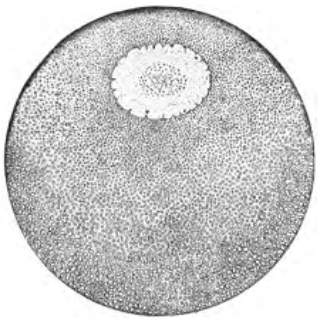


Amphibian egg

Fertilization

Fertilization is the fusion of sperm with egg resulting in the formation of zygote. It is characterized by the following events.

1. Fertilization is external.
2. It is monospermy, i.e. only one sperm fuses with the egg.
3. The fertilized egg rotates in such away that the animal hemisphere goes above.
4. The jelly coat swells and increases in thickness.
5. The second meiotic division is completed resulting in the release of the second polar body'
6. The sperm enters the egg in the animal hemisphere at an angle of 40^0 from the centre of animal pole.
7. Immediately after the entry of the sperm into the egg, the vitelline membrane becomes elevated. This membrane is now called **fertilization membrane**. The space between this membrane and the surface of the egg is called **perivitelline space** filled with a fluid called **perivitelline fluid**. In this fluid, the fertilized egg can rotate freely. The rotation of the egg is inevitable for the normal process of development. Immediately after fertilization, the black pigmented animal pole placed above and the yolk-laden vegetal pole below.
8. Before the release of egg into the water' the jelly coat remains thin. As the egg is released into the water, the jelly coat absorbs water and begins to swell until the thickness of the jelly becomes twice the diameter of the egg.
9. The second maturation division is completed immediately after fertilization. As a result, the fertilized egg releases the second polar body.
10. The egg pronucleus and sperm pronucleus fuse together to form the zygotic nucleus. This process is called amphimixis.

11. On one side just below the equator, a crescent like area appears; it will be grey in colour. This area is called **grey crescent**. It appears opposite to the point of sperm entry. The region of the grey crescent will become the posterior side and the opposite region will become the anterior side of the future embryo. This leads to the formation of a definite bilateral symmetry in the fertilized egg. .The unfertilized egg is radially symmetrical.

12. The sperm penetrates the egg perpendicular to the cortex. After penetration, the sperm moves in the cortex perpendicularly, along the radius of the egg. This path of the sperm is marked by pigment granules. This path of the sperm in the egg cortex is called **penetration path**. After crossing the cortex, the sperm changes its direction and moves towards the egg nucleus. This changed path is also marked by pigment granules and is called **copulation path**.

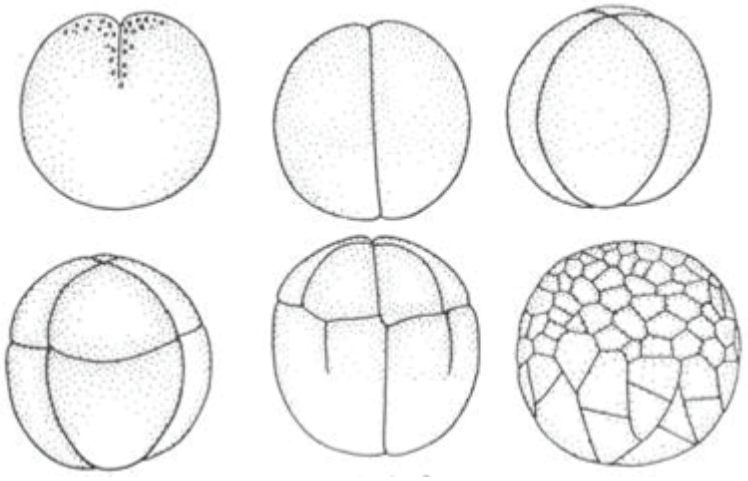
Grey Crescent (Gray Crescent)

1. Grey crescent is a crescent-like and grey colored area developing on the surface of amphibian egg opposite to the point of sperm entry.
2. It is a surface feature developing as a result of cytoplasmic movements stimulated by the sperm entry in the egg.
3. It appears just above the margin where the yellow-white vegetal pole material merges with the darkly pigmented animal pole material.
4. It appears on the surface of the egg opposite to the point of sperm entry.
5. Grey crescent marks the future dorsal side of the embryo.
6. The first cleavage bisects the grey crescent into two equal halves and this plane represents the future median plane of the embryo.
7. The formation of grey crescent, thus fixes up the final symmetry of the egg and the future embryo
8. In the gastrula, the grey crescent materials are located on the dorsal lip of the blastopore.
9. The grey crescent materials function as the organizer because, when it is- removed from the embryo, the embryo fails to develop further. At the same time when a normal embryo is grafted with another grey crescent, two embryos develop.
10. In the late gastrula, grey crescent materials are incorporated in to the chordamesoderm.

