Module 5

Cleavage and the Blastula

After several spawned eggs have been fertilized externally, or one to few ovulated eggs fertilized internally, these eggs are now ready to develop into new beings, they are now activated (as you have learned in the previous module) to proceed with the next phases of development.

In this module, we will tackle two subsequent stages after fertilization, and these are cleavage and blastula stages. In terms of the process itself, we can refer to the cleavage stage as the actively dividing stage (thru mitosis), and the blastula stage is formed by the process blastulation.

On the other hand, if the egg is not fertilized, no new life form or being develops. None of these two stages of development proceeds. The failed fertilization process does not release the egg from a depressed metabolism right after they were spawned in most anamniotes or after ovulation in amniotes. For anamniotes failed fertilization of enough eggs is a rare occurrence. For mammals, the unfertilized ova will disintegrate and will be shed together with the endometrial lining during the menstrual phase of primate mammals.

Learning Objectives: At the end of this lesson, the student should be able to:

1. Describe the process of making a fertilized egg (zygote) become a multicellular embryo.
2. Differentiate the different patterns of cleavage seen in different animals
3. Explain how the process of cleavage is regulated in a fertilized zygote.
4. Discuss the formation of a blastula in the early stages of development.

CLEAVAGE and BLASTULA

Just to recall what we learned in fertilization, right after the male and female pronuclei fuse with each other, the homologous chromosomes align in the metaphase plate, and proceeds to anaphase wherein the replicated chromosomes move towards the opposite pole and by cytokinesis (formation of cleavage furrow in animal cells) two **daughter cells** are formed. So, in other words, the parent cell (which is the fertilized egg), divides by ordinary mitosis.

In the cleavage process of any animal, the repeated divisions of the daughter cells by mitosis increase the number of cells, each of which is called a **blastomere.** At about 16-cell to 32 cell-stage the embryo is now called a **morula** (derived from the Greek and Latin words meaning “mulberry”), a solid ball of blastomeres. More cleavage events happen until there are several hundreds of blastomeres and the embryo is now called or is in the **blastula** stage.

Cleavage is the stage of development that involves intense cellular assembly. Since cleavage utilizes the cellular division of mitosis, there is a heightened activity for DNA and protein synthesis, then packaging them into chromosomes. New plasma membranes must be available for all the daughter cells that results from the cytokinesis of parent cells.

The pattern of cleavage is affected by the amount and distribution of the yolk in the ovum.

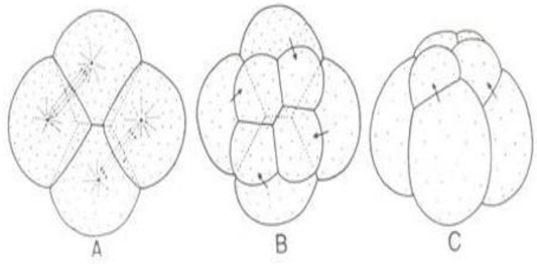
Recall in gametogenesis, specifically in oogenesis, one stage of egg development is the production of yolk. Yolk is a mixture of proteins, phospholipids, and fats that serves as food for the developing embryo. The amount of yolk in an egg cell varies in animal groups depending on the needs of the embryo. The table below summarizes the type of egg based on the amount and distribution of yolk, the type/pattern of cleavage and the representative animal group displaying the types.

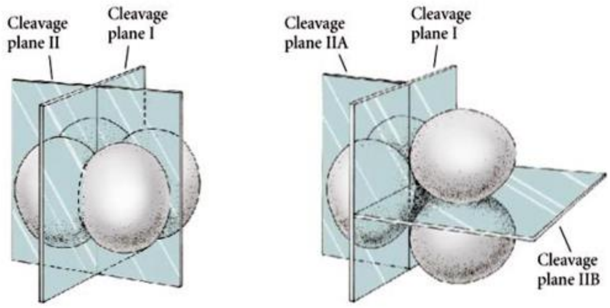
Amount of yolk	Distribution of yolk	Type of cleavage	Pattern of cleavage	Animal group
Oligolecithal/micro-lecithal	Isolecithal (uniformly distributed	Holoblastic equal cleavage (the entire egg divides and the blastomeres are of equal sizes	Radial cleavage	Echinoderms and amphioxus (deuterostomes)
			Spiral cleavage	Annelids and mollusks (protostomes)
Mesolecithal (moderate amount of yolk)	Telolecithal (yolk is concentrated toward the vegetal pole and the cells without yolk are in the animal pole	Holoblastic unequal cleavage (The cell division in the vegetal pole is slowed down by the presence of the inert yolk and the blastomeres are of unequal sizes (**micromeres** in the animal pole **/macromeres** in the vegetal pole**)**	Radial cleavage	Amphibians, lampreys and lungfish
Macrolecithal (large amounts of yolk)	Telolecithal (the embryo forming cytoplasm is displaced into a small disc on one edge of the ovum)	Meroblastic cleavage (The yolk part never cleaves but only the **blastodisc** at the animal pole		Reptiles and birds, fishes
	Centrolecithal	Meroblastic		Insects and arthropods
Alecithal (no yolk _		Holoblastic equal at the start but later on becomes Meroblastic cleavage		Mammals

Question 5.1: What are deuterostomes? What are protostomes?

