

M. Sc II Semester (Biotechnology)

Cells, Molecules and Developmental Biology

Unit IV

Types of Gametogenesis: Spermatogenesis and Oogenesis

Gametogenesis is the process of formation and differentiation of haploid gametes (sperms and ova) from the diploid primary germ cells, gametogonia (spermatogonia and oogonia) present in primary sex organs called gonads (testes in male and ovaries in female respectively).

Gametogenesis is of two types:

I. Spermatogenesis and II. Oogenesis.

I. Spermatogenesis

Definition:

It is the formation of haploid, microscopic and functional male gametes, spermatozoa from the diploid reproductive cells, spermatogonia, present in the testes of male organism.

Period:

In the seasonally breeding animals, the testes undergo testicular cycle in which the testes and their spermatogenic tissue become functional only in the specific breeding season. So in some seasonally breeding mammals like bat, otter and llama, testes enlarge, become functional and descend into the scrotum in the breeding season as become heavier due to accumulation of sperms, while become reduced, non-functional and ascend into the abdomen in other seasons.

But in human male, lion, bull, horse etc., the testes lie permanently in the scrotum and spermatogenesis occurs throughout the year. In human male, testes descend into the respective scrotal sacs during seventh month of development under the stimulation of FSH of adenohipophysis.

But in some mammals e.g. elephant, echidna, dolphin, whale, seal etc., testes lie permanently in the abdomen (intra-abdominal) mainly due to presence of blubber (thick fatty layer beneath the skin). Spermatogenesis is a continuous process and is completed in about 74 days.

Mechanism:

Spermatogenesis is divided into two parts:

A. Formation of Spermatid:

It is divided into three phases:

1. Multiplicative or Mitotic phase:

It involves the rapid mitotic division of diploid primary or primordial germ cells, called gonocytes, present in germinal epithelium of the seminiferous tubules of the testes. These cells are undifferentiated and have large and chromatin-rich nucleus.

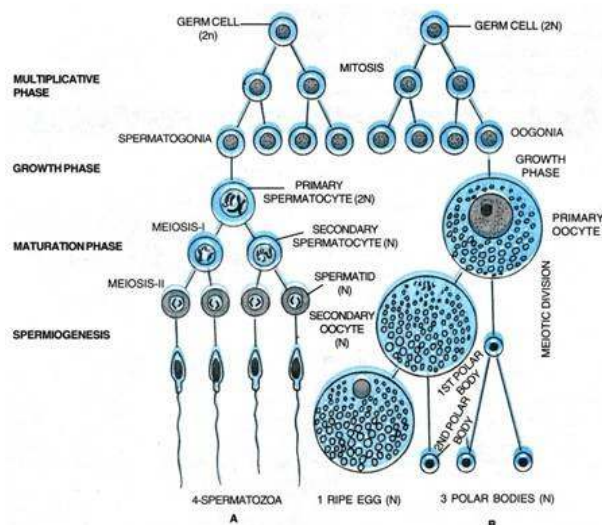


Fig. 3.13. Various stages in gametogenesis. A. Spermatogenesis. B. Oogenesis.

This forms large number of diploid and rounded sperm mother cells called spermatogonia (Gr. sperma = seed; gone = offspring). Each spermatogonial cell is about 12 μ m in diameter and has a prominent nucleus. Some spermatogonia act as stem cells (called Type A spermatogonia) and go on dividing and adding new cells by repeated mitotic divisions, so forming spermatogenic lineage, but some spermatogonia move inward and enter growth phase (called Type B spermatogonia).

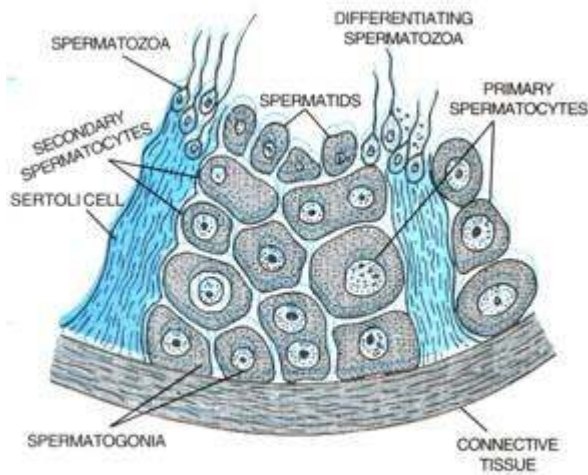


Fig. 3.14. T.S. A part of seminiferous tubule of testis showing spermatogenesis.

2. Growth phase:

It is characterized by spermatocytogenesis in which a diploid spermatogonium increases in size (about twice) by the accumulation of nutritive materials (derived from germinal cells and not synthesized) in the cytoplasm and replication of DNA, and forms diploid primary spermatocyte. Nutritive materials are derived from germinal cells. During this, the primary spermatocyte prepares itself to enter meiosis. Growth phase of spermatogenesis is of much shorter duration than that of oogenesis.

3. Maturation or Meiotic phase:

It is characterised by meiosis. The diploid primary spermatocyte undergoes meiosis-I (reductional or heterotypical division) and forms two haploid cells called secondary spermatocytes, each containing 23 chromosomes.

It is immediately followed by meiosis-II (equational or homotypical division) in each secondary spermatocyte to form two haploid spermatids, each of which has 23 chromosomes. So each diploid spermatogonium produces 4 haploid spermatids. Different stages of spermatogenesis are interconnected by cytoplasmic strands till spermiogenesis when the maturing and interconnected gametes separate from each other.

B. Spermiogenesis

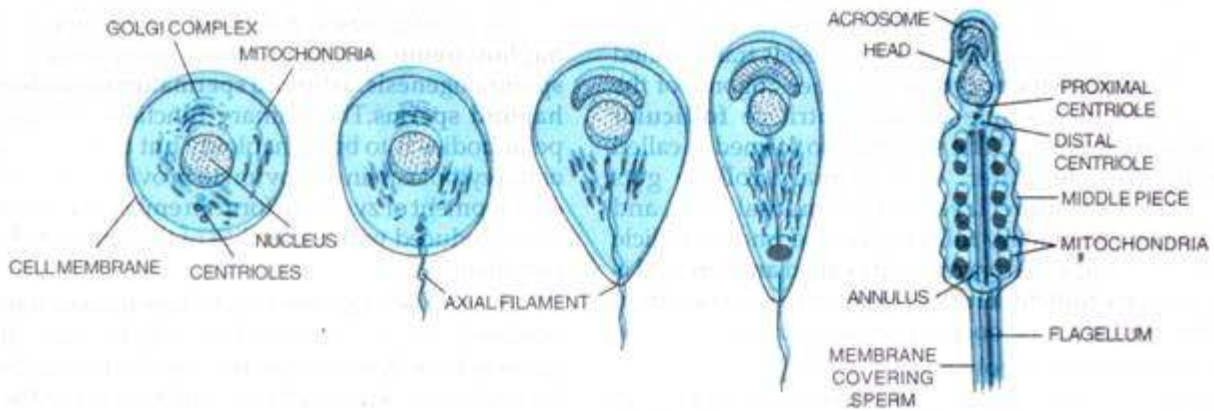


Fig. 3.15. Stages in spermiogenesis.

The transformation of a non-motile, rounded and haploid spermatid into a functional and motile spermatozoan is called spermiogenesis or spermioteliolysis. The main aim is to increase the sperm motility by reducing weight and development of locomotory structure.

It involves the following changes:

1. Nucleus becomes condensed, narrow and anteriorly pointed due to loss of materials like RNAs, nucleolus and most of acidic proteins.
2. A part of Golgi body of spermatid forms the acrosome, while the lost part of Golgi body is called Golgi rest.
3. Centrioles of spermatid form the neck of sperm.
4. Distal centriole gives rise to axoneme.
5. Mitochondria form a spiral ring behind the neck around the distal centriole and proximal part of axoneme. This is called nebenkern.
6. Most of cytoplasm is lost but some cytoplasm forms sheath of tail of sperm.

The spermatids mature into spermatozoa in deep folds of the cytoplasm of the Sertoli cells (nurse cells) which also provide nourishment to them. Mature spermatozoa are released in the lumen of seminiferous tubules, called spermiation. The two testes of young adult form about 120 million sperms each day.

Changes in spermatid to form sperm during spermiogenesis.

Structure of spermatid	Changes in the sperm
1. Nucleus	Shrinks and elongated.
2. Golgi complex	Changes to acrosome.
3. Distal centriole	Forms axial filament of sperm tail.
4. Mitochondria	Form mitochondrial spiral of sheath called nebenkem.
5. Cytoplasm	Generally lost except a thin sheath called manchette.

Control:

In human male, spermatogenesis starts only at the age of puberty due to increased secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus of brain. GnRH stimulates adenohypophysis to secrete two gonadotropins: FSH and ICSH. ICSH stimulates the Leydig's cells of testis to secrete male sex hormones, called androgens, most important of which is testosterone. Testosterone stimulates the spermatogenesis especially spermiogenesis. FSH stimulates the Sertoli cells of testis to secrete certain factors which helps in the process of spermatogenesis. It is called physiological control.

Types:

In man and a large number of other animals having XY mechanism in male, there are two types of sperms: 50% Gynosperms having X-Chromosome and 50% Androsperms having Y-Chromosome.

Significance:

- (a) Produces haploid sperms.
- (b) Crossing over may occur during meiosis-I, so producing variations.
- (c) Proves evolutionary relationship.

II. Oogenesis:

Definition:

It involves the formation of haploid female gametes called ova, from the diploid egg mother cells, oogonia, of ovary of female organism. It involves 2 biological processes: Genetical programming and packaging.

Period:

Period of oogenesis is different in different animals. In human female, there are about 1,700 primary germ cells in the undifferentiated female gonad at one month of foetal development. These proliferate to form about 600,000 oogonia at two months of gestation period and by its 5th month, the ovaries contain over 7 million oogonia; however, many undergo atresia (degeneration of germ cells) before birth. At the time of birth, there are 2 million primary follicles, but 50% of these are atretic.

Atresia continues and at the time of puberty each ovary contains only 60,000-80,000 primary follicles. Oogenesis is completed only after the onset of puberty and only one out of 500 is stimulated by FSH to mature. So oogenesis is a discontinuous and wasteful process.

Mechanism:

Like the spermatogenesis, oogenesis is formed of three phases:

1. Multiplicative phase:

In this certain primary germ cells (larger in size and having large nuclei) of germinal epithelium of ovary undergo rapid mitotic divisions to form groups of diploid egg mother cells, oogonia. Each group is initially a chord and is called egg tube of pfluger which later forms a rounded mass, egg nest.

2. Growth phase:

Growth phase of oogenesis is of very long duration than that of spermatogenesis e.g., only three days in Drosophila, 6-14 days in hen, 3 years in frog and many years (12-13 years) in human female. During growth phase, one oogonium of egg nest is transformed into diploid primary oocyte while other oogonia of the egg nest form a single-layered nutritive follicular epithelium around it. The structure so formed is called primary follicle. Later, each primary follicle gets

surrounded by more layers of granulosa cells and changes into secondary follicle. Soon secondary follicle develops a fluid-filled antral cavity called antrum, and is called tertiary follicle. It further changes to form Graafian follicle. So not all the oogonia develop further.

Growth phase involves:

(a) Increase in size of oocyte (2000 times in frog; 43 times in mouse; 90,000 times in *Drosophila*; 200 times in hen and about 200 times in human female) by the formation and accumulation of yolk (vitellogenesis) by a special mitochondrial cloud lying close to nucleus and called yolk nucleus.

(b) Nucleus becomes bloated with nucleoplasm and is called germinal vesicle.

(c) A thin vitelline membrane is secreted around the oocyte.

(d) Increase in number of mitochondria, amount of ER and Golgi body.

(e) Formation of lampbrush chromosomes in fishes, amphibians, reptiles, birds, insects, etc. for rapid yolk synthesis.

(f) Gene-amplification or redundancy of r-RNA genes for rapid synthesis of r-RNA.

3. Maturation phase:

It is characterized by meiosis. In this, the diploid and fully grown primary oocyte undergoes meiosis-I (reductional division) to form two unequal haploid cells. The smaller cell is called first polar body (Polarocyte) and has very small amount of cytoplasm. The larger cell is called secondary oocyte and has bulk of nutrient-rich cytoplasm. Both of these are haploids and each has 23 chromosomes. Secondary oocyte undergoes meiosis-II (equational division) to form two unequal haploid cells. The smaller cell is called second polar body and has very little of cytoplasm, while the larger cell is called ootid. It has almost whole of cytoplasm and differentiates into an ovum. Meanwhile, first polar body may divide into two.

So in oogenesis, a diploid oogonium forms one haploid ovum and two or three polar bodies while in spermatogenesis, a diploid spermatogonium forms four haploid sperms. The primary function of formation of polar bodies is to bring haploidy but to retain the whole of the

cytoplasm in one ovum to provide food during the development of zygote to form an embryo. The number of ova is reduced with the ability of the female to bear and rear them.

