GERMINAL STAGE

FIRST WEEK OF HUMAN DEVELOPMENT

The germinal stage involves several different processes that change an egg and sperm first into a zygote, and then into an embryo. The processes include fertilization, cleavage, blastulation, and implantation.

DAY ONE: FERTILIZATION

Key Terms - fertilization: the act of fecundating or impregnating animal or vegetable gametes

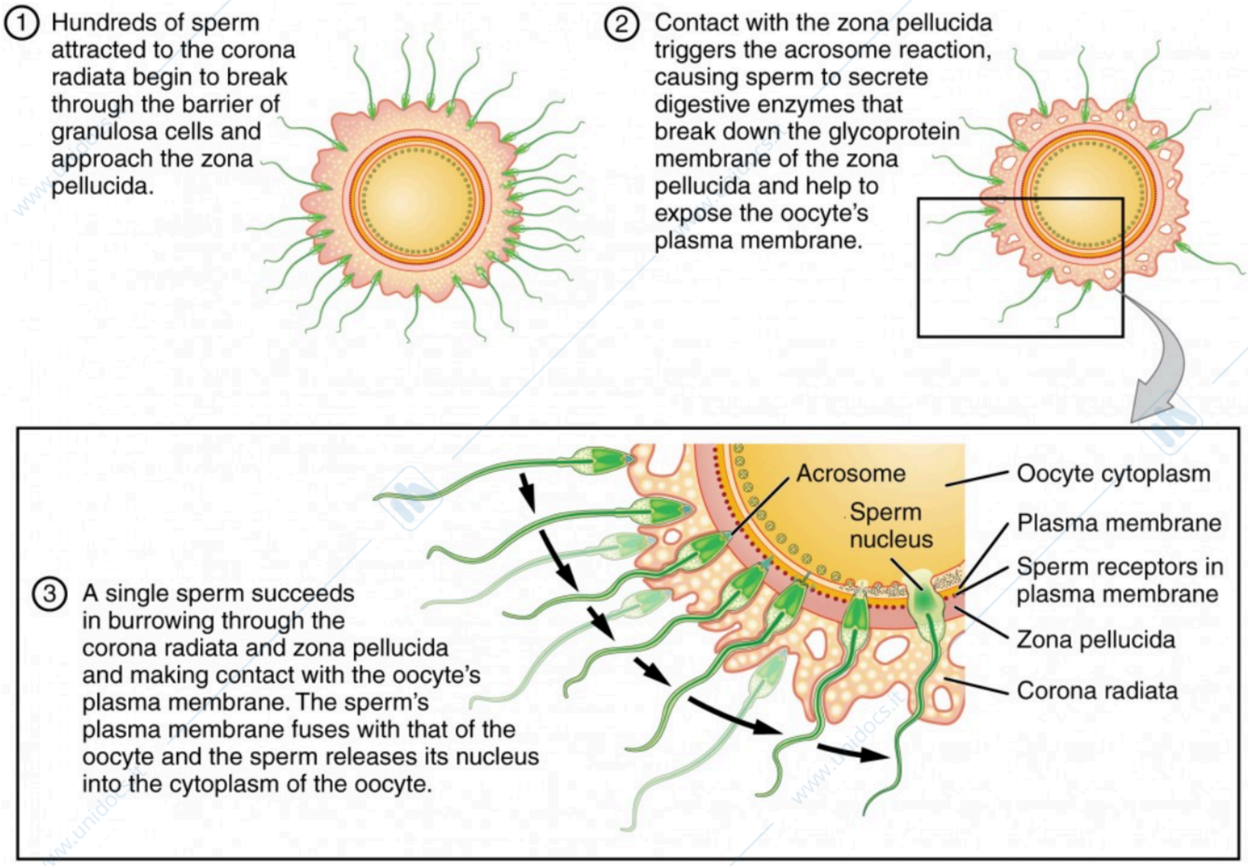


Figure: This drawing represents a mechanism of sperm–egg interaction.

Development begins at fertilization when a sperm penetrates an oocyte to form a zygote. A zygote is a highly specialized, totipotent cell, which has the ability to differentiate into any type of cell. It contains chromosomes and genes derived from the mother and father. The zygote divides many times and is progressively transformed into a multicellular human being through cell division, migration, growth, and differentiation.

DEFINITION: fertilization is the union of the male-sperm and female-oocyte gametes to form a zygote and marks the beginning of pregnancy and this process requires about 24 hours. If the oocyte is not fertilized, it slowly passes along the tube into the cavity of the uterus, where it degenerates and is resorbed.
Is important to remember that embryonic life begins with fertilization. Fertilization ends with the intermingling of maternal and paternal chromosomes at metaphase (a stage of mitosis) of the first mitotic division of the zygote.