Question 5.2: Differentiate radial cleavage from spiral cleavage.

After you have differentiated radial cleavage from spiral cleavage, label the figures below as to what pattern of cleavage each shows:

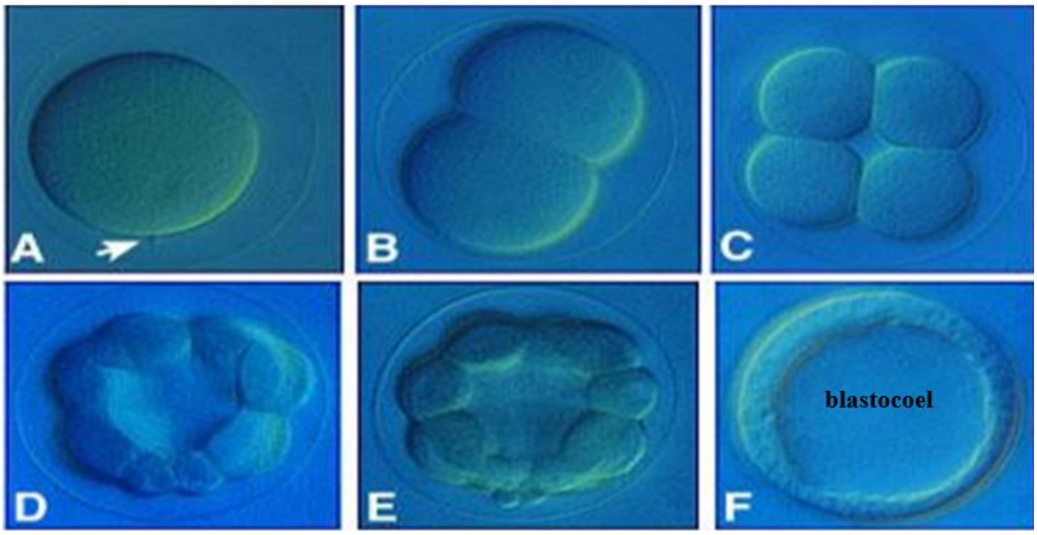




_________________ cleavage   ____________________ cleavage

Let us just look at some of these types and patterns of cleavage to give you some knowledge on the variety of this stage of development simply because of the amount of yolk present in the egg. And again, at the end of cleavage stage, the embryo as said earlier is now at the blastula stage.

Echinoderms (e.g., sea urchins)



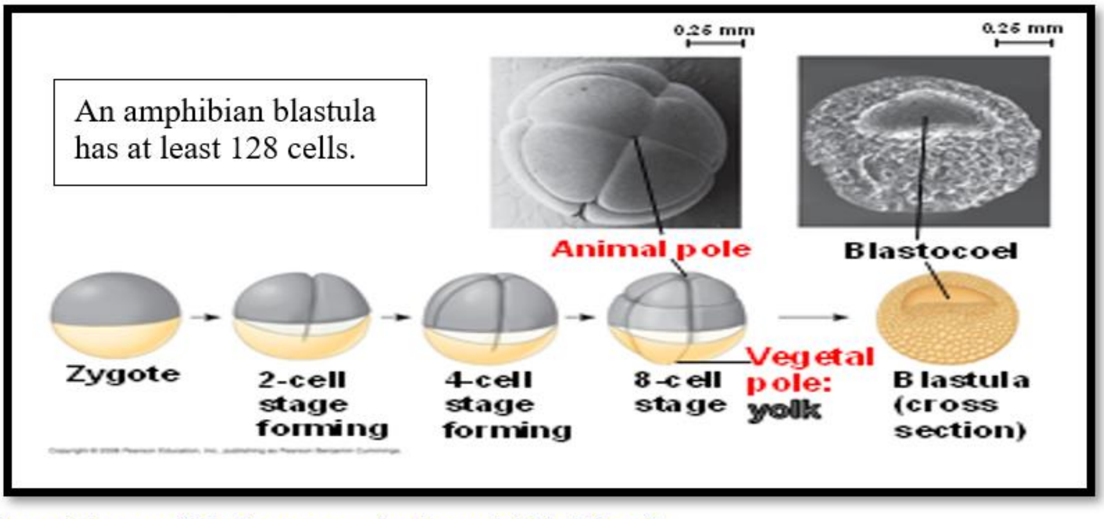
The cleavage process in sea urchins is very rapid because there is little amount of yolk and because of this little amount of yolk it should develop into a form that it can swim and look for its own food. Because of this little amount of yolk, the fertilized egg divides by **holoblastic equal cleavage.** The first two divisions are meridional, forming 2 then 4 blastomeres (from animal pole to vegetal pole), and the third is equatorial that produces 8-cell embryo (A to C). Note the arrow in A, this arrow shows the point of entry of the sperm cell. By D, a distinct cavity, **blastocoel** is forming at the center of the embryo and can now be referred to as the **blastula.** Note that the blastomeres are of equal sizes and are found surrounding the blastocoel.