In most of organisms including human female, the ovulation occurs at secondary oocyte stage in which meiosis-I has been completed and first polar body has been released. Meiosis-II is completed only at the time of sperm-entry.

Significance:

- (a) It produces haploid ovum by releasing 2 or 3 haploid polar bodies.
- (b) Most of cytoplasm is retained in functional ovum.
- (c) Variations may appear due to crossing over during Meiosis-I.
- (d) Proves evolutionary relationship.

Ref:

1.<http://www.yourarticlelibrary.com/biology/human-reproduction/types-of-gametogenesis-spermatogenesis-and-oogenesis-biology/26910>

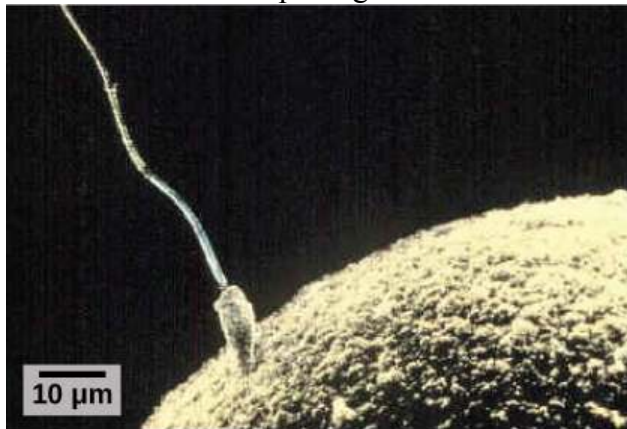
2.[https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_\(Boundless\)/43%3A_Animal_Reproduction_and_Development/43.3%3A_Human_Reproductive_Anatomy_and_Gametogenesis/43.3C%3A_Gametogenesis_\(Spermatogenesis_and_Oogenesis\)](https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/43%3A_Animal_Reproduction_and_Development/43.3%3A_Human_Reproductive_Anatomy_and_Gametogenesis/43.3C%3A_Gametogenesis_(Spermatogenesis_and_Oogenesis))

Fertilization and Early Embryonic Development

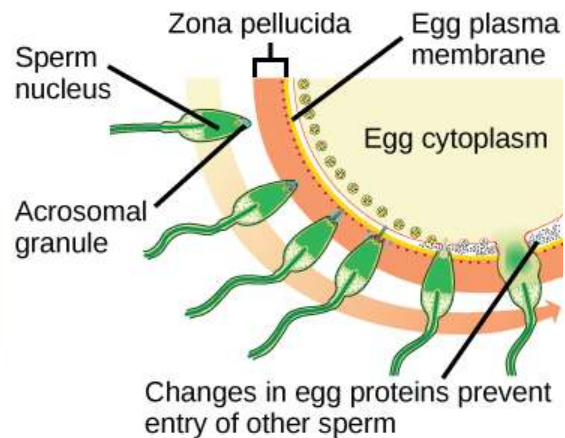
The process in which an organism develops from a single-celled zygote to a multi-cellular organism is complex and well-regulated. The early stages of embryonic development are also crucial for ensuring the fitness of the organism.

Fertilization

Fertilization is the process in which gametes (an egg and sperm) fuse to form a zygote. The egg and sperm each contain one set of chromosomes. To ensure that the offspring has only one complete diploid set of chromosomes, only one sperm must fuse with one egg. In mammals, the egg is protected by a layer of extracellular matrix consisting mainly of glycoproteins called the **zona pellucida**. When a sperm binds to the zona pellucida, a series of biochemical events, called the **acrosomal reactions**, take place. In placental mammals, the acrosome contains digestive enzymes that initiate the degradation of the glycoprotein matrix protecting the egg and allowing the sperm plasma membrane to fuse with the egg plasma membrane. The fusion of these two membranes creates an opening through which the sperm nucleus is transferred into the ovum. The nuclear membranes of the egg and sperm break down and the two haploid genomes condense to form a diploid genome.



(a)



(b)

(a) Fertilization is the process in which sperm and egg fuse to form a zygote. (b) Acrosomal reactions help the sperm degrade the glycoprotein matrix protecting the egg and allow the sperm to transfer its nucleus. (credit: (b) modification of work by Mariana Ruiz Villareal; scale-bar data from Matt Russell)

To ensure that no more than one sperm fertilizes the egg, once the acrosomal reactions take place at one location of the egg membrane, the egg releases proteins in other locations to prevent other sperm from fusing with the egg. If this mechanism fails, multiple sperm can fuse with the egg, resulting in **polyspermy**. The resulting embryo is not genetically viable and dies within a few days.

Cleavage and Blastula Stage

The development of multi-cellular organisms begins from a single-celled zygote, which undergoes rapid cell division to form the blastula. The rapid, multiple rounds of cell division are termed cleavage. Cleavage is illustrated in following [Figure a](#)). After the cleavage has produced over 100 cells, the embryo is called a blastula. The blastula is usually a spherical layer of cells (the blastoderm) surrounding a fluid-filled or yolk-filled cavity (the blastocoel). Mammals at this stage form a structure called the blastocyst, characterized by an inner cell mass that is distinct from the surrounding blastula, shown in [Figure b](#). During cleavage, the cells divide without an increase in mass; that is, one large single-celled zygote divides into multiple smaller cells. Each cell within the blastula is called a blastomere.

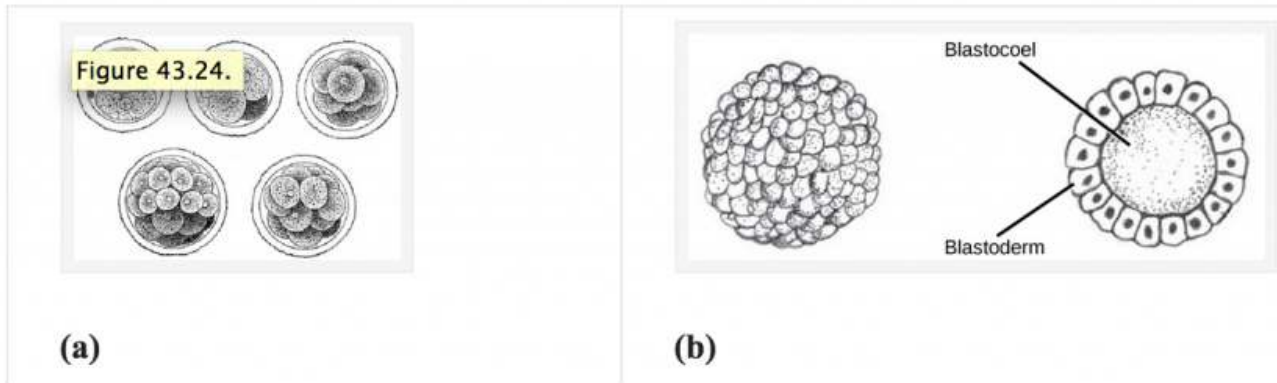
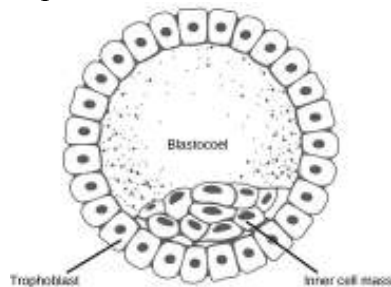


Figure (a) During cleavage, the zygote rapidly divides into multiple cells without increasing in size. (b) The cells rearrange themselves to form a hollow ball with a fluid-filled or yolk-filled cavity called the blastula. (credit a: modification of work by Gray's Anatomy; credit b: modification of work by Pearson Scott Foresman, donated to the Wikimedia Foundation)

Cleavage can take place in two ways: **holoblastic** (total) cleavage or **meroblastic** (partial) cleavage. The type of cleavage depends on the amount of yolk in the eggs. In placental mammals (including humans) where nourishment is provided by the mother's body, the eggs have a very small amount of yolk and undergo holoblastic cleavage. Other species, such as birds, with a lot of yolk in the egg to nourish the embryo during development, undergo meroblastic cleavage.

In mammals, the blastula forms the **blastocyst** in the next stage of development. Here the cells in the blastula arrange themselves in two layers: the **inner cell mass**, and an outer layer called the **trophoblast**. The inner cell mass is also known as the embryoblast and this mass of cells will go on to form the embryo. At this stage of development the inner cell mass consists of embryonic stem cells that will differentiate into the different cell types needed by the organism. The trophoblast will contribute to the placenta and nourish the embryo.



The rearrangement of the cells in the mammalian blastula to two layers—the inner cell mass and the trophoblast—results in the formation of the blastocyst.

Gastrulation

The typical blastula is a ball of cells. The next stage in embryonic development is the formation of the body plan. The cells in the blastula rearrange themselves spatially to form three layers of cells. This process is called **gastrulation**. During gastrulation, the blastula folds upon itself to form the three layers of cells. Each of these layers is called a germ layer and each germ layer differentiates into different organ systems.

The three germ layers, the endoderm, the ectoderm, and the mesoderm. The ectoderm gives rise to the nervous system and the epidermis. The mesoderm gives rise to the muscle cells and connective tissue in the body. The endoderm gives rise to columnar cells found in the digestive system and many internal organs.

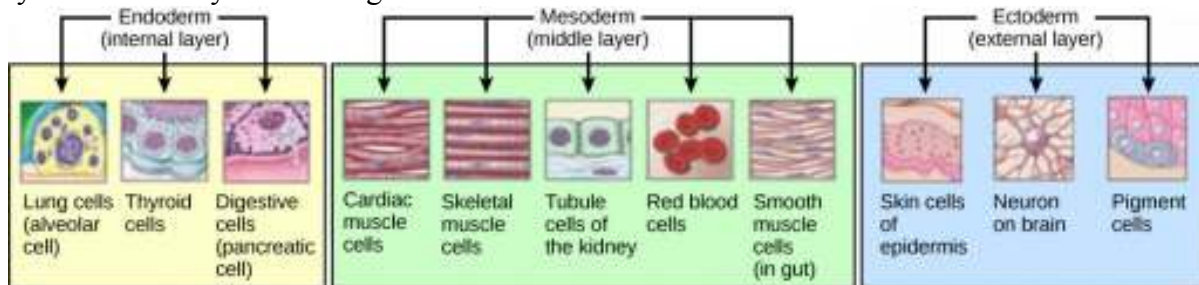


Figure : The three germ layers give rise to different cell types in the animal body.

Summary: The early stages of embryonic development begin with fertilization. The process of fertilization is tightly controlled to ensure that only one sperm fuses with one egg. After fertilization, the zygote undergoes cleavage to form the blastula. The blastula, which in some species is a hollow ball of cells, undergoes a process called gastrulation, in which the three germ layers form. The ectoderm gives rise to the nervous system and the epidermal skin cells, the mesoderm gives rise to the muscle cells and connective tissue in the body, and the endoderm gives rise to columnar cells and internal organs.

Reference:

<https://opentextbc.ca/biology/chapter/24-6-fertilization-and-early-embryonic-development/>

Human embryogenesis

Embryogenesis, the first eight weeks of development after fertilization, is an incredibly complicated process. It's amazing that in eight weeks we're transforming from a single cell to an organism with a multi-level body plan. The circulatory, excretory, and neurologic systems all begin to develop during this stage. Luckily, like with many complex biological concepts, fertilization can be broken down into smaller, simpler ideas. The big idea of embryogenesis is going from a single cell to a ball of cells to a set of tubes.

- **Step 1:** a *zygote* is the single cell formed when an egg and a sperm cell fuse; the fusion is known as fertilization
- **Step 2:** the first 12-to 24-hours after a zygote is formed are spent in *cleavage* – very rapid cell division

The zygote's first priority is dividing to make lots of new cells, so it's first few days are spent in rapid mitotic division. With each round of division, it doubles in cell number, so the cell number is increasing at an exponential rate! This division is taking place so quickly that the cells don't have time to grow, so the 32 cell stage known as the *morula* is the same size as the zygote. At this point, the zona pellucida (a protective membrane of glycoproteins that had surrounded the egg cell) is still intact, which also limits how big it can grow.

