Journal of Applied Biological Sciences 1 (2): 33-35, 2007

Development of the Yolk Nucleus of Previtellogenic Oocytes in Rainbow Trout, *Oncorhynchus mykiss,* Studied by Light Microscopy

Nagihan GÜLSOY

Marmara University, Science Faculty, Biology Department, 34722, Göztepe, Istanbul, TURKEY

Corresponding Author
e-mail: nagehan@marmara.edu.tr

Received : 14 June 2006
Accepted : 30 November 2006

Abstract

In this study, the development of the yolk nucleus in cytoplasm of previtellogenic oocytes was examined by light microscopy during oogenesis in trout, *Oncorhynchus mykiss*. We determined that oocytes 20-30 μm in diameter have much basophilic (electron dense) cytoplasm and an area of flocculent appearance begins to emerge around the nucleus. The yolk nucleus was first recognized within this area as a few fragments dense granular thread. These fragments were observed gradually increase in number and length until they assemble into a spherical mass. When oocytes develop to greater than 107 μm in diameter, the yolk nucleus was dispersed throughout the cytoplasm and the cytoplasm was observed more homogenous.

Key words: *Oncorhynchus mykiss, previtellogenic oocytes, yolk nucleus, trout*

INTRODUCTION

The yolk nucleus has been known as the Balbiani's body, nuage, nucleolus-like bodies or electron dense material in various animal species for many years [1-3]. It is found in the cytoplasm of previtellogenic oocytes during oogenesis in fish. The basophilic yolk nucleus is proliferated by growing oocyte of fish. This substance may not always play a part in yolk formation but this term has widely been used for a long time in fish literature [2-5]. The fine structure of the yolk nucleus is described in different fish species [1,5-7]. The yolk nucleus substance includes endoplasmic reticulum, ribosomes, free ribosomes (RNA), mitochondria, Golgi bodies and lipid bodies [2,3,5,8,9].

According to Iwamatsu and Nakashima, the yolk nucleus plays a role determining the vegetal pole of the oocyte [10]. Azevedo believes that required for cytoplasmic ribosomal maturation during previtellogenesis stages in *Xiphorous helleri* and he called it as the nucleolus-like bodies [2].

In this report, we have examined the morphological changes of the yolk nucleus during oogenesis in trout oocytes by using the light microscopy techniques.

MATERIALS AND METHODS

Research materials, *Oncorhynchus mykiss*, were sampled randomly on a monthly basis between July 2001 and June 2002 were used at Sarıkız stream in Kütahya, Turkey. Each month 7-10 fishes were sampled for a total 103 individuals throughout the year. After anesthetization, fishes were measured to total length to the nearest 0.5 cm, body weight to the nearest 5 g and ovary mass 0.01 g.

The ovaries for light microscopy were fixed in Bouin solution immediately. After dehydration tissues were embedded in Histo sec 56-58 pF (Merck) and sectioned at 5-7 μm. Transverse and longitudinal sections were obtained in ovaries. Samples were stained with toluidine blue and PAS- hematoxylin.

In each section, number of the different stages during each month was determined per microscopic field in the interior, middle and posterior parts of ovary for each individual. The number of different development stages was determined in the sections of interior, middle and posterior part of ovary. Oocytes sectioned through the nucleus were measured using an image analyzer (Media Cybernetics, Silver Spring, MD). Mean oocyte diameter was determinated as the mean diameter of the 10 largest oocytes within one cross-section of the ovary.

RESULTS

Female gonads were revealed in our microscopic examination like the ovary development of *O. mykiss* is the group-synchronous type as described by Iwamatsu & Nakashima and Tyler & Sumpter [10,11]. Several oocyte stages were observed in the same time (Fig. 1a). Progressive changes in cellular diameters during oogenesis are given in Table 1. When oocytes were developing, the diameters of oocytes increased dramatically. The small and rounded oogonia were found in the vicinity of the germinal epithelium. They were recognized by their size and by presence of the only one nucleolus (Fig. 1b). In small oocytes with large nucleus was not recognizable yolk nucleus (Fig 1c). When oocytes developed 20-107 μm in diameter, the yolk nucleus could have been clearly identified with light microscopy techniques (Fig. 1d). The previtellogenic oocyte was subdivided into chromatin nucleolar, perinucleolar and later perinucleolar stages. In the perinucleolar stage the cytoplasm of oocyte started to be more intensely basophilic and more easily stained with hematoxylin (Fig. 1d). As the oocytes grown further, a continuous or discontinuous, irregular ring-shape basophilic area of flocculent appearance in the cytoplasm begun to emerge around the nucleus (Fig 1d, e). The yolk nucleus in the trout oocyte was firstly recognized within a flocculent area as a few dense granular threads. These thread-like fragments expanded towards the cortical cytoplasmic area and finally occupy the whole cytoplasm (Fig 1d-f).

