ANA 301H1
EMBRYOLOGY LECTURE #2

THE FIRST TWO WEEKS

Objectives:

- Describe the interaction between sperm and ovum at fertilization including acrosome reaction, cortical reaction, and zona reaction.
- Describe the role of male and female pronuclei and the significance of the zygote.
- Describe cleavage, compaction, and blastocyst formation
- Describe the developmental potential of the blastomere, morula, inner cell mass, and trophoblast
- Understand the timing of tubal transit and onset of implantation.
- Understand the role of the zona pellucida during transit of the embryo in the uterine tube
- Understand the significance of ectopic pregnancy and tubal pregnancy in particular.
- Describe the formation of the syncytiotrophobast, cytotrophoblast, bilaminar embryonic disc, amniotic membrane and cavity and yolk sac membrane and yolk sac.

FERTILIZATION

SPERM - ZONA BINDING

- [redacted]binding sites on the sperm cell membrane bind with species specific sperm receptor molecules (the ZP3 glycoprotein) on the zona pellucida.

- Sperm - zona binding triggers acrosome reaction.

ACROSOME REACTION

- During acrosome reaction, the outer part of the acrosome membrane fuses with the sperm cell membrane and disappears. As a result, the enzymes that are contained within the acrosome are released.

- Acrosomal enzymes especially hyaluronidase and acrosin facilitate zona penetration by the sperm cell.

SPERM - EGG BINDING

- After penetration of the zona, a ligand on the sperm membrane binds with its receptor on the membrane of *the secondary oocyte*. This interaction is not as species - specific a phenomenon as that between the zona and the sperm cell.

- fertilin in sperm cell membrane binds to integrin on egg cell membrane; fertilin allows sperm membrane to break down at site of attachment to oocyte
4. fertilin is in the post-acrosomal part of the sperm cell, so sperm cell must "parallel park"

- The membrane of the sperm cell initially becomes incorporated into the membrane of the secondary oocyte as the head of the sperm and the sperm cell organelles are taken up into the oocyte. Eventually, the sperm cell membrane and organelles will be broken down. only genetic material survives

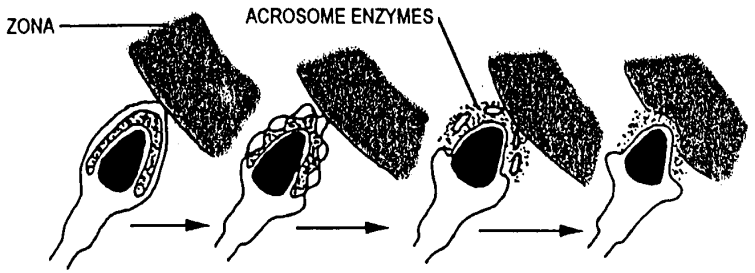
THE BLOCK TO POLYSPERMY

- The fusion of a single sperm with the oocyte is a critical event in development that results in an equal genetic contribution from each parent and the restoration of the chromosome number to the diploid value.

- Contact between the sperm cell membrane and the membrane of the secondary oocyte leads to the release of the contents (exocytosis) of the cortical granules.

- The contents of the cortical granules are thought to modify the zona so that no further sperm cells can penetrate it and no more sperm cells can bind with it. This is called zona reaction.

cortical granules also harden the zona so that other sperm that have penetrated the zona can't get any deeper
- zona reaction is to block polyspermy



1. Sperm - zona binding, a species - specific interaction between the sperm receptor on the zona pellucida and zona binding sites on the sperm cell membrane

