

Lesson

1

Stem Cell Development

Objectives

Students will be able to:

- Identify stages of early embryonic development.
- Compare and contrast embryonic developmental stages in terms of the potency of their cells (totipotent, pluripotent, and multipotent).
- Distinguish between ‘adult’ and ‘embryonic’ stem cells.
- Understand where cells used to create stem cell lines come from.

Class Time

1 class period

Prior Knowledge Needed

- Stem cells differentiate to give rise to different types of cells.
- Each type of cell has a unique look and function, (bone cells vs muscle cells for example).

Common Misconceptions

- Embryos are fetuses.
- Embryonic stem cells come from a woman’s uterus, a baby, or umbilical cords.
- Adult stem cells are only found in adults.

Introduction

In this activity, students make play dough models of an embryo through the early stages of development. They use their models to visualize where stem cells come from, and to understand that stem cells are totipotent, pluripotent and multipotent at different stages of development. Students fill out Student Handout 1.1 as they make their models. Students can work individually, or in groups of 2-3. Review/homework sheets and procedures for lesson extensions are also included.

Key Concepts

- Stem cells are totipotent, pluripotent or multipotent at different stages of development.
- Embryonic stem cells come from the inner cell mass of the blastula stage and are pluripotent. Removing the inner cell mass from the blastula destroys the blastula.
- Adult stem cells are multipotent and are already committed to one of the three tissue layers developed during the gastrula stage.

Materials

- 4 tubs of different colors of modeling clay or play dough (at least 3 tablespoons) per student or group
- Paper plates to represent Petri dishes
- Paper clips
- Straws
- If making play dough from scratch (see adaptations), you will need flour, salt, water, cream of tartar, oil and food coloring
- Student Handouts
 - 1.1 –*Modeling Stem Cell Development*
 - 1.2 –*Review: Stem Cell Notes*
 - 1.3 –*Stem Cell Comparison Charts*
 - 1.4 –*Sea Star Stem Cells Extension*
- Teacher Guides for Student Handouts 1.1 and 1.2
- A PowerPoint presentation to accompany this lesson can be found at nwabr.org.

Internet Resources

Pictures of each early stage of development (as well as pictures of *in vitro* fertilization) can be found through the Florida Institute for Reproductive Sciences and Technologies:

http://www.firstivf.net/laboratory_tour.htm#ICSI_Pictures

More sources for pictures of early development can be found in the “Additional Sources” section at the end of this lesson.

Introduce the Lesson:

Refer back to the planaria’s ability to regenerate. This regeneration depends on a type of cell that can

1. Self-renew (make more of themselves by dividing) and
2. Differentiate (give rise to daughter cells that can develop into many types of cells).

Stress that **these two characteristics are the hallmarks of a stem cell.**

In this lesson, students will learn where embryonic stem cells are found and how their potential to develop into different types of cells changes over time.

Procedure:

The students receive Student Handout 1.1 and fill it in as they are they directed by the teacher. **The teacher will demonstrate each step along the way** as the students make their own clay models. Ideally each student will have at least 3 tablespoons of each color. They can also work in teams of 2-3. Instruct students that they will need to divide up their clay and save some for later stages.

1. **Zygote (zye-goht):** To build the zygote model, use a single color to make both an egg (about the size of a ping pong ball) and a much smaller sperm cell. Mix them together to form a zygote. (The ‘tail’ of the sperm drops off and does not enter the egg). Place it on the “Petri dish” (paper plate) to represent in-vitro fertilization. The zygote is totipotent; this single cell will give rise to every cell type in the body and the placenta.
2. **Early Cell Divisions:** Divide the single-cell zygote in half, making two spheres. Divide each of those two cells in half, then each of those in half again, until there are 16 cells.
3. **Morula (mor-yoo-la):** Push the 16 cells together to form a sphere. This represents the morula stage. Have the students set this aside on the Petri dish (paper plate) to compare to the stages which will follow.
4. From the one-celled zygote to the 16-celled morula, the cells are considered totipotent. That means that any of the cells the students have just made could differentiate into any tissue in the body or placenta. In fact, identical twins arise from this stage of development; one or more cells from the morula (or earlier) can split off and become a separate, new organism.
5. Fill in the totipotent column on Student Handout 1.1
6. **Blastula (blast-yoo-la):** Explain that the cells will continue to divide and we will “fast forward” through the blastula stage, which occurs from the 3rd through the 14th day after fertilization. At this point some cells have differentiated into cells which will become the placenta. The pre-placental cells will form a hollow ball surrounding the embryonic cells.

