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Submission date: 09-Apr-2023 12:15PM (UTC+0700)
Submission ID: 2059375802
File name: EARLY_IN_VITRO_OOGENESIS_FULL_PAPER.pdf (213.31K)
Word count: 1631
Character count: 8501

EARLY IN VITRO OOGENESIS OF THE GIANT GOURAMY (*Osphronemus gouramy* Lac)

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Introduction

The giant gouramy is one of the indigenous species of Indonesia which had been cultured due to its high economical value. Many studies had been conducted to improve its production, yet more efforts need to be carried out especially those related to the reproductive biology of giant gouramy. One of the important aspects of reproduction is gametogenesis since this complicated process is the only way to produce oocyte and sperm and subsequently produce offspring.

Several aspects of gametogenesis in giant gouramy had been studied *in vivo*. Histological evaluation indicated the giant gouramy is an asynchronous batch spawner with several stages of oogenic development. The giant gouramy ovary has oogonia, two stages of previtelogenic oocyte (PV1 and PV2), six stages of vitelogenic oocyte (V1-6) and post vitelogenic oocyte (Wijayanti *et al*., 2009).

In vivo study, however, was hampered by the necessary to provide a large number of fish; therefore *in vitro* model was adopted in this study. *In vitro* oogenesis could be studied by culturing a particular type of oogenic cells (Salamat *et al*., 2010) or ovarian fragments (Miura *et al*., 2007). Miura *et al*. (2007) successfully culture the ovarian fragment of Japanese huchen (*Hucho perryi*) and common carp (*Cyprinus carpio*). In their experiment the ovarian fragments of 1 x 1 x 0.5 mm were cultured in agarosa gel placed in the middle of 24-*well plate* containing 1 mL medium.

This experiment was designed to study the early oogenesis in the giant gouramy by culturing oogonia and ovarian fragments as well as to determine the suitable culture condition to promote oogenesis.

Presented at the International Seminar: **Advance Technology on Veterinary and Life Science**. Faculty of Veterinary Science, Gajah Mada University Yogyakarta March 12, 2011

Material and Method

The giant gouramy oogonia and ovarian fragments were isolated from juveniles with body length of 16-24cm purchased from the local farmer. At the day of the experiment, the fish were decapitated; the ovaries were aseptically removed and placed in a glass dish containing DMEM (Caisson *Laboratories* Inc.) and 5% (v/v) pen/strep.

In the experiment 1, the ovaries were cut into 2mm pieces then were digested using 0.05% trypsin-EDTA (Gibco, Invitrogen) in DMEM for 30 minutes in an agitated water bath (37°C). The trypsin was inactivated by adding DMEM containing 5% (v/v) antibiotic and 10% (v/v) Foetal bovine serum (FBS). Following three consecutive washing in the inactivating medium, the cell suspension was passed on 50/30% percoll gradient and centrifuged at 1250 rpm for 20 minutes. The oogonia were recovered from the interface of percoll gradient. The viability of oogonia was evaluated using trypan blue. The oogonia (0,2 x 10^5/mL culture media/well) were grown as monolayer in 24-well plate (Nunc A/S, Denmark) in DMEM supplemented with 5% (v/v) pen/strep, 5% (v/v) L-glutamine and either giant gouramy serum (GS), FBS or their combination (each with four different concentrations v/v: 0, 5, 10, and 15 %).

In the experiment 2, the ovaries were cut into approximately $3mm^3$ pieces and were cultured as ovarian fragments under the same culture condition to those of oogonia. The oogonia and the ovarian fragments were cultured for 7 days with 5% CO_2, 31°C and saturated humidity.

The oogenic development was evaluated based on: 1) morphology and proliferation of oogonia in monolayer, 2) morphological and histological examination of the ovarian fragments. The most suitable culture condition was determined by analyzing the increase on oogonia and oocyte number using two ways ANOVA.

Results and Discussion

The viability of oogonia after percoll gradient centrifugation as assed by trypan blue exclusion test was 100%. The oogonia were survive and proliferated in culture. The proliferation rate was affected by the serum in dose dependent manner (Figure 1). The highest oogonia proliferation was found in culture supplemented with the GS (p<0.05). The GS used in this study contained 57.84 ng/mL estradiol 17β equivalent

to 2.89 – 8.67 ng/mL culture medium. Therefore it was suggested that the estradiol 17β stimulate oogonia to proliferate as the case in Japanese hutchen and common carp in which addition of estradiol 17β 0.1 ng/mL medium induced mitotic activity of oogonia (Miura *et al*., 2007)