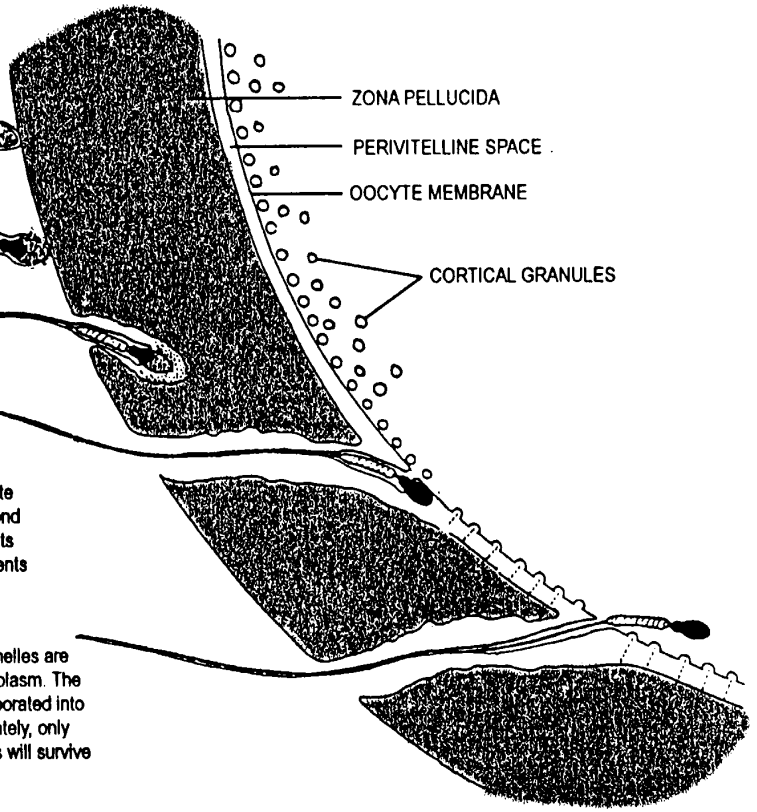
2. Sperm - zona binding triggers acrosome reaction

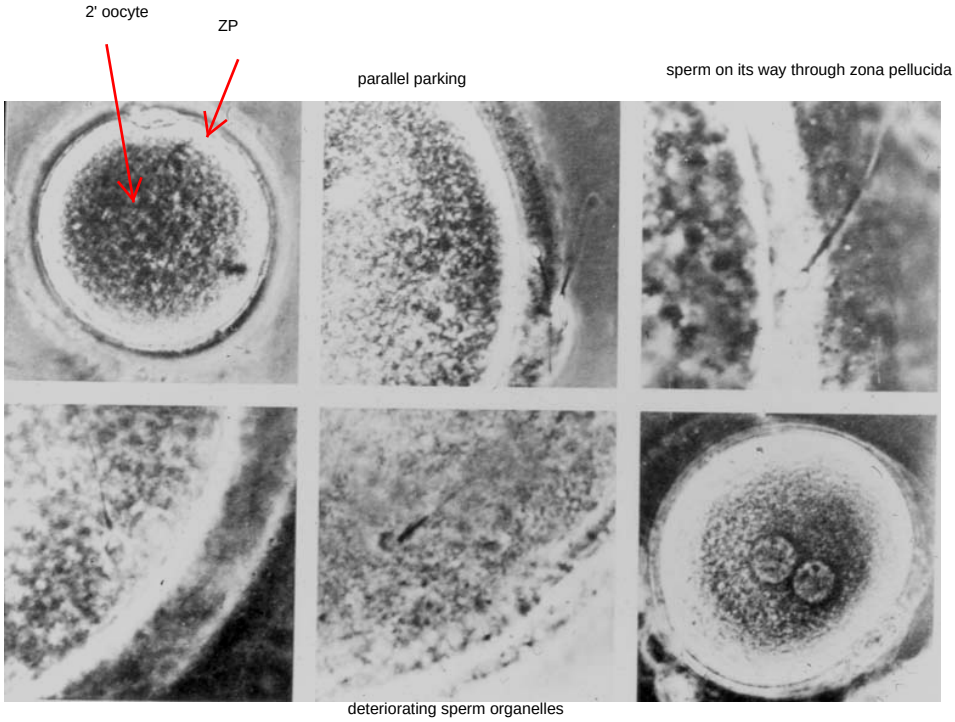
3. Acrosomal enzymes facilitate penetration of the zona pellucida

4. Sperm head crosses the perivitelline space and binds with sperm receptors on the oocyte membrane

5. Sperm - egg binding stimulates the secondary oocyte to complete meiosis II with the production of a second polar body. It also triggers the release of the contents of the cortical granules (cortical reaction). The contents contribute to the zona reaction.

