Sea Urchin Fertilization and Embryology Lab

Timing
The initial lab exercise requires a minimum of one 1hr and 30-minute period. You will want to leave ample time to document your methodologies and make all of your observations. Please recall that this laboratory will require you to prepare a lab report. This experiment requires some second day activities which require about 30 minutes and to analyze observations.

Background
Studies of sea urchins provide information on fertilization and development that apply to all organisms from jellies to humans. These eggs, then, provide a model embryo for understanding development in all forms. It is important to observe early embryonic development because it is during this time that the patterns of development of the organism originate. Sea urchin gametes are the same size as human gametes and development is very similar through the gastrula stage. Both sea urchins and humans are deuterostomes, meaning that patterns of cleavage are radial and the mouth arises at a site distant from the site of gastrulation. The name deuterostomes means second mouth. In contrast protostomes follow a spiral cleavage pattern and the mouth forms near the blastopore, the origin of gastrula. Notice the relationship between humans and sea urchins, which make sea urchin a good model to study embryological problems such as fetal alcohol syndrome, toxicity’s of any form etc.

Methodology
IMPORTANT - to maximize observations the first day, cultures of embryos should be started directly at the start of the lab.

Materials
• 0.5M potassium chloride solution (3.73g of KCL in 100ml of distilled water)
• sea water (or Instant Ocean, or artificial sea water)
• fertile sea urchins, see also keeping adults
• 1-5cc syringe
• plastic or glass pasteur pipets or eyedroppers
• small tubes to store sperm (micro-centrifuge tubes work great) (1-5ml)
• beakers a little smaller in diameter than the diameter of female urchins
• microscopes (at least 10x and 40x objectives)

Procedure
A. Spawning Animals:
To insure that there is at least one male and one female sea urchin it is best to have approximately 10 sea urchins on hand. (If sea urchins are being shared with multiple classes then save eggs and sperm and inject only one sea urchin for demonstration purposes.)

Inject 2 ml 0.5 M potassium chloride into the sea urchin by inserting the needles of the syringes
into the peristomial membrane surrounding the mouth (the only soft surface of the body), turn the animal upside and allow it to sit on a beaker (tripous, plastic) (Males: sperm are a milky white color and should be collected dry; Females: eggs are yellow/orange and should be collected into a full beaker of seawater)

- After injection, place the male urchin mouth side down onto a small dish, like a petri dish. (NOT UNDER WATER).
- In a minute or two you will start to see the white sperm appearing on the surface of the urchin.
- Collect the sperm "dry" in a pipet or eyedropper and place it into a small test tube. [Seawater "activates" the sperm reducing their life span from days to minutes! Keep concentrated until just before use.]
- After you have collected all that you can, place the tube into a refrigerator (not freezer) or an ice bucket with ice. Sperm will keep at 4 C for 2-5 days.
- After injection place a female urchin mouth up onto a beaker filled with seawater. Eggs will not she unless the female is in contact with seawater, so be sure to fill the beaker nearly up to the rim. The beaker should be slightly larger than the diameter of the sea urchin.
- The eggs will shed into the seawater and collected at the bottom of the beaker. This can take between 10-30 minutes and should be followed up with a minimum of two rinses of the eggs.
- Be sure to store eggs at the same temperature as the sea urchins (room temperature).

B. Fertilization:
Appropriate sperm dilution is essential for successful fertilization. Too low a sperm concentration results in fewer eggs being fertilized (safer than too high). Too high a sperm concentration will result in polyspermy (more than one sperm per egg) and abnormal development of the embryo. There are a number of built in mechanisms to help prevent polyspermy, but they are not full proof and at very high sperm concentrations they can be overwhelmed.

see animations Just right sperm, Too many sperm, Too few sperm

Sperm Dilution:
A sperm dilution series is a useful way of showing just how few sperm are needed to fertilize eggs. For comparison purposes, it is important to use the same sperm concentration for each fertilization. Use a pasteur pipet to make the initial dilution. Use a dilution series of 1 drop in 1-10 ml. Determine which dilution gives you optimal fertilization. Always use this quantity of sperm as the starting point for dilutions.

Eggs:
1. Pour the egg suspension gathered from spawning into a 500 ml beaker.
2. Fill to 100ml with seawater, and let the eggs settle. Without centrifugation the eggs will swell (egg jelly swells) to about 2x their actual size.
3. Dilute to a single layer of eggs.
4. Alternatively suspend the eggs in a large flask no greater than 1 cm in depth to insure adequate exchange of gases. (concentrations as high as 1% can be used in this way to make it easier for students to find the eggs in early stages of development) Eggs can be re-concentrated to allow for easier viewing by letting them settle in a beaker or test tube and pouring off the excess seawater. To fertilize:
1. Place a drop of egg suspension on a glass depression slide under their microscope (a less that 1% egg concentration will allow students to see individual eggs).
2. Focus on the eggs.
3. Next, add a small drop of stock sperm suspension to the drop of eggs and add a cover slip. Focus on the eggs and watch the fertilization membranes rise.

Development:
To have developing embryos to observe the next day, fertilize the single layer of eggs with the optimal sperm concentration as determined above embryos to develop either on stir plates (no heat).

**Observations (Embryology report, the report is due on April 24, 2013 during lab)**

1. What would happen if the sperm could not get into the egg? Alternatively, what would happen if the fertilization membrane did not form? (2 pt)
2. Adult echinoids are pentameric or pentaradial in their symmetry. What is the symmetry of the larvae? What does that mean for the process of metamorphosis from larvae (pluteus) to adult echinoid? (2 pts)
3. Sea urchin larvae are used in toxicological studies as a model for human development yet when you look at adult urchins and humans they are quite different. How may the use of these larvae be justified as human model organisms? What are advantages of using a sea urchin model in toxicological studies? (2 pts)
4. Why do sea urchins have external fertilization? What are the adaptive advantages of external fertilization? What are the adaptive advantages of internal fertilization?
5. What would happen after 24 hrs if you were to dissect some of the two cell stages? Why? (2 pts)