Amphibians (e.g., frogs)

In amphibians, the period of cleavage and the formation of blastula is usually completed within 24 hours. Like in many other animals, the cleavage is asymmetric because of the uneven distribution of the moderate amount of yolk (telolecithal).

The first two cleavage furrows form parallel to the line (or meridian) connecting the vegetal pole and the animal pole. During these divisions, the main effect of the yolk is to slow down the completion of cytokinesis. Because of this, the first cleavage furrow is still dividing the yolky cytoplasm in the vegetal pole when the second cell division begins.

During the third division, the yolk begins to exert its effect on the size of the 8 blastomeres resulting from the equatorial division (perpendicular to the line connecting the poles). The blastomeres in the animal pole are smaller in size (**micromeres)** because there is no yolk to impede or slow down the cleavage process. On the other hand, the blastomeres in the vegetal pole are larger in size (**macromeres**) because of the impeding effect of the yolk in this pole. Subsequently, the **blastocoel** of the **blastula** is formed entirely in the animal hemisphere. See the figure below.



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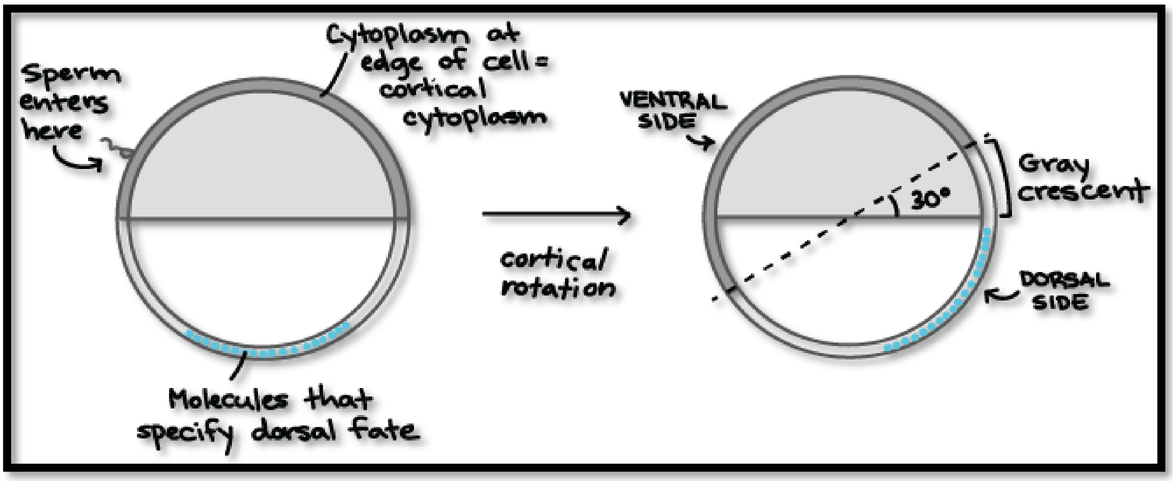
Before we proceed to the next pattern of cleavage seen in birds and reptiles let us focus a little bit on an important embryonic structure in amphibian development.

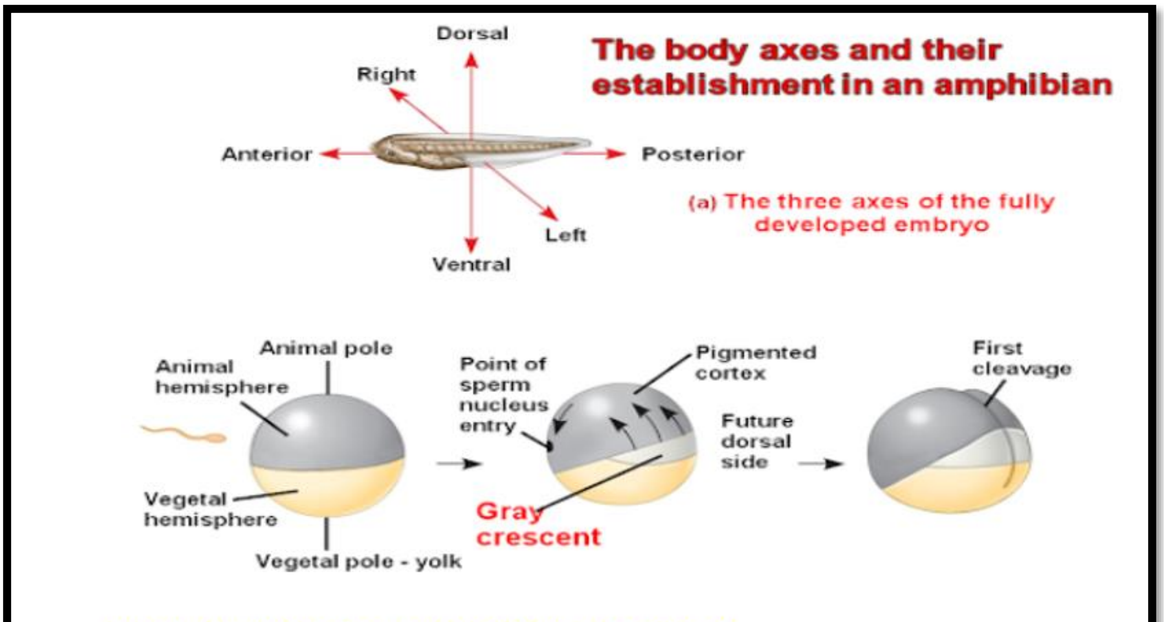
Recall the cortical region on the periphery of the unfertilized egg. As soon as a sperm enters, opposite to the entry of the sperm, microtubular structures pull and rotate the cortex and core of the vegetal pole 30° to the animal pole. This rotation or movement produces a lighter zone, called the **gray crescent**.