Cleavage

The first cleavage of frog's egg was observed by Swammerdam in 1738. The entire process of cleavage in frog's egg was studied by Prevost and Dumas in 1824. In frog's egg the cleavage is **holoblastic** and **unequal**. The cleavage occurs as follows.

1. The first cleavage plane is **meridional**. Initially, a furrow appears at the animal pole. It gradually extends towards the vegetal pole of the egg. It cuts the egg through its median animal-vegetal polar axis and results in two equal sized blastomeres.



Cleavage in frog's egg

2. The second cleavage furrow is again **meridional**. It bisects the first cleavage furrow at right angles. It is a holoblastic cleavage affecting both the blastomeres of the first cleavage. It results in the formation of four blastomeres.

3. In the next stage a **latitudinal/horizontal** furrow is formed above the equator nearer to the animal pole. Such a furrow is due to the influence of yolk concentration in the vegetal pole. The latitudinal furrow uniformly affects all the blastomeres. It results in the formation of eight blastomeres. Four of them remaining in the vegetal pole are large. They are named as **macromeres**. Another four blastomeres remain in the vegetal pole. They are named as **micromeres**. The micromeres are smaller in size than the macromeres.

4. The fourth set of cleavage planes are **meridional** and double in nature. They are unequal. They divide yolkless micromeres more rapidly than yolk-rich macromeres. These cleavages result in the production of 16 blastomeres.

5. The fifth cleavage is latitudinal /horizontal and double, dividing the micromeres as well as macromeres so that four tiers of blastomeres are formed.

6. As a result of further cleavages, a ball of several small blastomeres results. A closer observation reveals that, while the blastomeres above the equator are small and remain as micromeres, the blastomeres of the vegetal pole remain progressively larger. The larger blastomeres are called the macromeres.

Initially the continued division of blastomeres forms a ball like structure which is solid. It is called the **morula** stage, as this has superficial resemblance to a mulberry fruit. Very soon however the morula stage gives rise to a stage called the blastula which is a hollow ball like structure.

Blastulation

At the end of cleavage the solid ball of cells give rise to blastula which consists of a number blastomeres. The characteristic features of the blastula stage are the presence of a well defined cavity called the **blastocoel**. This is the beginning of the primary body cavity. The process of the formation of blastula is called **blastulation**. The blastula of frog is called amphiblastian as the cavity is confined to only the animal pole. The vegetal pole however is composed of a solid mass of non pigmented yolky cells.

In the thirty two cell stage, the blastula consists of a single layer of cells and is called the early blastula. The pigmented cells (micromeres) are found in the anterior half while the yolky megameres are present in the posterior half. As has been already pointed out, the blastocoel lies entirely in the anterior half. The blastula of frog is hollow and has a very well developed blastocoel. It is said to be a **coeloblastula**.

As segmentation proceeds, the number of cells in the blastula increase; so also the blastocoel. The floor of the blastocoel is flat while its top portion is arched. The roof is made up of three to four layers of pigmented micromeres while the floor is formed by yolky megameres. Between the micromeres and the megameres and along the equator is found a group of cells which are intermediate in size (between megameres and micromeres). These cells constitute the **germ ring**. The germ ring is formed in the region of the grey crescent.

Fate Map

Wather Vogt (1925) used vital staining method for the construction of fate maps of amphibians. Vital stains do not interfere with the normal processes. A piece of agar or cellophane

(stain carrier) is used and is pressed against the chosen area of blastula for a short period. Cellophane is better than agar as it can be cut easily into desired size and shape. The stain does not diffuse into the neighboring cells. The blastula of amphibian embryo is round and has three distinct regions:

1. The vegetal region is the pigment free macromere region. It represents **presumptive endoderm** and contains the material for the formation of midgut and hindgut of embryo.

2. Second region is that of animal pole of egg which consists of micromeres. It gives rise to future ectoderm of the animal and forms two main regions:

a. Region of **prospective ectoderm** which develops into the epidermis of skin.

b. Region of **prospective central nervous system** which forms brain, spinal cord and sense organs.

3. Third region is the marginal region of gray crescent. It forms the **presumptive mesodermal cells**. It consists of the following subregions:

a. **Presumptive notochordal** region which is present on the dorsal side and gives rise to notochord.

b. Below the notochordal area is the portion which forms the part of foregut.

c. Region of **presumptive somites** which develops on both the sides of notochordal area.

d. Ventrolateral mesodermal area which lies on lateral and ventral part of marginal zone and forms the **mesodermal lining** of the body cavity, kidney and reproductive organs.