7. To build the blastula model, pick a new color and make a sphere the size of a ping pong ball. The goal is to make a hollow ball, but since this will be difficult students can make the shape of a bowl. This will represent the cells that will become the placenta. Students can use the end of a straw or a pen cap to make indentations in the bowl that look like cells.
8. Use fresh clay or dough in the color used in the original zygote model, and make many small spheres (about the size of a pea or smaller) to represent the cells growing inside the hollow ball, or bowl. These represent the inner cell mass, or embryonic stem cells. Place these in a pile inside the bowl. Be sure to reinforce that the pre-placental cells (the ‘trophoectoderm’) would really form a “hollow ball” (trophoblast) completely surrounding the embryo, and that **even though they are now represented in a different color, they also originated from the morula.**
9. The cells of the blastula are pluripotent. The cells have already gone through one “fate decision”; the hollow ball can only become placenta, and the inner cell mass can become any type of cell in the body *except* placenta. The inner cell mass is the source of **embryonic stem cells**. If the inner cell mass is removed from the blastula, the blastula is destroyed and cannot continue to develop.
10. At this point, an **embryonic stem cell line** can be made. Cells from the inner cell mass of a four- to five-day old blastula are transferred into a plastic laboratory culture dish and grown in a medium that provides support and nutrients. When kept in this way, the inner cell mass (or embryonic stem cells) can continue to divide and proliferate for long periods of time without differentiating or losing pluripotency. A stem cell line, directed to differentiate into specific cell types, offers the possibility of treating diseases including Parkinson’s and Alzheimer’s diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis. (*Note: Many of the early embryonic stem cell lines that have been used for research in the last decade have been grown on mouse feeder cells and would be inappropriate for therapeutic uses in humans.*)
11. Set the blastula in the Petri dish (paper plate), and tell the students that in real life, the embryo would go into a freezer if not implanted immediately in a woman using *in vitro* fertilization techniques to become pregnant.
12. Fill in the pluripotent column on Student Handout 1.1
13. **Gastrula (gass-troo-la):** Explain that we are fast forwarding in time again and that the embryonic cells have continued to divide. At about 14 days after fertilization, the cells will begin to differentiate and form three layers (endoderm, mesoderm, and ectoderm). The differentiation is triggered in part by the attachment of the pre-placental cells to the uterine wall.
14. To build the gastrula model, make a new early placenta “bowl” in the same color as the previous one, using roughly a ping-pong ball sized sphere. Take a small bit more of the original embryo color and form a ball about the size of a small pea. Flatten a marble-sized piece of a new color and wrap that around the ball. Then add another layer in a new color around the outside. Now open

a paper clip, and use it to cut through the center of the gastrula. The three different tissue layers will be clearly visible on the inside. The three-layered ball can be somewhat flattened before cutting through it with the paper clip, if desired. This would more accurately represent the shape of the embryonic disc, from which the three tissue layers form. Place the gastrula on the Petri dish next to the morula and blastula. Optional: Have students bring their blastulas in their Petri dishes and place them in the freezer, where they will be stored indefinitely!

15. The gastrula is multipotent. The early placenta cells can still only become placenta. The inner cell mass has undergone another “fate decision” and has differentiated into three layers, the endoderm, mesoderm and ectoderm. The endoderm cells will become, in part, the digestive and respiratory tracts; the mesoderm will become bones, blood cells and the heart; the ectoderm cells will become the skin and central nervous system. Once the cells have differentiated into three layers, they are considered “adult” stem cells and can only make the type of cell determined by that layer.
16. Fill in the multipotent column on Student Handout 1.1.
17. Although the three layers of the embryo will be difficult to separate, students can take apart the remainder of the model and store the clay for further use.

