PRIMARY CULTURE OF GERM CELLS FROM THE JAPANESE OYSTER *Magallana gigas*

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Introduction

The Japanese oyster *M. gigas* is a key aquaculture species whose production is severely affected by infectious diseases that cause high mortality. Germ cells are valuable due to their self-renewal and gamete-forming capacity; however, protocols for their primary culture in bivalves are limited.

Objective

To establish a protocol for the isolation and primary culture of germ cells from the japanese oyster *M. gigas*.

Methodology

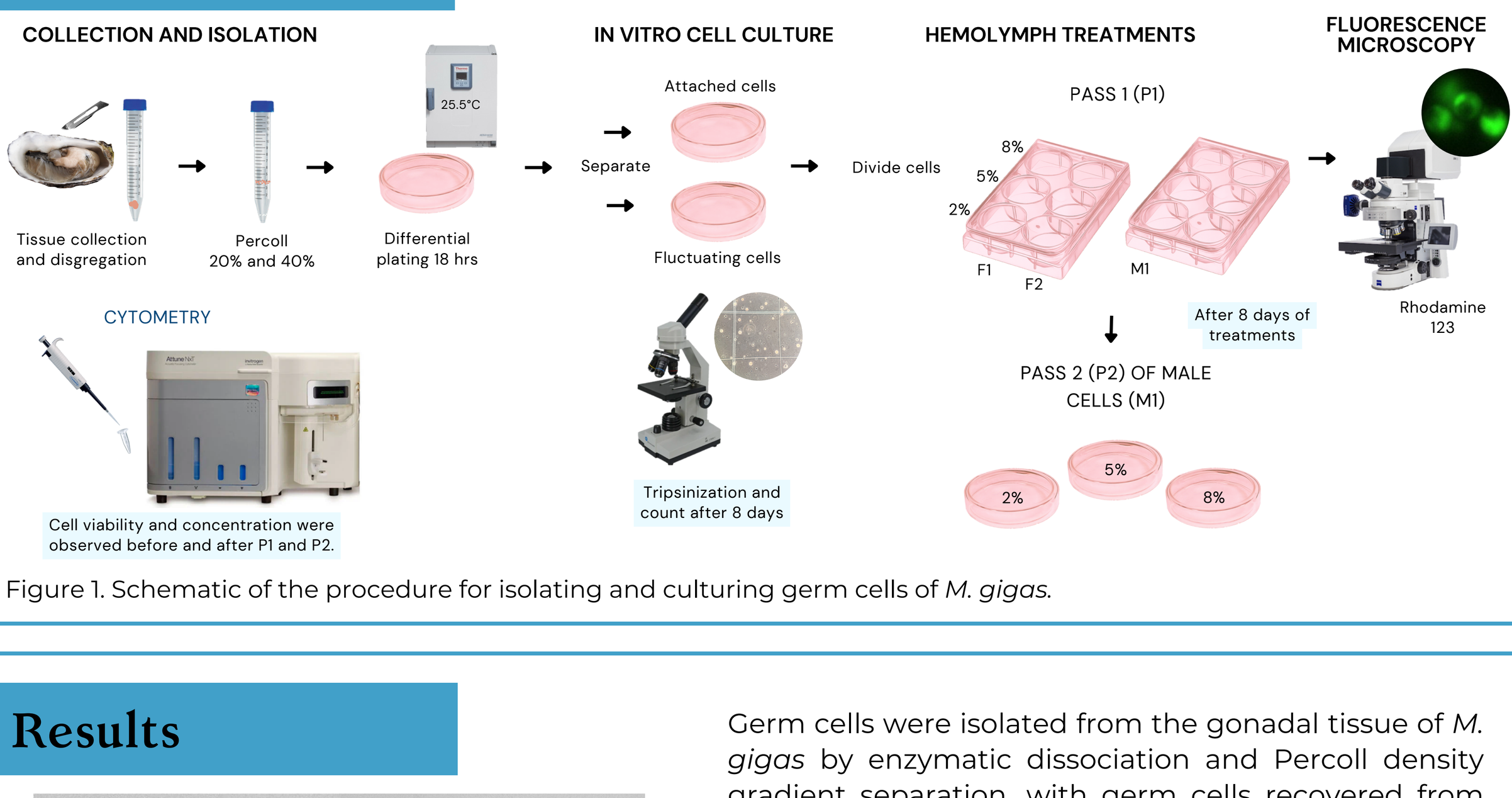

Figure 1. Schematic of the procedure for isolating and culturing germ cells of *M. gigas*.