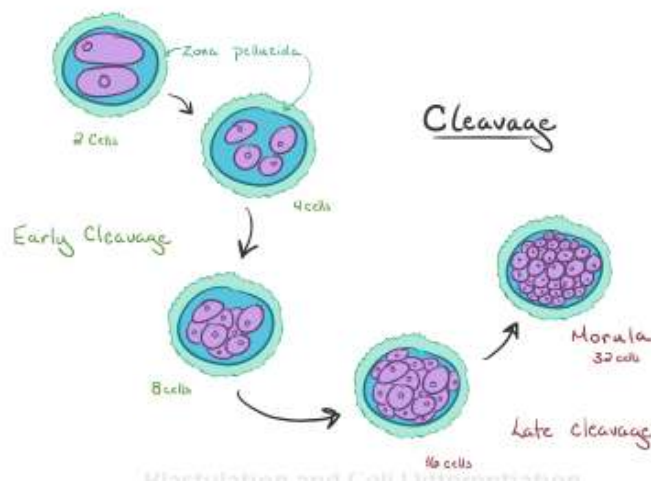


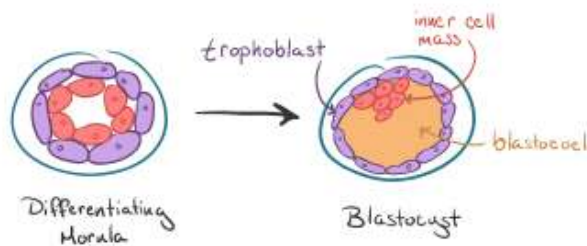
Illustration of early and late cleavage

Blastulation and Cell Differentiation

- **Step 3:** during *blastulation*, the mass of cells forms a hollow ball
- **Step 4:** cells begin to differentiate, and form cavities

Around day 4, cells continue to divide, but they also begin to differentiate and develop more specific forms and functions. When a cell differentiates, it moves down a certain path toward being a specific type of cell (e.g. an ear cell or a kidney cell), and this process (99% of the time) only goes in one direction. Two layers develop: an outer shell layer known as the *trophoblast*, and an inner collection of cells called the inner cell mass. Rather than being arranged in a solid sphere of cells, the *inner cell mass* is pushed off to one side of the sphere formed by the

trophoblast. The rest of the fluid-filled cavity is called the *blastocoel*, and the whole setup resembles a snow globe. The outer trophoblast will develop into structures that help the growing embryo implant in the mother's uterus. The inner cell mass will continue to differentiate and parts of it will eventually become the embryo, so it is sometimes called the *embryoblast* (the suffix "blast" means "to make"). This is also the time when the zona pellucida begins to disappear, allowing the ball of cells, now called a *blastocyst*, to grow and change shape. In non-mammal animals, the term for this stage is "*blastula*", but we will stick with terms that apply to human development for the purposes of this discussion.



At this point, cells in the inner cell mass are *pluripotent*, meaning they can eventually turn into the cells of any body tissue (muscle, brain, bone, etc). During the second week, these cells differentiate further into the *epiblast* and the *hypoblast*, which are the two layers of the *bilaminar disc*. This disc is a flat slice across the developing sphere, and splits the environment into two cavities. The hypoblast is the layer facing the blastocoel, while the epiblast is

Illustration of differentiating morula transforming into blastocyst

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Quick recap: the outermost layer of the sphere is the trophoblast. Inside the sphere are two spaces that are each lined by either the hypoblast or the epiblast. The point where the epiblast

and hypoblast press up against each other is known as the bilaminar disc, and this disk is what splits the sphere to make the two cavities.



Tree chart of zygote structures including blastula, trophoblast, inner cell mass, epiblast and hypoblast

The hypoblast does not contribute to the embryo, so we will now turn our focus solely on the epiblast.

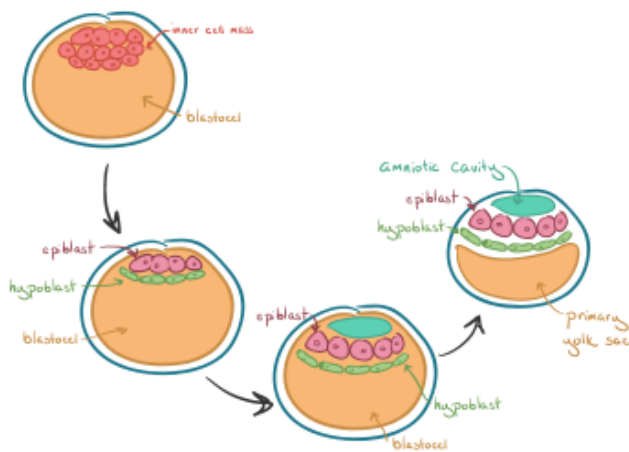


Diagram showing formation of epiblast, hypoblast, amniotic cavity and yolk sac

Making Tubes

- **Step 5:** During *gastrulation* the three germ layers form; the cell mass is now known as a gastrula
- **Step 5a:** The *primitive streak* forms

- **Step 6:** The *notochord* is formed

Week 3 of development is the week of *gastrulation*. A germ layer is a layer of cells that will go on to form one of our organizational tubes. Our anatomy can really be boiled down to an inner tube (our digestive tract), and a series of tubes that wrap around it. The three germ layers that will translate into these tubes are the *ectoderm*, the *mesoderm*, and the *endoderm*.

Germ Layer	What does the prefix mean?	Goes on to form:
Ectoderm	Outer, external	Epidermis (outer layer of skin), hair, nails, brain, spinal cord, peripheral nervous system
Mesoderm	Middle	Muscle, bone, connective tissue, notochord, kidney, gonads, circulatory system
Endoderm	Within	Epithelial lining of the digestive tract; Stomach, colon, liver, pancreas, bladder, lung

The first step of gastrulation is the formation of the primitive streak (~ day 16). Let's imagine the bilaminar disc as two tier cake. Imagine taking a knife and cutting into just the top layer (the epiblast) like you're going to cut a slice.

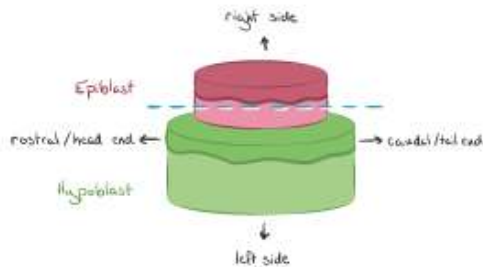


Figure of two tier cake representing epiblast and hypoblast with cut through epiblast layer

This cut is the primitive streak, and it cuts from the caudal (anus) end in toward the end that will eventually become the head (the rostral end). This streak determines the midline of the body, and separates the left and right sides. Like all deuterostomes, humans have bilateral symmetry, which means that there is a single across which we can split ourselves to make mirror images. What we are actually seeing when we look at a primitive streak are moving cells. They are going from the epiblast and moving down so they end up between the original epiblast layer and the hypoblast. I've always imagined the motion like water falling down a waterfall. The first layer to invaginate

dives the deepest and ends up closest to the hypoblast – this is the endoderm. The next layers will become the mesoderm, and the cells of the epiblast that continue to border the amniotic cavity are the ectoderm. We now have three germ layers, all of which will contribute to the developing embryo. In the picture below, the anus end is facing us.

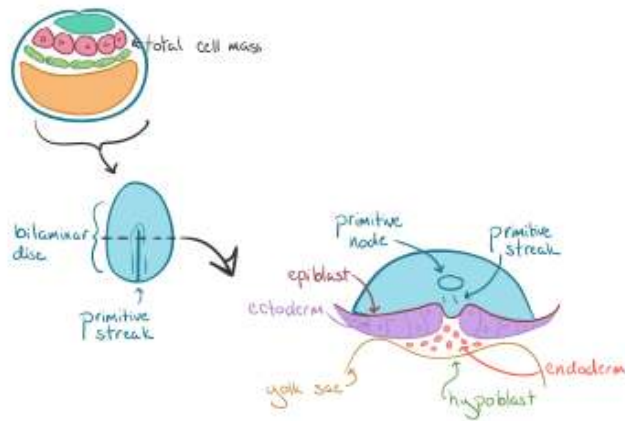


Figure of bilaminar disc

Directly beneath the primitive streak the mesoderm (the middle germ layer) forms a thin rod of cells known as the notochord. The notochord helps define the major axis of our bodies, and is important in inducing the next step of embryogenesis, when we finally start to make our tubes! The notochord is a defining feature of the Chordate phylum, and will eventually become our intervertebral discs.

Neurulation

- **Step 6:** Tubes form, making a *neurula*
- **Step 6a:** The notochord induces the formation of the *neural plate*
- **Step 6b:** The neural plate folds in on itself to make the *neural tube* and *neural crest*
- **Step 7:** The mesoderm has five distinct categories

All this and we still haven't made tubes! Now that we have successfully made the cell layers, we have to create the final 3D product. The first step in this rolling is the creation of the notochord. The notochord causes the ectoderm above it to form a thick flat plate of cells called the neural plate. The neural plate extends the length of the rostral-caudal axis. The neural plate then bends back on itself and seals itself into a tube known as the neural tube that fits underneath the

ectoderm. The borders of where the neural plate had been get pulled under with it, and become the neural crest. The neural tube will become the brain and spinal cord.

The neural crest is sometimes called the fourth germ layer, because the cells that become the sympathetic and parasympathetic nervous systems, melanocytes, Schwann cells, even some of the bones and connective tissue of the face.

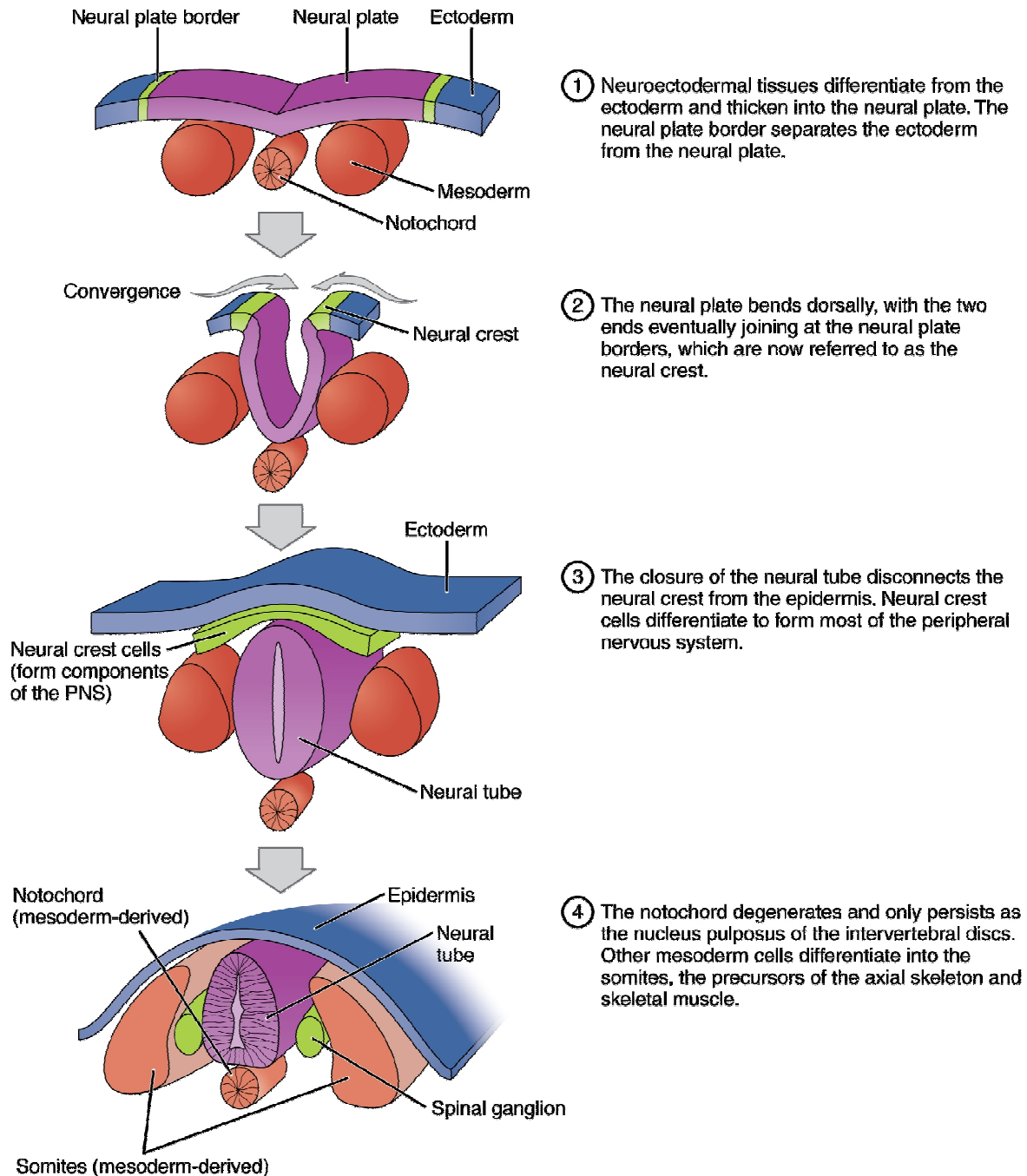


Illustration of neurulation process

Meanwhile, the mesoderm can be subdivided into the axial, paraxial, intermediate, and lateral plate mesoderms. The notochord came from the axial mesoderm. The paraxial mesoderm will give rise to somites, which will differentiate into muscle, cartilage, bone, and dermis. Somite derivatives create a segmented body plan (see right). The intermediate mesoderm is the origin of our urogenital system – our kidneys, gonads, adrenal glands, and the ducts that connect them. The lateral plate mesoderm will give rise to the heart (the first organ to develop!), blood vessels, the body wall, and the muscle in our organs.