Discussion

Clarify any questions that the students might have regarding the handout, models or terminology.

Discuss the LIMITATIONS of the model. These limitations can include:

- The simulation shows only discrete points in time rather than continuous development.
- The different colors may give the wrong idea about origins of cells—all of the “colors” originate from the original zygote.
- Students cannot see the spherical nature of trophoblast.
- Any other limitations students may suggest.

Important terms to emphasize include:

Totipotent, pluripotent, multipotent, zygote, blastula/blastocyst, embryonic stem cell, adult stem cell.

Suggested Extension—viewing of Sea Star Stem Cells

Working independently, students observe prepared slides of developing Sea Star embryos. Sea Urchin embryo slides can also be used. Students draw and label the zygote, morula, blastula and gastrula stages, reinforcing their understanding of early development.

Procedure:

Using microscopes, students examine prepared slides of the sea star embryos. They will sketch and label various stages of development, as described in Student Handout 1.4.

Materials:

Class set of Sea Star embryology slides (fertilized egg, early cleavage, morula, blastula, gastrula) available from various biological supply houses.

- Student Handout 1.4
- Microscopes

More Extensions

The play dough can also be used to model “Therapeutic Cloning”, also known as Somatic Cell Nuclear Transfer (SCNT). Use two colors of dough, to make an unfertilized egg cell. The nucleus would be one color inside the cytoplasm of another color (it might look a bit like a fried egg). Have the students inscribe the number 23 in the nucleus using an open paper clip to represent the number of chromosomes. Then have them build another cell to represent a somatic (body) cell with a different colored nucleus, and the number 46 inscribed on it. Have the students cut out both nuclei with the paper clip. The nucleus removed from the egg cell will be destroyed, and the nucleus from the somatic cell will replace the egg’s nucleus. Explain that since the egg now has 46 chromosomes, it will behave as if it had been fertilized, and begin to divide and grow. This is how cloning is done!

Adaptations

An excellent way to show continuous differentiation of the embryo, is to use homemade play dough, and add food coloring at each appropriate step. Recipe makes about 1 ½ cups of play dough.

- 1 cup flour
- 1 cup warm water
- 2 teaspoons cream of tartar
- 1 teaspoon oil
- ¼ cup salt

Mix in saucepan. Stir over medium heat until thick.
Remove, kneed until smooth.

In this version of the activity, the students will use the same play dough from beginning to end. They will take part of the dough from the morula stage, and add food coloring to the part which will become the placenta. The embryo will then differentiate inside the placenta, and can be divided into three layers, each a different color to form the gastrula.

Homework

The Student Handout 1.3 *Review: Stem Cell Notes* can be assigned as homework. Students can use their notes from the play dough activity as well as from the background information sheet Student Handout 1.4 *Stem Cell Comparison Charts* to guide them in completing this summary.

Additional Sources

A series of pictures showing early sea urchin embryo development from the 1-cell stage through to the late blastula stage:

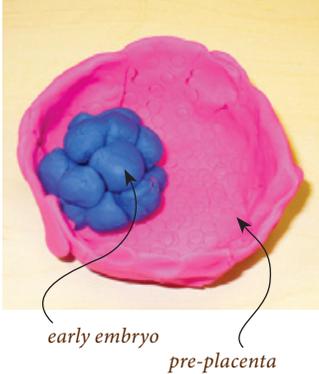
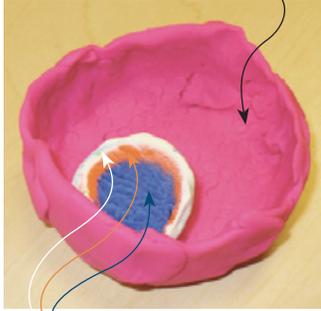
<http://www.luc.edu/faculty/wwasser/dev/urchindv.htm>

Drawings of early human development:

<http://www.biology.iupui.edu/biocourses/n100/2k4ch39repronotes.html>

The Visible Embryo is a visual guide through fetal development from fertilization through pregnancy to birth: <http://www.visembryo.com/baby/index.html>

