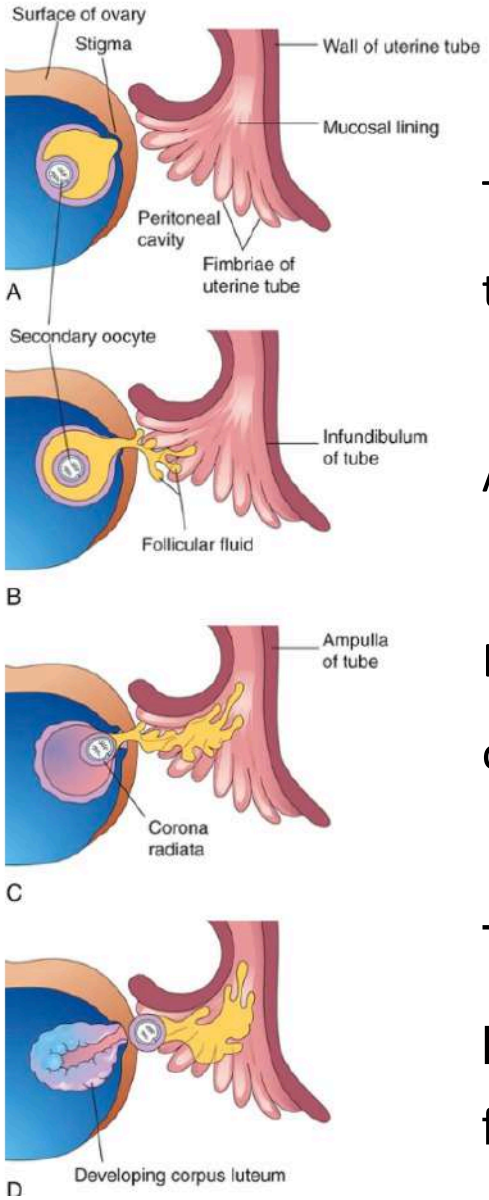
Embryology
Lesson II-
Fertilization and
I week

Prof. Mattia Lauriola

Ovulation



The follicle continues to enlarge and soon forms a bulge on the surface of the ovary.

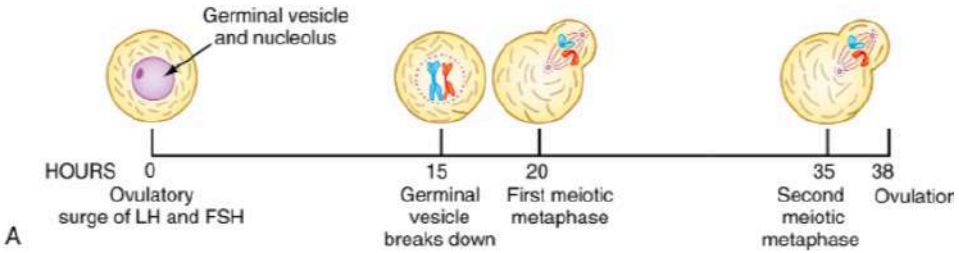
A small oval, avascular spot, the **stigma**, soon appears on this bulge.

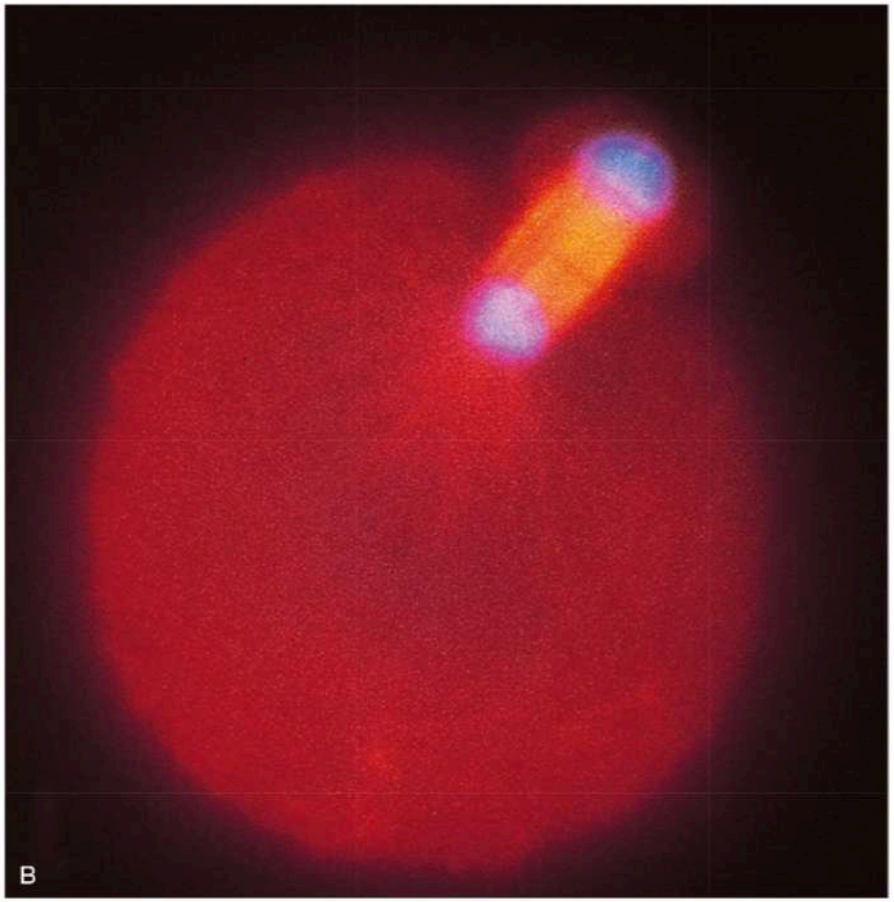
Before ovulation, the secondary oocyte and some cells of the cumulus oophorus detach from the interior of the distended follicle.

The expelled secondary oocyte is surrounded by the **zona pellucida**, an acellular glycoprotein coat, and one or more layers of follicular cells, which are radially arranged to form the **corona radiata** and cumulus oophorus

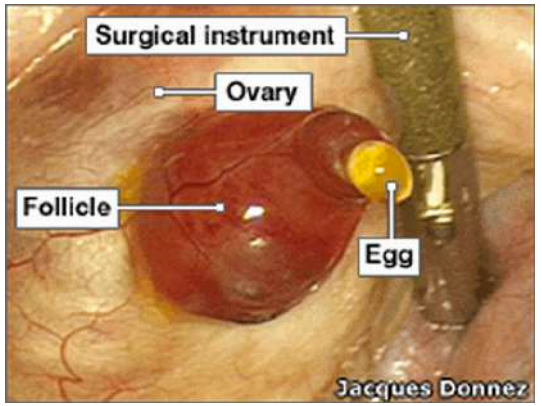
38 h onset to Ovulation

By twenty hours, the chromosomes are lined up in metaphase- Cell division to form the secondary oocyte and the first polar body rapidly





The secondary oocyte promptly begins the meiotic division but about <u>three hours before ovulation</u> is arrested at the second meiotic metaphase.

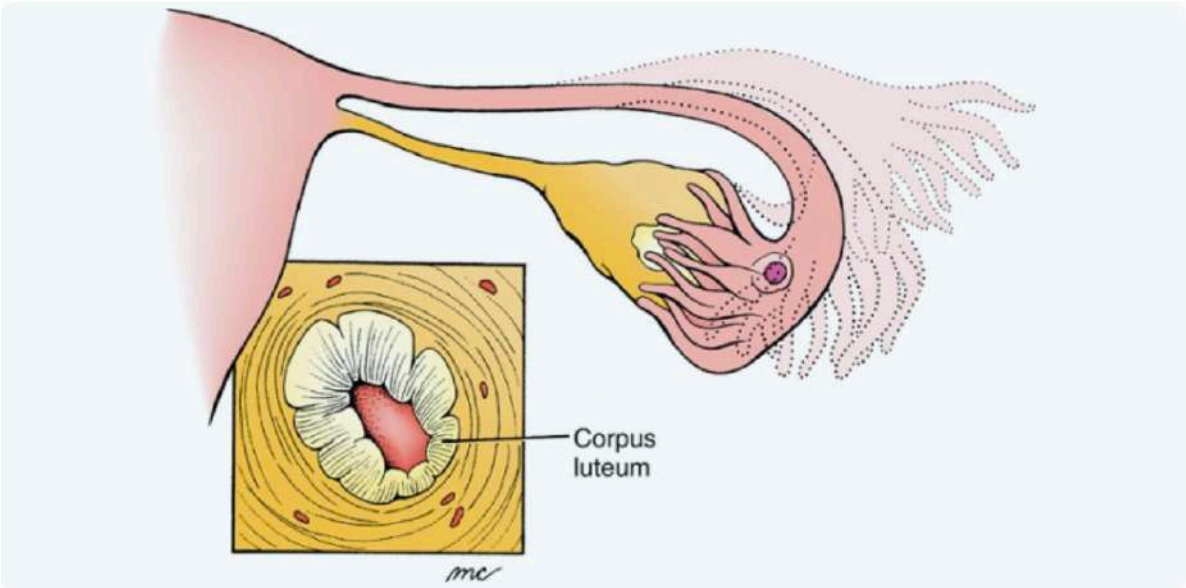


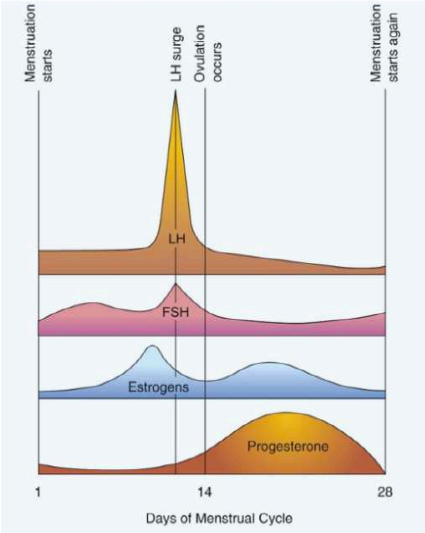
Ovulation follows within 24-36 hours of a surge of LH

which appears to be the result of signaling molecules from the granulosa cells (feedback).

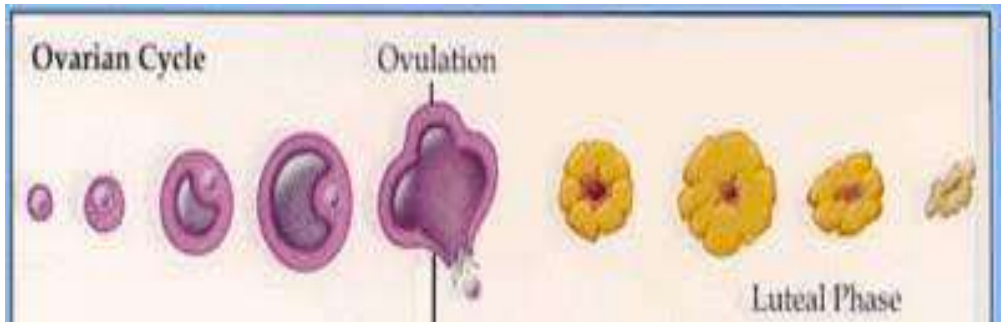
This surge, elicited by a high estrogen level in blood, appears to cause the stigma to rupture, expelling the secondary oocyte along with the follicular fluid.