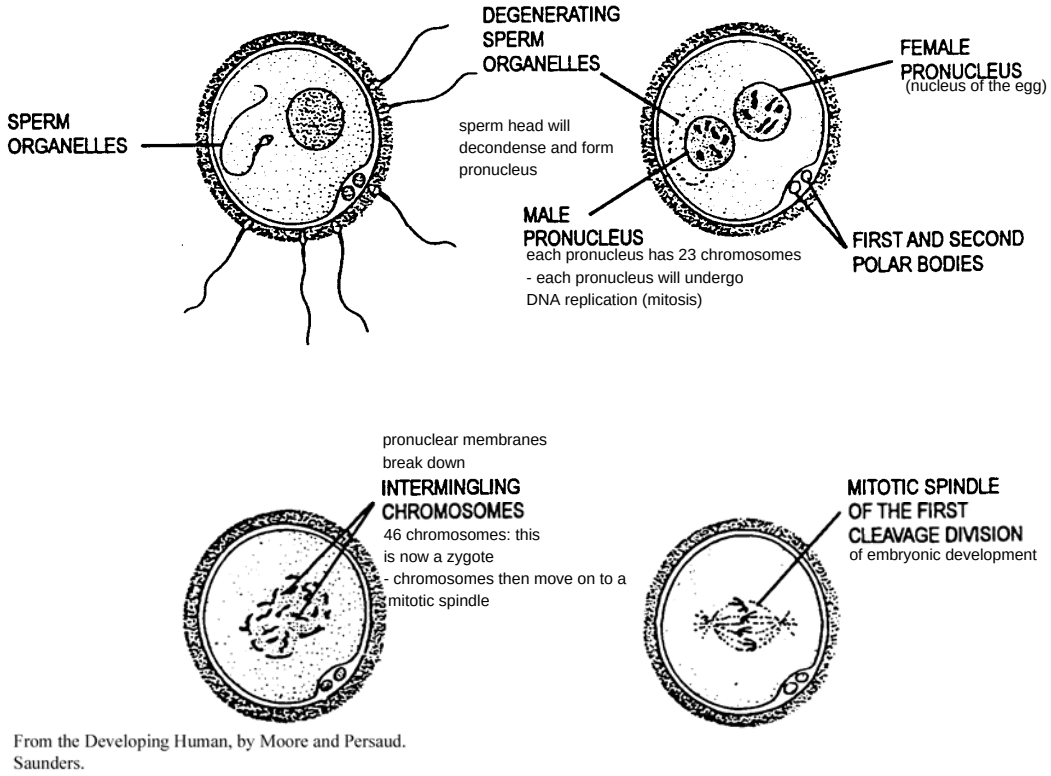
6. The sperm head and organelles are taken up into the egg cytoplasm. The sperm membrane in incorporated into the egg membrane. Ultimately, only the paternal chromosomes will survive



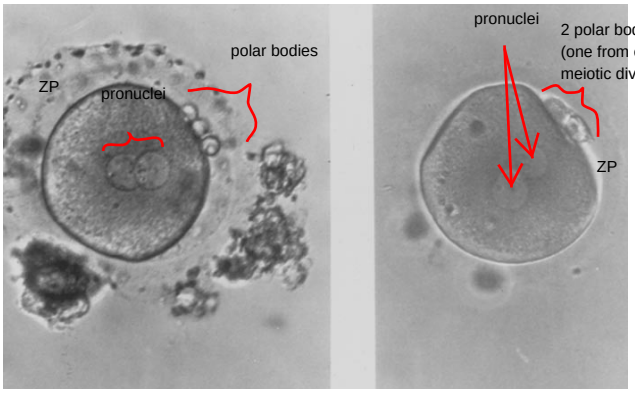


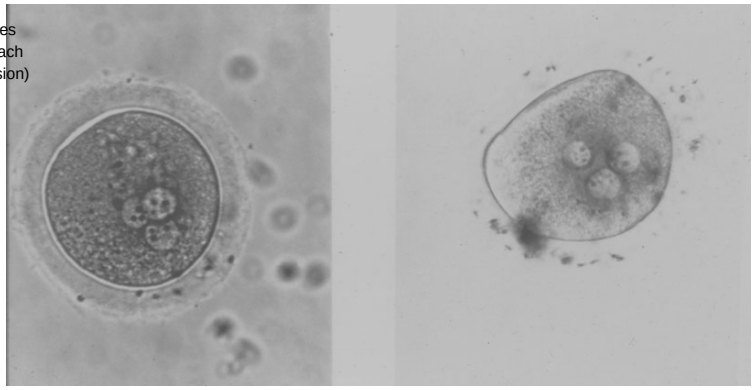
FORMATION OF THE ZYGOTE

- During the first 12 hours following entry into the secondary oocyte, the nucleus of the sperm cell decondenses and forms the male pronucleus.
- The secondary oocyte completes the second meiotic division and casts off the second polar body. The nucleus of the oocyte is now called the female pronucleus.
- DNA replication occurs in the chromosomes of the male and female pronuclei. This step is akin to the replication of DNA that precedes any mitotic division
- The male and female pronuclei approach each other.
- The pronuclear membranes break down and the 23 chromosomes from the sperm nucleus join the 23 chromosomes from the oocyte nucleus on a mitotic spindle for the first cleavage division of the zygote. *The pairing of the 23 paternally derived chromosomes with the 23 maternally derived chromosomes restores the diploid number of 46.*



From the Developing Human, by Moore and Persaud. Saunders.