When the oocytes increased in volume larger than 107 µm in diameter, cytoplasm gradually loses its basophilia and become more granular. Formation of the yolk nucleus dispersed in to the oocyte and their components were distributed in the ooplasm (Fig. 1f).

Table 1. Changes in the diameter of maturating and mature oocytes during oogenesis female trout, n, number counted.

Stage	N	Diameter
Oogonia	20	5 µm
Chromatin nucleolar	50	10-20 µm
Early Perinucleolar	30	20-107 µm
Late Perinucleolar	40	100-410 µm
Cortical alveolar	10	411-502 µm
Early Vitellogenesis	50	551-1055 µm
Late Vitellogenesis	50	902-3145 µm
Mature	100	4.46-5.27 mm

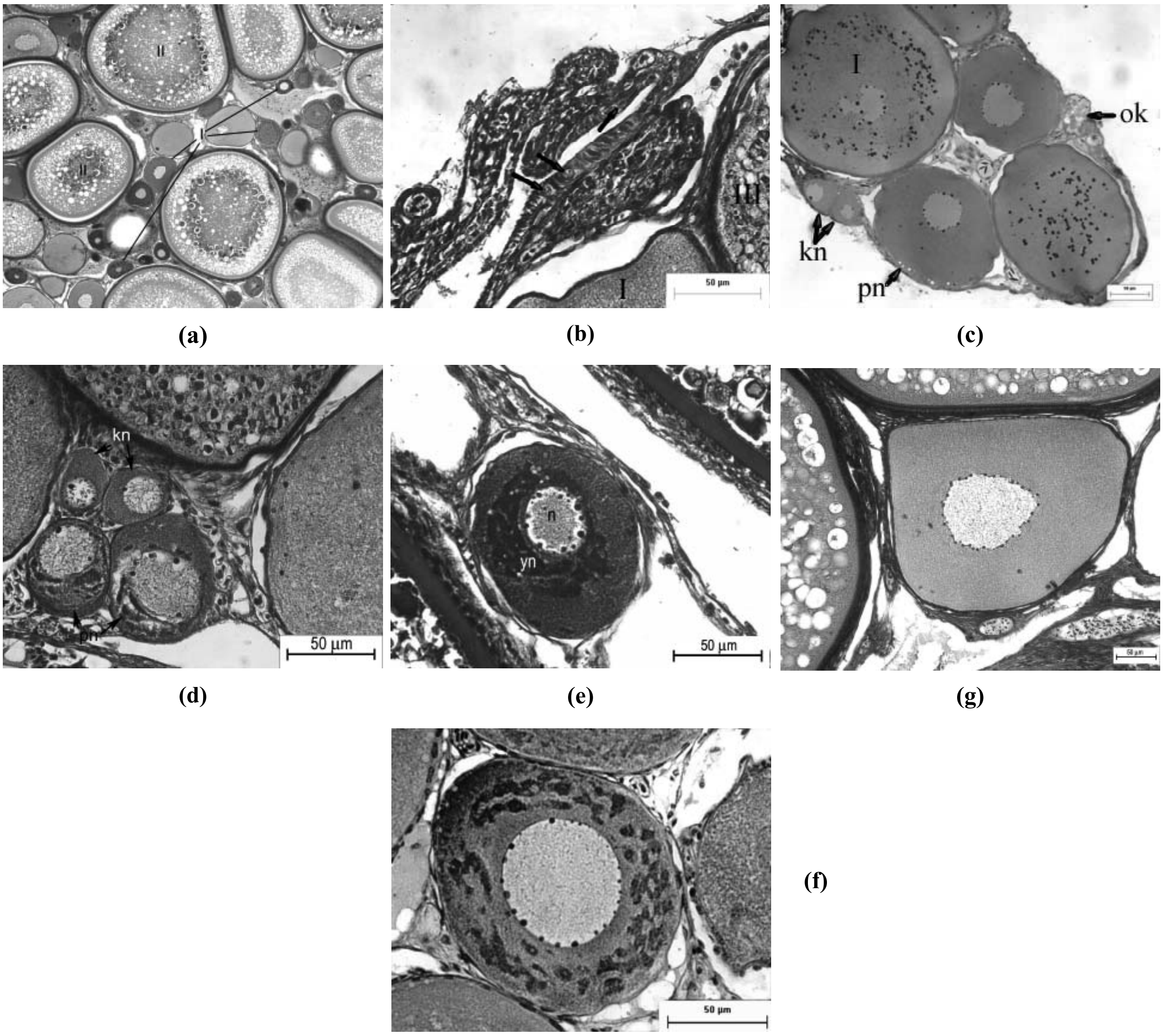

Figure 1. The development of the yolk nucleus (yn) in previtellogenic oocytes in trout. (a) General view of female gonad in different stage of oocytes. I, previtellogenic oocytes, II, vitellogenic oocytes. (b) Oogonia are seen (arrow) in vicinity of the germinal epithelium. (c,d) different oocytes in previtellogenic stage. ok-oocyte groupe, pn- perinucleolar oocyte, pn- perinucleolar oocytes with toluidin blue and heamalum. Yolk nucleus is seen near the perinucleolar cytoplasm. (e) In late perinucleolar oocyte is contain yolk nucleus (yn) and nucleus (n) and yolk nucleus which thread-like fragments expanding towards the cortical cytoplasmic area. (f) The cytoplasm of large previtellogenic oocyte gradually loses its basophilia and the yolk nucleus dispersed in to the oocyte. (g) Late perinucleolar stage of oocyte is seen which the yolk nucleus wholly disappeared.

DISCUSSION

In the present study, we examined the development of the yolk nucleus of previtellogenic oocytes in rainbow trout, *Oncorhynchus mykiss*. The basophilic cytoplasm was shown as important characteristic in early perinucleolar oocytes which were differentiated from oogonia and indicated the beginning of the oogenesis as described by Bielenska-Osuchowska [13]. In advanced perinucleolar oocytes, the yolk nucleus appears as a conspicuous, strongly basophilic structure that is initially located next to the nucleus [3]. The results of in this study were confirmed that the yolk nucleus is a spherical body that has a strong affinity for hematoxylin and toluidine blue and extensive networks of thread-like structures as previously shown in earlier studies [8, 9,10,12]. According to formerly histochemical studies in various animals, the granular components of the yolk nucleus may consist of RNA, proteins or both [2,3]. It is formed containing numerous Golgi bodies, endoplasmic reticulum (ER), mitochondria and granules in ultrastructural studies [3, 13]. These observations seem to be in agreement with the results in teleost and amphibian expressed by [3,5,7]. Kobayashi & Iwamatsu and Beams & Kessel stated that the exact role of the yolk nucleus is still not clear but it probably forms essential precursor substance (perhaps RNA) needed for oocyte growth and vitellogenesis [5,7]. No morphological significant changes were noted in the development of the yolk nucleus in *O.mykiss*.