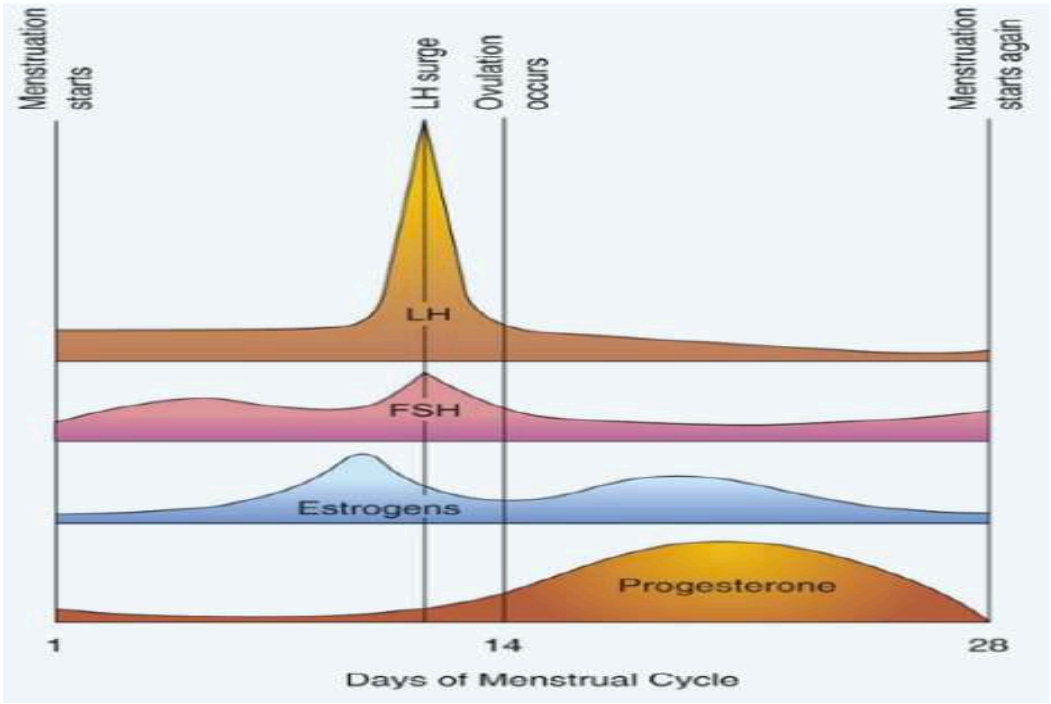
Plasmins and matrix metalloproteinases (MMPs) also appear to have some control over stigma rupture

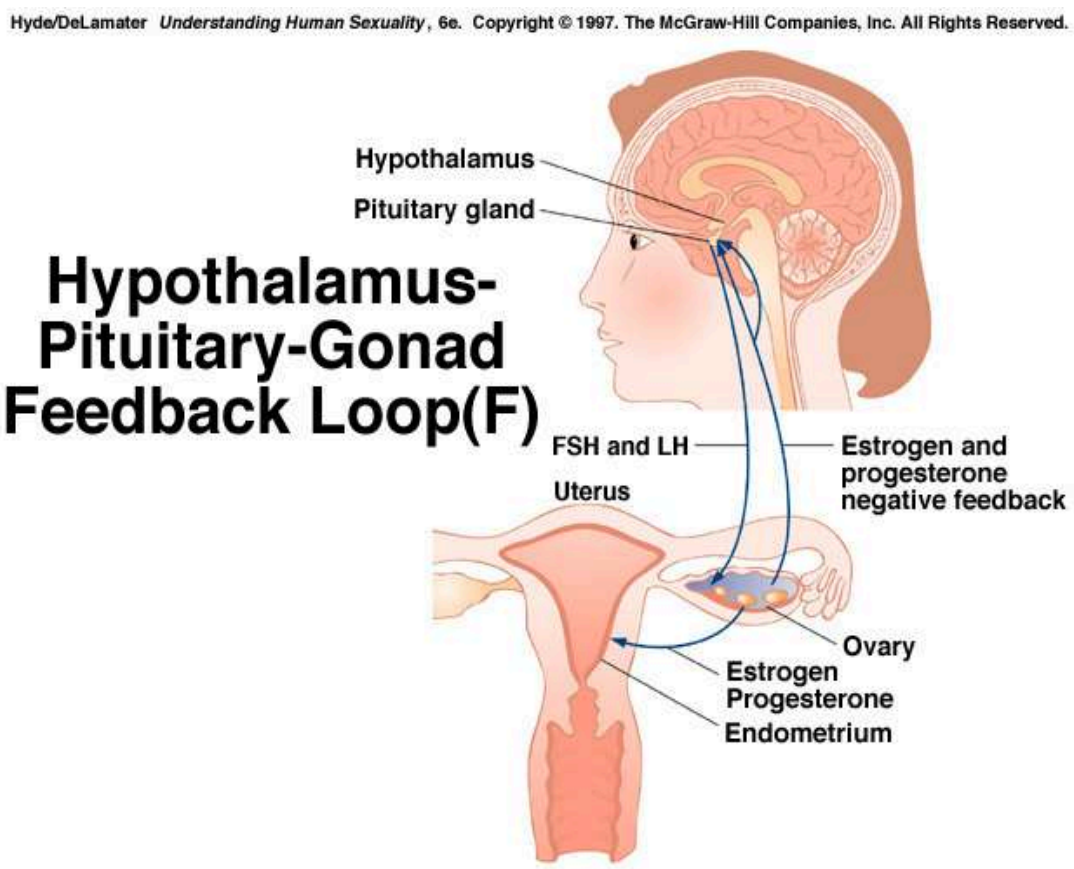




Hormonal Profile



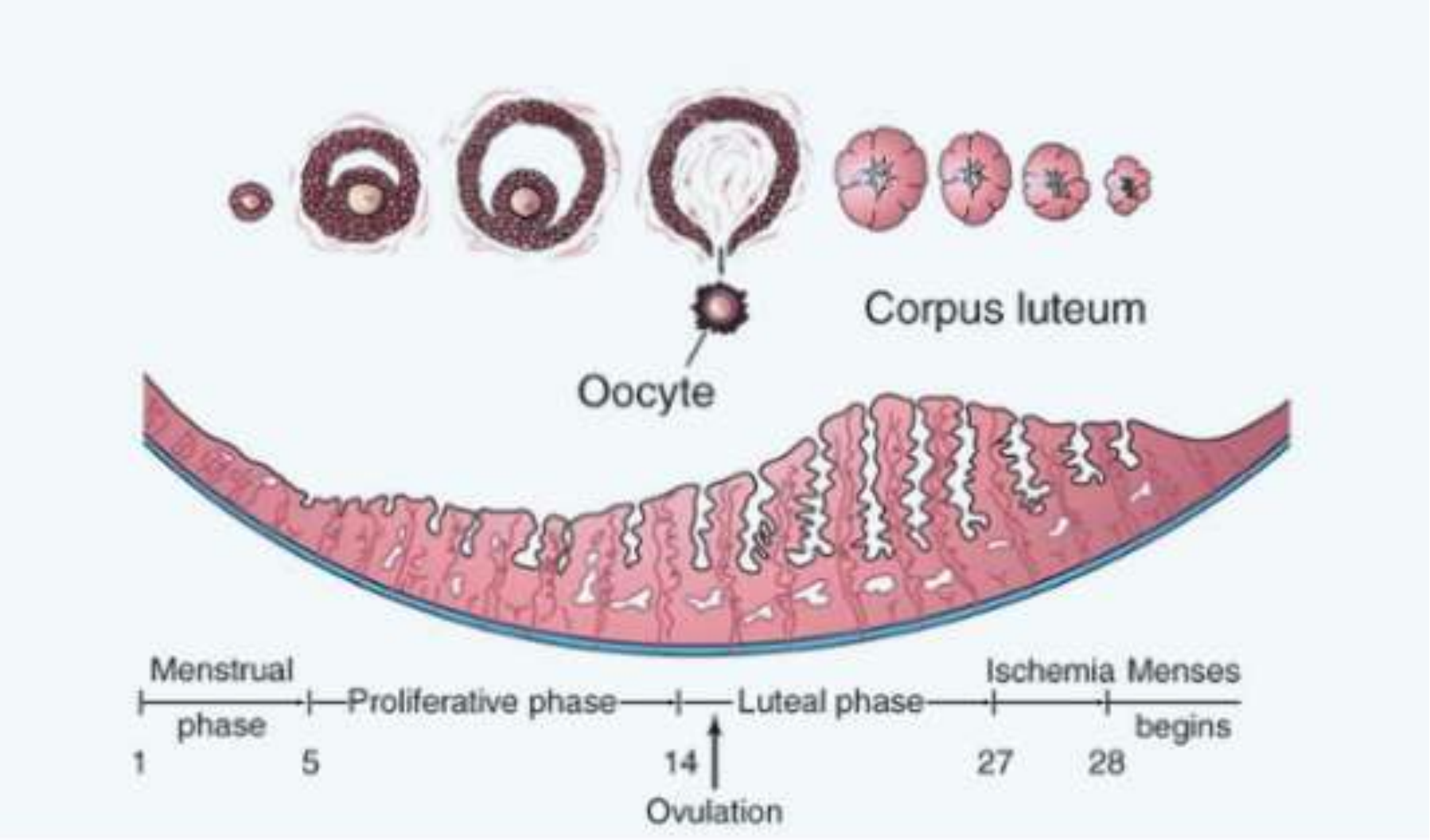




Menstrual Cycle
The cycle is the period during which the oocyte matures, is ovulated, and enters the uterine tube.

Estrogen and progesterone produced by the ovarian follicles and corpus luteum cause cyclic changes in the endometrium of the uterus. These monthly changes in the uterine lining constitute the menstrual cycle.

UTERINE CYCLE



Viability of Oocytes and Sperms

Oocytes in the uterine tube are usually fertilized within 12 hours of ovulation.

In vitro observations have shown that oocytes cannot be fertilized after 24 hours, and they degenerate shortly thereafter. Most sperms do not survive for more than 24 hours in the female genital tract. Some sperms are captured in folds of the mucosa of the cervix and are gradually released into the cervical canal and pass through the body of the uterus into the uterine tubes.

Semen and oocytes can be frozen and stored for many years to be used in assisted reproduction.