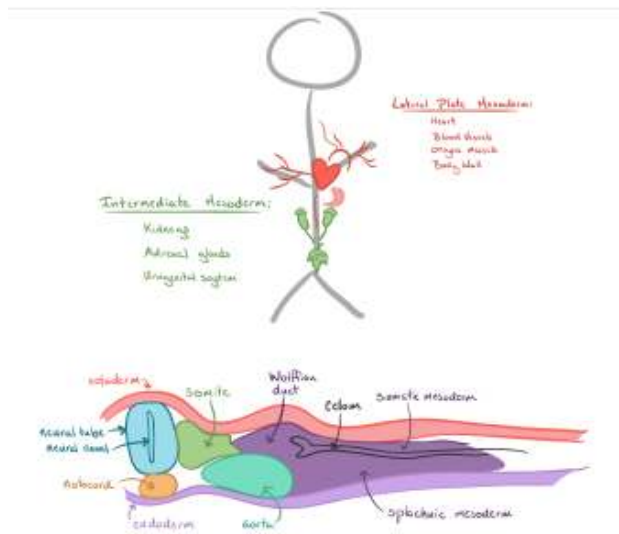


Figure of stick figure labeled with urogenital system, heart, blood vessels, the body wall, and organ muscle

Figure of endoderm transforming into the digestive tract

Also at the same time, the endoderm is rolling into a tube as well – the digestive tract. The digestive tract is subdivided into the foregut, midgut, and hindgut. Each subdivision has its own nerve and blood supply. Organs related to the GI tract actually start off as outpouchings of this tube. The foregut gives rise to the esophagus, stomach, part of the duodenum, and the respiratory bud, which will eventually develop into the lungs. The second half of the duodenum through to the transverse colon arise from the midgut. The remainder of the GI tract, including the rest of the transverse colon, the descending colon, the sigmoid colon, and the rectum are formed from the hindgut.

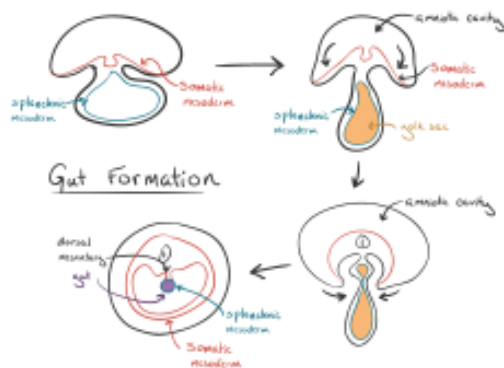


Diagram of gut formation

That's what is going on with each of the three layers. While this is happening, the mesodermal layers are circling around the endoderm, and the part of the ectoderm that will become the skin is circling around both of the other layers. Some tubes, like the neural tube, are closing, while the gut tube is connecting to the ectoderm to form the mouth and the anus. By the time eight weeks have passed, all of our tubes are in order, the primitive heart has been beating for almost five weeks, and development is well on its way!

Consider the following

The gut tube is the only developmental tube that is supposed to remain an open cylinder. If the neural tube does not close, it creates a life-threatening condition known as spina bifida. Spina bifida can occur due to genetic factors, but may also be caused by a lack of folic acid during pregnancy or if the mother has uncontrolled diabetes. Spina bifida can lead to weakness and paralysis of the legs, bladder and bowel control issues, and other physical problems. Children with spina bifida often struggle academically, potentially due to problems in the development of the central nervous system. While there is no known cure for spina bifida, the introduction of folic acid into everyday foods like cereal and bread has drastically reduced the incidence of neural tube defects in newborns.

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DEVELOPMENT OF EMBRYO SAC OR FEMALE GAMETOPHYTE

INTRODUCTION

The plant life cycle is characterized by the alternation of generations between a diploid sporophyte and a haploid gametophyte. The sporophyte functions to produce spores, which then develop into gametophytes. The differentiated gametophytes in turn produce either the male gametes (sperm) or female gametes (egg cells). In contrast to lower plant species, in which the gametophyte is the dominant, free-living generation, gametophytes of angiosperms are smaller and less complex than the sporophyte and are formed within specialized organs of the flower. The male gametophyte (pollen or microgametophyte) develops within the anther, whereas the female gametophyte (embryo sac or megagametophyte) is a product of the ovule. Sexual reproduction requires the delivery of the sperm nuclei, via the pollen, to the embryo sac, where fertilization occurs and the new diploid sporophyte is formed. These unique features of the angiosperm reproductive process illustrate one of the most fundamental problems in plant biology. That is, how does the transition from sporophytic to gametophytic development occur within the context of the flower? How do the gametophytes interact with each other and the maternal plant to produce a seed? A wealth of information has been obtained regarding sexual reproduction in plants by genetic and molecular analyses of pollen development and pollen-stigmakyle interactions. By comparison, less is known about the regulation of female gametophyte development and the relationship of the embryo sac to the ovule before and after fertilization. The ovule is the source of the megagametophyte and the progenitor of the seed. Specification of the megasporocyte, production of a functional megaspore (megasporogenesis), formation of the embryo sac (megagametogenesis), and embryogenesis all occur within the ovule. Because embryo sacs, unlike pollen, maintain physical contact with the parent sporophyte throughout their development, this association of the female gametophyte and the sporophyte provides an opportunity to examine interactions between cells, tissues, and genomes.

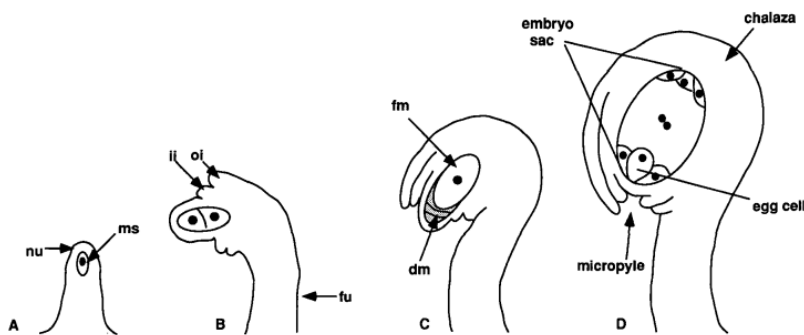


Figure 1. Ovule Development.

Stages are shown for an anatropous ovule with *Polygonum*-type embryo sac development. For more details, see text.

(A) Ovule shortly after initiation, showing a single megasporocyte (ms), nu, nucellus.

(B) Ovule after both integuments have been initiated. At this time, the megasporocyte has undergone the first meiotic division. The axis of the nucellus is transiently perpendicular to the axis of the funiculus (fu), ii, inner integument; oi, outer integument.

(C) Ovule after meiosis. The functional megaspore (fm) at the chalazal end has expanded, and the nonfunctional megaspores are degenerated. The axis of the nucellus is now parallel to the funiculus due to unequal growth, primarily of the integuments. dm, degenerate megaspores.

(D) Ovule after megagametogenesis. The mature embryo sac contains seven cells and eight nuclei.

OVULE AND EMBRYO SAC DEVELOPMENT

Ovule Development: Ovules are specialized structures, derived from the placenta of the ovary wall, that produce the megasporocyte and are the site of embryo sac formation, fertilization, and embryogenesis. The ovule consists of three basic structures: a nucellus, one or two integuments, and a funiculus. The nucellus is derived from the apical portion of the ovule primordium and functions as the megasporangium. That is, the nucellus produces the megasporocyte, which will undergo meiosis to form the megaspores. Shortly after ovule initiation, a single subdermal nucellar cell enlarges and displays a prominent nucleus. This cell represents the archesporium, or spore-bearing tissue, and typically occupies a position directly below the apex of the nucellus. Progeny obtained from periclinal chimeras show that the megasporocyte is derived from the subdermal (L2) histogenic layer. The single cell of the archesporium may function directly as the megasporocyte, or it may undergo one mitotic division to produce a megasporocyte and a somatic cell. The archesporium is not always represented by a single archesporial cell. Some species, including soybean and *Brassica campestris*, have a multicellular archesporium containing several archesporial cells, only one of which gives rise to the megagametophyte. Factors that control the development of the archesporium and determine the identity of the megasporocyte have not been identified. The location of the megasporocyte, directly below the apex of the nucellus, suggests that position may be an important factor in megasporocyte specification. The integuments are initiated at the base of the nucellus during megasporogenesis. The inner integument is most often dermal (L1) in origin, whereas the outer integument is usually derived from both dermal and subdermal layers. The two integuments are considered to have distinct evolutionary origins. Periclinal divisions in the integuments generate an increase in the number of cell layers, whereas anticlinal divisions and cell elongation are responsible for growth parallel to the nucellus. As the embryo sac develops, the integuments continue to enlarge, typically overgrowing the nucellus. The amount of ovule curvature varies with the extent of differential growth of the integuments and funiculus; the degree of curvature forms a basis for classification of ovule morphology. Thus, the mature anatropous ovule shows extensive curvature such that the long axis of the nucellus is parallel to the axis of the funiculus. In ~65% of the species examined, most of the nucellus degenerates before the embryo sac reaches maturity. The embryo sac is then in direct contact with the inner integument. In these situations, the innermost cell layer of the inner integument may differentiate into a unique cell layer termed the endothelium. Radial cell expansion, endopolyploidy, and prominent nuclei are observed in the endothelial cells and also the anther tapetum, which is thought to be involved in secretion and nutrition of the pollen. Maheshwari (1950) speculated that the cytological features shared between the endothelium and tapetum could indicate a similar function for both tissues. In species in which the nucellus does not degenerate, the inner integument does not differentiate an endothelium, and the embryo sac may receive nutrients from the nucellus directly. The ovule is connected to the ovary wall by the funiculus, a stalklike structure extending from the lowermost part of the chalaza to the placenta. Usually, a single vascular strand runs through the funiculus from the placenta terminating at the base of the embryo sac. The mature ovule displays a polarity with respect to the axis determined by the location of the chalaza and micropyle. Esau (1977) defined the chalaza as the region extending from the base of the integuments to the point of attachment of the funiculus. The micropyle is located at the point where the integuments

terminate and is the site where pollen tubes enter the ovule. The possible significance of this ovular polarity in embryo sac development is discussed below. Embryo Sac Development The process of embryo sac development can be divided into two stages: megasporogenesis and megagametogenesis. In general, during megasporogenesis, the megasporocyte undergoes meiosis and four megaspore nuclei are produced. Subsequent mitotic divisions, nuclear migration, and cytokinesis during megagametogenesis produce the mature embryo sac. Considerable diversity in the pattern of embryo sac development is found among plant species. Haig (1990) proposed a model for embryo sac development whereby different patterns could be generated by variations in meiosis, cytokinesis, and the timing and number of mitotic divisions.

TYPE	MEGASPOROGENESIS				MEGAGAMETOGENESIS			
	mega-sporocyte	meiosis I	meiosis II	functional megaspore	mitosis	mitosis	mitosis	mature gametophyte
MONOSPORIC (Polygonum)								
MONOSPORIC (Oenothera)								
BISPORIC (Allium)								
TETRA-SPORIC (Adoxa)								

Figure 2. Patterns of Embryo Sac Development.

Some examples of patterns of embryo sac development are illustrated schematically. Representative genera are indicated in parentheses. Comprehensive descriptions of the variation and complexity of embryo sac development can be found in several reviews (Maheshwari, 1950; Willemsse and van Went, 1984; Haig, 1990).

Development of the Polygonum-Type Embryo Sac:

The Polygonum-type pattern illustrated in Figure 2 is the most commonly observed form of embryo sac development. Approximately 70% of the species examined, including *Arabidopsis* and maize, have this form of embryo sac. Polygonum type embryo sacs originate from a single chalazally located megaspore that undergoes three successive mitotic divisions. During the first meiotic division, the spindle is oriented parallel to the micropylar-chalazal axis of the nucellus. Wall formation occurs perpendicular to this axis, creating a dyad of megaspores. Frequently, the

dyad cell closest to the micropyle degenerates without undergoing a second meiotic division. After the second meiotic division, another transverse wall is made, resulting in a linear arrangement of four megaspores. The megaspore closest to the chalaza enlarges before undergoing mitosis. The three nonfunctional megaspores degenerate and are eventually crushed by the expanding functional megaspore. Tetrahedral arrangements of megaspores have also been observed in *Arabidopsis* and T-shaped tetrads have been seen in maize. The linear array is, however, most common. Callose, a P-1,3-glucan, is thought to function in the selection of a functional megaspore. During megasporogenesis, callose accumulates first in the cell walls of the megasporocyte and then in the megaspore walls. After meiosis, callose walls become thinner or absent in the functional megaspore. The presence of callose in the walls of the nonfunctional megaspores probably ensures that only the functional megaspore receives nutrients from the nucellus. The pattern of callose deposition is variable, reflecting the pattern of megasporogenesis. For example, in *Oenothera*, a monosporic species, callose is thinner at the micropylar end of the ovule, where the functional megaspore is located. In tetrasporic species, meiosis occurs without cytokinesis, and callose does not accumulate in the walls of the single tetranucleate megaspore. The female gametophyte is generated from the functional megaspore via a process termed megagametogenesis. In *Polygonum*-type embryo sacs, the functional megaspore at the chalazal end enlarges prior to the first of three free nuclear divisions. After the first mitosis, the two nuclei migrate to opposite poles and the smaller vacuoles coalesce into a large central vacuole. Cass et al. (1985) suggested that formation of this central vacuole plays an important role in positioning the nuclei before subsequent mitotic divisions. Each of the two nuclei then divides two more times, resulting in an eight-celled coenocytic megagametophyte. Wall formation, nuclear migration, and differentiation follow, forming a mature embryo sac.