SITE: the usual site of fertilization is in the ampulla, a saccular dilation of the uterine tube (in the ovary end of a Fallopian tube).

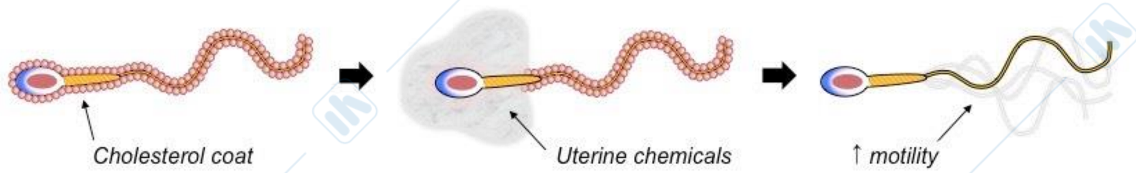
PHASES: fertilization is a multi-step process whereby multiple sperm bind to the corona radiata, but only a single sperm usually fertilizes the egg. The phases of fertilization follow:

→ At the beginning the **passage of a sperm through the corona radiata of the oocyte**.
Successful fertilization is enabled mainly by these processes: chemotaxis, adhesion, and digestion.

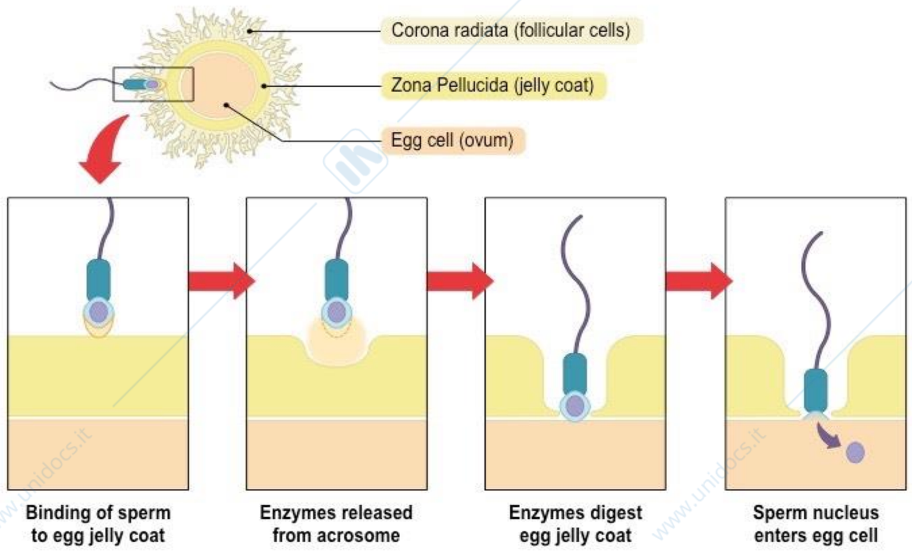
- Chemotaxis is a chemical process in which chemical signals from the egg direct the movement of sperm toward the egg.
- Once a sperm reaches the egg, adhesion occurs. In this process, a sperm "sticks" to the egg, which is enabled by sperm-receptor proteins on the egg. Adhesion causes reactions that block the entry of additional sperm.
- After a sperm adheres to the egg, digestive enzymes in the acrosome on the head of the sperm break down the zona pellucida of the egg. The zona pellucida is a protein layer surrounding the cell membrane of the egg. Digestion of the zona pellucida allows the sperm to enter the egg. The zona pellucida will continue to contain the developing zygote until the end of the germinal stage.

Key Terms - acrosome: a structure forming the end of the head of a spermatozoon

The process of fertilization in humans involves a number of key processes, including:
CAPACITATION – biochemical changes which occur post ejaculation to improve sperm motility. Capacitation occurs after ejaculation, when chemicals released by the uterus dissolve the sperm's cholesterol coat. This improves sperm motility (hyperactivity), meaning sperm is more likely to reach the egg (in the oviduct). It also destabilases the acrosome cap, which is necessary for the acrosome reaction to occur upon egg and sperm contact

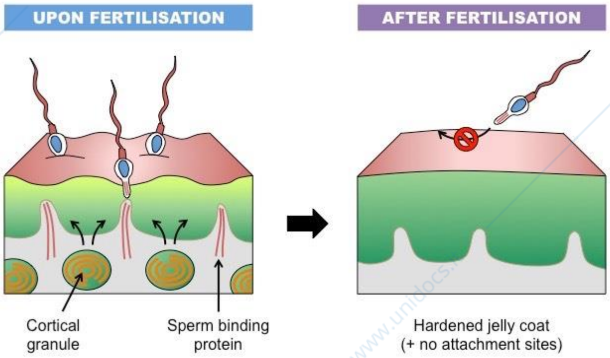


ACROSOME REACTION – the release of hydrolytic enzymes which softens the zona pellucida (jelly coat). In particular when the sperm reaches an egg, the acrosome reaction allows the sperm to break through the surrounding jelly coat. The sperm pushes through the follicular cells of the corona radiata and binds to the zona pellucida (jelly coat). The acrosome vesicle fuses with the jelly coat and releases digestive enzymes which soften the glycoprotein matrix. The sperm then pushes its way through the softened jelly coat and binds to exposed docking proteins on the egg membrane. The membrane of the egg and sperm then fuse, and the sperm nucleus (and centriole) enters the egg.