Summary

- Gametogenesis
- Ovarian Cycle

https://studentconsult.inkling.com/read/larsen-human-embryology-schoenwolf-5/videos/animation-1-1

Fertilization

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.*

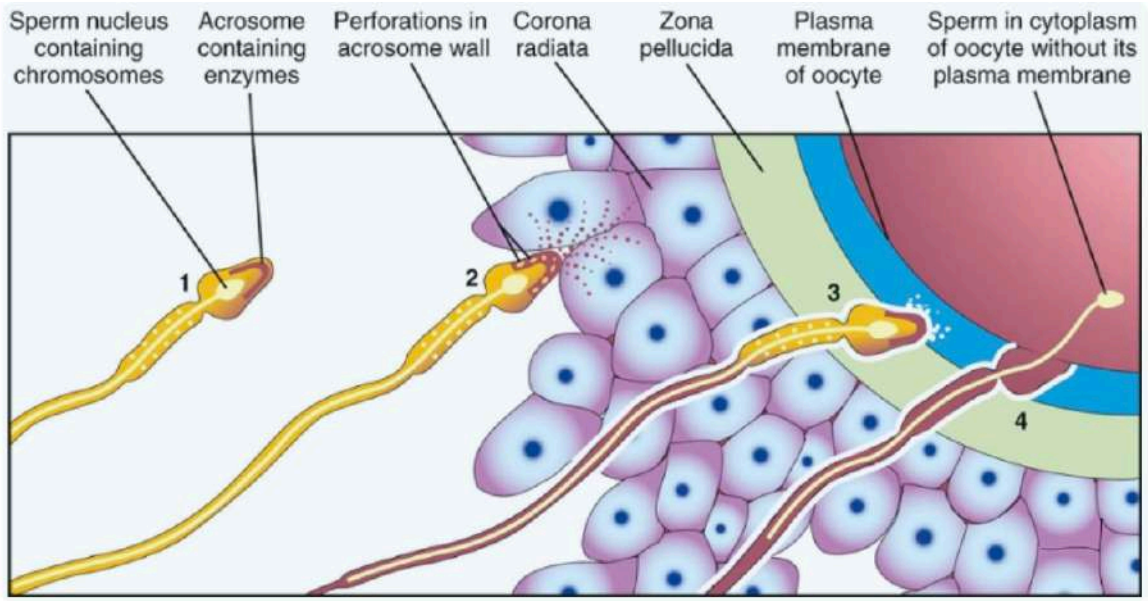
It contains chromosomes and genes derived from the mother and father.

The zygote divides many times and is progressively transformed into a multicellular human being through cell division, migration, growth, and differentiation

Site of Fertilization

The usual site of fertilization is the **ampulla**, a dilation of the **uterine tube**.
If the oocyte is not fertilized, it slowly passes along the tube into the cavity of the uterus, where it degenerates and is resorbed.

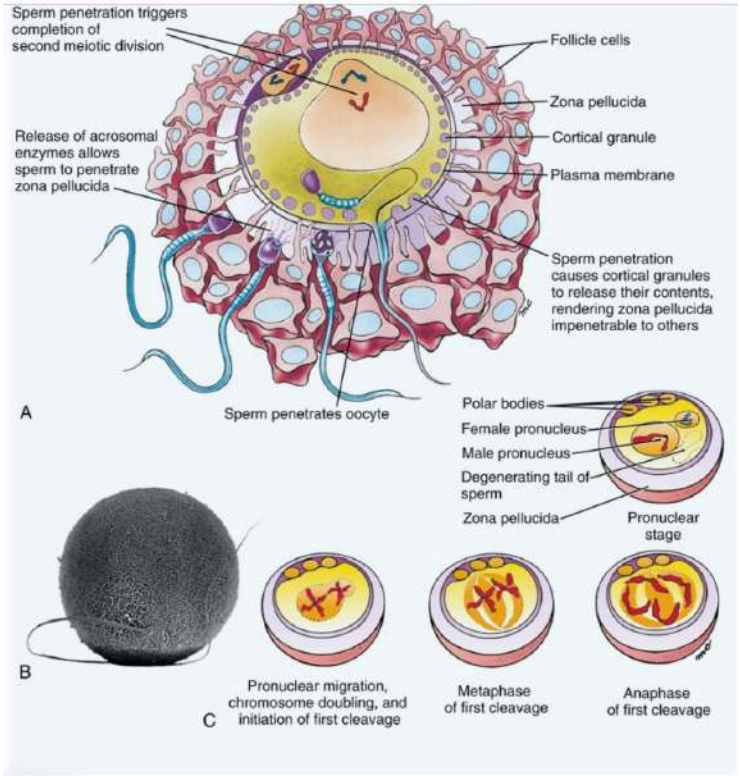
Fertilization is a complex sequence of coordinated molecular events that begins with the contact between a sperm and an oocyte.



Fertilization

In vitro evidence suggests that the ovulated follicle contains a currently unknown **sperm chemotropic factor** and that only capacitated sperm are able to respond to this factor by directed swimming toward the mature oocyte.

Oocytes are attractive



Fertilization

Fertilization ends with the intermingling of maternal and paternal chromosomes at **metaphase** of the first mitotic division of the **zygote**.

Carbohydrate- and protein-binding molecules on the surface of the **gametes** (oocyte or sperm) are involved in sperm **chemotaxis** (movement of cells) and gamete recognition, as well as in the process of fertilization.

Phases of Fertilization. 1

Passage of a sperm through the corona radiata of the oocyte.

Dispersal of the follicular cells of the corona radiata results mainly from the action of the enzyme *hyaluronidase*, which is released from the acrosome of the sperm.

Tubal mucosal enzymes also appear to assist hyaluronidase. Additionally, movements of the tail of the sperm are important during penetration of the **corona radiata**.

Phases of Fertilization. 2

Penetration of the zona pellucida.

The formation of a pathway through the **zona pellucida** for the sperm results from the action of enzymes released from the acrosome.

The proteolytic enzyme *acrosin*, as well as *esterases* and *neuraminidase*, appears to cause lysis of the zona pellucida, thereby forming a path for the sperm to follow to the oocyte.

Phases of Fertilization. 2

Penetration of the zona pellucida.

When a spermatozoon reaches the zona pellucida surrounding the oocyte, it binds in a *species-specific interaction* with a **glycoprotein sperm receptor molecule** in the zona pellucida (ZP3, one of three glycoproteins composing the zona pellucida).

Binding to ZP3 is mediated by a sperm surface protein called **SED1.**

Binding of human sperm to eggs involves a sequence of sugar molecules, called sialyl-Lewis^{x} at the ends of the oligosaccharides of the ZP proteins.

As a result of this binding, the acrosome is induced to release degradative enzymes that allow the sperm to penetrate the zona pellucida.

Phases of Fertilization. 2

CD9 (tetraspanin) is a transmembrane receptor on the oocyte membrane and will bind IZUMO (IgG superfamily) from the sperm.

IZUMO was named after the Japanese shrine to marriage

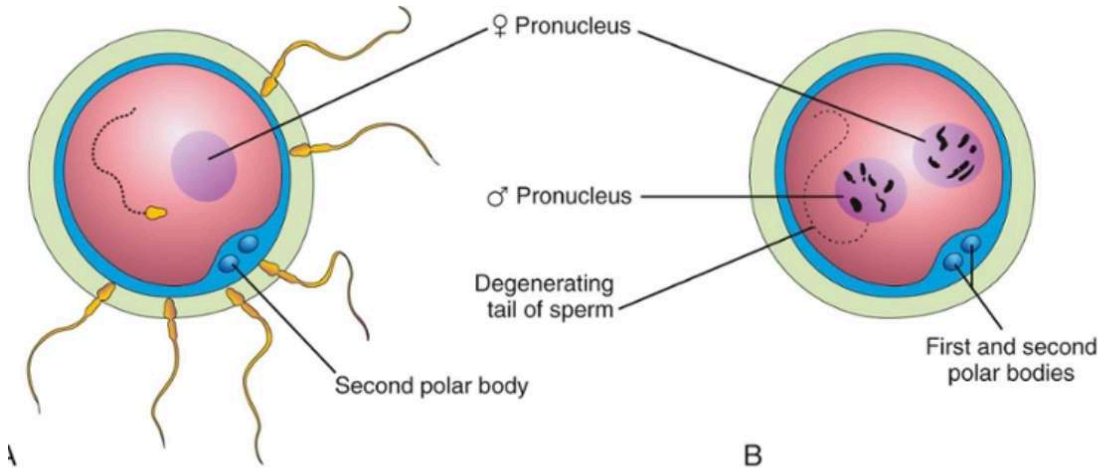
Integrin on the egg will bind the FERTILIN-beta present on the sperm.

And several factors from the ADAM superfamily (extracellular matrix proteins).

Phases of Fertilization.3

Fusion of the plasma cell membranes of the oocyte and sperm. Once the fusion occurs, the contents of cortical granules from the oocyte are released into the **perivitelline space, between the oocyte and zona pellucida**, resulting in changes in the zona pellucida. These changes prevent other sperms from entering (polyspermy). The cell membranes break down at the area of fusion. The head of the sperm then enter the cytoplasm of the oocyte, but the plasma membrane and mitochondria of the sperm remain behind.

DAY 0



Phases of Fertilization.3

Membrane fusion immediately causes two events to occur:

- formation of a **calcium wave** that radiates over the surface of the egg from the point of sperm contact;
- release of the contents of thousands of small **cortical granules**, located just beneath the oocyte cell membrane, into the **perivitelline space** between the oocyte and the zona pellucida.

These two events alter the sperm receptor molecules, causing the zona to become impenetrable by additional spermatozoa. Therefore, these changes prevent **polyspermy** or fertilization of the oocyte by more than one spermatozoon. Because a few hundred spermatozoa reach the vicinity of the egg, the need to block polyspermy is extremely important.

Phases of Fertilization.3

The cortical granules contain

proteases that clip perivitelline tether proteins,

peroxidases that harden the vitelline envelope,

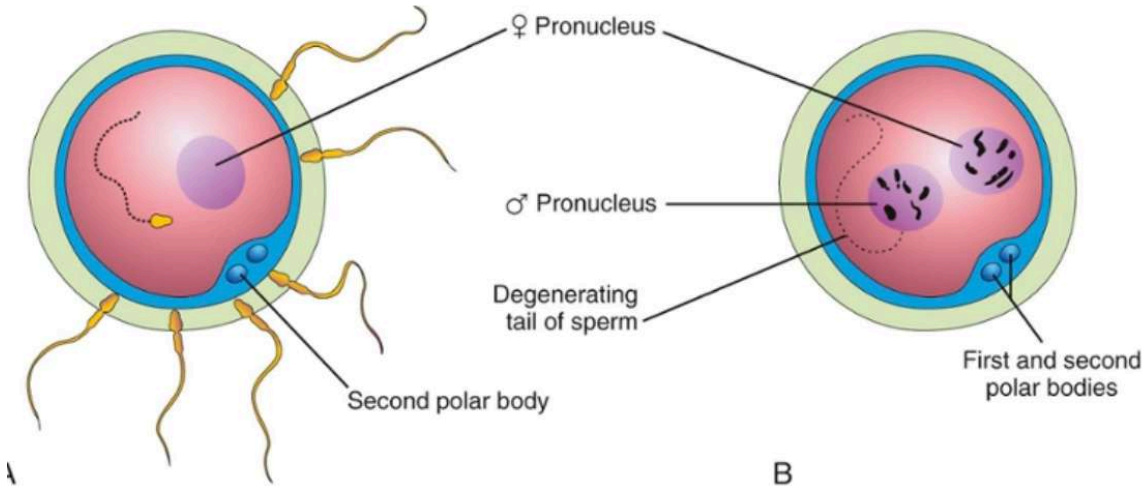
glycosaminoglycans that attract water into the perivitelline space, causing it to expand and form the hyaline layer.