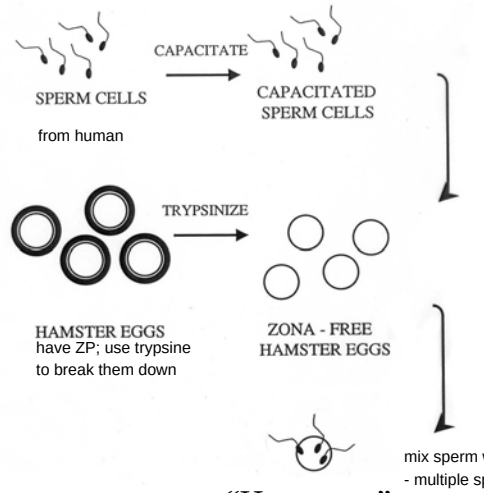


3 pronuclei; 1 from egg; 2 sperm cells have fertilized this egg; this egg is not viable (polyspermy)

ZONA PENETRATION ASSAY

(Zona Free Hamster Egg Test)

FERTILIZATION TEST: why can't a couple get pregnant?

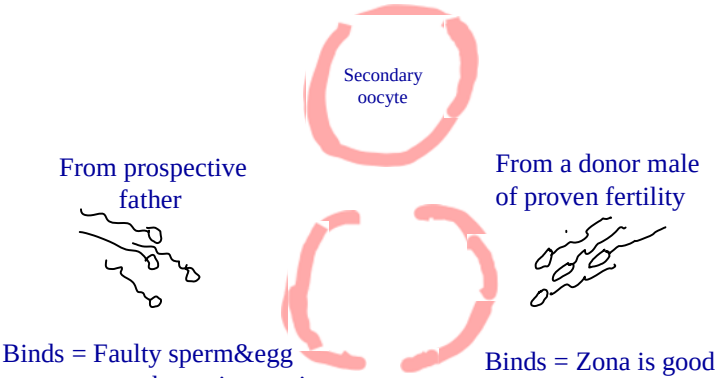


"Humpsters"

mix sperm w/ hamster eggs
- multiple sperm cells will fertilize b/c ZP is gone
- ovum will divide 1x or 2x then die
- successful fertilization tells you problem is likely w/ sperm-ZP interaction

HEMIZONA ASSAY

- take egg and cut ZP in half
- capacitate sperm cells from prospective father and capacitate sperm cells from a proven male



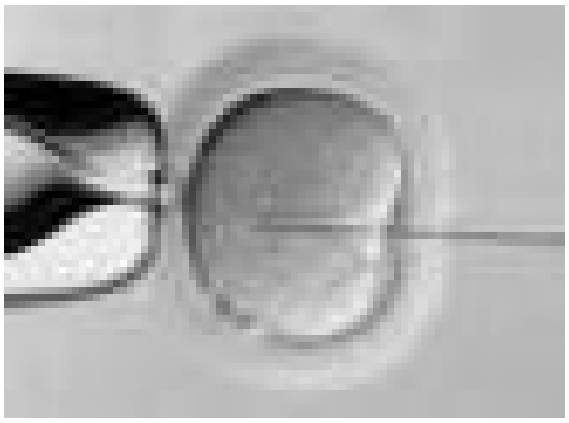
Binds = Faulty sperm&egg membrane interaction

Doesn't = Faulty sperm&zona interaction

Binds = Zona is good

Doesn't = Faulty zona

Intra-cytoplasmic Sperm Injection (ICSI)