Acknowledgments

This study was funded within the framework of a research program financed by Marmara University, Scientific Research Found (Project number, FEN-BSE-085/0512019).

REFERENCES

[1]. Droller MJ, Roth TF. 1966. An electron microscope study of yolk formation during oogenesis in *Lebistes reticulatus uppyi*. Cell Biology. 28: 209-232.

[2]. Azevedo, C. 1984. Development and ultrastructural autoradiographic studies of nucleolus-like bodies (nuages) in oocytes of viviparous teleost (*Xiphophorus helleri*). Cell & Tissue Research, 238: 121-128.

[3]. Guraya, SS. 1986. The cell and molecular biology of fish oogenesis. (Monographs in Developmental Biology, Vol 18) Basel,New York: Karger. 223 p.

[4]. Raven, CP. 1961. Oogenesis: the storage of developmental information. Oxford, Pergamon Press. 274 p.

[5]. Kobayashi H, Iwamatsu T. 2000. Development and fine structure of the yolk nucleus of previtellogenic oocytes in medaka *Oryzias latipes*. Development Growth Differantiation, 42:623-631.

[6]. Yamamoto, M. 1964. Electron microscopy of fish development. III Changes in ultrastructure of nucleus and cytoplasm of oocytes of goldfish during the first growth phase. Memoirs of the Faculty of Fisheries, Hokkaido University, 13:79-106.

[7]. Beams HW, Kessel RG. 1973. Oocytes structures and early vitellogenesis in the trout, *Salmo gairdneri*. American Journal of Anatomy, 136:105-122.

[8]. Kjesbu OS, Kryvi H. 1998. Oogenesis in cod, *Gadus morhua* L., studied by ligth and electron microscopy. Journal of Fish Biology, 34:735-746.

[9]. Muñoz M, Sàbat M, Mallol S, Casadevall M. 2002. Gonadal structure and gametogenesis of *Trigla lyra* (Pisces: Triglidae). Zoological Studies, 41:412-420.

[10]. Iwamatsu T, Nakashima S. 1996. Dynamic growth of oocytes of medaka, *Oryzias latipes*– Stages of oocytes development. Zoological Science, 5: 353-373.

[11]. Tyler CA, Sumpter JP. 1996. Oocyte growth and development in teleost, Reviews in Fish Biology and Fisheries. 6: 287-318.

[12]. Wallace RA, Selman K. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. American Zoology, 21:325-343.

[13]. Bielańska-Osuchowska, Z. 2006. Oogenesis in pig ovaries during the prenatal period: ultrastructure and morphometry. Reproductive Biology, 6 (2):161-193