

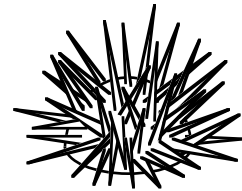
SEA URCHIN FERTILIZATION AND DEVELOPMENT

Introduction

A sexually reproducing organism begins as a zygote (the cell produced when an ovum is fertilized by a sperm). The events that occur next are quite similar throughout the animal kingdom. Three principal phases can be recognized.

1. **Cleavage:** Rapid nuclear and cytoplasmic divisions (without cell growth) partition the zygote into many smaller cells (blastomeres).
2. **Gastrulation:** Cell migrations result in the formation two (diploblastic) or three (triploblastic) primary germ layers. This brings into contact cells from different regions of the embryo, allowing important cell-cell interactions to occur.
3. **Organogenesis:** The primary germ layers differentiate into tissues and organs. Details vary, but if three germ layers are present, each gives rise to the same types of structures in all organisms.
 - a. **Ectoderm** forms the outer covering of the organism, the nervous system and special sensory structures.
 - b. **Mesoderm** forms muscles, skeletal components, blood, connective tissue and the reproductive and excretory organs.
 - c. **Endoderm** forms the lining of the alimentary canal, digestive glands, and respiratory structures (when present).

Since you chose this topic for your individual project, you must be interested in acquainting yourself with fertilization and early development in an animal—the sea urchin. The most exciting way to do this is by watching it happen in living urchins. To give you a better sense of the cell migrations and the dynamics of early development, there is microcinematography—time lapse movies taken through the microscope.



Of all the "spiny skins" (echinoderms), none lives up to its name so well as the sea urchin. This interesting marine animal occurs in tremendous numbers on rocky shores, in sandy areas and, occasionally, on mud-bottomed areas. The sexes are separate and fertilization is external. These animals release their gametes (millions of eggs by each female and billions of sperm by each male) into the sea where fertilization and development occur. Because they develop externally and are nearly transparent, sea urchin embryos are ideal for observing the early stages of development.

When fertilization occurs, the chromosome number (halved during the formation of the gametes) is restored and the subsequent nuclear divisions are mitotic. Keep this in mind as you observe the early cleavage stages—all the phases of mitosis take place each time a cell divides.

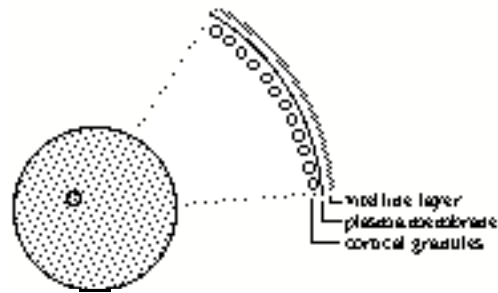
Sea Urchin Sperm



Except for a few differences in size and shape, the sperm cells of most animals (including sea urchins) are essentially similar. Perhaps this similarity stems from their common mission, which is to move about and penetrate the egg. Each sperm is composed of a head, mid-piece and tail. The head contains chromatin (chromosomal DNA and its related proteins) and an enzyme-laden acrosome, which helps the sperm penetrate the egg. The midpiece contains mitochondria along with the bases of the microtubules that make up the flagellum of the tail. The mitochondria generate the ATP needed to sustain the action of the flagellum.

Sea Urchin Egg

The sea urchin egg completes meiosis within the ovary (in some animals, meiosis is completed only after fertilization occurs). The unfertilized egg is a single, spherical cell about $90\ \mu\text{m}$ in diameter. Like any cell, it contains a nucleus and cytoplasm. Since the egg has completed meiosis, the egg is haploid. The difference in the size of the egg compared to a normal diploid cell is entirely due to the cytoplasm, which is filled with ribosomes, mRNAs, mitochondria, etc., along with yolk, which is used as a source of energy for the embryo until it can feed on its own. Outside the plasma membrane is a special, semi-permeable vitelline membrane, or “vitelline layer.” Directly under the plasma membrane is a compact layer of special granules about $1\ \mu\text{m}$ in diameter called “cortical granules.”

