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A Method for Karyotyping Zebrafish Oocytes

Guang Huang,[1,*] Ruizhen Li,[2,*] Jincai Xiao,[1] Tao Du,[1] and Hua Shao[1]

Abstract

In this study, zebrafish follicles containing stage III oocytes were isolated and incubated *in vitro* to allow oocyte maturation. A minicell containing the intact oocyte spindle was excised after proteolytic digestion of the micropyle area of the follicle. The minicell was subjected to hypotonic treatment in deionized water for 10 min, and then spread on glass slide for karyotyping. Dyads at metaphase II and sister chromatids at anaphase II are clearly shown. Also, cytochalasin B treatment inhibited chromosomal segregation. This simple and inexpensive chromosome preparation protocol could be readily applicable to other fish species.

Keywords: chromosome spread, oocyte, zebrafish

KARYOTYPING OOCYTE CAN DETECT changes in number of chromosomes, structure, and chromosome-attached proteins, which may be associated with infertility, developmental delay, and abnormalities of sexual differentiation and development. Zebrafish has already been an extensively studied vertebrate model organism in developmental biology, reproductive biology, and stem cells. No protocol for karyotyping zebrafish oocytes has yet been developed. The large volume of the gamete rich in yolk materials makes it difficult to apply chromosome spread methods developed in the much smaller mammalian oocytes.

In this study, zebrafish follicles at the size of 0.65 to 0.7 mm in diameter were isolated and cultured *in vitro* in oocyte maturation medium containing 90% Leibovitz L-15 medium (L-15) supplemented with 5 mg/mL bovine serum albumin (BSA) (V900933; Sigma), 1 μg/mL 17α,20β-dihydroxy-4-pregnen-3-one (DHP), pH 9.0, in a 35-mm culture dish at 28°C for 3 h.[1] The follicle-enclosed oocytes underwent germinal vesicle breakdown, visualized after ooplasmic clearing resulted from proteolytic cleavage of vitellogenin (Supplementary Fig. S1). At 4.5 h after the initiation of oocyte maturation, a glass needle with an inner diameter of 50 μm, filled with the crystallized protease, was placed over the micropyle region of the follicle. The setup was transferred into 90% Leibovitz L-15 medium (L-15) containing 5 mg/mL BSA and 5 μg/mL cytochalasin B (CCB, A606580; Sangon Biotech) and incubated at 25°C for 5 min. Positive pressure was applied to the needle using a microinjector. Three to five minutes later, the micropyle was digested, leaving a small hole with a diameter of 60 μm. The ooplasmic membrane under the micropyle started to extrude through the hole and formed a minicell including the spindle. The minicell was excised using a mouth-operated pipette, followed immediately by transferring into a 40 μL water droplet containing 1 mg/mL BSA (hypotonic solution) (Supplementary Fig. S2). Ten minutes later, the minicell was carefully transferred onto a glass slide prewet with

[1]Laboratory of Aquatic Animal Reproduction and Breeding, College of Fisheries, Guangdong Ocean University, Zhanjiang, China.
[2]Ottawa Hospital Research Institute, Ottawa, Canada.
*Cofirst authors.