Cellular Anatomy of the Mature Embryo Sac

The *Polygonum*-type embryo sac has one egg cell, two synergids, three antipodal cells, and a central cell that contains two nuclei. These cells comprise four groups that function in fertilization, embryogenesis, and nutrition of the embryo sac and embryo.

Egg Cell: The egg cell is located at the micropylar end of the embryo sac and ultimately fuses with a sperm nucleus to produce a zygote. The egg cell lies adjacent to the two synergids, separated from them by either partial cell walls or the plasmalemma alone. The distribution of cytoplasm within the egg cell is highly polarized, due to the presence of a large vacuole at the micropylar end that restricts the nucleus and most of the cytoplasm to the chalazal end.

Synergids: The synergids, which are located on either side of the egg cell, play an important role in fertilization (Jensen, 1965a; Schulz and Jensen, 1968a; see Russell, 1993, this issue). The pollen tube discharges its contents into one of the synergids prior to incorporation of the sperm nuclei into the egg and central cells.

Central Cell: Positioned in the center of the embryo sac, this cell contains two nuclei, a large vacuole, and many cytoplasmic organelles. The polar nuclei originate at both the micropylar and chalazal ends of the coenocytic megagametophyte and migrate to the center after cellularization. The polar nuclei may partially fuse with each other before they are fertilized by a single sperm nucleus, generating the triploid primary endosperm nucleus (Cass et al., 1985). The mature endosperm will provide nutrients for the developing embryo or seedling.

Antipodal Cells: Three antipodal cells are located opposite the egg at the chalazal end of the embryo sac. No specific function during reproduction has been attributed to the antipodals, but they are thought to be involved in the import of nutrients to the embryo sac. Cytological characteristics of cells within the embryo sac as well as cytochemical localization of proteins, starches, lipids, and nucleic acids have been used to assess the physiological state of the embryo sac and suggest relative rates of metabolic activity. For example, the presence of numerous ribosomes and mitochondria in the synergids, central cells, and antipodals suggests a high metabolic activity. By contrast, the egg cell has fewer ribosomes, plastids, and other organelles and appears to be relatively quiescent.

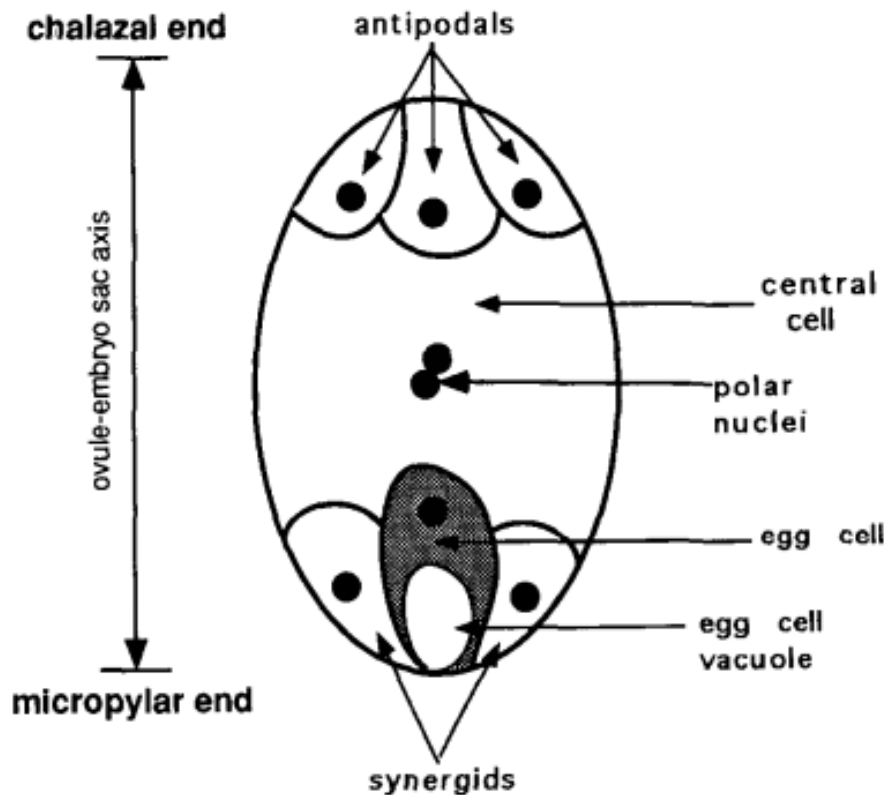


Figure 3. Organization of the Embryo Sac.

The orientation of the embryo sac with respect to the chalazal–micropylar axis of the ovule is indicated by the vertical arrow on the left. The egg apparatus, including the egg cell and synergids, is located at the micropylar end, where the pollen tube enters the embryo sac. The central cell contains two nuclei. Three antipodal cells are located at the chalazal end of the embryo sac. The egg cell is actually adjacent to, rather than between, the two synergids. Note the position of the vacuole in the egg cell.

Reference: Leonore Reiser and Robert L. Fischer². The Ovule and the Embryo Sac. *The Plant Cell*, Vol. 5, 1291-1301, October 1993.

Double Fertilization

Key Points

- Double fertilization involves two sperm cells; one fertilizes the egg cell to form the zygote, while the other fuses with the two polar nuclei that form the endosperm.
- After fertilization, the fertilized ovule forms the seed while the tissues of the ovary become the fruit.

- In the first stage of embryonic development, the zygote divides to form two cells; one will develop into a suspensor, while the other gives rise to a proembryo.
- In the second stage of embryonic development (in eudicots), the developing embryo has a heart shape due to the presence of cotyledons.
- As the embryo grows, it begins to bend as it fills the seed; at this point, the seed is ready for dispersal.

Key Terms

- **double fertilization:** a complex fertilization mechanism that has evolved in flowering plants; involves the joining of a female gametophyte with two male gametes (sperm)
- **suspensor:** found in plant zygotes in angiosperms; connects the endosperm to the embryo and provides a route for nutrition from the mother plant to the growing embryo
- **proembryo:** a cluster of cells in the ovule of a fertilized flowering plant that has not yet formed into an embryo

Double Fertilization

After pollen is deposited on the stigma, it must germinate and grow through the style to reach the ovule. The microspores, or the pollen, contain two cells: the pollen tube cell and the generative cell. The pollen tube cell grows into a pollen tube through which the generative cell travels. The germination of the pollen tube requires water, oxygen, and certain chemical signals. As it travels through the style to reach the embryo sac, the pollen tube's growth is supported by the tissues of the style. During this process, if the generative cell has not already split into two cells, it now divides to form two sperm cells. The pollen tube is guided by the chemicals secreted by the synergids present in the embryo sac; it enters the ovule sac through the micropyle. Of the two sperm cells, one sperm fertilizes the egg cell, forming a diploid zygote; the other sperm fuses with the two polar nuclei, forming a triploid cell that develops into the endosperm. Together, these two fertilization events in angiosperms are known as double fertilization. After fertilization is complete, no other sperm can enter. The fertilized ovule forms the seed, whereas the tissues of the ovary become the fruit, usually enveloping the seed.

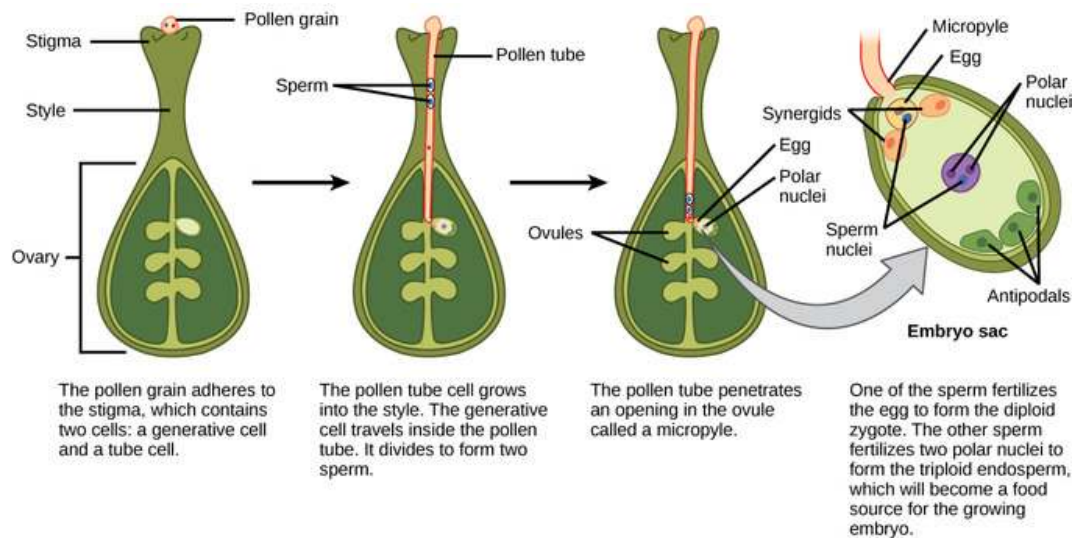


Figure Double fertilization: In angiosperms, one sperm fertilizes the egg to form the $2n$ zygote, while the other sperm fuses with two polar nuclei to form the $3n$ endosperm. This is called a double fertilization.

After fertilization, embryonic development begins. The zygote divides to form two cells: the upper cell (terminal cell) and the lower cell (basal cell). The division of the basal cell gives rise to the suspensor, which eventually makes connection with the maternal tissue. The suspensor provides a route for nutrition to be transported from the mother plant to the growing embryo. The terminal cell also divides, giving rise to a globular-shaped proembryo. In dicots (eudicots), the developing embryo has a heart shape due to the presence of the two rudimentary cotyledons. In non-endospermic dicots, such as *Capsella bursa*, the endosperm develops initially, but is then digested. In this case, the food reserves are moved into the two cotyledons. As the embryo and cotyledons enlarge, they become crowded inside the developing seed and are forced to bend. Ultimately, the embryo and cotyledons fill the seed, at which point, the seed is ready for dispersal. Embryonic development is suspended after some time; growth resumes only when the seed germinates. The developing seedling will rely on the food reserves stored in the cotyledons until the first set of leaves begin photosynthesis.

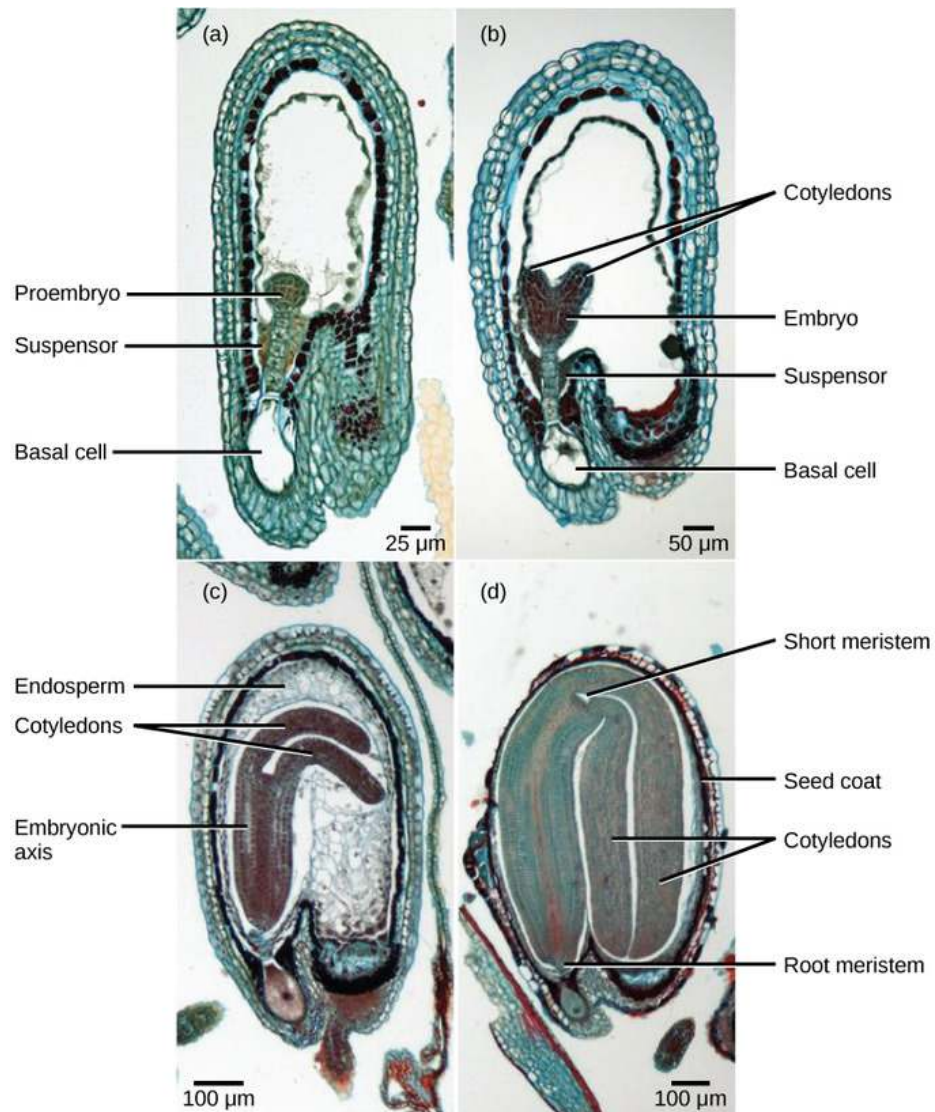


Figure 32.2D.132.2D.1: **Embryo development:** Shown are the stages of embryo development in the ovule of a shepherd's purse (*Capsella bursa*). After fertilization, the zygote divides to form an upper terminal cell and a lower basal cell. (a) In the first stage of development, the terminal cell divides, forming a globular pro-embryo. The basal cell also divides, giving rise to the suspensor. (b) In the second stage, the developing embryo has a heart shape due to the presence of cotyledons. (c) In the third stage, the growing embryo is crowded and begins to bend. (d) Eventually, it completely fills the seed.