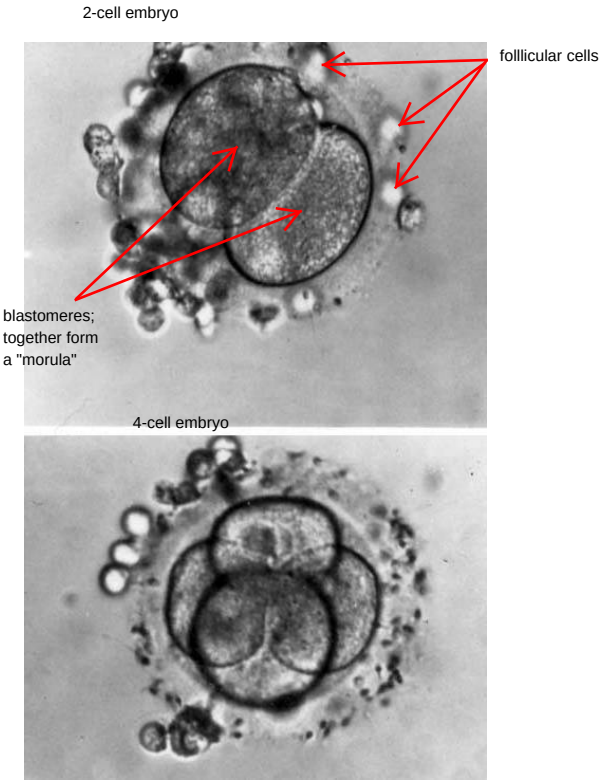


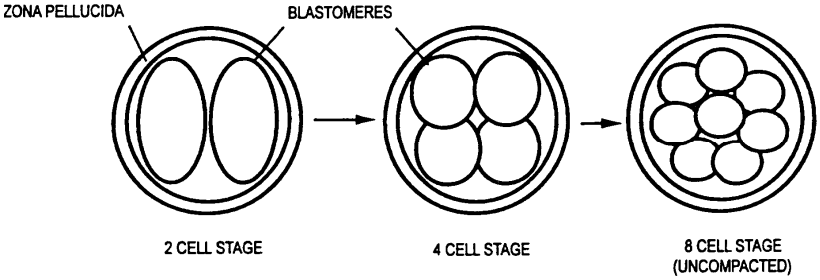
THE FIRST TO WEEKS OF DEVELOPMENT

The first week of development is taken up with the transport of the embryo from the site of fertilization in the ampulla of the uterine tube to the site of implantation in the uterus and with the development of an embryonic form called a blastula. During the second week, the embryo implants in the lining of the uterus and the amniotic cavity and primitive yolk sac are formed.

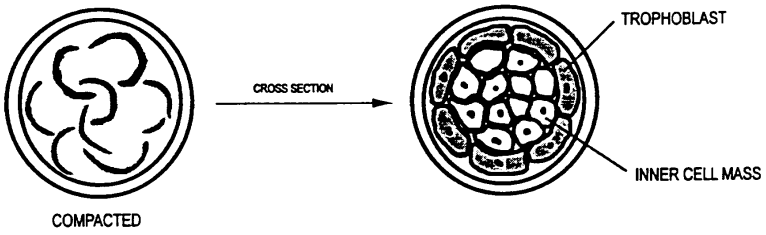
CLEAVAGE

- A series of comparatively rapid mitotic divisions resulting in progressively larger numbers of progressively smaller cells.
- The first cleavage division occurs approximately 24 hours following the production of the second polar body.
- Subsequent divisions are asynchronous and follow at intervals of 12 to 24 hours.
- The cells produced by cleavage are called blastomeres. The spherical cluster of blastomeres produced by these early divisions is called a morula.
- The blastomeres produced by the first few cleavage divisions are totipotential. Each has the potential to develop into a complete organism. As development proceeds the potentiality of the blastomeres becomes progressively restricted.

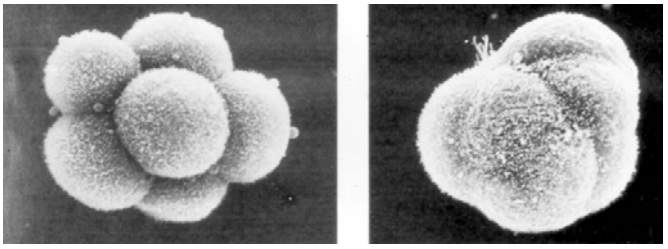




at 8-cell stage, it's safe to sample a blastomere and analyze its DNA (via PCR) to see if it's a mutant (if so, can choose not to implant)



Compaction



- At about the 8 cell stage, the outer cells flatten out forming the trophoblast. The trophoblast is responsible for implantation. and forming the placenta
- The inner cells form the inner cell mass. The inner cell mass forms the embryo and its membranes.