What is the significance of this gray crescent in the cleavage of amphibian eggs?

- The first cleavage division bisects the gray crescent (see figure above)
- The gray crescent marks the future dorsal side of the embryo as well as the other axes (see the figure at the lower part).
- Take note also of the blue colored molecules that also moves to the future dorsal side. These molecules as we will know later are important determinants for proper development of the organism during gastrulation.



https://www.khanacademy.org/science/biology/developmental-biology/signaling-and-transcription-factors-in-development/a/frog-development-examples



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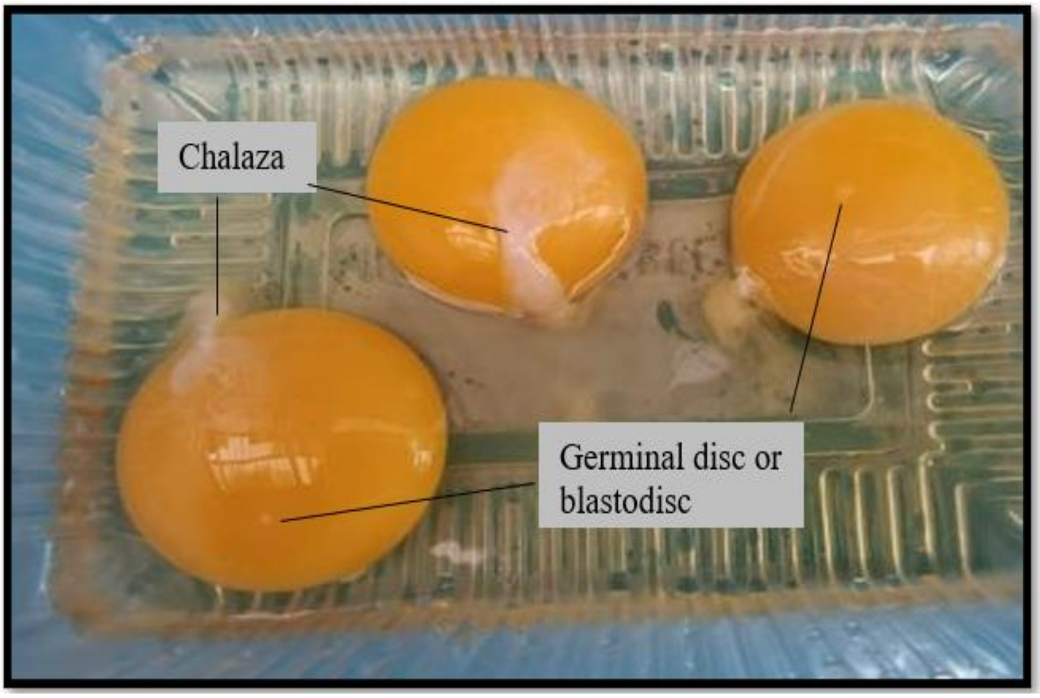
Birds and Reptiles

If you have cracked open an egg for your breakfast or for baking or for mukbang 😊, you should have noticed the very large yolk and that white spot on top of the yolk. So the whole yolk and the white spot is the egg cell of birds. What kind of egg is it based on the amount of yolk? __________. How about the type of egg based on the distribution of the yolk? _______.