Reference:

[https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_\(Boundless\)/32%3A_Plant_Reproductive_Development_and_Structure/32.2%3A_Pollination_and_Fertilization/32.2D%3A_Double_Fertilization_in_Plants](https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/32%3A_Plant_Reproductive_Development_and_Structure/32.2%3A_Pollination_and_Fertilization/32.2D%3A_Double_Fertilization_in_Plants)

Establishment of symmetry in plants

Meristematic Plant Development

Phyllotaxis is known as the study of plant patterns. Diversity is present, still similar patterns can be observed in many different types of plants. A common pattern consists of two sets of spiral that form a lattice i.e., observed in the stamens and carpels of flowers, the florets of compound flowers, the scales of pine cones, cycads and seed ferns. This type of pattern is known as Spiral Phyllotaxis. The apex is known as the tip of a plant shoot. The region that contains undifferentiated stem cells is apex. This region is called as apical meristem. The production of plant organs such as leaves, thorns, tendrils, sepals, petals etc. is carried by apical meristem. Apical ring is present near the boundary of apical meristem. The process is observed under the microscope. When extensive cell division start it leads to the formation of plant organ in the spot along the apical ring. Primordia exhibit the phyllotactic pattern which is preserved and developed in various plant organs.

Fibonacci numbers appear naturally as a consequence of the dynamical systems approach. Many research work shows that Fibonacci numbers promoted the survival of plants. The prevalence of the Fibonacci numbers was then seen as a consequence of Darwinian Theory of Evolution; survival of the fittest is the consequence of the Fibonacci numbers. The adult plants preserve the pattern of the early development. This does not contradict the theory of evolution of course. Natural selection is acting to promote various but some typical types of developmental processes.

Two important processes in plant growth at the cellular level are:

1. Cell division
2. Cell expansion

These two processes are responsible for the change in the shape and size of a plant and its various organs. These two processes share no casual relationship. Growth of plant cells depend mainly on water consumption although they consume many other compounds also the cell division of plant cells consist of the duplication of nucleus and the building of cell wall to separate the two new nuclei. The parent cell gives rise to complete two daughter cells. A plant shoot is approximately a cylinder so it is convenient to use a cylindrical coordinate system to indicate position on a shoot. Cell division is classified into three types according to the orientation of the new cell wall with respect to the three mutually orthogonal directions of the cylindrical coordinate system. In transverse cell division, the new cell wall is roughly orthogonal to the axis of the shoot, in periclinal cell division the new cell wall is parallel to the surface of the plant and in anticlinal cell division the new wall is contained in a plane passing through the axis of the shoot.

There are two basic types of development in plants:

1. Embryonic

2. Meristematic

The embryonic development results in seed or spore. After dormancy period is over seed begins to absorb water and cells undergo expansion, this results in the bursting of new plant, this is known as germination. After germination, if all goes well, the seedling has a root growing down into the ground and a shoot growing up from the ground. The line formed by the root and shoot form the main axis of the plant. In meristematic development, some species of plants have meristems that are present before germination while in other species the meristems form after germination. In the plant, there may be present a clear boundary between embryonic and meristematic development. The entire plant organ develops from the primodium. The type of cells that develop is not completely determined until after the formation of the primordium. A variety of factors decide which type of plant organ develops.

Development of seedling Plant embryogenesis is followed by germination. Germination marks a switch from an anabolic phase of nutrient accumulation to a catabolic phase of nutrient consumption and growth. There are two stages separated by period of quiescence, where embryo become desiccated and is stored in seed.

Environmental influences to which seedling respond:

Gravity: It ensures shoot grows upward and root grows downwards.

Light: It enables seedling to extend through soil in dark and then switch to vegetative growth once it has emerged at surface.

Transition to germination

It is controlled by abscisic acid and gibberlic acid. Abscisic acid inhibits growth and gibberellic acid promotes growth. There are number of genes which affect transition when mutated but are not involved in hormone synthesis. Instead these genes encode regulatory proteins that control downstream embryo specific and seedling specific gene expression. Mutations in these genes cause appearance of trichomes on cotyledons. Trichomes are epidermal hair usually found on leaves. For example LEC1 and FUSCA 3

Dark development or Skotomorphogenesis

It is the first stage of the germination. It is characterized by rapid extension of shoot upwards through soil. Shoot is pale and spindly, condition described as etiolated. Light development or Photomorphogenesis It is the type of development where seedling emerges from soil and is exposed to light. The seedling turns green as it begins to accumulate chlorophyll. Photosynthesis begins and shoot apical meristem is activated leading to the formation of true leaves.

Shoot and root development

The vascular plant begins its existence as a single cell, the zygote. The zygote grows into embryo; the early embryo is globular whereas a mature embryo has a defined apical basal growth axis. This polar structure has two distinct zones during longitudinal axis formation. The two zones have the capacity of continuous growth and are set apart at opposite poles. These regions are the apical meristems, one producing the shoot system, the other producing the root system. These are open ended indeterminate growth systems from which the same kinds of organs and/or tissues are produced continuously and which result in the primary plant body.

In response to environmental conditions, such as photoperiod and low temperature, the shoot apical meristem may undergo transition to a floral state. In some cases, the meristem become determinate and ceases to produce new organs and most root meristems remain indeterminate, although lateral roots which branch off a primary axis can become determinate. Shoot buds containing meristematic cells give rise both to terminal apices and to lateral. Roots also branch profusely, but from meristematic tissue deep within the root axis, so generating extensive root systems typical of most land plants. In monocotyledons, an intercalary meristem located at each node of the stem provides the facility for continued longitudinal growth if the shoot tip is destroyed. Plants with extended lifespans have additional meristem layers called cambium which develop within roots and stems, and lead to an increase in girth along the plant's longitudinal axis. Vascular cambium generates extra conducting tissue; cork cambium produces protective tissue, replacing the functions of epidermis in stems, and cortex and epidermis in roots. Cambial meristems and their derivative tissues are referred to as the secondary plant body. Although no new organs are produced by these lateral meristems, the secondary plant body may constitute the bulk of the plant, for example a tree's trunk, branches and roots. The group of cells which generates the vascular tissues including the pericycle in the roots of higher plants is called procambium. It is a meristematic tissue that is concerned with providing the primary tissues of the vascular system.

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Seed development

The seed development process, from ovule fertilization to physiological maturity, can be divided into four phases. Phases I and II comprise cell division and expansion. Reserve accumulation occurs in Phase III as seed dry mass increases. At the end of this phase, seed moisture loss is intensified (Phase IV). After fertilization, there is a period of seed structure formation as a result of cell division, expansion and differentiation (histodifferentiation) in which seed structure primordia are formed and future embryo parts can be visualized. During this phase, there is a significant increase in seed size forming the embryonic cells that receive assimilates from the parent plant. During this period, seed moisture content remains constant and high. The significant decrease in seed moisture content occurs at the end of maturation when changes in cell membrane structure organization occurs as well as increases in enzyme synthesis in preparation for successful germination. Recalcitrant seeds usually do not show this transition period between maturation and germination.

Pollination and fertilization

Pollination is the transfer of pollen from the anther to the stigma after it germinates. The pollen grain has two cells, the tube nuclei and the generative cell. The generative cell divides mitotically and produces two sperm nuclei. When the female part of the flower is matures, the stigma secretes a sugary solution. This promotes the germination of the pollen grain, if viable, compatible pollen comes in contact with this moist stigma. Sexual reproduction in plants is centered in flowers. Co-evolution of flowering plants and their pollinators started about 225 million years ago. The sexual cycle includes development of the male (pollen) and female (embryo sac) structures of the flower for angiosperms. In this part of the cycle reduction division of the chromosomes occurs to produce the haploid (n) chromosome number i.e., pollen grains and an eight-celled embryo sac contain haploid ($1n$) male and female gametes, respectively. The germinated pollen grain on the stigma produces a tube (*pollen tube*) that carries two sperm nuclei (n) down the style into the ovary until it reaches the embryo sac. In the embryo sac, fertilization takes place and hence one sperm fertilizes the egg to form a zygote ($2n$). The other sperm fuses with the large central cell of the embryo sac to produce a triploid ($3n$) cell, a process called triple fusion.

Ovule development

The next step after fertilization is the development of the ovule (containing the zygote and the $3n$ central cell) into a seed. In gymnosperms and angiosperms, seeds develop from ovules. Ovules consist of a stalk that bears the nucleolus (equivalent to the mega sporangium; diploid maternal tissue). The nucleus is enveloped by one (gymnosperms) or two (angiosperms) covering layers (diploid maternal tissue), called the integuments. An ovule is therefore, in a developmental sense, an unfertilized, immature seed precursor and, in a morphological and evolutionary sense, a mega sporangium surrounded by integuments. These integuments develop into the test a (seed coat), of which in mature seeds the outer cell layers of the outer integument usually forms a dead covering layer, while inner cell layers may remain alive. Within the nucleolus, a megaspore

develops into a haploid mega gametophyte (female gametophyte). The mega gametophytes of gymnosperms and angiosperms differ considerably. The mature gymnosperm mega gametophyte is multi cellular, usually several archegonia develop within the mega gametophyte and one egg forms in each archegonium. In most angiosperm species, the mega gametophyte, in its mature state also called the embryo sac, is seven-celled and eight-nucleate, referred to as the Polygonum-type.⁷ The gymnosperms have naked seeds; their seeds are not enclosed by an ovary and are usually found naked on the scales of a cone. In a typical mature gymnosperm seed, the embryo has two covering layers: the haploid maternal mega gametophyte with stored nutrients and the diploid integument tissue that develops into the test a. In contrast to the gymnosperms, the angiosperm ovules and seeds are covered; they are enclosed inside the ovary. The ovary is the base of a modified leaf (carpel) or the fusion between several carpel's in a pistil. A mature ovary contains one or more mature seeds and is called a fruit; a pericarp (fruit coat) develops from the ovary wall and can contain additional flower parts. Both seeds and fruits can be the dispersal units of angiosperms. (Figure 2) A hallmark /characteristics of angiosperm reproduction are double fertilization (Figure 3); that is, in addition to the egg cell fertilization, a second fertilization event occurs in which the central cell nucleus of the mega gametophyte is targeted by a second sperm cell nucleus. This leads to the formation of the endosperm. Since the central cell of most angiosperm species has either one or two nuclei, the resulting fertilized endosperm is either diploid or triploid.

General parameters to characterize seed maturation

During the 1960s and 1970s, there was a significant effort by seed technologists to clarify the maturation process and to define the primary changes occurring during seed development. The following changes occur during seed development:

Seed moisture content: Ovule moisture content at the time of fertilization is approximately 80% (fresh weight basis), both for monocots and dicots. That value decreases during maturation although it remains relatively high throughout most of the maturation period because water is the vehicle for transferring nutrients from the parent plant to the developing seeds. The initial phase of dehydration is slow, and is accelerated from the time the seeds reach maximum dry weight; at that time, seeds possess 35% to 55% moisture content for orthodox monocot and dicot seeds, respectively, produced in dry fruits. This decrease in moisture content proceeds until hygroscopic equilibrium is attained. From that point on, moisture content changes are associated with variations in relative humidity. However, seeds produced in fleshy fruits have a lower decrease in moisture content than seeds produced in dry fruits. Developing recalcitrant seeds do not show marked changes in desiccation at the end of maturation, possessing moisture contents usually over 60% (fresh weight basis).

Seed size: The fertilized ovule is a small structure with respect to final seed size. Plant species with large seeds have an advantage under low light conditions, when their greater protein and lipid reserves, or their more advanced development, can facilitate growth.¹³ However, large seeds usually come at the cost of seed number per flower or fruit.¹⁴ In addition, large seeds cannot be

physically borne on small plants because of the weight of the seed, which may partly explain the association between plant size and seed size.

Seed dry weight: After sexual fusion, the developing seeds begin to increase in weight as a result of nutrient accumulation and water uptake. Seed fill is initially slow because cell division and elongation are occurring during this stage. Soon after, dry mass accumulation increases until seeds reach their maximum dry weight.