CORTICAL REACTION – hardening of the jelly coat post fertilization to prevent potential polyspermy. The cortical reaction occurs once a sperm has successfully penetrated an egg in order to prevent polyspermy.

Cortical granules within the egg's cytoplasm release enzymes (via exocytosis) into the zona pellucida (jelly coat). These enzymes destroy sperm binding sites and also thicken and harden the glycoprotein matrix of the jelly coat. This prevents other sperm from being able to penetrate the egg (polyspermy), ensuring the zygote formed is diploid.



What was described since here was the passage of a sperm through the corona radiata of the oocyte, as a result of enzymatic action of tubal mucosa and semen.

→ After the sperm penetrates the zona pellucida: digests a path by action of enzymes released from its acrosome. Sperm tail movements also help penetration of corona and zona pellucida.
Key Terms - zona pellucida: a glycoprotein membrane surrounding the plasma membrane of an oocyte

→ Then the sperm head attaches to surface of the oocyte, plasma membranes of oocyte and sperm fuse, and then break at contact point.
At this time there is an important reaction, the **Acrosome Reaction,** were sperm bind to ZP proteins in the zona pellucida; this initiates the release of enzymes from the sperm allowing it to burrow through the zona pellucida.
Head and tail of sperm enter oocyte cytoplasm with sperm's plasma membrane being attached to oocyte's plasma membrane. Once inside the cytoplasm of the oocyte, the sperm tail degenerates.

→ Oocyte responds by Zonal reaction - **Zona Rx**, were the binding of the sperm and egg plasma membranes initiates Ca+ influx into the egg and release of cortical granules from the egg that block other sperm from fertilizing the egg. Secondary oocyte completes second meiotic division and its chromosomes (22 plus X) arrange themselves in a vesicular nucleus called the *female pronucleus*. The second polar body is extruded.

→ Sperm head enlarges and forms the *male pronucleus*. The male and female pronuclei approach each other in the oocyte center, meet, and lose their nuclear membranes. They resolve their chromatin into a complete single haploid set of chromosomes which become organized on a spindle.

→ After the maternal and paternal chromosomes intermingle, metaphase of the first cleavage mitosis takes place, and the normal chromosome number is reconstituted.

→ Anaphase of the first cleavage mitosis then occurs.

→ The first 2 *blastomeres* are next seen, following cell division, and they are surrounded by the zona pellucida.

ABOUT DAY TWO: STARTING CLEAVAGE OF ZYGOTE

All these previous phases of fertilization cause metabolic activation of the oocyte, which initiates cleavage of the zygote. A zygote undergoes rapid cell divisions to form a spherical ball of cells: the blastula; this will further develop into a blastocyst.

DEFINITION: cleavage consists of repeated mitotic divisions of the zygote, resulting in a rapid increase in the number of cells that are called blastomeres.
Key Terms - blastomere: any cell that results from division of a fertilized egg

SITE: division of the zygote begins approximately 30 hours after fertilization and usually occurs in the uterine tube medial to the ampulla.