Modeling Stem Cell Development

	Totipotent Stem Cells	Pluripotent Stem Cells	Multipotent Stem Cells
Diagrams of play-dough creations	<p>Zygote:</p>  <p>Morula:</p> 	<p>Blastula/Blastocyst:</p> 	<p>Gastrula:</p> 
Approximate days cell division occurs	Before 3 days	3-14 days	After 14 days
Approximate number of cells	1-16	Up to several hundred	Several hundred and more
Definitions of important terms	<p>Totipotent: Stem cells that can differentiate into any type of cell.</p> <p>Zygote: Single cell formed when sperm cell fertilizes the egg.</p> <p>Morula: The mass of up to 16 undifferentiated cells produced by the first four divisions after fertilization.</p> <p>(Teacher note: Some researchers have proposed using cells from early cleavage/morula stages to create stem cell lines, allowing the embryo to continue to develop.)</p>	<p>Pluripotent: Stem cells that can differentiate into most types of cells. (Here, the embryonic cells can become anything except a placenta).</p> <p>Blastula: The “hollow ball” stage where the pre-placental cells form the ball with the early embryo inside. This is also referred to as the Blastocyst stage.</p> <p>Embryonic stem cell: Stem cell taken from blastocyst (or earlier stages). Currently, researchers use cells from the inner cell mass at the blastocyst stage.</p> <p>Embryonic Stem cell line: Embryonic stem cells, which have been cultured under <i>in vitro</i> conditions that allow proliferation without differentiation for months to years.</p>	<p>Multipotent: Stem cells that can differentiate into a limited range of cell types.</p> <p>Gastrula: The embryo develops three cell layers. Stem cells are limited to forming tissues only from that layer.</p> <p>Adult stem cell: Any stem cells taken after the three cell layers have formed. Stem cells taken from umbilical cord blood, and from anyone after birth are considered adult stem cells.</p> <p>(Teacher note: the three layers are ectoderm – which becomes skin/nervous system, mesoderm – which becomes muscle/bone, and endoderm, which becomes lining of gut and internal organs)</p>
Other Notes:	Totipotent cells are used for Pre-Implantation Genetic Diagnosis	Pluripotent cells from the inner cell mass are used to make embryonic stem cell lines	Multipotent ('Adult') cells are already committed to a certain tissue layer

Review: Stem Cell Notes

What are two main characteristics of stem cells?

1)

2)

What is the major difference between adult and embryonic stem cells?

Embryonic stem cells:

“Adult” stem cells:

Describe what each of these terms means in reference to stem cells and their capabilities:

Totipotent-

Pluripotent-

Multipotent-

Terms associated with development:

Zygote-

Blastula/Blastocyst-

Embryo-

Fetus-

Stem Cell Notes

What are two main characteristics of stem cells?

1)

Self-renew (make more of themselves by dividing)

2)

Differentiate (give rise to daughter cells that can develop into many types of cells)

What is the major difference between adult and embryonic stem cells?

Embryonic stem cells:

Embryonic stem cells: Can become any type of cell in the body

“Adult” stem cells:

“Adult” stem cells: Current research indicates that these cells can become cells of a particular tissue type (i.e. blood)

Describe what each of these terms means in reference to stem cells and their capabilities:

Totipotent-

Capable of becoming any cell in the body or placenta. Capable of regenerating an entire new organism

Pluripotent-

***Capable of becoming almost any cell in the body (except placental tissue).
Not capable of regenerating an entire new organism. ("Embryonic stem cells")***

Multipotent-

Capable of becoming a cell of a particular tissue type. (see "Adult" stem cells)

Terms associated with development

Zygote-

Fertilized egg

Blastula/Blastocyst-

***Hollow ball stage where some cells begin to differentiate from others.
Cells from the 'inner mass' of this stage are used to make embryonic stem cell lines***