Germination: Seeds of various cultivated species are able to germinate a few days after ovule fertilization. In this case, germination refers to protrusion of the primary root, not the formation of a normal seedling because histo-differentiation has not been completed and reserve accumulation is still incipient at this phase. Therefore, this germination does not lead to the production of vigorous seedlings. Theoretically, it is possible to consider that the percentage of germinable seeds increases during maturation, reaching a maximum around the time when seeds attain maximum dry weight. This is only found in species where dormancy does not occur, because the imbalance in the germination promoters/inhibitors induced during the reserve accumulation period may directly affect seed germinability.

Vigor: Seed vigor changes are usually parallel to nutrient reserve transfer from the parent plant. This means that the proportion of vigorous seeds increases during maturation, reaching a maximum near to or at the same time as seed maximum dry weight.

Determination of physiological maturity

The identification of the time of physiological maturity has been a controversial subject among different authors studying seed maturation. Among the differing physiological maturity concepts, three are dominant:

- a. Physiological maturity is identified as maximum seed dry matter accumulation
- b. Physiological maturity is reached when there is no further significant increase in seed dry weight
- c. Physiological maturity occurs when seeds reach maximum dry weight, germination and vigor

According to the prevailing concept, seed development ceases when physiological maturity is achieved, but this idea remains controversial because this expression is frequently used with different meanings. For example, the first concept of physiological maturity was proposed by Shaw and Loomis in 1950 as the stage in which the seed possesses maximum dry weight and yield. Physiological maturity has also been termed “relative maturity” by Aldrich in 1943, morphological maturity” by Anderson in 1955 and “mass maturity” by Ellis and Pieta Filho in 1992 while others prefer to use terms such as “harvest maturity”, “agronomic maturity”, and “time of harvest” to identify this same stage. Consequently, physiological maturity and harvest time are distinct events. Seed maturation is a process characterized as an organized sequence of

several physiological events culminating at physiological maturity, while harvest time is a decision based on technological and economic parameters associated with seed yield/quality. The determination of physiological maturity usually requires differing measurements of seed dry weight during the seed filling period which is often affected by sampling variation. This makes it difficult to determine the precise time of physiological maturity, i.e., to identify the moment when the transfer of plant reserves to the seed ceases. This is perhaps the primary reason why some authors suggest the identification of physiological maturity as the “period in which no more significant increases occur in seed dry weight”. Seed dry weight is usually determined at different intervals during maturation. The most frequent method is drying seeds in an oven immediately after harvest followed by weighing the dried seeds for a moisture content determination. Probably the most accurate method of determining physiological maturity is the measurement of ^{14}C assimilate uptake by the developing seed. This was demonstrated by TeKrony et al. (1979) with soybeans, Lee et al. (1979) with oats and Hunter et al. (1991) with maize seeds. Changes in seed color or other visual changes in seed or fruit structure are also excellent morphological indicators of physiological maturity. For example, physiological maturity was closely associated with yellow color in *Dovyalis caffra* fruit. Debate is also found in the research literature regarding the simultaneous expression of maximum germination, vigor and seed dry weight at physiological maturity. Harrington proposed that these three parameters occurred at the same time and could be considered together as markers of physiological maturity. However, although some studies have confirmed this hypothesis, several observations have shown biochemical changes leading to metabolic “adjustments” after seeds reach their maximum dry weight. As a result, the existence of these differing studies about physiological maturity create confusion in the literature that can be attributed primarily to the wide variation in experimental designs and varying concepts of physiological maturity. Finally, the definition of physiological maturity based on seed maximum dry weight should be considered a reference point to characterize the end of seed development and the physiological independence of the seed from the parent plant.

Seed physiological maturity and harvest time

Physiological maturity identifies the moment seeds possess or are close to their maximum physiological potential. As a result, the decision to identify this time as the optimum time for harvesting a seed production area should be logical. Any harvest delay from the point in which the seed becomes independent of the mother plant may have drastic consequences on seed quality as a result of seed exposure to less favorable environmental conditions for relatively long periods of time resulting in rapid deterioration of the seeds. In the latter collection of fruit of *Dovyalis caffra* may not be convenient, and also fungal attack may interfere with the germination process, particularly under laboratory conditions. Generally, visual identification of physiological maturity can help identify the time in which seeds reach their greatest quality or are very close to this time.

Environmental factors affecting seed development

Components of the environment factors that influence seed performance include soil fertility, water, temperature, light, and seed position on the plant.

Soil fertility: In general, plants that have been fertilized with the three major elements (N, P, and K) produce larger seeds than those which have not been fertilized. The increase in seed size is due to an enhanced seed development rate during the seed filling period as a consequence of increased nutrient availability. According to Copeland and McDonald when the effects of individual fertilization elements on seed development are considered, nitrogen has the greatest influence on seed size, seed germination, and vigor.

Water: Water deficits reduce plant metabolism and seed development. Research has reported decreases in leaf area, photosynthetic rate, and other effects that promote flower abortion and negatively influence assimilate production and translocation to developing seeds; one of the most important effects is the decrease in carbohydrate supply caused by a reduction in photosynthesis rate. Prolonged droughts and reduced soil water availability cause decreases in seed size, particularly when these effects occur during seed filling. If water deficits occur during flowering, its primary effect is on a reduction in seed number.

Temperature: High temperatures during seed development produce smaller seeds, while low temperatures retard seed growth. Seed germination and vigor are also adversely affected by exposure to low temperatures during development. High temperatures are considered the principal reason for the “forced maturation” of some plants. This phenomenon is also caused by water deficits or desiccant application at inappropriate times during maturation. The occurrence of greenish seeds is undesirable because this abnormality results in decreases in seed germination and vigor.

Light: The seasonal distribution of solar radiation is a fundamental factor in assuring adequate plant development. In general, reduced light to the parent plant results in smaller seeds.

Seed position on the plant

The position in the inflorescence can affect seed development rate. For example, distal seeds in a wheat spike have slower growth rates and shorter seed filling periods than proximal seeds. Corn seeds at the tip of the ear are smaller than those at the base which has been attributed to inadequate photosynthetic supply. Soybean pods located in lower plant branches are produced before those located in the upper nodes and are affected by different environmental conditions during development and this causes differences in seed performance. Smaller seeds are also produced from smaller fruits or those that mature later in the growing season or are exposed to unfavorable environmental conditions. The usual consequence is decreased seed germination and vigor.

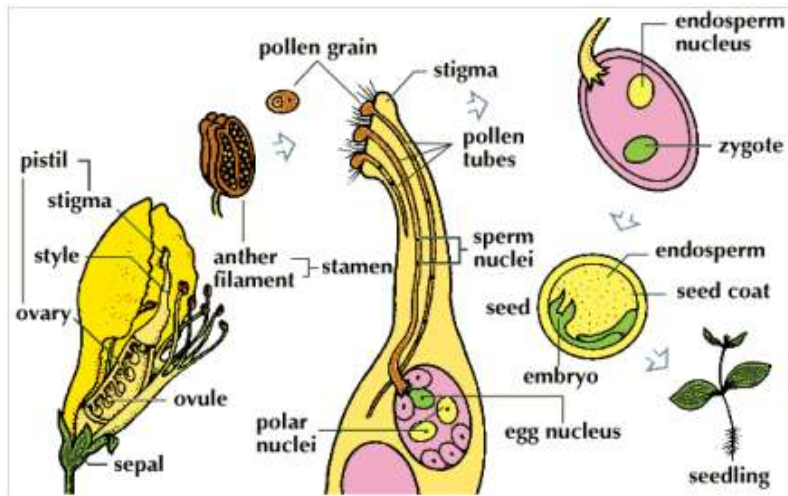
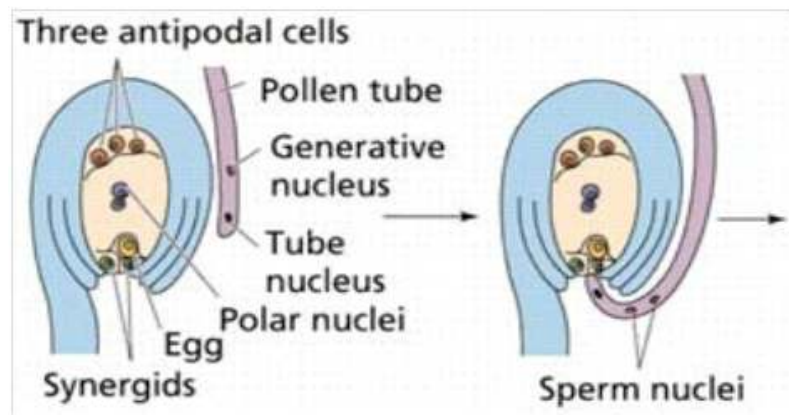


Figure 2 The seed reproduction (pollination and seed embryo, endosperm and seed coat development (<http://agritech.tnau.ac.in/seed/seedconcepts.html>).



Fertilization in higher plants is different from lower plants and animals.

The pollen has 3 nuclei rather than one:

- one to drive pollen tube expansion, one to fertilize the egg cell and form the embryo and
- one to fertilize the polar nucleus to form the endosperm

Figure 3 Embryo development and double fertilization.

Seed germination/sexual propagation

Plant propagation is the sexual and asexual multiplication of plants, and has three aspects;

1. Knowledge on the chemical, physical, and environmental manipulations, as well as command of the needed technical skills.
2. Fundamentals of plant biology and physiology.
3. Expertise on specific plant species.

Sexual propagation involves the union of pollen from a male flower part with the egg of a female ovary to produce a seed. Seed can be recalcitrant, inter mediator orthodox seed. The longevity of orthodox seeds is increased over a wide range of environmental conditions by decreasing storage temperature and seed water content without causing significant damage to seed metabolism. Orthodox seeds are, therefore, considered desiccation tolerant. Recalcitrant seeds, however, decrease in viability when their water content is reduced below a relatively high value. They are, therefore, considered desiccation intolerant. The seed is made up of three parts: the outer seed coat, which protects the seed; the *endosperm*, which is a food reserve; and the embryo, which is the young plant itself. When a seed is mature and put in a favorable environment, it germinates (begins active growth). (Figure 4) Seed germination is a critically important juncture in the plant life cycle and the decision made by an imbibing seed to initiate germination can be considered to be a critical regulatory step in plant development.

Stages of seed germination

1st Stage

1. Imbibition - initial absorption of water to hydrate seed
2. Activation of metabolism - increased respiration and protein synthesis

2nd Stage

1. Digestion of stored food- for example, starch to sugars in cotyledon or endosperm
2. Translocation to embryo- sugars move to embryo for growth

3rd Stage: cell division and growth-development of seedling.

Following initial water uptake, this phase of development is characterized by relatively little change in seed water content until it is terminated by the initiation of embryo growth. During this time, energy metabolism resumes, respiration processes are activated, and the cell cycle may be initiated, while events associated with seed maturation are suppressed. Sometimes, removal of the developing seed from its surrounding fruit tissue is sufficient to permit germination, but germination of the seed cannot occur even under this situation until near maturation. The ability of seeds to germinate readily when conditions are suitable for successful growth and the ability

to avoid germination at inappropriate times are thus essential to the survival of a species. The time between seed imbibitions and radical emergence is the period of germination in the strict sense. According to Hartmann et al., three conditions are required for germination to be initiated:

1. The seed must be viable,
2. Dormancy of the seed must be overcome,
3. The seed must be subjected to the appropriate environmental conditions: available water, proper temperature regimes, a supply of oxygen, and sometimes light as well as various types of fungi and/or other microorganisms that are needed to decompose seed coat to allow germination. ([Figure 5](#))

After the seeds have germinated, they require favorable environment for their establishment. All these factors become available if the seeds have chance to germinate in their original ecology or if the original ecology is not seriously disturbed.

Factors affecting seed germination

Germination potential of seeds of many plants can be influenced by various environmental and the seed internal factors. Without understanding of such factors and appropriate knowledge of plant propagation techniques and their seed biology of indigenous trees, production of seedlings from seeds would be difficult. This is due to the existence of unique survival and developmental strategy of each indigenous tree species through long time of evolutionary processes. One of the major strategies in seed physiology is: some seeds of indigenous tree species undergo the period of dormancy to pass unfavorable environmental or internal situations. The germination of seeds in a particular situation and season is determined by the interaction between the dormancy releasing factors, which influence on the termination of dormancy or initiation of germination and seedling growth in many plant species like Phyto-hormones, light, temperature, water, nutrients, moisture or mechanical cues.