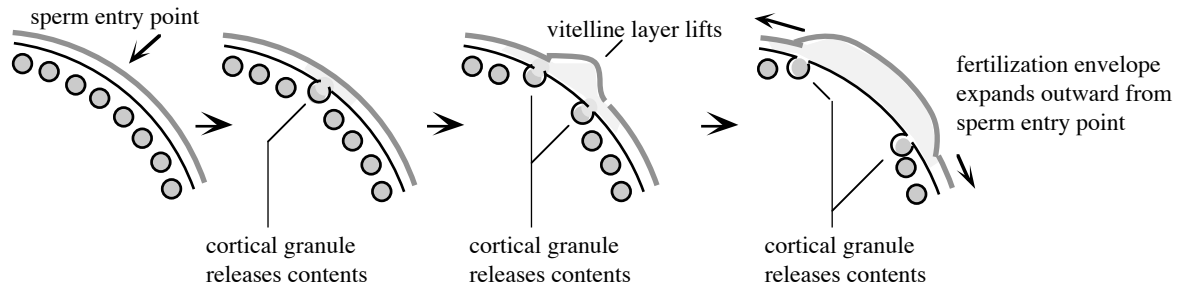


The Fertilization Event

Why, when millions of eggs and billions of sperm are present, does only one sperm fertilize one egg? Why is it important that only one sperm fertilize an egg? In sea urchins, if more than one sperm—each with the haploid complement of chromosomes—should enter the egg (polyspermy), too many chromosomes would be present and development would be abnormal. Furthermore, each sperm contributes a centriole, so the first mitosis of a di-spermic egg would have three spindle poles. When this happens (which it does on rare occasions) the chromosomes cannot segregate properly! There are two types of "blocks" to prevent more than one sperm from fertilizing an egg.

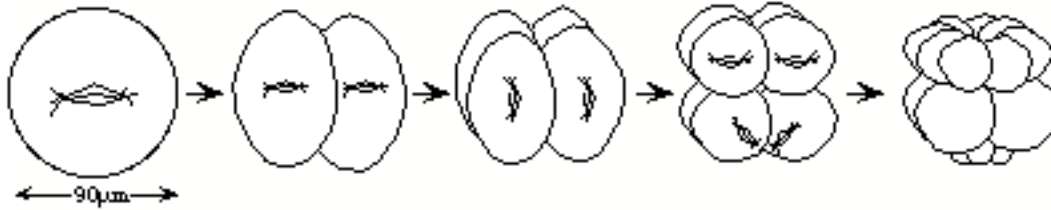
A "fast block" to polyspermy is induced within 1 second after contact of the fertilizing sperm and persists for about 60 seconds. It is an electrical effect: the plasma membranes of cells usually maintain an electrical potential of about 70mV by pumping ions from one side to the other. On fertilization, the membrane is “depolarized,” resulting in the transient dissipation of this electrical difference. This change helps prevent a second sperm from entering the egg.

The contact of the fertilizing sperm is followed, after about 20 seconds, by a sequence of events called the "slow block." Sperm entry induces the membranes of the cortical granules nearest the sperm entry point to fuse with the plasma membrane and discharge their contents into the space between it and the vitelline layer. The fusion of one cortical granule induces the neighboring cortical granules to fuse, also spilling their contents into the perivitelline space. The "cortical reaction" thus, begins at the point of sperm entry and spreads in all directions to the opposite pole of the egg.