The trigger for the cortical granules to exocytose is the release of calcium ions from cortical smooth endoplasmic reticulum in response to sperm binding to the egg.

Phases of Fertilization.4

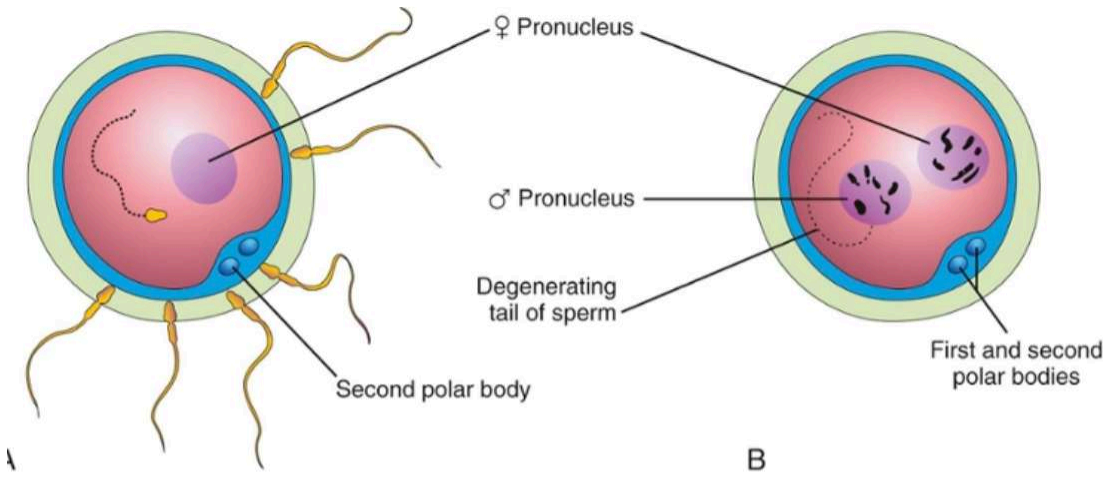
Completion of the second meiotic division of the oocyte.

The oocyte completes the second meiotic division and forms a **mature oocyte** and a second polar body. The nucleus of the mature oocyte becomes the female pronucleus.



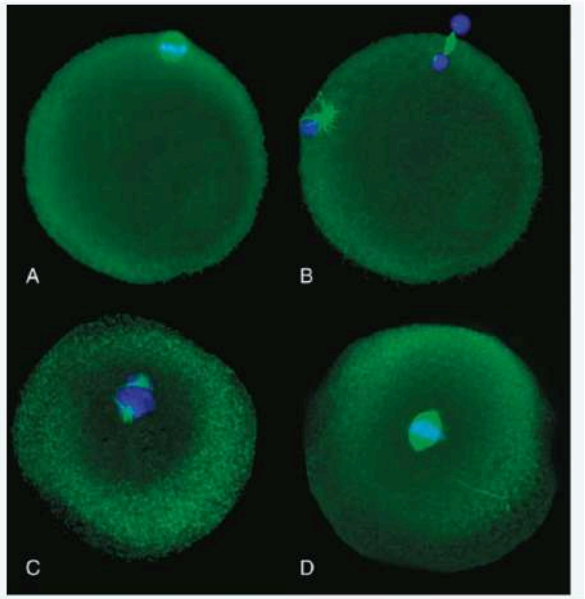
Phases of Fertilization.5

Formation of the male pronucleus. Within the cytoplasm of the oocyte, the nucleus of the sperm enlarges to form the male pronucleus. The tail of the sperm degenerates. During growth, the male and female pronuclei replicate their DNA.



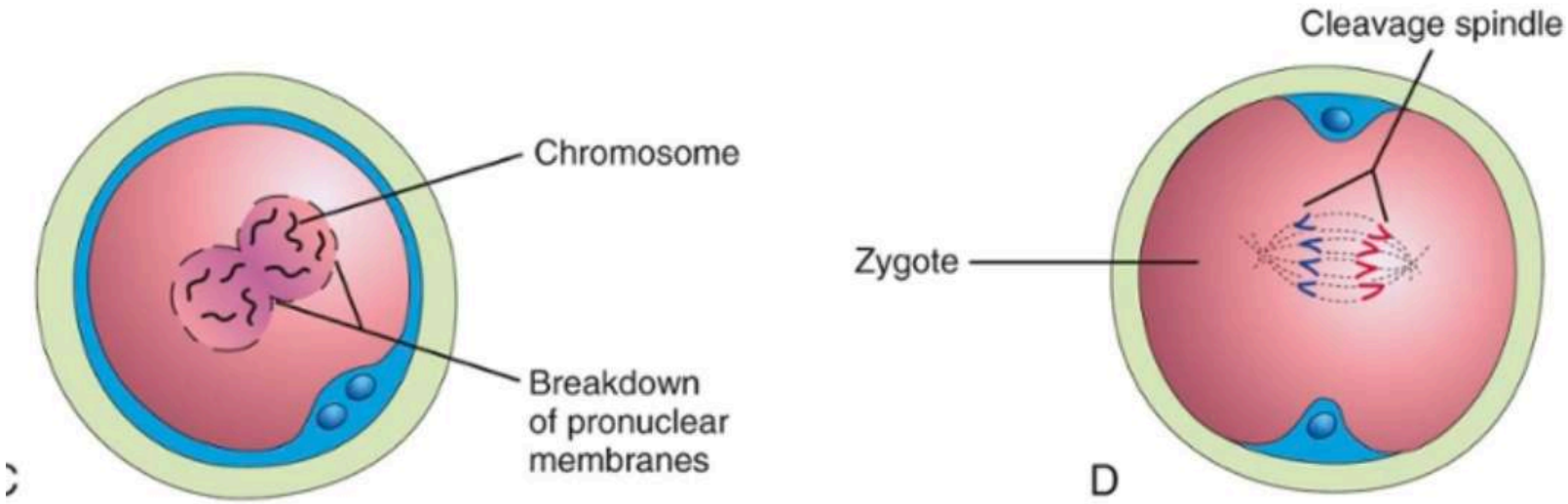
Phases of Fertilization.5

After penetration of the oocyte by the sperm, the nuclei of the oocyte and sperm swell within the zygote and are called the **female** and **male pronuclei**. Their nuclear membranes quickly disappear as both maternal and paternal chromosomes are replicated in preparation for the first cleavage



Phases of Fertilization.6

Breakdown of the pronuclear membranes. Condensation of the chromosomes, arrangement of the chromosomes for mitotic cell division, and the first cleavage division of the zygote occur. The combination of 23 chromosomes in each pronucleus results in a zygote with 46 chromosomes.



Results of Fertilization

-Stimulates the secondary oocyte to complete the second meiotic division, producing the second polar body.

-Restores the normal diploid number of chromosomes (46) **in the zygote**

-Results in variation of the human species through mingling of maternal and paternal

https://studentconsult.inkling.com/read/larsen-human-embryology-schoenwolf-5/videos/animation-1-2

https://www.youtube.com/watch?v=7G2rL5Cutd4

https://www.youtube.com/watch?v=_krJsK5Dxj4 is **ICSI**

Chimeric contribution of human extended pluripotent stem cells to monkey embryos *ex vivo*

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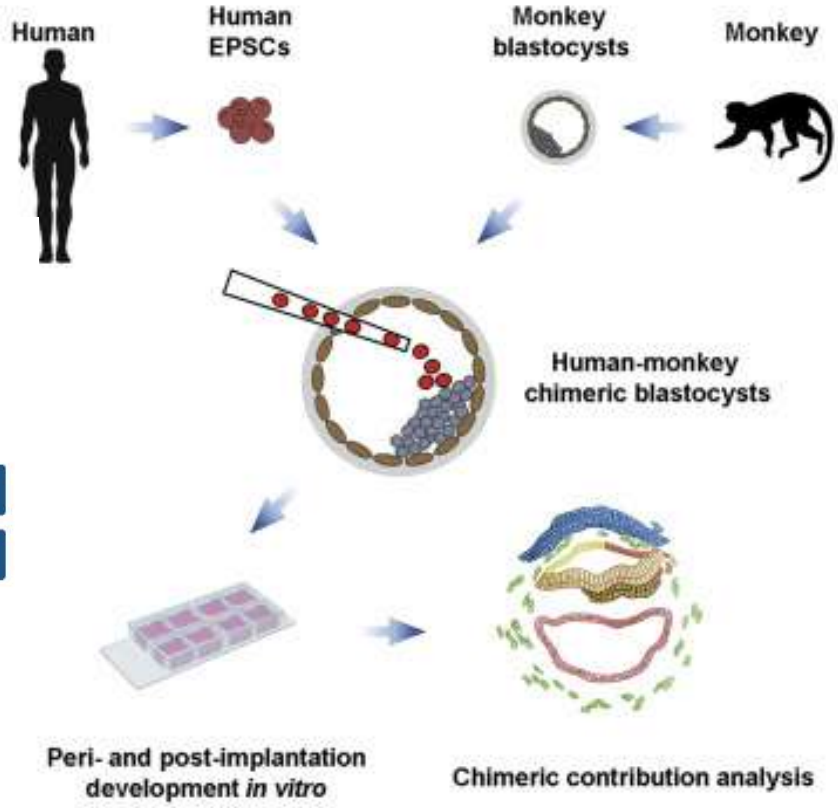
> Cell. 2021 Apr 15;184(8):2020-2032.e14. doi: 10.1016/j.cell.2021.03.020.

Chimeric contribution of human extended pluripotent stem cells to monkey embryos ex vivo

Tao Tan [1], Jun Wu [2], Chenyang Si [3], Shaoxing Dai [3], Youyue Zhang [3], Nianqin Sun [3], E Zhang [3], Honglian Shao [3], Wei Si [3], Pengpeng Yang [3], Hong Wang [3], Zhenzhen Chen [3], Ran Zhu [3], Yu Kang [3], Reyna Hernandez-Benitez [4], Llanos Martinez Martinez [5], Estrella Nuñez Delicado [5], W Travis Berggren [4], May Schwarz [4], Zongyong Ai [3], Tianqing Li [3], Concepcion Rodriguez Esteban [4], Weizhi Ji [6], Yuyu Niu [7], Juan Carlos Izpisua Belmonte [8]



Do not consistently and robustly contribute to chimera formation when the host animal has a high evolutionary distance from humans.