Seed maturity and dormancy

Propagations of many indigenous tree species from seeds had been difficult due to lack of precise knowledge on their seed biology and germination physiology. Many native plant species developed survival strategies through evolutionary processes for millions of years. So they must simultaneously sense a number of environmental conditions and fix time of seed maturation and germination and seedling emergence to particular periods of the year according to their habitat locations for successful establishment and survival. Seed set and releases of seeds into the soil occur at the pick of the dry season and emergence of seedlings under this condition could jeopardize survival. Hence a structural or physiological adaptive mechanism called dormancy prevents seed germination. A seed becomes dormant when growth potential of the embryo falls below the restraining force of its covering structure. Seed dormancy is defined as the state in which otherwise mature and viable seeds will not germinate even when exposed to favorable

growth conditions. Dormancy is a resting stage. Seeds will only germinate when the conditions are suitable. Some causes of seed dormancy are:

- a. Hard seed coat
- b. Embryo dormancy
- c. Immature embryo- the embryo is not fully developed when the seed is dispersed
- d. Chemical inhibitor

Types of dormancy

1. Quiescent seed dormancy -even a non-dormant or quiescent seed has a unique ability to revert to a dormant state under stressful conditions. Dormancy is desired in the wild, where plants depend entirely on nature for survival. Seeds dormancy has intrigued biologists and frustrated horticulturists for many years because matured seeds fail to germinate if planted immediately without a rest period.
2. Structural dormancy is imposed *via* seed coat, which prohibits the entry of water, air, outward diffusion of possible endogenous germination inhibitors, as well as through mechanical restriction of embryo growth. Species with hard, impervious seed coverings occur in trees such as *Podocarpus falcatus*, *Olea europaea* and various *Acacia*. To help such seeds to germinate, mechanical methods were employed to loosen the seed covering by cracking the seed coat. Similarly seeds of *Prunus africana* were found to germinate by seed coat scarification.
3. Physiological dormancy (embryo dormancy) occurs when the embryo requires a special treatment to induce it to start active growth. A cold temperature application of about 1 to 7°C is commonly required to break the dormancy. In some plant species, embryo dormancy can be broken by a certain quality of light. For example, exposure of lettuce seeds to red light (about 660 nm) induces germination, but far red light (730nm) inhibits it. It was indicated long years ago that if lettuce seeds received red light after exposure to far red light, germination occurred and the germination of seeds of this species can occur only as long as the last treatment before sowing was red light.

Way of dormancy breakage

Seeds germinate only after the dormancy is overcome or broken either through;

1. Natural means such as animal gut activities (Manzano *et al.*, 2005), wild fire, rainfall or through
2. Artificial means such as scarification, seed coat cracking, removing chemical inhibitors through leaching by water etc.

For example, Acacia seeds dormancy removed by scarifying seed coat, Podocarpus falcatus dormancy removed by cracking away the latter using a piece of clean basalt rock (ca 200-300).

1. **Scarification:** Scarification is when the seed coat is rubbed with something such as sandpaper. This makes the seed coat thinner so water can enter more easily. This method is best for smaller seeds that are hard to cut for example kaka beak. To do this easily you can shake the seeds forcefully in a container lined with sandpaper
2. **Chipping seeds:** Chipping is cutting a small piece out of seed coat with a sharp knife. Water can then enter the test a through the cut. This is useful on large seeds for example, sweet pea
3. **Soaking seeds:** Soaking seeds in water overnight will soften the test a. This can be useful for seeds such as wattle, sweet pea and kowhai. Commercial growers use diluted acids on some seeds
4. **Immature embryo:** the embryo is not fully developed when the seed is dispersed. Therefore, Store seeds before planting. Waiting for a period of time will allow the embryo to mature
5. **Chemical inhibitors:** Some seeds contain chemicals that prevent germination. This is common in fleshy fruit but it depends on the seed type as to what will get rid of the inhibitor

The ability of seeds to germinate readily when conditions are suitable for successful growth and the ability to avoid germination at inappropriate times, through the maintenance of dormancy, can also be controlled by endogenous chemicals. Seed of some fleshy fruits, such as strawberry and tomato, will not germinate in the fruit because of the presence of chemical germination inhibitors. Some desert plant seeds germinate only after a heavy rain, the seedlings assured adequate moisture for survival and development until their roots are developed enough to absorb moisture. A number of chemicals in plants inhibit germination of seeds while they are still embedded in the pulp of the fruit (example, in tomato and strawberry). Seed maturity can also affect seed germination and the emergence of seedlings in many cases. In some species, such as *Pinus* and *Ranunculus*, the fruits are shed before the embryo fully matures. Such physiologically immature seeds must undergo certain enzymatic and biochemical changes to attain maturity. These changes are collectively called after ripening. Immature embryos cannot germinate. But such seeds can be matured artificially by storing fruits or cones for a certain period of time to allow embryos to mature completely and to germinate. Investigation of the germination physiology of seeds of several indigenous tree species of Ethiopia have shown that a certain level of maturity must be reached for the successful germination of the seeds to produce the required amount of seedlings for mass propagation of forest trees. For example, in *P. falcatus*, fruit collection should be commenced when at least 60-70% of them become yellow to get high quality viable seeds. Seed germination studies of *Bixaorellana* by Hill et al., indicated that the more matured seed germinated faster, had a higher total germination, and produced more

vigorous seedlings. For a given species, it is often the case that either the presence of an impermeable, leathery or hard seed coat or chemical inhibitors within the embryo or within the stored food of the seed or the embryo immaturity prevent seed germination. However, it can sometimes happen that all the three factors can combine and prevent seed germination. Therefore, the problem associated with seed germination that prevent successful seedling production, depend much on the understanding of the biology of seed dormancy and the seed maturity condition of the species of interest.

Storage time and temperature: Seed germination can be determined by many factors including seed storage time and temperature. Legesse Negash (2003) indicated that germination of *P. falcatus* had shown to be declined with increased storage time. Temperature is the most important environmental factor that regulates the aging of seeds, partly due to the increased deterioration following long-term storage under high temperature or under poor storage conditions. Storage time and temperature affects both germination percentage and rate of germination. Germination percentage, unlike germination rate, may remain relatively constant, at least over the middle part of the temperature range if sufficient time is allowed for germination to occur. With proper handling and storage, seed longevity may be maintained for significant periods. However, the processing, the rate of physiological change, storage and germination conditions for many indigenous tree seeds are poorly understood and hence they may look healthy but fail to germinate when planted due to seed short viability conditions as well as lack of the knowledge of their reproductive biology. Viability at the end of storage depends on:

- a. The initial viability at harvest, as determined by factors of production and methods of handling; and
- b. The rate at which deterioration takes place. Basic features of storage that increases the viability of seeds²⁶ include
- c. Protection from water,
- d. Avoidance of mixture with other seed,
- e. Protection from rodents, insects, fungi, and others. Retention of viability also varies with the climatic factors of the area in which storage occurs.

Viability is a measure of the propagation of seeds in a lot that are capable of germinating while longevity is a measure of how long seeds remain viable. The relative storability index depends on the kind of seeds (recalcitrant or orthodox), condition of seeds at the beginning of storage and environmental conditions of storage, primarily temperature and humidity and it indicates the storage time where 50% or more of seeds can be expected to germinate. Species with recalcitrant or short-lived seeds normally retain viability for little as few as days, months, or at most a year following harvest. But if properly stored recalcitrant seeds will retain viability for longer periods up to four to five years. Storage conditions that reduce seed deterioration are those that slow respiration and other metabolic processes without injuring the embryo. Poorest conditions are found in warm, humid climates; best storage conditions occur in dry, low storage temperature

and modification of the storage atmosphere. Of these, the moisture-temperature relationships have the most practical significance. Recalcitrant seeds owe their short life primarily to their sensitivity to low moisture and they can lose their viability when the seed moisture content dropped below 30%.

Seed treatments related to fire

Seeds of many fire-adapted plants are very difficult to germinate, and some species have been impossible to propagate by seeds. Numerous species, which inhabit fire dependent ecosystem, have evolved reproductive strategies to adapt to factors associated with fire and many of them have evolved barriers to seed germination that are overcome only by fire-related cues. Recurring fires are an integral part of many ecosystems, and when such areas are protected from forest fire; their local ecology becomes severely disturbed. Plant species use this strategy is described as “Hard seeded” with a prominent waxy cuticle that enforces dormancy by forming a water-impermeable barrier. The positive effect of smoke on seed germination is not limited to species native to fire-prone habitats. Smoke has been used to break dormancy and improve germination of seeds of common vegetables without obvious need of fire for their germination such as lettuce and celery. Seeds of many recalcitrant species were found to break dormancy upon contact with smoke, or even with wood ash.

Gibberellic acid (GA3)

Gibberellins are a group of plant growth regulators which play an important role in the regulation of seed germination and breaking dormancy. According to Taiz & Zeiger, seed germination may require gibberellins for one of several possible steps: the activation of vegetative growth of the embryo, the weakening of a growth constraining endosperm layer surrounding the embryo, and the mobilization of stored food reserves of the endosperm. Seed germination can be stimulated by applying artificially produced Phyto-hormones or by natural means. In a natural means of breaking dormancy, the gibberellins synthesizing mechanism is activated and the actual synthesis of gibberellins takes place when the seeds are transferred to a suitably higher temperature. Consequently, accumulation of GA results in germination of seeds. Gibberellins promote growth by increasing plasticity of the cell wall followed by the hydrolysis of starch to sugar which reduces the potential in the cell, resulting in the entry of water into the cell causing elongation, hence, causing germination of seeds. Releasing from dormancy can be affected by a variety of environmental and chemical stimuli. It is mediated through a common signal transduction chain that coordinates diverse cellular responses but that may differ between the seeds of different species and dormancy types. Gibberellic acid is known to break dormancy of several types of plant seeds: these are

- a. Light promoted seeds, such as grand rapids lettuce seed (*Lactuca sativa* L. var. Grand Rapids)
- b. Light inhibited seeds, such as the seed of the honey bee plant (*Phacelia tanacetifolia* Benth)

- c. Seeds requiring stratification (storage at low temperatures in a moist condition), such as the hazel nut (*Corylusavellana*)
- d. Seeds requiring after ripening (storage at room temperature in dry condition), such as the wild oat (*Avenafatua*) as reported by Chen & Change.

Potassium nitrate (KNO₃)

Nitrogenous compounds, especially nitrates promote the seed germination of a wide range of plant species. The effect of such a nitrate compounds on promotion of seed germination is best realized in combination with other factors such as temperature manipulations or light. In assessment of the interaction of nitrate with growth regulators, the phenomena initiated by many environmental factors, that interacts through altering the availability of endogenous hormones. Plant hormones and environmental factors can often bring out identical responses in seeds, but a causal relationship between the two remains a matter of much controversy. However, potassium nitrate (KNO₃) was most widely used chemical for promoting germination and for breaking seed dormancy. For this purpose solutions of 0.1 to 0.2% KNO₃ were common in usual germination testing and are recommended by the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA) for germination tests of many species. Both higher and lower concentration of KNO₃ has effect on germination of seeds of many plants. Yucel & Yilmaz, reported that lower concentration of KNO₃ (<1%) inhibit germination of *Salvia cyanescens* seeds. The optimum germination rate was better in 1% concentration series and when KNO₃ exceeds 2% in concentration, it becomes a germination inhibitor.

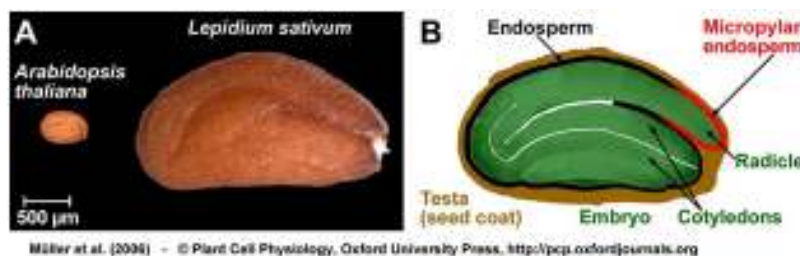


Figure 4 Seed parts.

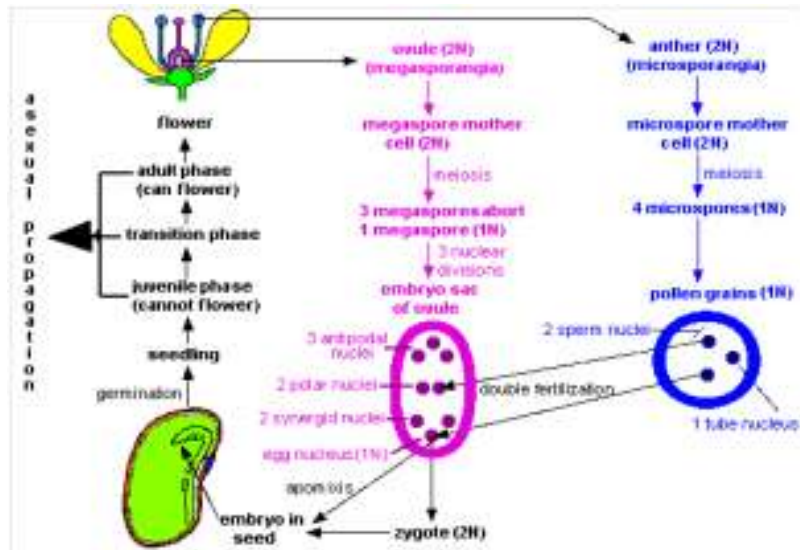


Figure 5 Seed germination process with stages of development.

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