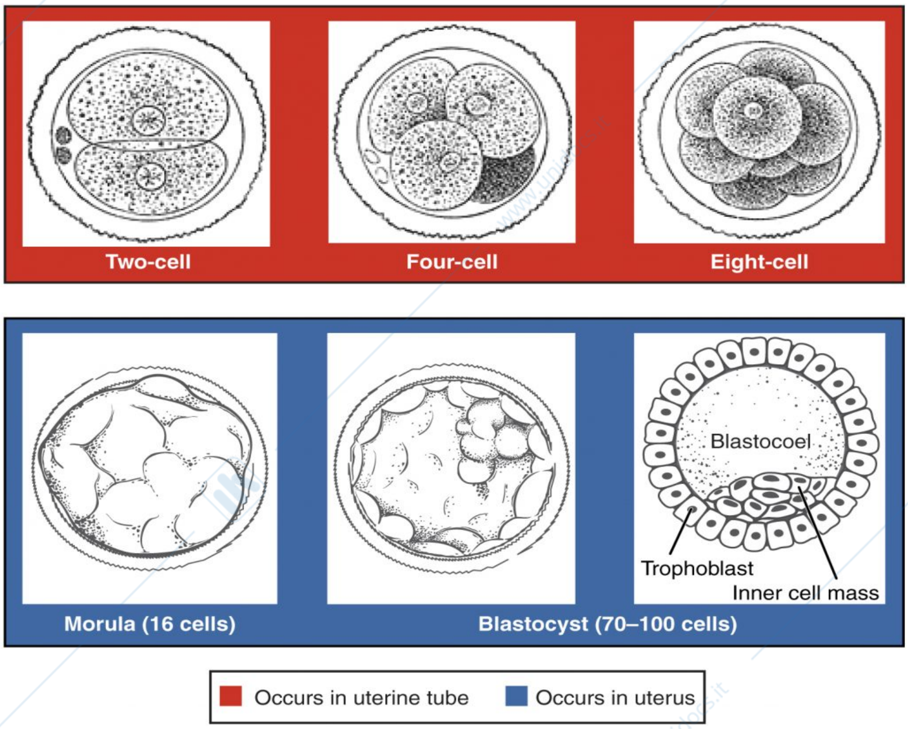


Image credit: OpenStax Anatomy & Physiology

STAGES OF CLEVAGE: the beginning of the cleavage process is marked when the zygote divides through mitosis into two daughter cells, the blastomeres, and takes place by 30 hours.
Further divisions follow rapidly upon one another, forming progressively smaller and smaller blastomeres: 4 are seen in 40-50 hours, 8 by 60 hours, and 12-16 by day 3 or 4.
The 12-16 blastomere stage, arrived at by cleavage of the fertilized ovum, is a solid ball resembling a mulberry and is called a morula (morula stage). As it forms, the morula enters the uterine cavity from the tube.

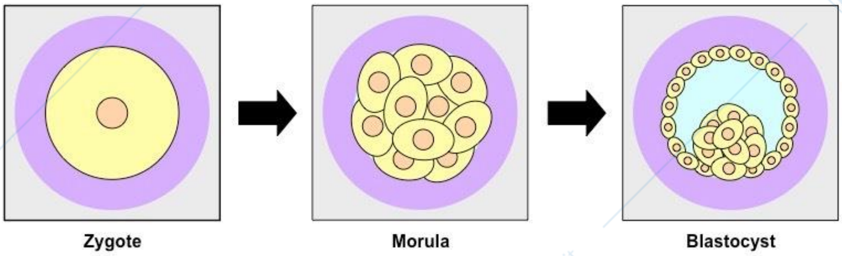
Initially the dividing cells, called blastomeres ("blastos" Greek for sprout), are undifferentiated and aggregated into a sphere enclosed within the membrane of glycoproteins (termed the zona pellucida) of the ovum. When eight blastomeres have formed they begin to develop gap junctions, enabling them to develop in an integrated way and co-ordinate their response to physiological signals and environmental cues.

When the cells number around sixteen the solid sphere of cells within the zona pellucida is referred to as a morula. At this stage the cells start to bind firmly together in a process called compaction, and cleavage continues as cellular differentiation.

ABOUT DAY FOUR: MORULA

DEFINITION: a morula (from the Latin word “morus”) is an early-stage embryo consisting of 16 cells (called blastomeres) in a solid ball contained within the zona pellucida. It enters the uterus nearly 3 days after fertilization.

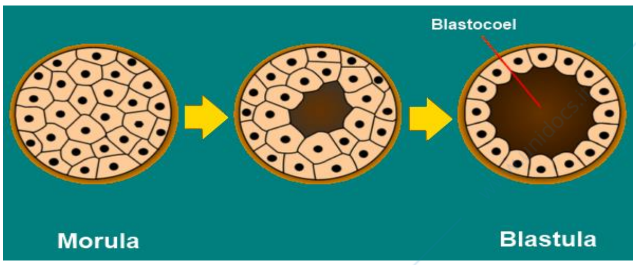
The morula is produced by a series of cleavage divisions of the early embryo, starting with the single-celled zygote. Once the embryo has divided into 16 cells, it begins to resemble a mulberry, hence the name morula.



Within a few days after fertilization, cells on the outer part of the morula become bound tightly together with the formation of desmosomes and gap junctions, becoming nearly indistinguishable. This process is known as compaction. The cells on the outside and inside become differentially fated into trophoblast (outside) and inner cell mass (inside) progenitors. A cavity forms inside the morula, by the active transport of sodium ions from trophoblast cells and osmosis of water. This results in a hollow ball of cells known as the blastocyst.

DAY FIVE: BLASTULATION

About day 5, fluid enters the morula from the uterine cavity and occupies the intercellular spaces. The fluid-filled spaces fuse to form a single, large cavity, the *blastocele*, and the morula is now called a *blastocyst* (blastocyst stage). Day 5 have 2 identifiable cell types and a fluid-filled cavity (blastoceol).