Xenogeneic barriers between hPSCs and evolutionarily distant host animal species have been suggested to account for limited chimerism

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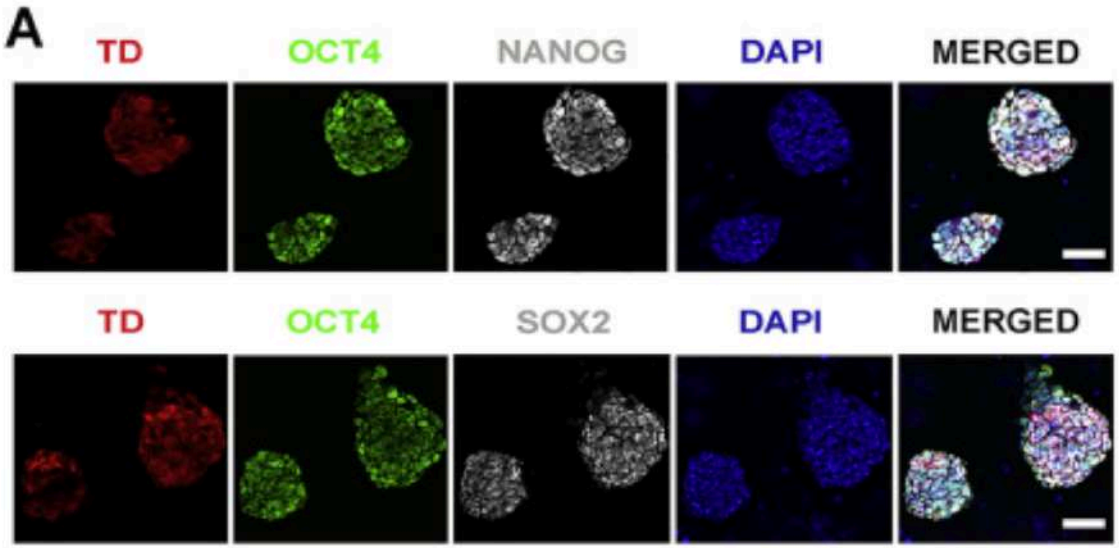
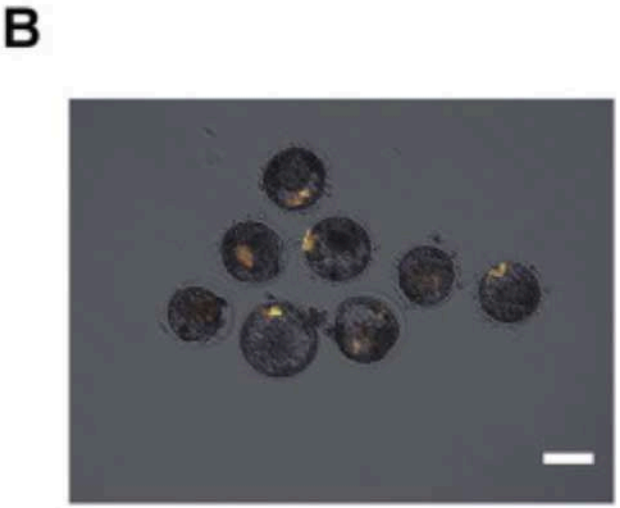
https://www.sciencedirect.com/science/article/pii/S0092867421003056#mmc4

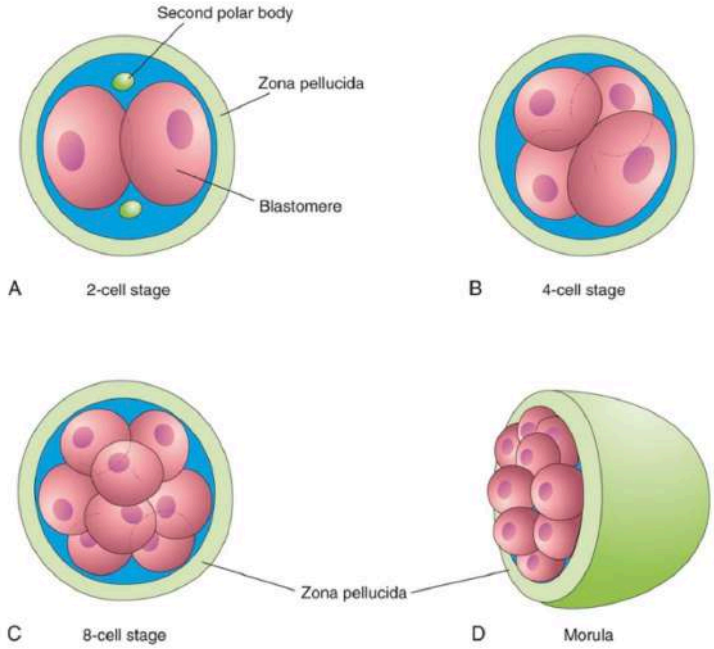




https://www.sciencedirect.com/science/article/pii/S0092867421003056#mmc4

Cleavage of Zygote

Cleavage consists of repeated mitotic divisions of the zygote, resulting in a rapid increase in the number of cells—**blastomeres**.
Division of the zygote begins approximately 24-30 hours after fertilization.
These blastomeres become smaller with each cleavage division. During cleavage, the zygote is still surrounded by the zona pellucida



Cleavage of Zygote

Within 24-30 hours after fertilization, the zygote initiates a rapid series of mitotic cell divisions called **cleavage**.

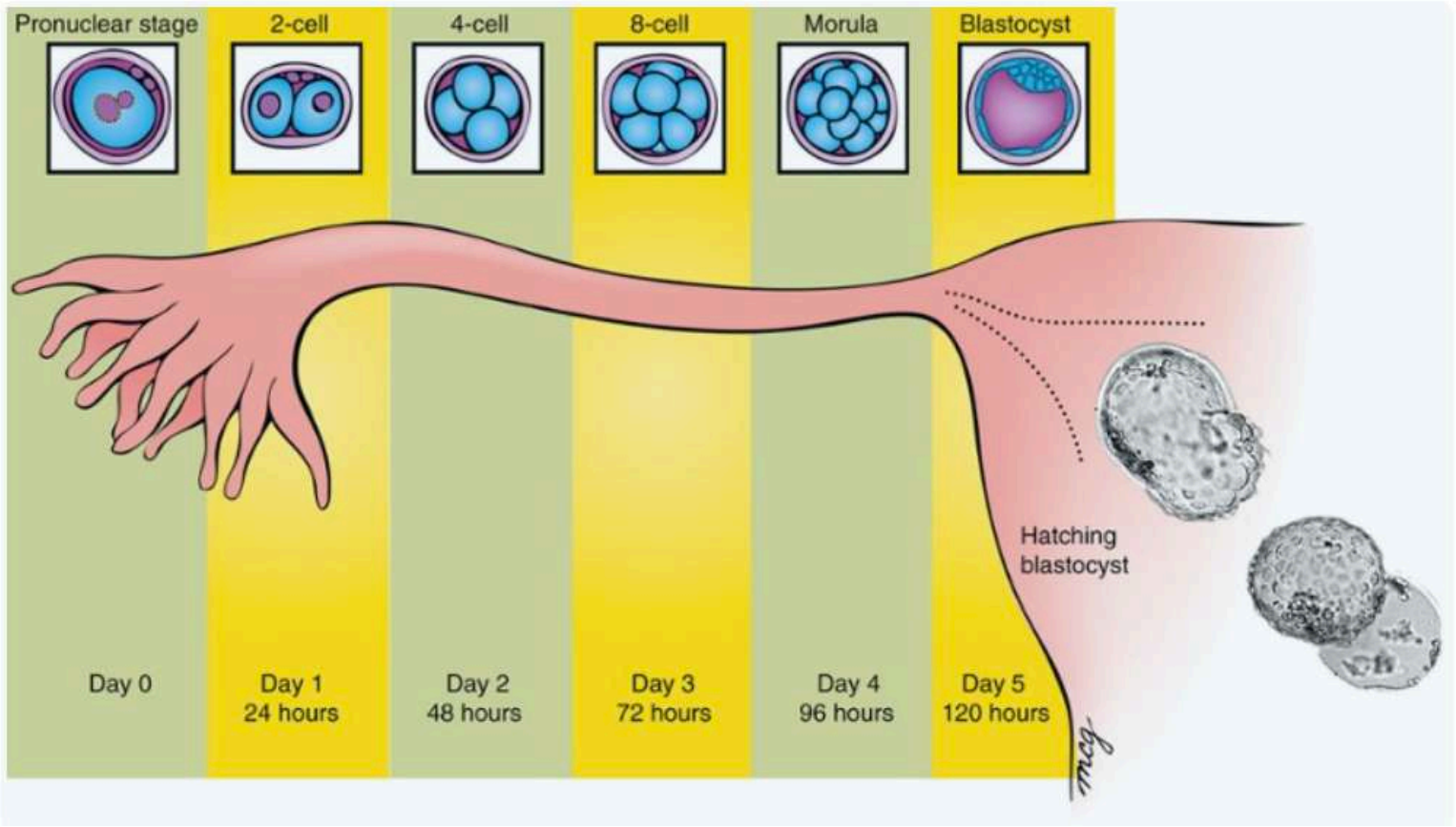
These divisions are not accompanied by cell growth, so they subdivide the large zygote into many smaller daughter cells called **blastomeres**.

The embryo as a whole does not increase in size during cleavage and remains enclosed in the zona pellucida.

The first cleavage division divides the zygote to produce two daughter cells.