Embryo-

Early stages of development, prior to 8 weeks

Fetus-

Later stages of development, from 8 weeks to birth

Stem Cell Comparison Charts

Stem Cells and Potency

Potency	What can they become?	When do they occur?	Where do they come from?	What are they referred to?
Totipotent (Toti=total)	Able to make all the cells in the human body and the placenta	Before 3 days	From cells of first few cell divisions	Early embryonic cells (blastomeres)
Pluripotent (Pluri=more)	Able to make most of the cells in the human body, with the exception of placental tissues	3-14 days (before 'gastrulation', the development of 3 germ layers in the embryo)	From inner cell mass of blastula	Embryonic stem cells (if cultured <i>in vitro</i>) Pluripotent stem cells (cells within the inner cell mass)
Multipotent (Multi=many, much)	Able to make a range of cells within a particular tissue type (such as blood)	After 14 days	From cells of the developing individual as well as adult	Cord blood stem cells Adult stem cells

Adult vs. Embryonic Stem Cells

Type	Where are they obtained?	How flexible are they?	Advantages	Disadvantages
Embryonic	From inner cell mass of blastocyst of: Donated fertilized eggs (IVF) or donated eggs fertilized by researchers Product of "Somatic Cell Nuclear Transfer" (genetically identical to donor nucleus)	Pluripotent* *very early embryonic cells are totipotent, but these are not used to make stem cell lines	Can become most cells/tissues of the body Easier to culture in lab Great potential for developing future therapies to cure diseases	Potentially ethically problematic: blastocyst must be destroyed when cells are removed, egg donation also an issue
Adult	Often from adult tissues/organs (note: this term is also often used for multipotent cells found in fetuses or younger individuals, including newborns and children)	Multipotent* *some studies suggest certain adult stem cells may be able to be reprogrammed to become pluripotent	Less ethically problematic - no destruction of blastula involved Already used in therapies (bone marrow transplants)	Hard to culture in lab Most are limited to become specific tissue types

Sea Star Stem Cells Extension

Using the prepared slides of sea star or sea urchin embryonic development, find the following stages of development. **Draw and label** at least one example from each stage in the space provided.

1. **Zygote** or fertilized egg. The fertilized egg can be distinguished by the presence of a fertilization membrane surrounding the cell. Also, the nucleus in a fertilized egg is quite indistinct.

2. **Early cell divisions** ("cleavage"). Find two-cell, four-cell, and eight-cell stages. Note that the appearance of the embryo in each case will depend on its orientation with respect to the surface of the slide. Some embryos may be seen in end view, other in a side view at various angles.

3. **Morula**. Find one or more 16-cell stages. (You may be able to count the number of cells by careful focusing at different levels. Even if you are unable to count the exact number of cells, you can make a rough estimate.) Look for a cluster **without** a central cavity. This is the morula. (Some 16-cell clusters might already show the beginnings of a blastocoel, the central cavity.)

4. **Blastula**. 32-cell, 64-cell and later stages will usually show a central cavity, the blastocoel. The cells can be referred to as '**blastocysts**'.