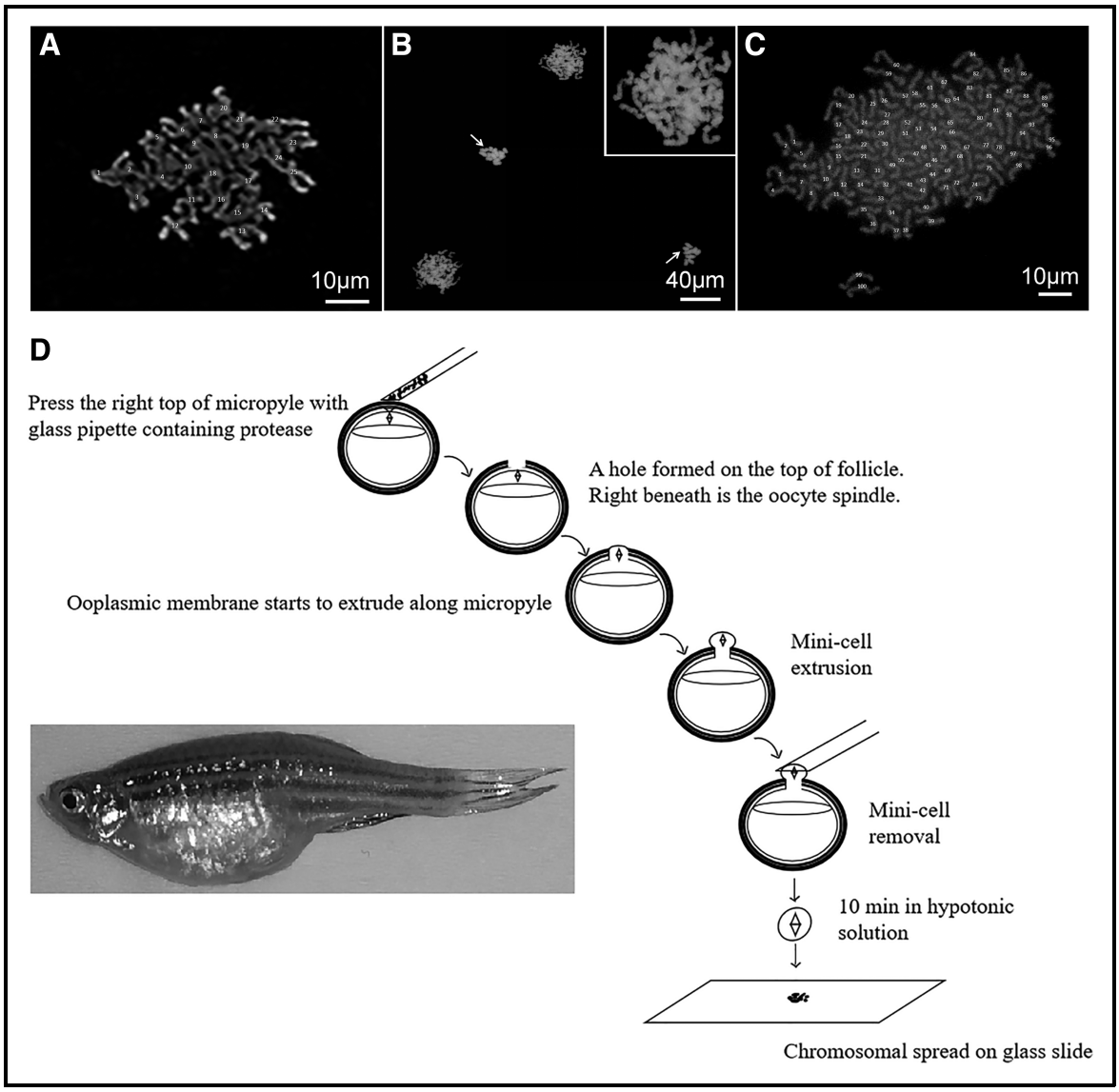

FIG. 1. Karyotyping zebrafish oocytes at metaphase II and anaphase II. **(A)** Typical image of zebrafish oocyte chromosomes with 25 dyads at metaphase II. Bar: 10 μm. **(B)** A minicell derived from anaphase II oocytes exhibits two sets of segregating sisters (the *arrows* show the polar body). Bar: 40 μm. **(C)** Anaphase II spread consists of 100 clearly identifiable chromosomes in the cytochalasin B-treated oocyte. Bar: 10 μm. **(D)** Schematic illustration of karyotyping the zebrafish oocyte.

fixative, 1% paraformaldehyde (PFA) (V900894; Sigma) in water, pH 9.2, containing 0.15% Triton X-100 and 3 mM DL-dithiothreitol (DTT) (V900830; Sigma) (see a detailed protocol in Supplementary Data). A total of 67% of minicells (179/266) karyotyped were able to show the identified chromosomes. Figure 1A shows the typical image of zebrafish oocyte chromosomes with 25 dyads at metaphase II. Minicells derived from anaphase II oocytes exhibit two sets of segregating sisters (Fig. 1B, the arrows show the polar body). The inset in Figure 1B depicts the enlarged picture with the clear sisters in anaphase II. If the mentioned procedure was repeated with the exception that 25 μg/mL cytochalasin B was added into the medium 1 h after the initiation of maturation, anaphase II spread consists of 100 clearly identifiable chromosomes, indicating failure of cytokinesis in both meiosis I and meiosis II. Figure 1D is a schematic illustration of karyotyping zebrafish oocytes.

To our knowledge, this is the first report of a method for karyotyping oocytes in zebrafish, and indeed in teleost. Two critical steps in this method are (1) we loaded crystallized protease in the microinjector needle to ensure highly localized protease activity in the area of micropyle and (2) we excised a minicell containing the spindle apparatus from the host oocyte. The minicell approach to karyotype large oocytes was

first developed by the authors in *Xenopus* oocytes.[2] This method could be used for other fish oocyte karyotyping. In addition to karyotyping, the samples can be further used to analyze chromosome-associated proteins, as we have done in our previous study.[2]

Authors' Contributions

H.S. conceived and designed the study. G.H., R.L., J.X., T.D., and H.S. conducted the experiments. H.S. and R.L. analyzed the data, and wrote the article with assistance from G.H. All authors read and approved the final article.

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Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Data
Supplementary Figure S1
Supplementary Figure S2

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Address correspondence to:
Hua Shao, PhD
Laboratory of Aquatic Animal Reproduction and Breeding
College of Fisheries
Guangdong Ocean University
Zhanjiang 524088
China

E-mail: hua_shao@hotmail.com