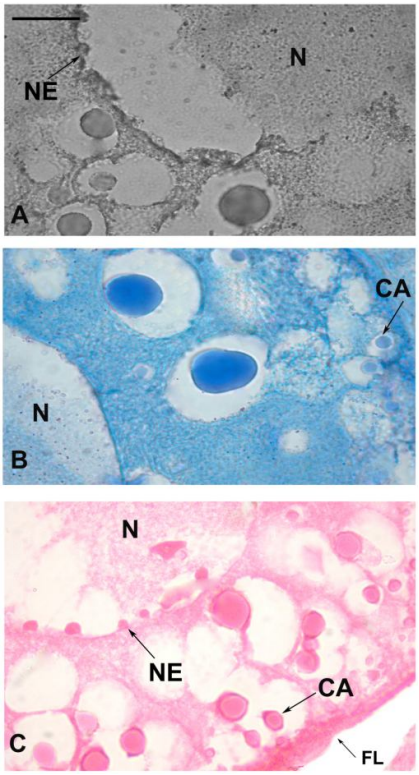

Fig. 3A. Yolk granules contributed from nuclear extrusions that stained positive to Sudan black B, B: Yolk granules incorporated through peripheral vacuoles, C: Yolk granules contributed from nuclear extrusions that stained positive to Oil red O. NE: nuclear extrusions, CA: cortical alveoli, N: nucleus. Scale bar: 25µm.

Careful observations revealed that the yolk droplets originate from two different sources (i) yolk material contributed from the nuclear extrusions (nuage) that stained positive to Sudan black B and Oil Red O (Fig. 3A and C) and (ii) those granules incorporated into the oocytes extraneously through peripheral vacuoles i.e. plasma membrane of the oocyte which stained positive to nile blue (Fig. 3B). The size of the yolk lipid granules increased from the site of their incorporation to the storage site or final position (Figs. 1-3).

DISCUSSION

There is a great diversity in the pattern and reproductive strategies adapted by teleosts inhabiting marine and freshwater aquatic systems (Ravaglia and Maggese, 2002). Zebrafish is a freshwater reverine fish originating from India, Pakistan and Bangladesh. The adult fish exhibits presence of persistent oogonia, asynchronous oogenesis and lays eggs year round. On an average a female is known to oviposit 200-300 eggs once in 2-3 months under laboratory conditions when fed regularly (Rajapurohit and Pancharatna, 2007; David and Pancharatna, 2009ab). In this study we observed that adult ovary had possessed oogonial nests, dividing oogonia and newly formed oocytes indicating oogonial proliferation and oogenesis are persistent and prevalent in the adult ovaries (Figs 1A-C). In an earlier study Selman *et al*., (1993) reported the presence of oogonia but without evidence for dividing oogonia suggesting oogonial proliferation is not apparent in adult ovary. Further, in our observation, germ cells were clearly distinguishable from the somatic/follicular cells by their larger size, spherical shape and greater nucleo-cytoplasmic ratio. Binucleate oocytes were also observed although, not frequently in our study.

The growing oocytes have been classified into 5 stages in the adult ovary of zebrafish by Selman *et al*., (1993). All the types i.e. from stage I – stage V oocytes were observed in the present study. Primary oocytes exhibited the ooplasm that stained positive to proteins, mucopolysaccharides and negative to lipids (Fig. 2). With the onset of vitellogenesis the yolk granules accumulated in the ooplasm; vitellogenesis proceeded in two directions: (i) lipid yolk containing neutral lipids originated from nucleus and extruded into peripheral ooplasm (Fig. 3) while (ii) yolk granules that stained positive for proteins, carbohydrates, and acidic lipids were incorporated from the peripheral ooplasm and were distributed interior (Fig. 3). The size of these lipid droplets increased from the site of their incorporation to the site of storage in the oocyte. In conclusion, the paper presents relevant findings on zebrafish ovarian follicular growth, such as, (i) in adult zebrafish, oogonia (ovarian stem cells) are persistent (ii) oogonial mitosis (proliferation) and their transformation (differentiation) into oocytes (oogenesis) are prevalent in adult ovary. Thus this fish forms suitable animal to study the regulatory mechanisms underlying proliferation and differentiation of germ line stem cells, as the adult ovary of the fish has persistent germ line stem cells/oogonia unlike adult mammalian ovary, (iii) yolk originates from two sources, from nucleus and from the exterior and (iv) yolk granules are composed of protein yolk, lipid (neutral and acidic lipids) and carbohydrate yolk.

REFERENCES

David, A. and Pancharatna, K. 2009a. Developmental anomalies induced by a non-selective COX inhibitor (ibuprofen) in zebrafish (*Danio rerio*). Environ. Toxicol and Pharmacol. 27(3): 390-395.

David, A. and Pancharatna, K. 2009b. Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, *Danio rerio*. J. App. Toxicol. 29(7): 597-602.

Fenske, M. and Segner, H. 2004. Aromatase Modulation Alters Gonadal Differentiation in Developing Zebrafish (*Danio rerio*). Aqua. Toxicol. 67: 105-126.

Garg, R. A. 1998. Cloning and mRNA expression of the Activin Type IIB Receptor and Role of Activin in Final Oocyte Maturation in Zebrafish, *Danio rerio*. York University, Department of Biology, Toronto.

Maack, G. 1964. Estrogen Related Alterations of Gonad Development and of Reproduction in the Zebrafish, *Danio rerio*. Ham. Buc. Hamburg.

Nagahama, Y. and Yamashita, M. 2008. Regulation of oocyte maturation in fish. Develop. Growth Differ. 50: 195-219.

Nazan Deniz Koc (Yon)., Aytekin, Y. and Yuce, R. 2008. Ovary Maturation Stages and Histological Investigation of Ovary of the Zebrafish (*Danio rerio*). Braz Arch of Biol and Tech.51(3):513-522.

Orn, S., Holbech, H., Madsen, T., Norrgren, L. and Peterson, G. 2003. Gonad Development and Vitellogenin Production in Zebrafish (*Danio rerio*) Exposed to Ethinylestradiol and Methyltestosterone. Aqua Toxicol. 65(4), 397-411.

Rajapurohit, S.V. and Pancharatna, K. 2007. Development and hatching success of zebrafish *Danio rerio* under laboratory conditions. J of Adv Zool. 28(1): 21-25.

Ravaglia, M. and Maggese, M. C. 2002. Oogenesis in the swamp eel *Synbranchus marmoratus* (Bloch, 1795) (Teleostei; Synbranchidae). Ovarian anatomy, stages of oocyte development and micropyle structure. Biocell. 26(3): 325-337.

Raz, E. 2003. Primordial germ-cell development: The zebrafish perspective. Nature Reviews. 4: 690-700.

Selman, K., Wallace, R. A., Sarka, A. and Qi, X. 1993. Stages of Oocyte Development in the Zebrafish, *Brachydanio rerio*. J Morpho. 218: 203-224.

Van Ree, G. E. 1977. *In vitro* induction in nuclear breakdown in oocyte of the zebrafish, *Brachydanio rerio* Ham. Buc., Effects of the composition of the medium and of protein and steroid hormones. Proc. Kon.Ned.Akad. Wetensch.Series C.,80:353-371.

Weber, L. P., Hill, R. L. and Janz, D. M. 2002. Developmental Estrogenic Exposure in Zebrafish (*Danio rerio*): II. Histological Evaluation of Gametogenesis and Organ Toxicity. Aqua Toxicol. 62:431-446.

****