DEFINITION: as fluid increases in the cavity, the blastomeres are separated into two major areas called trophoblast and embryoblast. In mammals the blastula is called a blastocyst.

• The trophoblast is the thin outer cell layer that forms the wall of a blastocyst during early pregnancy, providing nutrients to the embryo and later developing into the embryonic part of the placenta.

• The embryoblast is a discrete group of blastomeres that is the primordium of the embryo. The embryoblast is a group of centrally located cells.

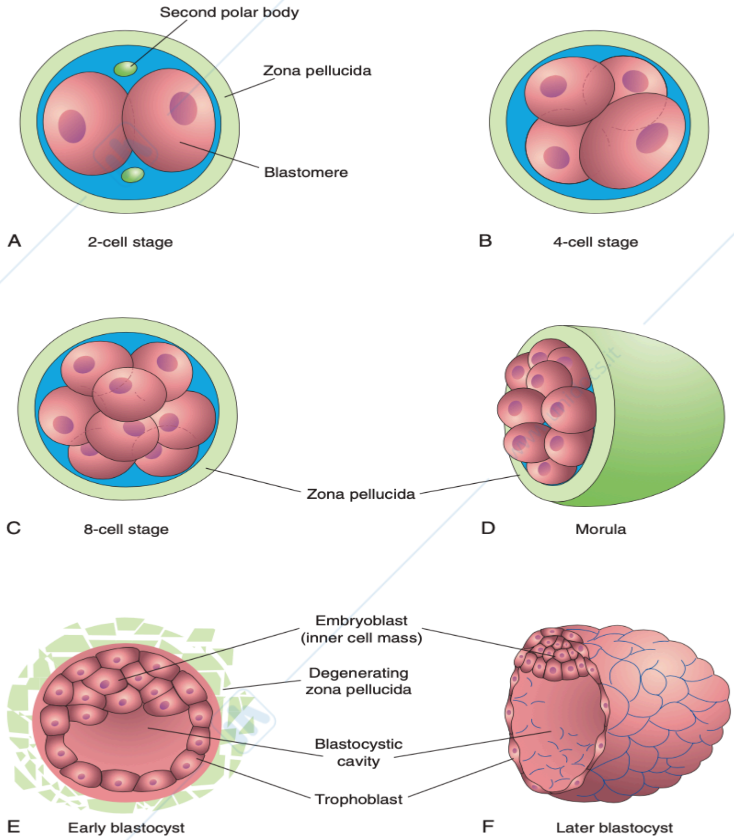
The free blastocyst is seen in the uterine cavity on day 4 or 5, from this time the zona pellucida disappears rapidly. During blastulation, cells continue to divide and begin to differentiate. The outer cells of the morula are polarized and give rise to trophoblast while the inner, apolar cells become the inner cell mass.

For the human, the blastocyst is formed by days 5 to 6 after fertilization. At this time, the blastocyst has reached the uterus, but has not yet implanted into the uterine wall.

On day 5, preimplantation human embryo contains 200 to 250 cells and only 30 to 34 of which are inner cell mass. In subsequent development, the cells of the inner cell mass will give rise to all tissues of the embryo's body.

In particular the inner cell mass will give rise to the pre-embryo, the amnion, yolk sac and allantois, while the fetal part of the placenta will form from the outer trophoblast layer. The embryo plus its membranes is called the conceptus, and by this stage the conceptus has reached the uterus. Exposed cells of the trophoblast allow the blastocyst to attach itself to the endometrium, where it will implant. The formation of the hypoblast and epiblast, which are the two main layers of the bilaminar germ disc, occurs at the beginning of the second week.

The embryoblast and the trophoblast then turn into two sub- layers, in particular the inner cells will turn into the hypoblast layer, which will surround the other layer, called the epiblast, and these layers will form the embryonic disc that will develop into the embryo. The trophoblast will also develop two sub-layers: the cytotrophoblast, which is in front of the syncytiotrophoblast, which in turn lies within the endometrium. Next, another layer called the exocoelomic membrane or Heuser’s membrane will appear and surround the cytotrophoblast, as well as the primitive yolk sac. The syncytiotrophoblast will grow and will enter a phase called lacunar stage, in which some vacuoles will appear and be filled by blood in the following days.



Figures showing cleavage of the zygote and formation of the blastocyst. A to D show various stages of cleavage. The period of the morula begins at the 12- to 32-cell stage and ends when the blastocyst forms. E and F show sections of blastocysts. The zona pellucida disappears by the late blastocyst stage (5 days). Although cleavage increases the number of blastomeres, note that each of the daughter cells is smaller than the parent cells. As a result, there is no increase in the size of the developing embryo until the zona pellucida degenerates. The blastocyst then enlarges considerably (D).