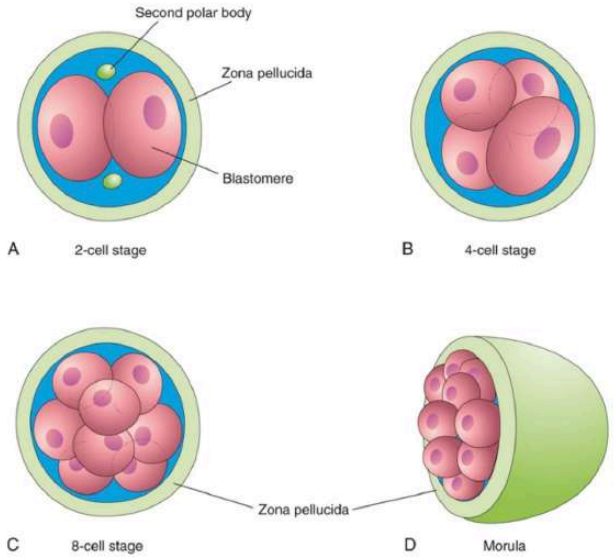
The second division, which is complete at about forty hours after fertilization, produces four equal blastomeres. By three days, the embryo consists of six to twelve cells, and by four days, it consists of sixteen to thirty-two cells. The embryo at this stage is called a **morula.**

From Blastomere to Morula



Morula Formation

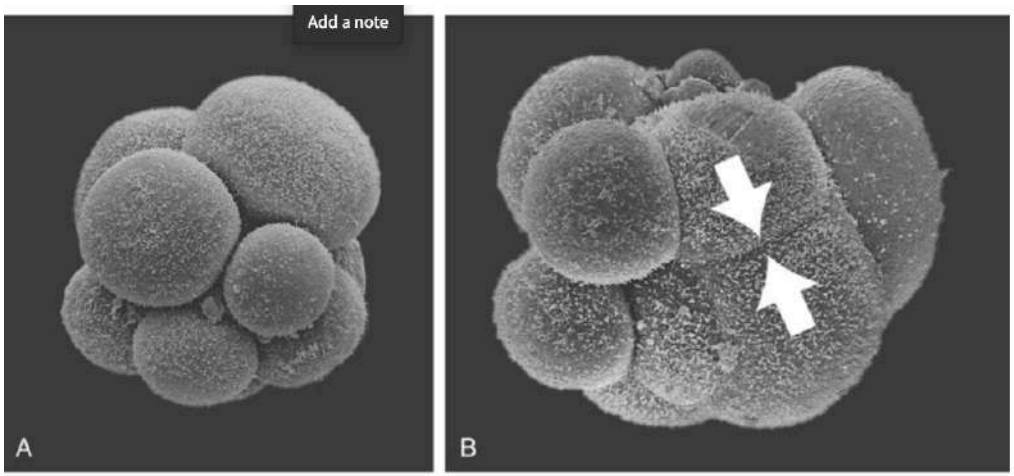
After the eight-cell stage, the blastomeres change their shape and tightly align themselves against each other—**compaction**.
This phenomenon may be mediated by cell surface adhesion glycoproteins.
Compaction permits greater cell-to-cell interaction and is a prerequisite for segregation of the internal cells that form the inner cell mass.



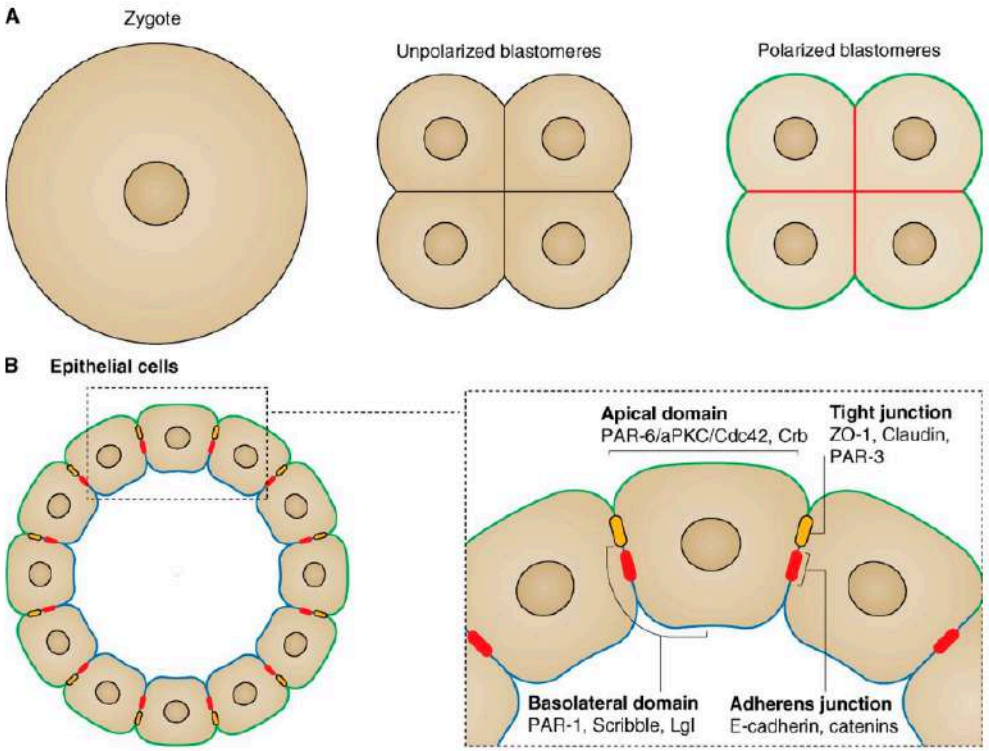
Compaction

The cells of the morula will give rise not only to the embryo proper and its associated extraembryonic membranes but also to part of the placenta and related structures.

Starting at the eight-cell stage of development, the originally round and loosely adherent blastomeres begin to flatten, developing an inside-outside polarity that **maximizes cell-to-cell contact among adjacent blastomeres**. As differential adhesion develops, the outer surfaces of the cells become convex and their inner surfaces become concave. This reorganization, called **compaction**, also involves changes in the blastomere cytoskeleton.



Blastomeres and epithelial cells polarity

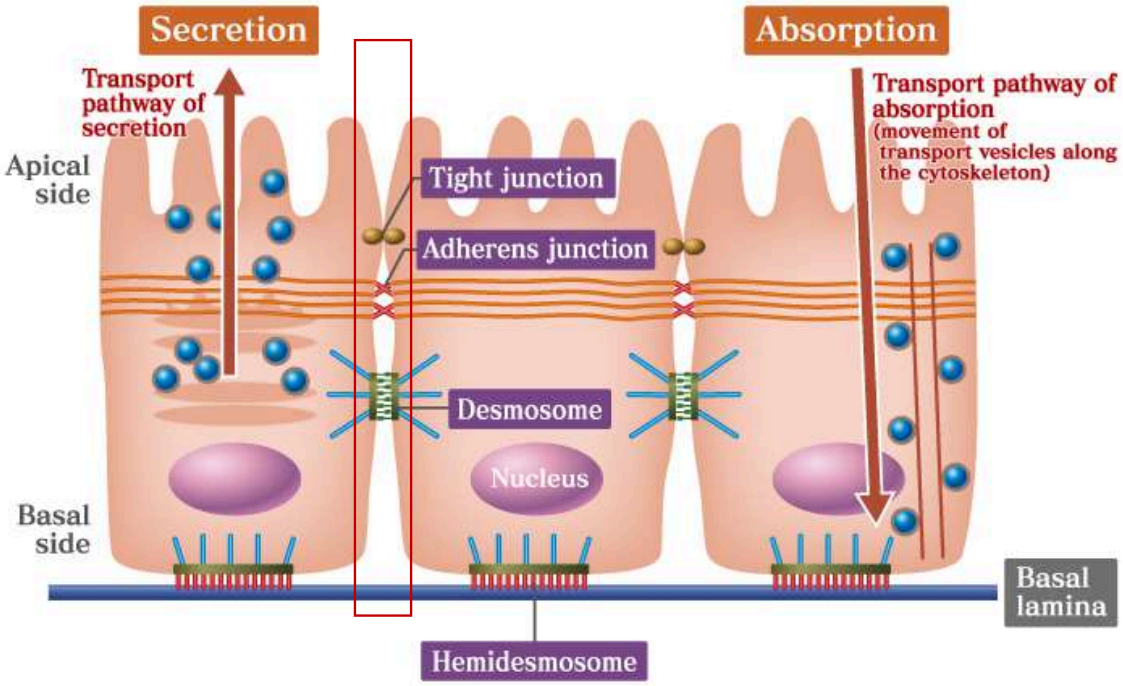


Getting to know your neighbor: cell polarization in early embryos

The zygote undergoes cleavage to first produce unpolarized blastomeres. Blastomeres subsequently develop radial polarity by differentiating their contacted (red) and contact-free (green) surfaces.

How cells define polarity

Terminal bars



Apical domain facing the lumen containing Aquaporins channels, responsible for the water balance

Basolateral domain in contact with the basal lamina

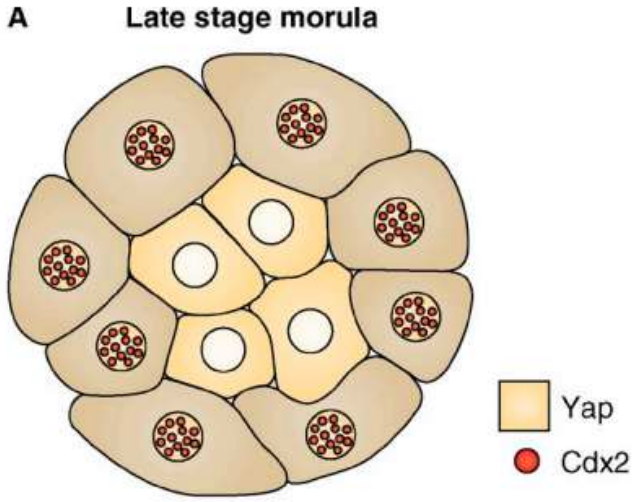
Embryoblast Formation

The inner cells of the morula—the **embryoblast** or **inner cell mass**—are surrounded by a layer of flattened blastomeres that form the trophoblast. *Hippo signaling is an essential factor in segregating the inner cell mass from the* ***trophoblast***.

Embryoblast Formation

The Hippo pathway is a signaling cascade that can respond to cellular interactions to regulate the nuclear localization of transcriptional coactivator Yap.

When Hippo signaling occurs, active **Yap is phosphorylated** and localizes to the **cytoplasm;** otherwise Yap is localized to the nucleus and functions as a co-TF



How does the Hippo signaling pathway interpret cell position?

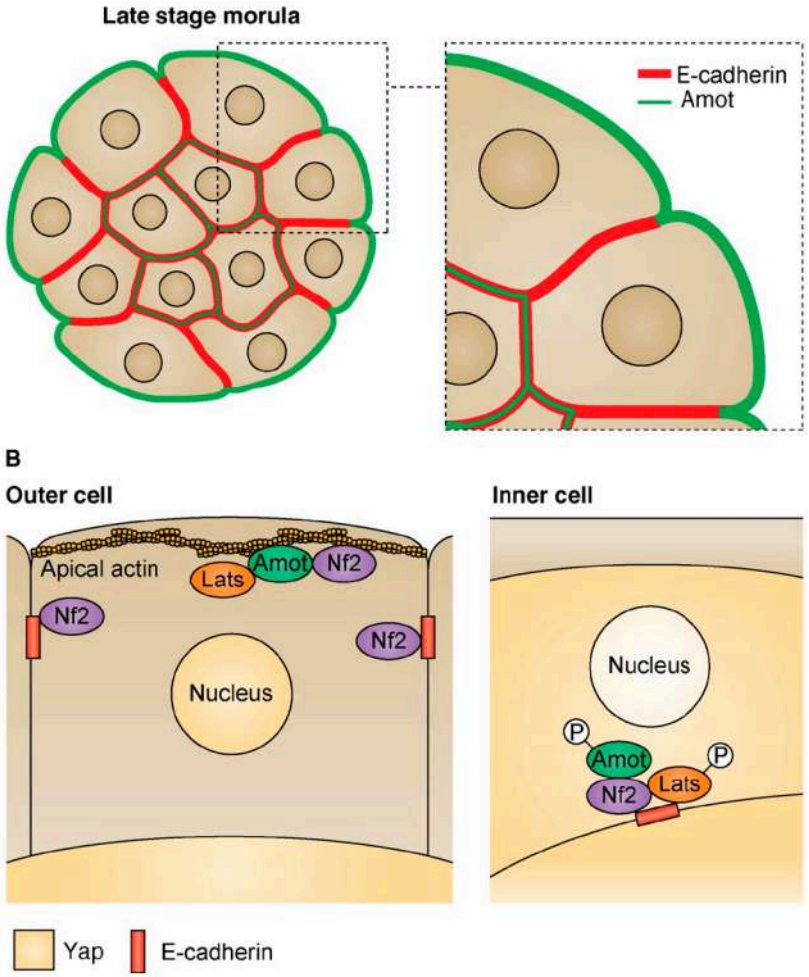
Several upstream components of the pathway have polarized distributions in outer cells.