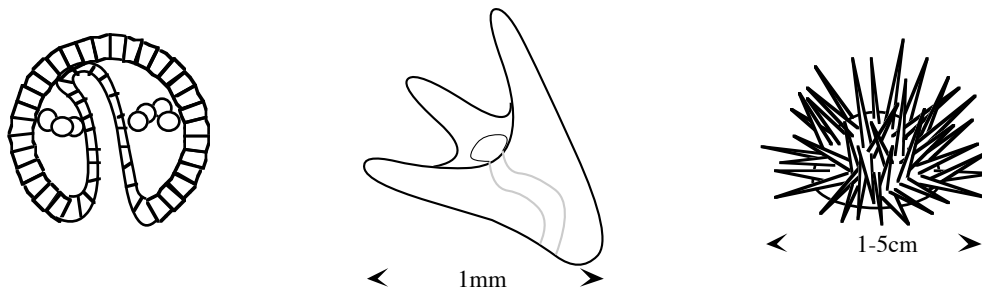
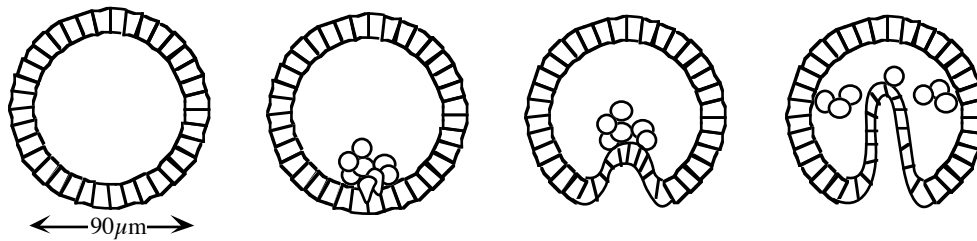
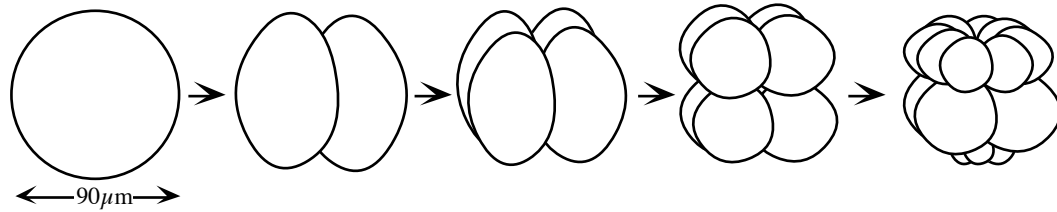
Rupture of the cortical granules releases a mixture of enzymes, structural proteins and mucopolysaccharides into the perivitelline space. One of the enzymes modifies the vitelline layer so that extra sperm are detached. Another breaks down the proteins that bind the vitelline layer to the plasma membrane. The mucopolysaccharides cause the diffusion of water into the perivitelline space, elevating the vitelline layer. The vitelline layer receives structural proteins from the discharged granules, transforming it into the "fertilization envelope." Other proteins associate with the fused plasma membrane-cortical granule membrane, to form the "hyaline layer." Sperm that had bound to the egg surface, but had not penetrated before rupture of the cortical granules, now begin to detach in a wave that follows the progress of this breakdown. Detachment of all but the successful sperm is complete 60 seconds after fertilization.

After fertilization, the zygote begins cleavage. In typical sea urchins, cleavage occurs in a stereotyped way, producing progressively smaller cells (blastomeres). In the early cleavages, the orientation of the mitotic spindle changes in a specific manner, resulting in divisions that are either vertical or horizontal. [In some animals, the planes of cell division give a spiral cleavage pattern. Snails with right-handed spiral cleavages show a right-handed spiral shell; snails with left-handed spiral cleavages show a left-handed spiral shell. The asymmetry that determines adult morphology is apparently expressed even in the earliest stages of embryogenesis.] At the fourth cleavage, something unusual happens—the mitotic spindle of the lower four cells does not establish itself in the center of the cell; instead, it is off to one side. The resulting cell division produces a large cell and a small cell, instead of two equal cells. Both cells, of course, obtain equivalent sets of chromosomes, but one receives more cytoplasm than the other. While unequal cell division is highly unusual for normal mitotic cells, it is common in the early cleavages of embryos. After this division, the embryo is easily seen to be composed of three layers of cells: the top tier of medium-sized cells (mesomeres) produced by normal cell division, and a middle tier of large cells (macromeres) and a bottom tier of small cells (micromeres) that derive from the unequal cleavages.



By the time cleavage is complete, the embryo (the blastula) is a hollow ball of cells. After formation of the blastula, two things happen. One, which makes observation more difficult, is that the embryo “hatches” from the fertilization envelope. The cells have cilia on them, allowing the embryos to swim. You will find that you have to move your microscope slide around to follow them! The second, and fundamentally more important thing that happens is that the cell migrations of gastrulation begin. These are difficult to visualize from descriptions; the result is somewhat like sticking a finger into a balloon until the two sides meet. It begins with the descendants of the micromeres crawling individually into the hollow cavity inside the blastula (blastocoel) to become mesenchyme cells. Then, cells on the sides of the embryo migrate down, around, and up to force the ingression of the primitive gut, the archenteron. Eventually, the archenteron nears the opposite side of the blastula, bends, and contacts the side of the embryo, where the cell-cell interactions induce the formation of the mouth.