END OF WEEK ONE: IMPLANTATION

DEFINITION: the term "implantation" is used to describe process of attachment and invasion of the uterus endometrium by the blastocyst (conceptus) in placental animals. In humans, implantation of a fertilized ovum is most likely to occur around nine days after ovulation; however, this can range between six and 12 days. The implantation process continues through the second week of development.

SITE: the normal site for the implantation is the endometrium of the posterior wall of the fundus of the uterus in or near the middle line. The endometrium after implantation is called decidua.

In order that implantation can take its normal course, the blastocysts and the uterine mucosa must be able to interact. These two, independent structures must, therefore, undergo synchronous changes. During the implantation there are several changes in the uterine mucosa: implantation generally takes place on the 21st day of the menstrual cycle during the pregestational phase. At this time, the mucosa is thick, highly vascularized, and contains a large amount of glycogen. There are proliferation and predominance of secretion, congestion, and edema of the uterine wall. The blastocyst finds conditions in-the uterus very favorable for its implantation, especially for its nutrition.

STAGES: three implantation stages can be distinguished:

1. Adplantation of the blastocyst on the endometrium
2. Adhesion of the blastocyst to the endometrium
3. Invasion of the trophoblast and embedding

ADPLANTATION OF THE BLASTOCYST ON THE UTERINE MUCOSA

When the blastocyst emerges from the pellucid zone on the 5th day, it comes into contact with the maternal uterine mucosa in that it embeds itself in the endometrium with its embryonic pole.

The adhesion can occur when beforehand the uterus has entered its secretory phase (luteinizing phase).

This reception-ready phase of the endometrium lasts 4 days (20th -23rd day) and is usually termed the "implantation window". It follows around 6 days after the LH peak and is characterized by the appearance of small elevations at the apical pole of the epithelial endometrium cells.

One of the tasks of these elevations consists in the absorption of the uterine fluid, which brings the blastocyst nearer to the endometrium and immobilizes it at the same time. In this stage the blastocyst can still be eliminated by being flushed out.

There is also a hypothesis that the progesterone and the estrogen are responsible for an oedema that already fills the flattened out uterine cavity. This is also supposed to contribute to the blastocyst being pressed against the uterine epithelium.

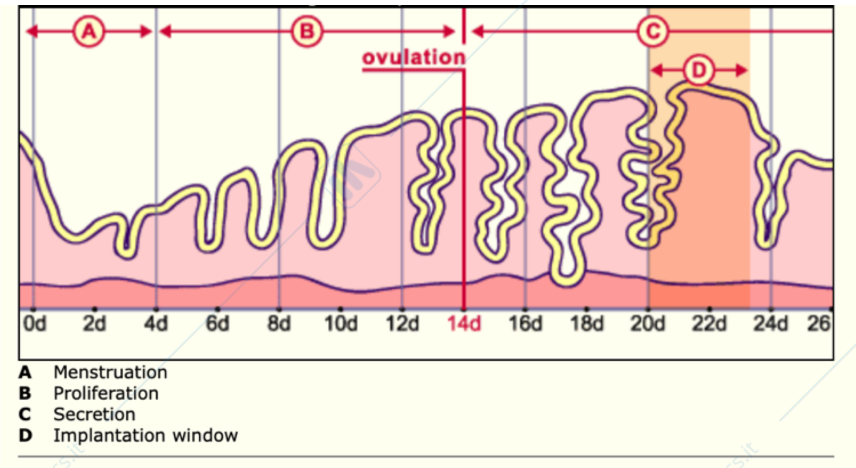


Figure: Menstruation cycle with the cyclic alterations of the endometrium. The "implantation window" that corresponds to the period of maximum receptivity is "D".

ADHESION OF THE BLASTOCYST TO THE ENDOMETRIUM

After the apposition of the free blastocyst at the uterine epithelium the microvilli on the surface of the outermost trophoblast cells interact with the epithelial cells of the uterus. In this stage the blastocyst can no longer be eliminated by a simple flushing out. The adhesion of the blastocyst on the endometrium arises through cell surface glycoproteins, the specific mechanisms of which, though, are not yet well understood.

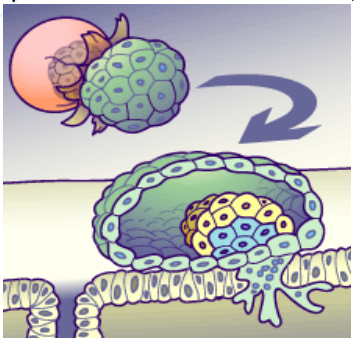


Figure: Hatching of the blastocyst and adhesion on the endometrium. One sees the cells of the syncytiotrophoblast that grow in between the cells of the uterine epithelium.

