

Fertilization and Culture of Embryos

Similar methods are used to fertilize *Phallusia* and *Ciona* eggs.

- For *Phallusia* large cultures: to fertilize eggs in a synchronous manner:
 - Add 2 mL of activated sperm to 10 mL of dechorionated eggs in SW.
 - Mix well.
 - You can judge if synchronous fertilization has been achieved by observing egg shape change: 80% of the eggs should lose their roundness and become pear shaped within 5 min of sperm addition.

- For *Phallusia* small cultures:
 - Add approximately 10 μ l of activated sperm mixture near some eggs in a Petri dish containing about 5 ml TAPS filtered sea water.
 - Once eggs are fertilized, wash extensively with SW and culture the embryos at 18–22°C, either spread in a monolayer (but not touching) in appropriately sized GF-coated Petri dishes or keep as a suspension in a GF-coated beaker equipped with a paddle rotating at 50 rpm. For *Phallusia* maintained around 20°C, first cleavage takes place about 50 min after fertilization, gastrulation after 3h and the embryo develops into a tadpole in 12h.

- For *Ciona*: to fertilize eggs in a synchronous manner:
 - Add 500 μ L of sperm activated with alkaline SW to 10 mL of dechorionated eggs in SW.
 - Mix well. With an appropriate stereo-microscope, one can see active sperm moving around eggs. Eggs start to spin and, when fertilized, become pear shaped.
 - After 10 min incubation, transfer eggs to another agarose coated Petri dish containing SW and culture them at 16–18°C. Information on *Ciona* embryonic stages, morphology and cell lineages can be found on FABA (<http://chordate.bpni.bio.keio.ac.jp/faba/1.4/top.html>) and ANISEED databases (<https://www.aniseed.cnrs.fr/aniseed/>).