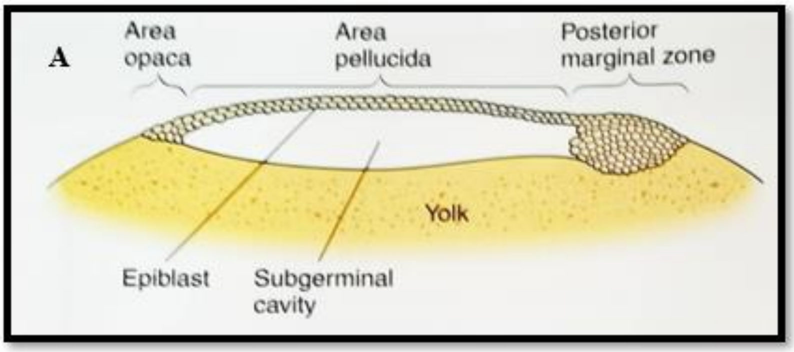
So where the yolk is found, is the vegetal pole, and that white small spot is the animal pole, which will be the only part of the cell that will divide. The cleavage process in birds and reptiles is called **meroblastic cleavage** wherein only the cytoplasmic disc in the animal pole will be cleaved by a series of mitotic divisions and the yolk will not divide at all.



Question 5.3: What is the chalaza (chalazae, plural form)? What is its role in the development of the bird's ovum or egg cell? Attach a photo of an egg YOU cracked yourself and check if there is a blastodisc or none and label the chalaza.

At the end of the cleavage process, the embryo is not called a blastula but, **blastodisc/germinal disc/blastoderm,** which may be composed of 50,000-60,000 cells by the time the egg is laid down (see the figure below which are three eggs I cooked scrambled style for breakfast 😊). Note the **chalaza**. So, if you see this white spot from the egg you will cook, most likely the egg was a fertilized egg and did not become a chick at all ☹.

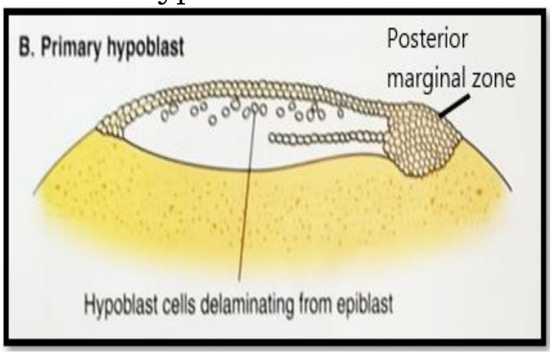
A sagittal section of the blastodisc reveals the following plan and structures at this stage of development in birds and reptiles. As shown below, there is a relatively transparent central region, the **area pellucida**. It is called as such, because the cells here have less yolk and do not lie directly on the yolk mass. The region of the blastodisc (edges in particular) that is lying above the yolk is called the **area opaca**. A space separating the yolk from the blastodisc is called **subgerminal cavity.**

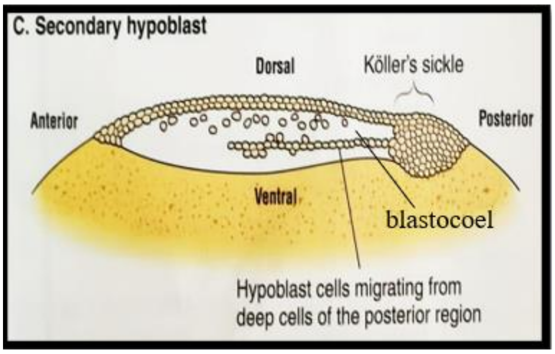


Sagittal section through the blastodisc of a newly laid egg (lifted from Wilt & Hake, 2004).

Some cells of the blastodisc detach from it (as it travels down the oviduct) and die within the subgerminal cavity. What is left is a layer of cells called **epiblast.** The **posterior marginal zone** is made of cells in the periphery of the blastodisc that meets with the yolk. What does this zone tell us? From the word posterior, this marks the future posterior end of the organism…duh! 😊

Look at the figure below, some cells of the epiblast separate or detach from it. The cells detach as a group, rather than singly, and this is called **delamination.** This delaminated or detached cells form a transient or temporary layer, the **primary hypoblast (B).** Later on, more cells from the deeper region of the posterior zone, called **Köller's sickle,** migrate forward and join with the cells of the initial hypoblast forming the **secondary hypoblast** or simply the hypoblast **(C).** The space between the epiblast and the hypoblast is the **blastocoel.**