INVASION OF THE TROPHOBLAST AND EMBEDDING

The trophoblast differentiates into two different cell masses, shortly before it comes into contact with the endometrium:

- the outer syncytiotrophoblast (ST)
- the inner cytotrophoblast (CT)

The cytotrophoblast, deep inside, consists in an inner irregular layer of ovoid, single- nucleus cells. This is also where intensive mitotic activity takes place. In the periphery the syncytiotrophoblast forms a syncytium, i.e., a multi-nucleic layer without cell boundaries that arises from the fusion of cytotrophoblast cells. The syncytiotrophoblast produces lytic enzymes and secretes factors that cause apoptosis of the endometrial epithelial cells. The syncytiotrophoblast also crosses the basal lamina and penetrates into the stroma that lies below, eroding the wall of capillaries. With the implantation of the blastocyst in the endometrium the syncytiotrophoblast develops quickly and will entirely surround the embryo as soon as it has completely embedded itself in the endometrium.
The uterine mucosa reacts to the implantation by the decidual reaction. The syncytiotrophoblast cells phagocytize the apoptotic decidual cells of the endometrium and resorb the proteins, sugars and lipids that have been formed there. They also erode the canals of the endometrial glands and the capillaries of the stroma.

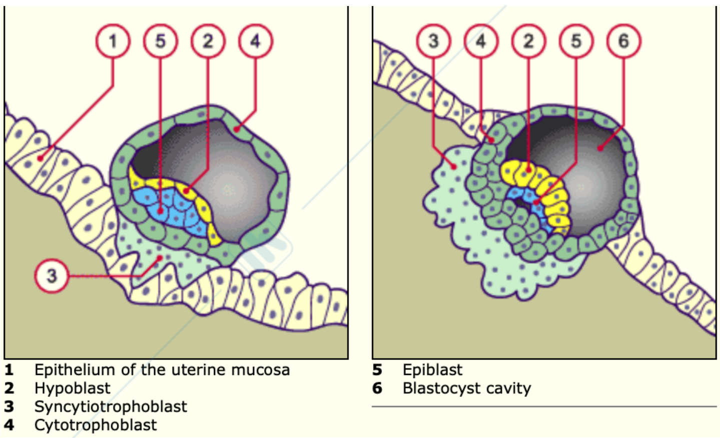
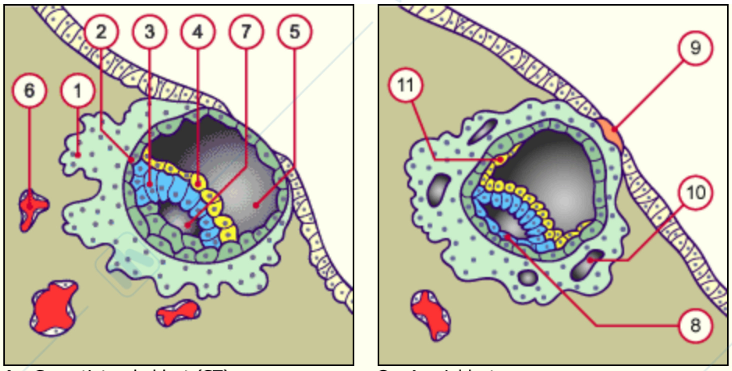


Figure: Free blastocyst (following the dissolution of the pellucid zone) in adplantation phase on the uterine wall (6th to 7th day). The trophoblast cells of the embryonic pole differentiate themselves, multiply, and form the invasive syncytiotrophoblast. The abembryonic pole consists of cytotrophoblast cells.
Figure: Didermic embryonic disk (hypoblast and epiblast) after 8 days. The ST continues its invasive, lytic activity into the maternal tissue.

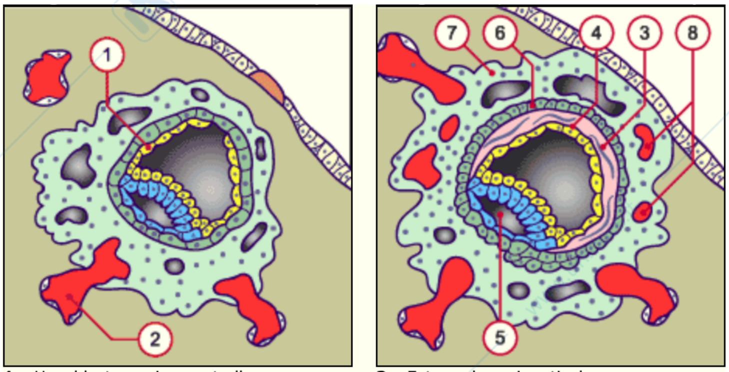


1 Syncytiotrophoblast (ST)
2 Cytotrophoblast (CT)
3 Epiblast
4 Hypoblast
5 Blastocyst cavity
6 Maternal blood capillary
7 Amniotic cavity

8 Amnioblasts
9 Fibrin plug
10 Trophoblast lacunae
11 Multiplying hypoblast

Figure: Complete implantation of the embryo into the endometrium and covering of the implantation location by a fibrin plug.
The amniotic cavity expands, and a cellular layer (amnioblasts) now separates it from the CT. The hypoblast cells also begin to multiply. Extracellular vacuoles appear in the ST and join to form lacunae.

