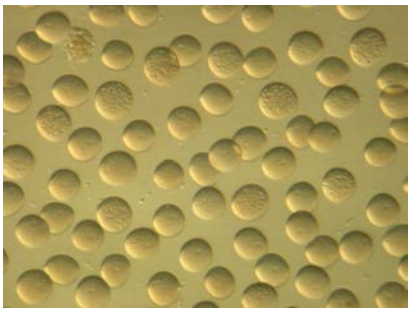
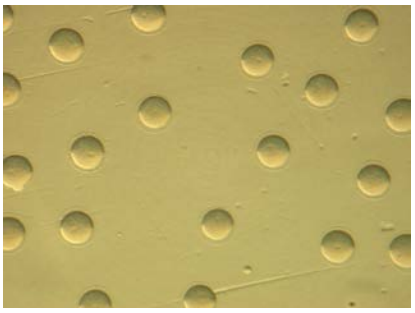
*F*ertilization *in* Paracentrotus lividus

1. Gametes from adult sea urchins can be obtained following the procedure described in the **ASSEMBLE-JRA1-Protocol-16.**
2. Transfer the suspension of eggs into a larger vessel (beaker). Allow the eggs to settle by gravity. Wash gently the eggs, removing the covering sea water and replacing it with fresh FSW; repeat.
3. Prepare a sperm dilution adding 1 drop of dry sperm in a test tube filled with 5 ml of FSW. Mix well to make an uniform suspension of sperm.
4. To inseminate eggs, add some drops of this sperm dilution to the eggs suspension and mix gently in order to achieve a synchronous and complete fertilization. Allow the eggs settle by gravity.
5. In order to remove excess sperm, wash gently the eggs, removing the covering seawater and replacing it with fresh FSW; repeat.
6. Transfer the eggs (actually zygotes) into Petri dishes, gently pouring the suspension from the beaker to the dish or using a pipet (use care to pre-wet the pipet). Place no more than a single layer of eggs resting on the bottom and fill the dish with fresh FSW.
7. Observe fertilized eggs placing the dish under the binocular microscope. The fertilization envelope (FE) is well visible and can serve as an indicator of successful fertilization (the elevation of the FE occurs within 30 s of insemination, from the point of sperm entry, and is completed very quickly). The FE is to be observed for the majority of the eggs; if less than the 90-95% fertilization is occurred, leave the dish out and start again (by using another female).



unfertilized eggs



fertilized eggs

Paola Cirino, Alfonso Toscano
Stazione Zoologica Anton Dohrn, Italy

Animals: Adult sea urchins (test diameter 5-7cm).
Apparatus: filtered seawater 0,22µ (FSW), Pasteur pipets and pipet bulbs, glass beakers, Petri dishes, eppendorf tubes, test tube.
A binocular microscope.
Parameters: T 20 °C

Additional information:

1. Spawned eggs can be checked for quality before the insemination observing them microscopically (at 10 x). The eggs should be of a uniform size with no germinal vesicle visible (mature sea urchins have completed meiosis).

2. If possible, do not mix eggs from different individuals.

3. Do not allow the eggs to sit overly long; the suspension can be stored for up to 5-6 hours in a refrigerator.

4. Dry sperm can be stored in a refrigerator for later use (for up to 24 hours). The diluted sperm can be used for only about 15minutes (to a maximum of 30) before they became inactive.

5. Avoid excess of sperm, which can lead into polyspermy and endangers development.

Contact e-mail: paola.cirino@szn.it