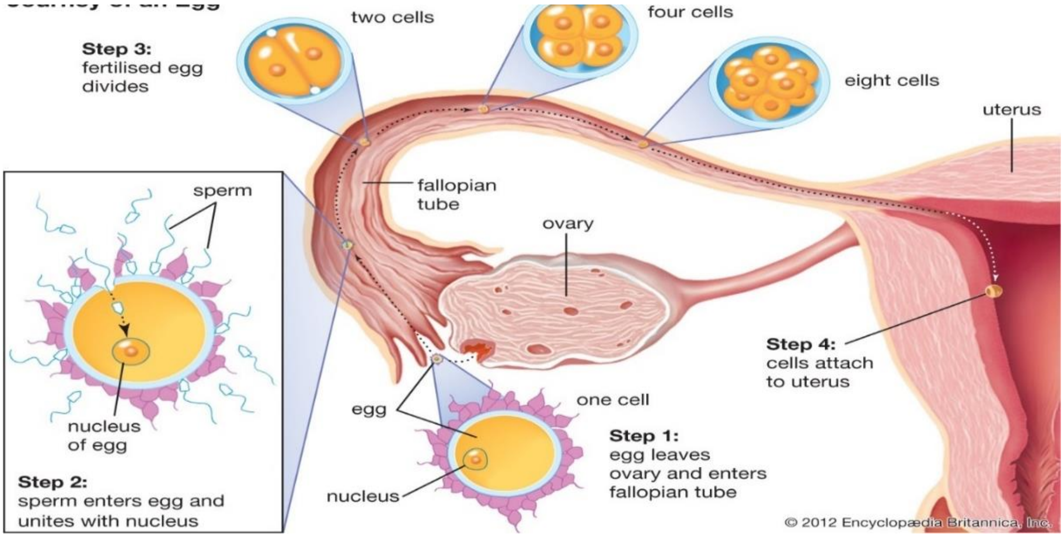




By 20 hours of incubation, gastrulation movements (discussed in the next module) have begun.

Mammals (humans)

To give you an idea of how cleavage in humans takes place, look at the figure below which depicts the journey of an ovulated egg. Again, based on the table earlier, in non-egg laying mammals, the fertilized egg divides by holoblastic equal cleavage but later divides by meroblastic cleavage.



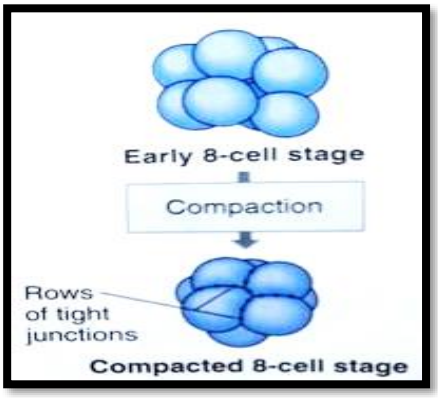
https://www.britannica.com/science/fertilization-reproduction

1. As soon as the egg is released from the ovary, the "suction" or the contraction of the fimbriae brings the ovulated egg into the fallopian tube and if;
2. sperm cells are there (and you know how they got there, right? 😊), fertilization happens. *Just to remind you, ovulated human eggs have only 24 hours to be viable while sperm cells are viable for at least 72 hours inside the female's body. So if you go back to the chart of the menstrual cycle, sperm cells which come to the fallopian tube 24 hours before or 24 hours after the egg is ovulated, a sperm cell can fertilize the egg* 😊
3. And right after fertilization, the zygote divides by mitosis into two daughter cells. And this commences a series of mitotic division as it travels down to the uterus (2 becomes 4, 4 becomes 8) and when it is made of 16 to 32 cells, it is called a **morula**.
4. By the time it reaches the uterus the embryo (58 to 120-cell) is now called a **blastocyst** (as will be discussed in a little while).

It can be said that cleavage in mammals takes place at a slower pace compared to the other nonmammalian animals. There are other differences that were observed during cleavage of a mammalian zygote:

1. As early as the 2-cell stage, a new gene transcription is detected, called zygotic gene transcription.
2. Limited maternal control of the organization of the egg or embryo.
3. There is almost no yolk (alecithal).
4. Elaborate extracellular membranes are formed very early in its development.
5. Some genes in the male and female may be expressed differentially during embryonic development, a phenomenon called **parental imprinting.**

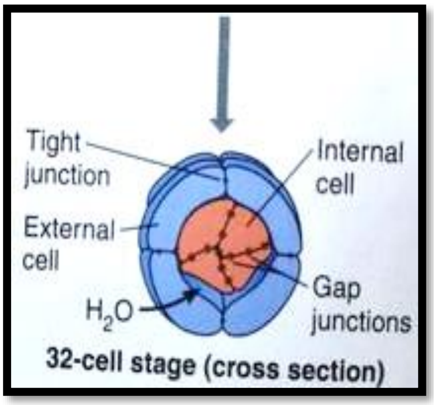
What is the mammalian **blastocyst**? Let us go back to the 8-cell stage of the mammalian embryo (images are from mouse development).



At this stage, **compaction** takes place. The blastomeres become tightly bound to each other.

The loosely attached blastomeres become a true polarized epithelium. The outer cell surfaces bear microvilli, and ***tight junctions*** develop between adjacent cells.

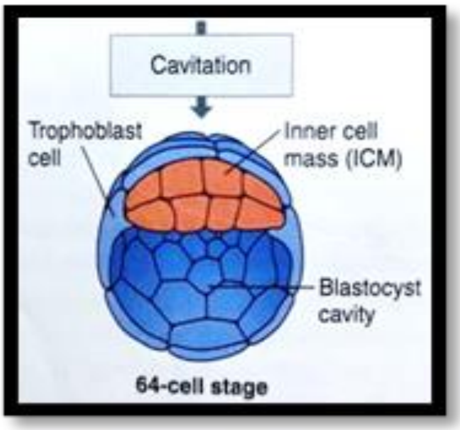
Polarized means the apical surfaces face the outside of the embryo and basal surfaces facing the inside (Carlson, 1994).