In the middle of the 2nd week extracellular vacuoles appear in the ST. They join together forming lacunae. Initially these lacunae are filled with tissue fluids and uterine secretions. Following the erosion of the maternal capillaries, their blood fills the lacunae that later develop further into intervillous spaces. The invasive growth of the ST ceases in the zona compacta of the endometrium. At around the 13th day the primitive utero-placental circulatory system arises.



1 Hypoblast growing ventrally
2 Eroded maternal capillaries

3 Extraembryonic reticulum
4 Heuser´s membrane
5 Amniotic cavity
6 Cytotrophoblast
7 Syncytiotrophoblast
8 Lacunae, filled with blood

Figure: The destructive lytic activity of the ST reaches the capillaries of the endometrium. The maternal blood flows into the lacunae. The ST envelops the maternal capillaries, expands its lacunae network, and forms an arterial inflow and a venous outflow system.

At the end of the 2nd week, when implantation has ended, the embryonic bud consists schematically of two hemispheric cavities that lie on one another: the amniotic cavity (dorsal) and the umbilical vesicle (ventral). The floor of the amniotic cavity is formed by the epiblast, and the roof of the umbilical vesicle by the hypoblast. These two layers, which lie on one another, form the embryo or the double-layered embryonic disc.

ABNORMALITIES

Many early embryos abort spontaneously. The early implantation stages of the blastocyst are critical periods of development that may fail to occur because of inadequate production of progesterone and estrogen by the corpus luteum. Clinicians occasionally see a patient whose last menstrual period was delayed by several days and whose last menstrual flow was unusually profuse. Very likely, such patients have had an early spontaneous abortion. The overall early spontaneous abortion rate is believed to be approximately 45%. Early spontaneous abortions occur for a variety of reasons, an important one being the presence of chromosomal abnormalities.

Abnormal fertilization

Parthenogenesis: oocyte is activated without sperm penetration and development may begin. No record of viable birth via this method. Cleaving oocytes in ovary may develop into an ovarian teratoma

Superfecundation may follow poly-ovulation. An oocyte is fertilized by spermatozoa from one male and another oocyte is fertilized by a second male. Seen in various mammals, not usual in man.

Superfetation: ovulation and fertilization occur during an established pregnancy.

Abnormal implantation sites

The human blastocyst normally implants in the endometrium along the posterior wall of the body of the uterus, where it becomes attached between the openings of the endometrial glands or occasionally in the mouth of a glandular duct.

Not infrequently, the blastocyst implants in abnormal locations outside the uterine body. This usually leads to the death of the embryo and severe hemorrhage of the mother during the second month of pregnancy. Such an implantation is called an extrauterine or ectopic pregnancy and may occur in the abdominal cavity, the ovary, the uterine tube or pelvis. Rarely does an extrauterine embryo come to full term.

Tubal pregnancy is the most frequent ectopic sit the tube usually ruptures during the second month of pregnancy, resulting in severe internal hemorrhaging.
Blastocysts sometimes implant outside the uterus and result in ectopic pregnancies; 95% to 98% of ectopic implantations occur in the uterine tubes, most often in the ampulla and isthmus. A woman with a tubal pregnancy has the usual signs and symptoms of pregnancy; however, she may also experience abdominal pain (from distention of the uterine tube), abnormal bleeding, and irritation of the pelvic peritoneum.
The causes of tubal pregnancy are often related to factors that delay or prevent transport of the cleaving zygote to the uterus (e.g., blockage of uterine tube). Ectopic tubal pregnancies usually result in rupture of the uterine tube and hemorrhage into the peritoneal cavity during the first 8 weeks, followed by death of the embryo.

Abdominal pregnancy: the peritoneal lining of the rectouterine cavity is the most frequent implantation sit also on peritoneum of the intestinal tract or omentum.

Occasionally, implantation in the uterus itself may lead to serious complications, particularly if implantation occurs near the internal os (low uterus). The placenta then bridges the os and we have what is called placenta previa which results in severe bleeding in the latter or second part of pregnancy and during delivery.
Fertilized ovum may abnormally move to contralateral tube.