



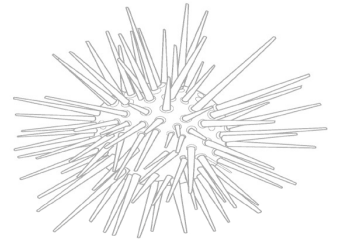
**EXPERIMENT**

**In vitro fertilization of Paracentrotus lividus through the stimulation with potassium chloride.**

"SCIENTISTS"

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CATEGORY:  
 Animal Biology



**What do we pretend to do?**

Study the IVF (In Vitro Fertilization) carrying out an animal experiment. Obtain gametes from a sea urchin stimulated by potassium chloride. Study the process of fertilization and follow the phases of the embryonic development until the larva stage. Know how some chemicals can affect in the spermatozoid mobility and therefore hinder the process fertilization.

**NECESERY MATERIALS**

Samples of alive Paracentrotus Lívidus.

**Products**

Sea Water, vinegar, Potassium Chloride, Ammonia, Detergent.

**Materials**

Beakers, Petri dish, Syringe, Pipette, Latex gloves, Optical Microscope, Digital camera.

**THE EXPERIMENT STEP BY STEP**

All the tools and materials to be used must be sterilized as well as the sea water. To sterilize the sea water, we will have to boil it at 100° Celsius during 45 minutes.

**Getting the sea urchin**

Keep the sea urchin alive before the experiment: Since the sea urchins are gotten, we will have to place them on a container with sea water. Due to the difficulty of the task, we will try to do the experiment as soon as possible to keep the sea urchin alive. Get ready a Potassium Chloride dilution in a concentration of 0.5 M.

**Extracting the gametes**

First of all, face the sea urchin with the oral zone looking up, and then inject (with the syringe) 5 ml of the Potassium Chloride dilution 0,5M. Secondly, shake gently the sea urchin to mix properly the Potassium Chloride dilution. In this stage, wait for the release of the gametes as we do not know the sea urchin sexuality. Take into account, the colour of the ovocytes is red and for the spermatozoids is white. The gametes ejaculation will be done through the *opposite the mouth* side. After that, place the male sea urchin in a Petri capsule without sea water and the female sea urchin will be placed in a beaker with sea water. Finally, wait for the sea urchin to release all the gametes.

**Gametes observation**

It must be taken into account that spermatozoids come in a very high concentration, so they must be diluted to be observed.

White liquid secreted by male sea urchin must be taken with a pasteur pipette to deposit one single drop in a petri dish. Add 10 sea water drops and then mix all the components. One drop of this solution must be placed in a microscope slide, in order to be observed. This action must be repeated using the red liquid from female sea urchin. The final step of this part of the experiment is taking photographs of microscope slide to keep a graphic sample of gametes observation.

**Fertilization**

Most of the sea organisms have an external fertilization. In other words, gametes are released to the environment and the cells joint takes place at the sea. This external fertilization makes the species able to recognize gametes from its own specie, in this case, ovocytes (oocytes) have developed a gelatine layer able to recognize and interact with sea urchins spermatozoids exclusively.

To make fertilization easier, wait for the decantation of ovules at the bottom of a beaker, discarding then the supernatant (yellow water) carefully and finally adding clean sea water. This procedure needs to be performed twice in order to remove the gelatine layer. Three samples of ovules (red liquid) must be extracted with a pasteur pipette and deposited in a beaker. Then, 150 ml of sea water should be added. After that, take a spermatozoids sample with a different pipette to join it inside the beaker with the ovules. Shake it softly and wait one minute to finally extract a drop of this solution. One more time, this solution must be placed in a microscope slide, in order to be observed and photographed.

**Stages during the development of the embryo**

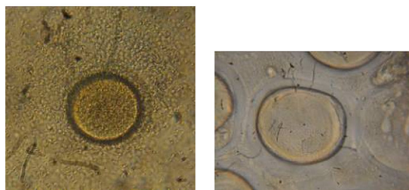
Keep the beaker that contains fertilized ovocytes in a secure place at room temperature. Following the timings shown below\*, take a sample off the beaker and deposit it in a microscope slide, to observe it and take final photos of the result.

*Stage	Time
First division	1,5 - 2 hours
Second division	2,5 - 3 hours
Blastula	24 hours
Gastula	2 days
Larva Pluteus	4 - 5 dias

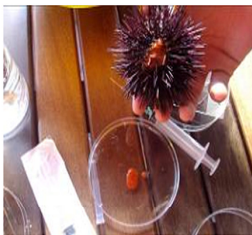
**Spermatozoids mobility at several environments**

Add 1 ml. of vinegar (ACID PH) to an spermatozoids solution, it will react with changes in its mobility.

The main difficulty of this research project is to get alive samples of sea urchins during the reproduction time (from January to March). Sea urchin fishing takes place at Algeciras's Bay (Strait of Gibraltar), where it is restricted.



FERTILIZATION OVOCYTES



TAKING THE OVUM OUT OF THE FEMALE SEA URCHIN

**FOR FURTHER INFORMATION**

<http://www.fivsalesianos.blogspot.com>