Results

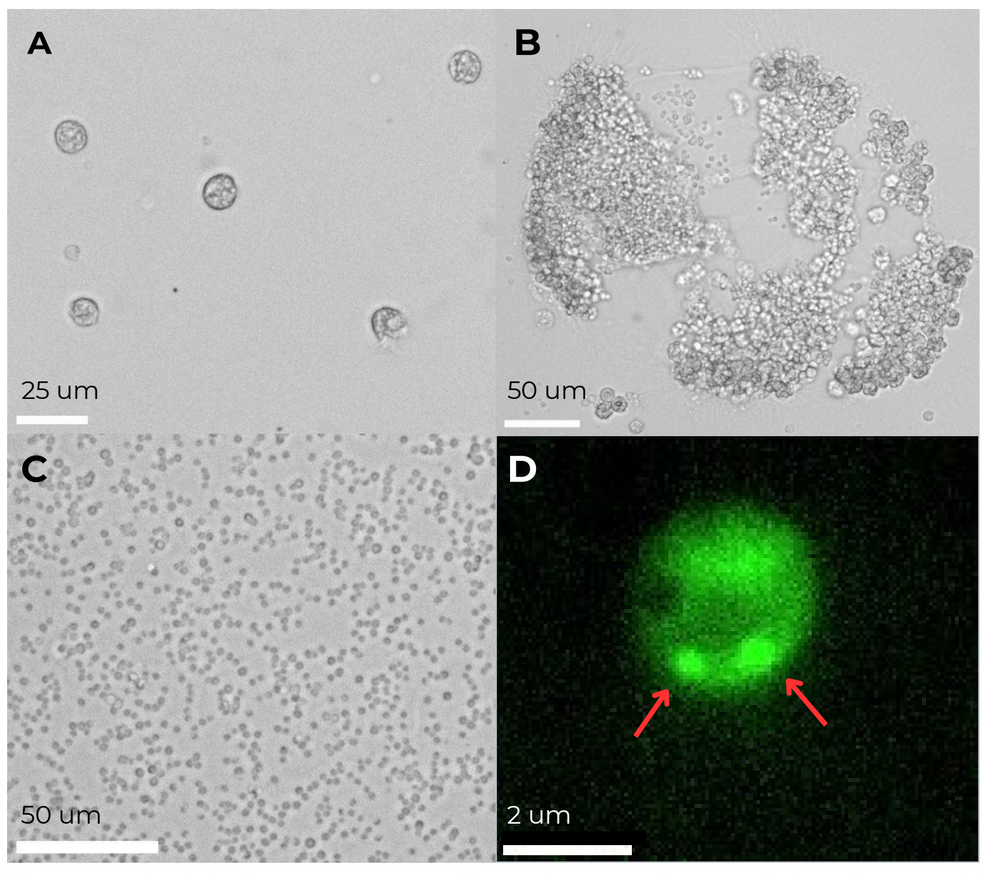

Figure 2. Male germ cell culture from *M. gigas*: (A) seeding (Day 0); (B) circular cell aggregates (Day 4); (C) small-cell predominance (Day 8); (D) Rhodamine 123–stained motile cell showing mitochondrial labeling (red arrows).

Germ cells were isolated from the gonadal tissue of *M. gigas* by enzymatic dissociation and Percoll density gradient separation, with germ cells recovered from the 20% Percoll layer. Of the three individuals used, two were presumptively female and one male; female-derived cultures showed limited survival and no proliferation.

Male-derived germ cells were maintained in primary culture and, after 8 days, a first passage (P1) was performed by seeding 1 × 10^5 cells per well in six-well plates with hemolymph treatments (2%, 5%, and 8%). At the end of P1, cultures reached 3.27 × 10^6 ± 6.35 × 10^5 cells, showing proliferation and possible differentiation, including transient circular cell aggregates and the appearance of small motile cells.

A second passage (P2) was performed in 60 mm plates using 8 × 10^5 cells per plate, resulting in rapid cell reorganization within 24 h and extensive growth with increased motility by day 3. Mitochondrial activity in motile cells was observed by Rhodamine 123 staining.

Conclusions

The culture conditions used allowed the maintenance of putative male germ cells for up to 20 days, with cell proliferation and the appearance of motile cells consistent with spermatogenic differentiation. Further studies are required to confirm cell identity and evaluate their fertilization capacity.

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