At the next (fourth) division, many of the cell division planes are angled such that the resulting cells remain on the surface, But some divisions take place with the division planes angled parallel to the surface, thus generating some three or four "internal" cells.

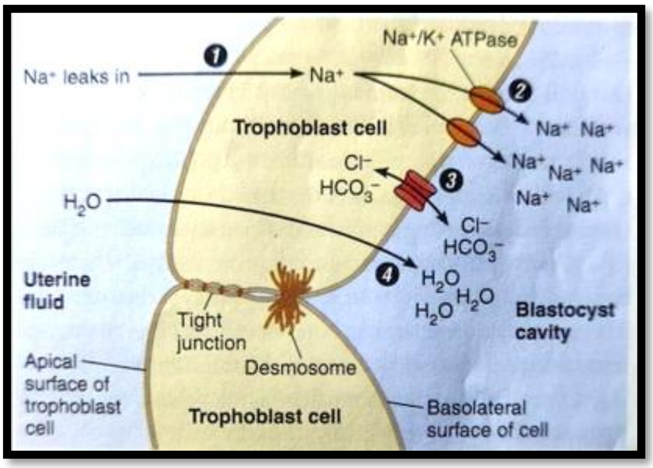
These internal cells are called **inner cell mass** while the external cells will make up the trophoblast.

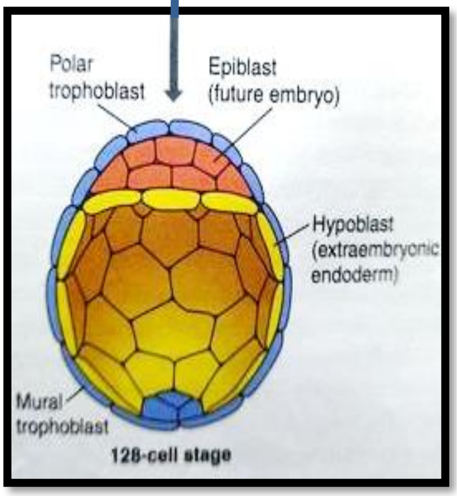
Note the ***gap junctions*** formed among the internal cells.



At the 32-cell stage, another important process occurs in the mammalian embryo, and that is **cavitation.** This process leads to the formation of the blastocyst cavity (**blastocoel).**

The gap junctions mentioned above are very important in the formation of the blastocyst cavity. Cavitation (see figure below) involves the build-up of fluid within the cavity. The fluid accumulates through the sodium transport system based on Na^+, K^+ -ATPase that develops in the outer blastomeres (Carlson, 2004). Image from Wilt & Hake (2004)



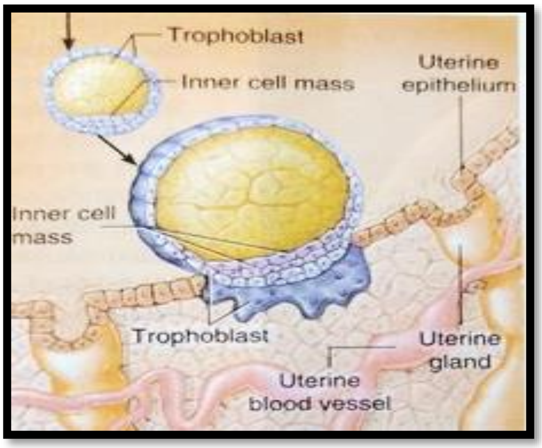


At the blastocyst stage, the embryo consists of two distinct groups of cells: the smaller group, called **inner cell mass** (ICM)**,** and the outer superficial layer, **trophoblast**, which surrounds the inner cell mass.

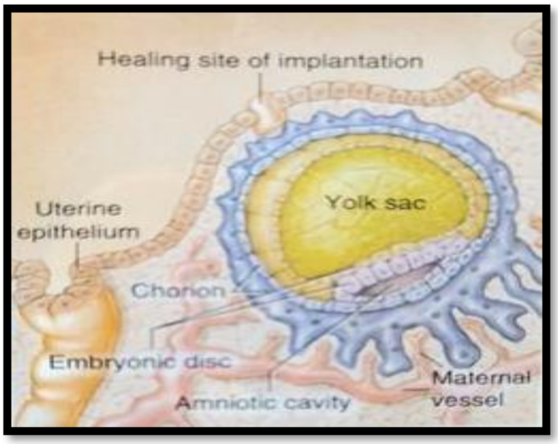
The inner cell mass give rise to the future embryo and looking at the figure on the left, the ICM by delamination forms the outer epiblast (give rise to the entire embryo) and inner hypoblast (from where the extra-embryonic membrane develops). See the similarity with birds and reptiles.