Lats1/2 kinases, which phosphorylate Yap to prevent its nuclear accumulation, are found at contact-free surfaces within outer cells but are mostly cytoplasmic within inner cells.

In outer cells, **Amot** is found at contact-free surfaces, whereas in inner cells, it is phosphorylated and enriched at all cell surfaces, eventually becoming expressed at high levels specifically in inner cells

E-cadherin is required to exclude Yap from the nucleus of some inner cells

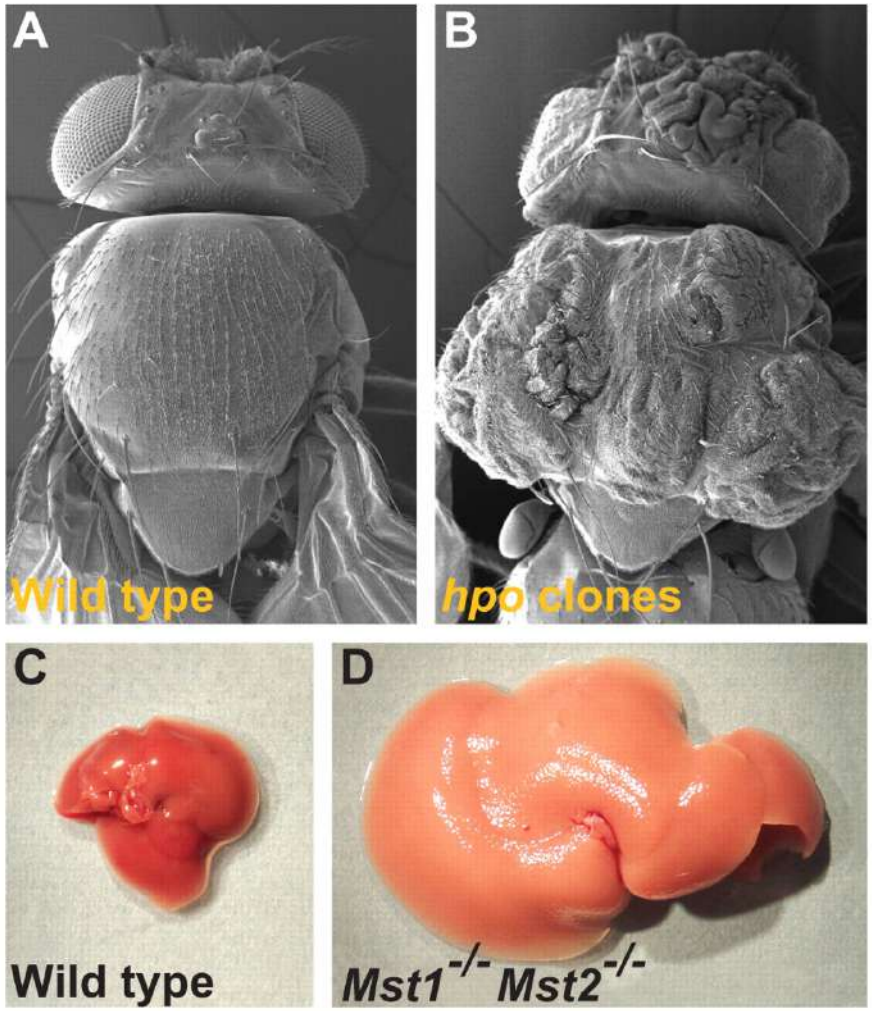
Importantly, Amot only colocalizes with E-cadherin within inner cells, where its function is required, because Amot is excluded from basolateral surfaces in outer cells



Hippo signaling pathway

Precise control of organ size is crucial during animal development and regeneration. Dysregulation of this pathway leads to massive overgrowth of tissue.

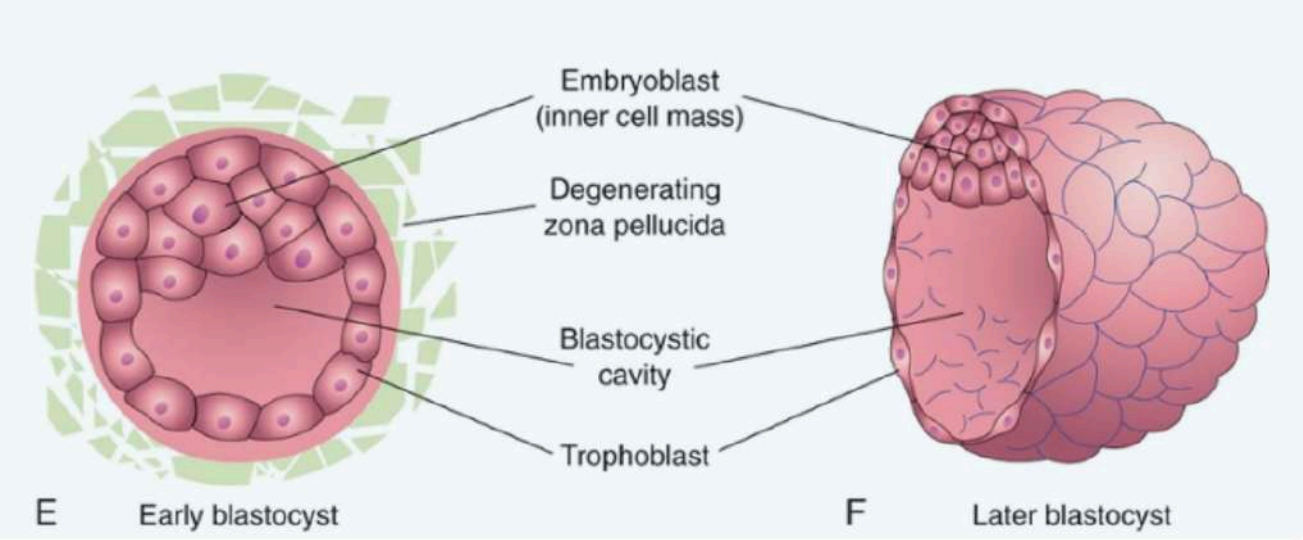
For example, when two-thirds of a mouse liver is surgically removed, the remaining one-third regenerates its original mass within 7–10 days and then ceases growth.



Embryoblast Formation

The inner cells of the morula—the **embryoblast** or **inner cell mass**—are surrounded by a layer of flattened blastomeres that form the **trophoblast**. *Hippo signaling is an essential factor in segregating the **inner cell** mass from the **trophoblast***.

An immunosuppressant protein—the **early pregnancy factor**—is secreted by the trophoblastic cells and appears in the maternal serum within 24 to 48 hours after implantation. The early pregnancy factor forms the basis for a pregnancy test applicable during the first 10 days of development



hCG

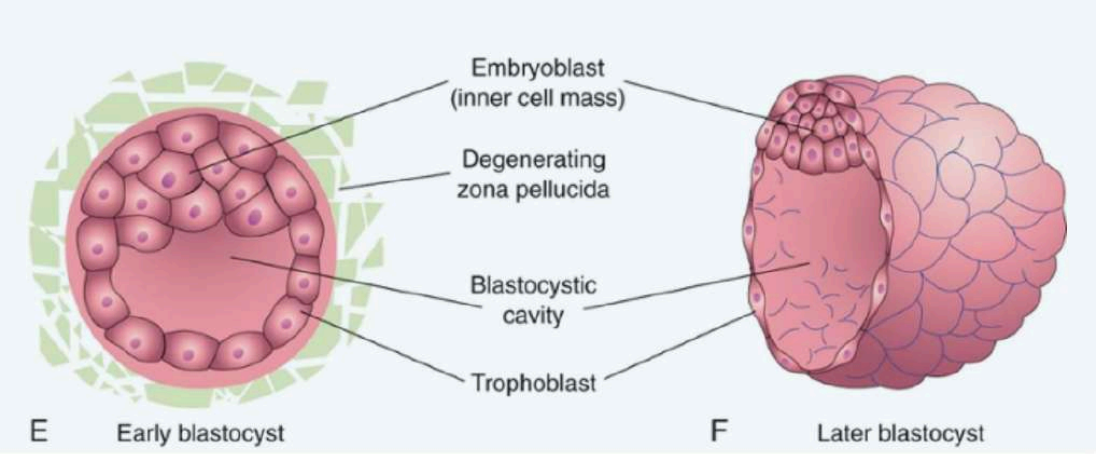
https://studentconsult.inkling.com/read/moore-before-we-are-born-9/chapter-3/cleavage-of-zygote

Formation of Blastocyst

Shortly after the morula enters the uterus (about 4 days after fertilization), uterine fluid passes through the zona pellucida to form a fluid-filled space—the **blastocystic cavity**—inside the morula.
As fluid increases in the cavity, the blastomeres are separated into two parts:

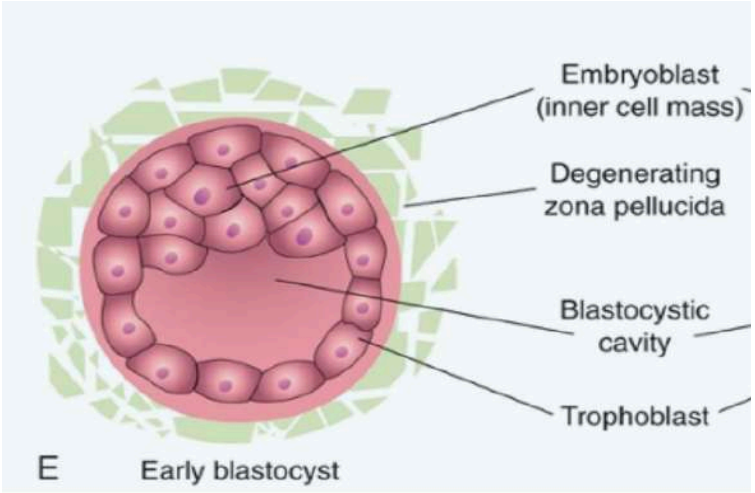
The **trophoblast**, the thin outer cells that give rise to the embryonic part of the placenta

The **embryoblast**, a discrete group of blastomeres that is the primordium of the embryo