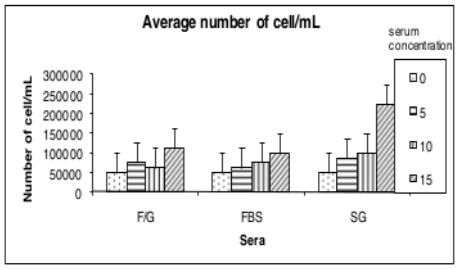

Figure 1. The number of giant gouramy oogonia cultured for 7 days in DMEM medium

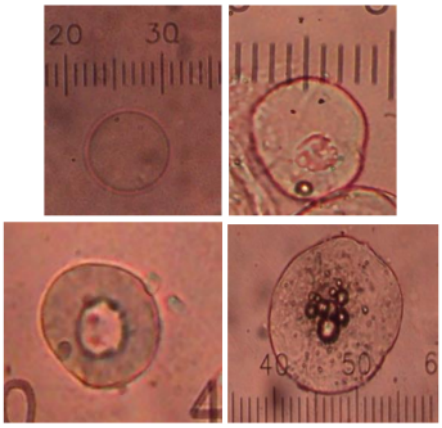

Figure 2. The morphology of oogenic cells of ovarian fragments cultured for 7 days in DMEM medium. A. oogonia, B. oocyte at PV-1 stage, C. oocyte at PV-2 stage, D. oocyte at V-1 stage

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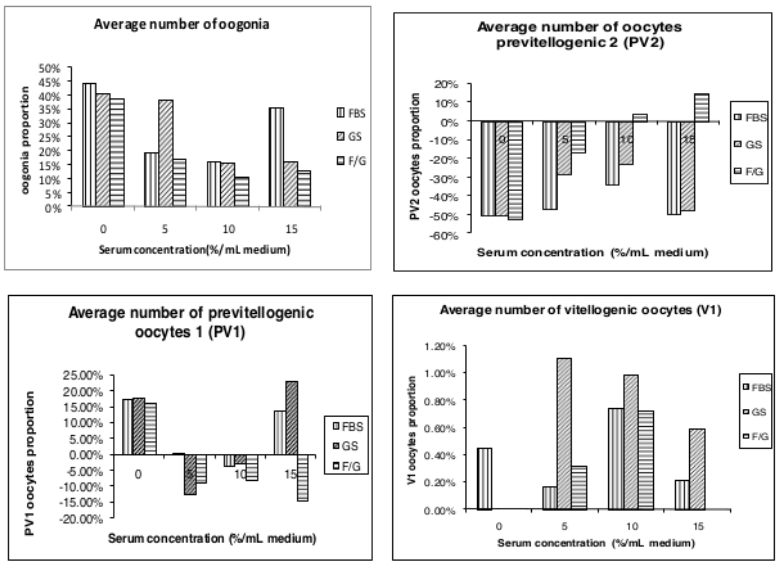

Figure 3. The proportion of oogonia, oocyte at PV-1, PV-2 and V-1in ovarian fragments cultured for 7 days in DMEM medium

The oogonia of the ovarian fragments proliferated but they responded differently to the isolated oogonia in which the presence of serum seems to reduce proliferation in dose dependent manner (Figure 3). The previtelogenic-1 (PV-1) and previtelogenic-2 (PV-2) oocyte proportion were reduced. It was suggested that the decreased of PV1 oocytes were due to oocyte atresia since the decreased of PV1 oocyte did not accompanied by the increased of PV2. Atresia in early oogenesis is very rare but it was reported to occur in few teleost species (Miranda *et al*., 1999). The decreased of PV2 was proposed due to recruitment into V1 stage as the V1 oocyte was not detected in the pre cultured ovary. The follicle of fish show a dynamic microenvironment, in which the somatic granulose/theca cells and the oocytes communicate with each other through a network or locally produced factors (reviewed by Hsueh *et al*., 2000; Hillier, 2001; Matzuk *et al*., 2002; Ge, 2005). This network facilitated signals from the somatic cells toward the oocyte, and also strengthens these signals through interactions with developmentally specific factors produced by the somatic cells and/or by the oocyte itself (Rosenfeld *et al*., 2007).

Conclusions

In conclusion, oogonia of giant gouramy survive and proliferated *in vitro* but the isolate oogonia responded differently to oogonia in the ovarian fragment to the presence serum. The giant gouramy serum promotes isolate oogonia proliferation and differentiation of PV2 oocyte into V1 oocyte.

Acknowledgement

This study was part of a research scheme "Hormonal Regulation on Gametogenesis in the Giant Gouramy" under coordination of GEW.

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