The trophoblast will form the placenta.

Just to give you a picture what happens to a fertilized human ovum, see the figures below that were lifted from Solomon, Berg & Martin, 2008)



About 7 days after fertilization, the blastocyst drifts to an appropriate site along the uterine wall and begins to **implant** using the polar trophoblast, or the layer of trophoblast closer to the ICM.



About 10 days after fertilization, the embryonic disc (= blastodisc) resulting from cleavage has 2 layers, the epiblast and the hypoblast. The space surrounded by the yolk sac is the **blastocoel.** The **chorion** has also formed from the trophoblast making the future embryo connected with the maternal blood vessels.

You might wonder why the human blastocyst has a yolk sac when its ovum has no yolk at all. This would be explained by common ancestry. Since mammals came from egg laying reptiles the yolk sac persisted in the line of mammals even yolk is absent. This is the reason why meroblastic cleavage occurs in fertilized mammalian eggs.

A few more other concepts related to cleavage would be good for you to know before we end this module 😊

1. Cleavage may distribute developmental determinants differently in animal groups such that the cleavage pattern is affected by the distribution (as well as by the yolk content). In terms of distribution of determinants, there are two kinds of development in animals:

   a. <u>**Mosaic development**</u> – The developmental patterns are relatively rigid, because the distribution of important materials every time the zygote divides are not homogenous or the distribution in each blastomere is not equal, the course of development of the cells run differently.

   b. <u>**Regulative development**</u> – The distribution of the determinants or important molecules are homogenous, or each cell of the cleaving cell receive the same determinants, the embryo develops as a self-regulating whole.

Since we cannot cover much on these two concepts, I am providing a link which you can read further (not to be included in the quiz 😊) regarding mosaic and regulative development.

https://www.cell.com/current-biology/comments/S0960-9822(06)01269-3

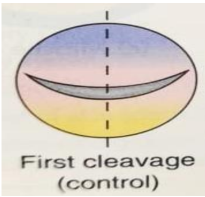
The gray crescent that was mentioned in the amphibian cleavage contain a wide array of determinants important in development. Shown below is an experiment done to see the importance of the gray crescent in amphibian development. Read through it and at the end follow the given instruction.

KEY EXPERIMENT

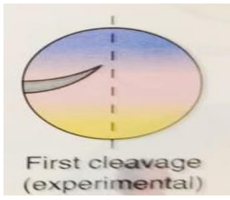
QUESTION: Is the development of a frog egg influenced by cytoplasmic determinants?

HYPOTHESIS: The gray crescent, which is normally bisected by the first cleavage division, thus establishing the right and left halves of the embryo, is the location of crucial developmental determinants.

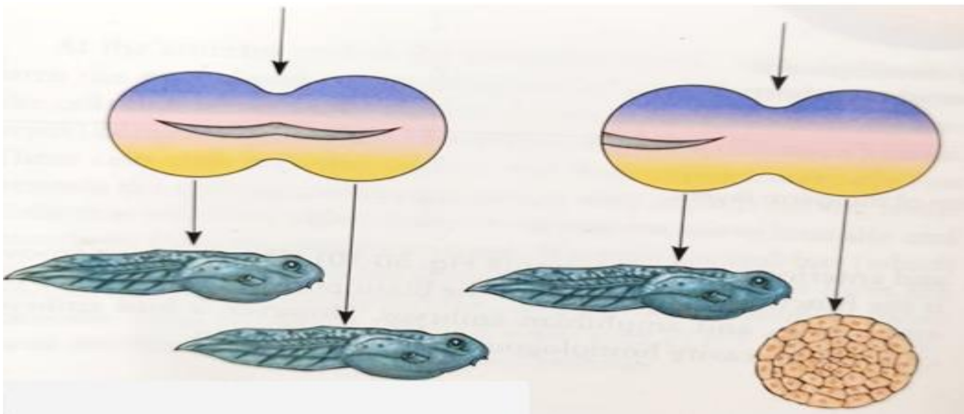
EXPERIMENT: The two blastomeres resulting from the first cleavage division are separated by the researcher and allowed to develop independently.



The first cleavage is allowed to occur normally, and each separated blastomere includes half of the gray crescent.



The plane of cleavage is altered by the experimenter such that only one blastomere contains the gray crescent.



QUESTION 5.4: Give the results of this experiment and state a conclusion based on the experiment done.

BOOK REFERENCES:

1. Carlson, B.M. 1996. Patten's Foundation of Embryology. 6th ed. McGraw Hill Book Company.
2. Carlson, B.M. 2004. Human Embryology and Developmental Biology. Mosby.
3. Solomon EP, Berg LR and Martin DW. 2008. Biology. 8th ed. Thomson Brooks/Cole.
4. Wilt, F & S. Hake. 2004. Principles of Developmental Biology. W.W. Norton & Company.

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