While the formation of the gut is proceeding, the mesenchyme cells move about inside the blastocoel, eventually to line up in positions where they will begin to secrete the calcium-phosphate skeleton of the sea urchin—the “spicules.” As the skeleton grows, the embryo changes shape into the larval form, called a “pluteus.”



What forms after all this doesn't look anything like the typical sea urchin with which we are familiar. Instead, it is a free-swimming pluteus larva that forms part of the ocean plankton until it grows enough in size to be ready to settle to the ocean floor and undergo metamorphosis. Metamorphosis occurs through the growth of a small adult urchin on one side of the gut of the pluteus; the miniature adult hatches, and crawls away from the cast-off pluteus skeleton.

When you come to the lab, you will have at least two goals. The first is to watch fertilization happen under the microscope, in order to observe what happens to the sperm, and see how it triggers the lifting of the fertilization envelope. Remember that this sequence of events is almost identical to your own start to life. The second is to identify, and place in the correct order, a series of embryos that have developed from eggs fertilized at various times over the previous several days. To do this, you will need to identify a variety of distinguishing characteristics that you can use to recognize different developmental stages.

Fertilization in the Lab

Place 2-3 drops of ova (eggs) in the well of a depression slide. Focus under the low power objective of your microscope to observe the intact, unfertilized eggs. Then add a drop of sea urchin sperm to add to the eggs. Immediately after the two kinds of gametes are mixed, gently place a cover slip over the well and immediately observe under the microscope. Look for live sperm swimming and attaching themselves to the eggs.

Motion picture analyses of sea urchin fertilization reveal that after sperm bind to the egg, they rotate about the axis of the binding point for 15 to 20 seconds after the initial attachment. You may be able to see this attachment and rotation.

You can identify the first sperm to penetrate because it becomes immobile and begins to enter the egg. The remaining sperm continue to move until they are detached (as described above). Look carefully for the one sperm that is beginning to penetrate an egg. When you have found it, you will soon see a fertilization membrane form at that point and spread outward over the entire egg.

Sea Urchin Embryogenesis

You will need to follow the developing embryos for several days, identifying, drawing and recording on tape the successive stages the animal undergoes. [Note: some organisms, such as the South African Clawed Frog, *Xenopus laevis*, have been assigned numbers for a variety of morphologically-defined stages.] Here, we are interested only in general identities—2-celled stage, 4-cell stage, 16-cell stage to blastula, early gastrula, mid-gastrula, late gastrula, early pluteus, etc.]

Look carefully at each stage, and try to determine the most striking characteristics of embryos at this developmental stage. When you see a particularly clear example, get it on tape and draw a picture; label the parts appropriately, and write a description of how you know what stage it is. Be prepared to explain to the class during your oral report what you have learned, either by re-drawing the distinguishing features on the board, or by pointing them out on the video microscope at the front of the room.

Stages of development—(possible titles for drawings):

- Sea urchin sperm
- Unfertilized egg
- Unfertilized egg with approaching sperm
- Unfertilized egg with fertilizing sperm attached
- Fertilized sea urchin egg
- Fertilized egg with fertilization membrane forming
- Fertilized sea urchin egg—zygote
- Diagrams of first four cleavages
- Results of third cleavage--8-cell stage
- Results of fourth cleavage--16-cell stage

Early blastula
Late (swimming) blastula
Early gastrula
Mid-gastrula
Late mid-gastrula (showing spicules)
Archenteron tip in contact with "mouth" invagination
Early pluteus, "mouth" fused with archenteron
Pluteus with four arms

Labels you should use wherever appropriate:

Sperm head
Sperm tail
Fertilizing sperm
Direction of rotation
Fertilization envelope
First cleavage plane
Second cleavage plane
Third cleavage plane
Mesomeres
Macromeres
Micromeres
Blastocoel
Cilia
Direction of swimming
Flattened area on blastula where invagination will begin
Mesenchyme cells
Blastopore
Archenteron
Spicules
Mouth invagination
Mouth
Anus
Gut
Pluteus arms