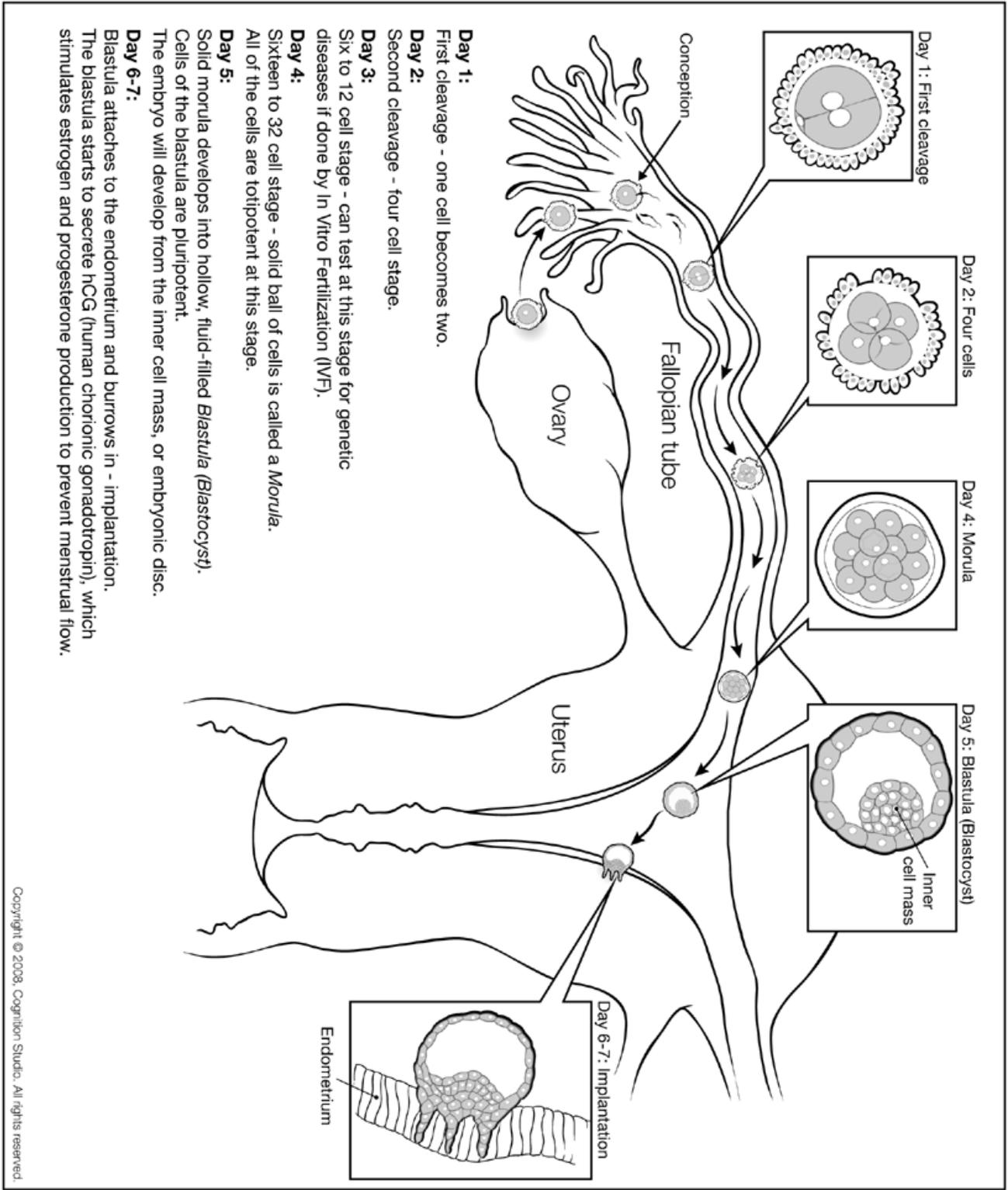
5. **Gastrula**. In the early gastrula, cells have just begun to push in from one end. In the middle gastrula stage, cells have pushed in sufficiently to produce an opening to the outside (archenteron – a primitive gut) by means of a wide blastopore. The formation of the archenteron is completed in the late gastrula.

6. **Circle the stages** at which the embryonic cells are totipotent, and underline the stages at which the embryonic cells are pluripotent:

Zygote – Early Cell Divisions – Morula – Blastula – Gastrula

Early Development

In the first days and weeks after conceptions, mitotic cell divisions begin, converting the one-celled zygote to a multicellular early embryo.



Day 1:
First cleavage - one cell becomes two.

Day 2:
Second cleavage - four cell stage.

Day 3:
Six to 12 cell stage - can test at this stage for genetic diseases if done by In Vitro Fertilization (IVF).

Day 4:
Sixteen to 32 cell stage - solid ball of cells is called a *Morula*. All of the cells are totipotent at this stage.

Day 5:
Solid morula develops into hollow, fluid-filled *Blastula (Blastocyst)*. Cells of the blastula are pluripotent. The embryo will develop from the inner cell mass, or embryonic disc.

Day 6-7:
Blastula attaches to the endometrium and burrows in - implantation. The blastula starts to secrete hCG (human chorionic gonadotropin), which stimulates estrogen and progesterone production to prevent menstrual flow.

Copyright © 2008, Cognition Studio. All rights reserved.

Early Development continued