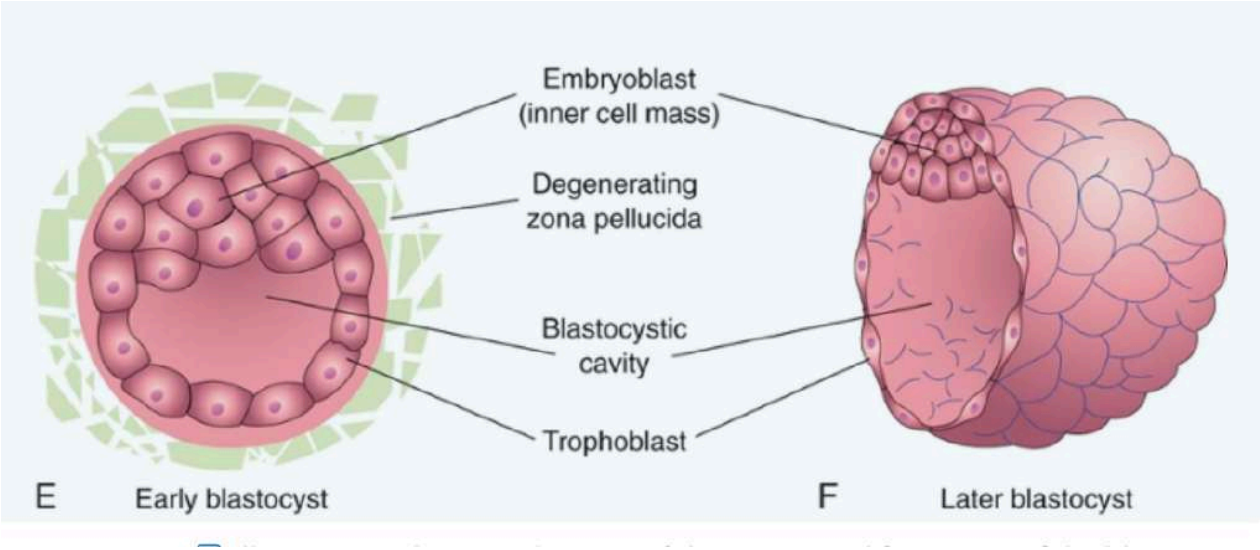
Embryoblast Formation

- The centrally placed blastomeres are now called the **inner cell mass**, whereas the blastomeres at the periphery constitute the **trophoblast**.
- Because the inner cell mass gives rise to the embryo proper, it is also called the **embryoblast**.
- The **trophoblast** is the primary source of the fetal component of the placenta



Formation of Blastocyst

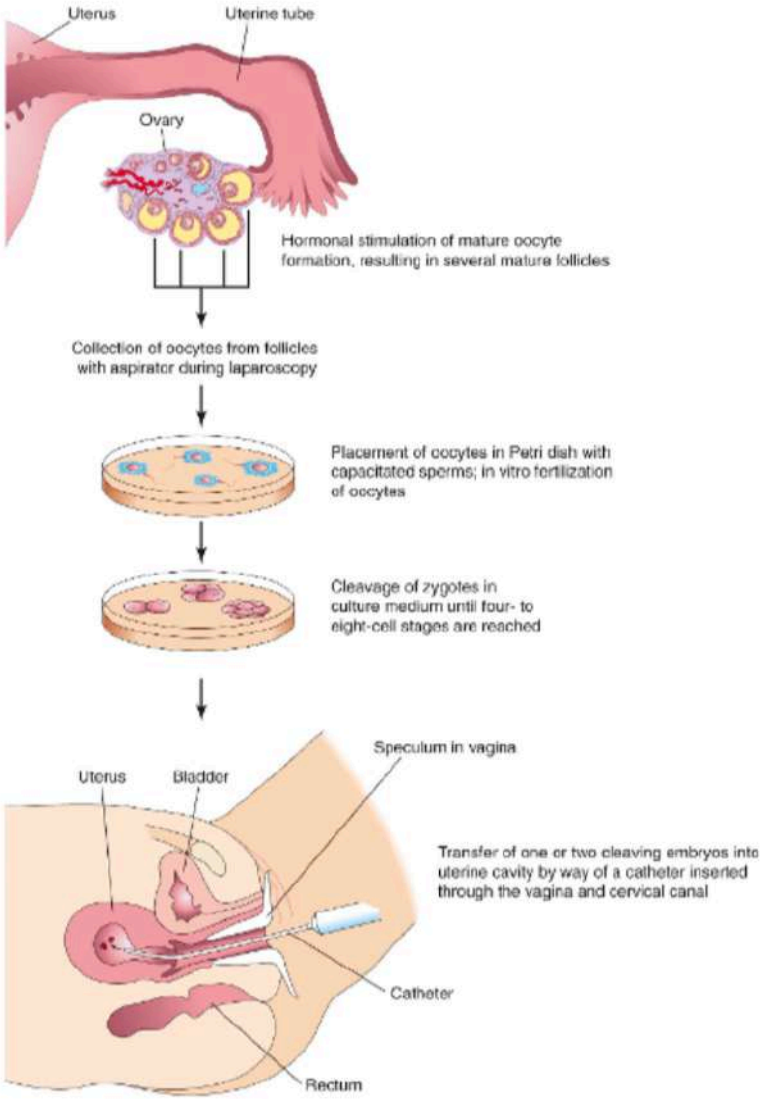
During this stage of development—*blastogenesis*—the conceptus is called a **blastocyst**. The embryoblast now projects into the **blastocystic cavity** and the trophoblast forms the wall of the blastocyst. After the blastocyst has floated in the uterine fluid for approximately 2 days, **the zona pellucida degenerates and disappears**. Shedding of the zona pellucida has been observed in vitro. The shedding permits the blastocyst to increase rapidly in size. While floating freely in the uterine cavity, the blastocyst derives nourishment from secretions of the uterine glands.

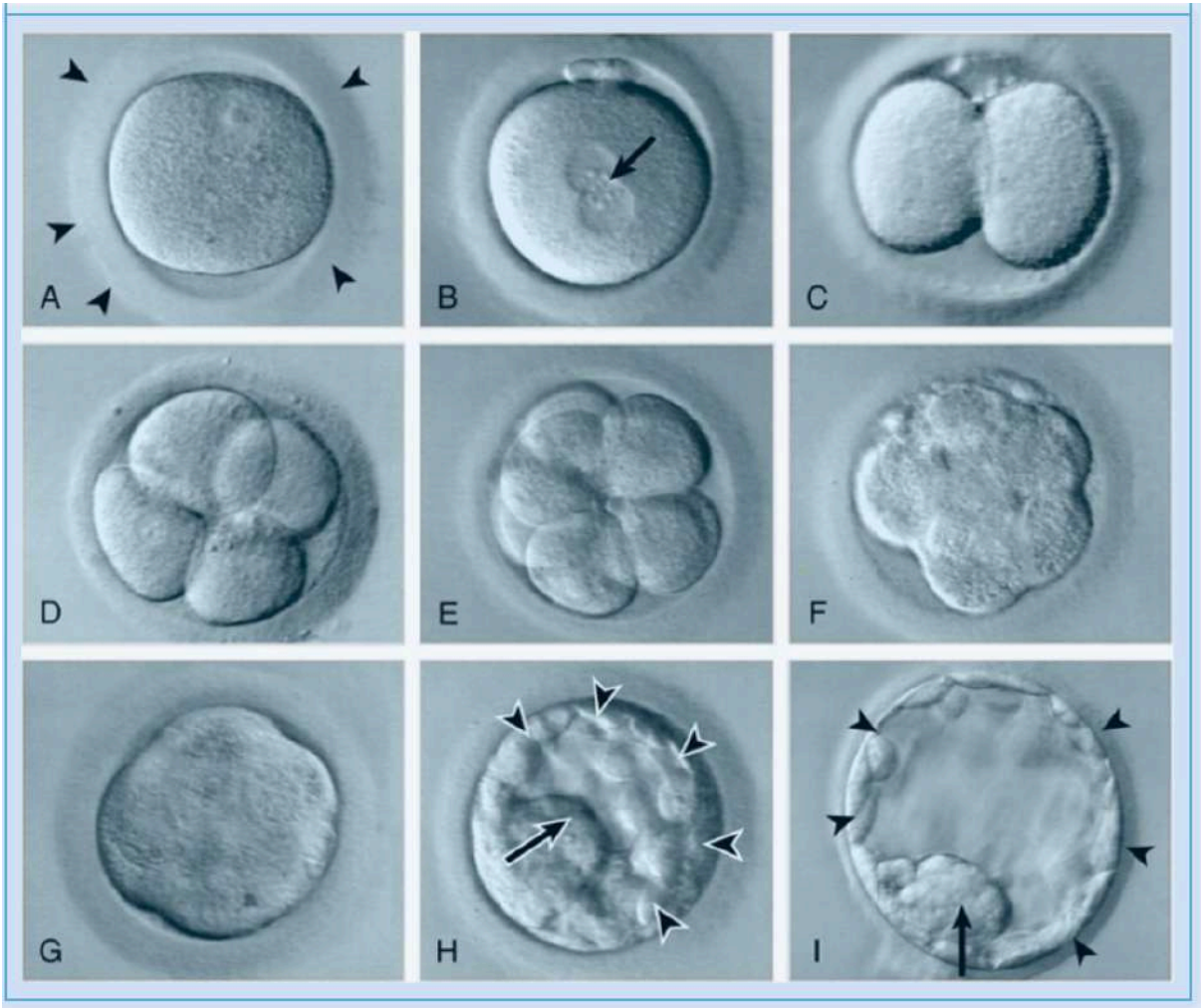


The side of the blastocyst containing the inner cell mass is called the **embryonic pole**

https://studentconsult.inkling.com/read/larsen-human-embryology-schoenwolf-5/videos/animation-1-3

IVF strategies





https://www.youtube.com/watch?v=_krJsK5Dxj4

https://www.youtube.com/watch?v=V6-v4eF9dyA

https://www.youtube.com/watch?v=uCn1PQP2yAo

IVF

- The procedure of **in vitro fertilization (IVF)** and **embryo transfer** is widely used in cases in which scarring of the oviducts (a common consequence of pelvic inflammatory disease, PID, a serious complication of sexually transmitted diseases such as gonorrhea) prevents either the sperm from reaching the ampulla of the oviduct or the fertilized oocyte from passing to the uterus.

- FSH sometimes combined with clomiphene citrate—a drug that blocks the ability of hypothalamic cells to detect estrogen in the blood. In the presence of clomiphene citrate, hypothalamic cells respond to the perceived deficiency of estrogen by signaling the pituitary to release high levels of FSH, which stimulates the growth of follicles and their secretion of estrogen. Once estrogen levels rise sufficiently, the pituitary gland rapidly releases LH, triggering maturation of oocytes. Sometimes to ensure that maturation of oocytes occurs, hCG is also given when follicles have attained optimal growth (determined by ultrasound examination of the ovaries and plasma estradiol concentration measurements).