- after compaction, can no longer sample blastomeres safely b/c of complexity of jxns b/w cells (damage if you try to sample)

COMPACTION

- Occurs at the 8 cell stage - ie) between the third and fourth cleavage divisions.

- Prior to compaction, the blastomeres are loosely associated. During compaction the blastomeres form a tightly packed ball of cells called a morula and the outermost cells maximize their contact with each other.

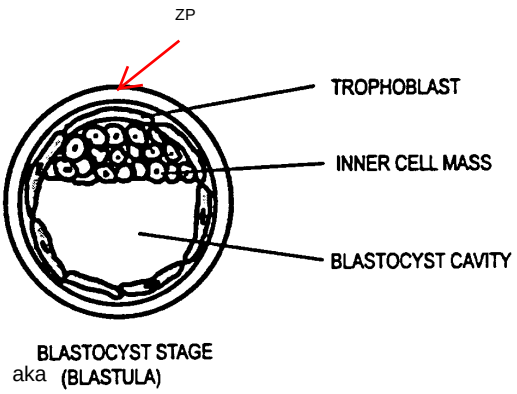
- The outer cells seal the interior of the morula. They form the trophoblast. The trophoblast makes no contribution to the embryo. Rather, it is responsible for the phenomenon of implantation and for the formation of the placenta.

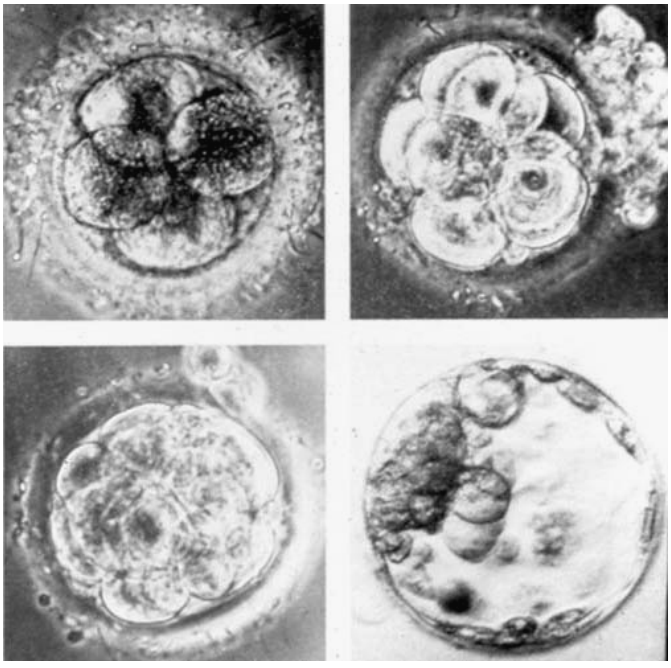
- The inner cells are referred to as the inner cell mass and will form the embryo, the amniotic membrane and the lining of the primitive yolk sac.

FORMATION OF THE BLASTOCYST

- Shortly after arriving in the uterus at approximately the 32 cell stage (4 days after fertilization), a fluid filled cavity (the blastocyst cavity) appears within the morula. As a result the cells of the inner cell mass are displaced to one pole

- It is still contained within the zona pellucida.

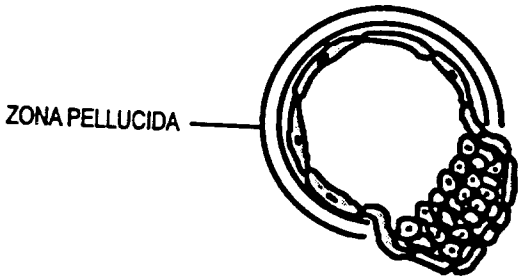


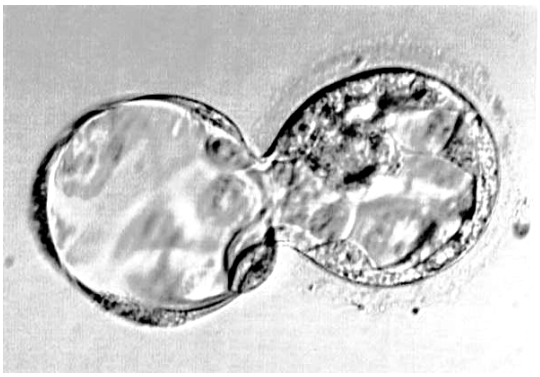


IMPLANTATION

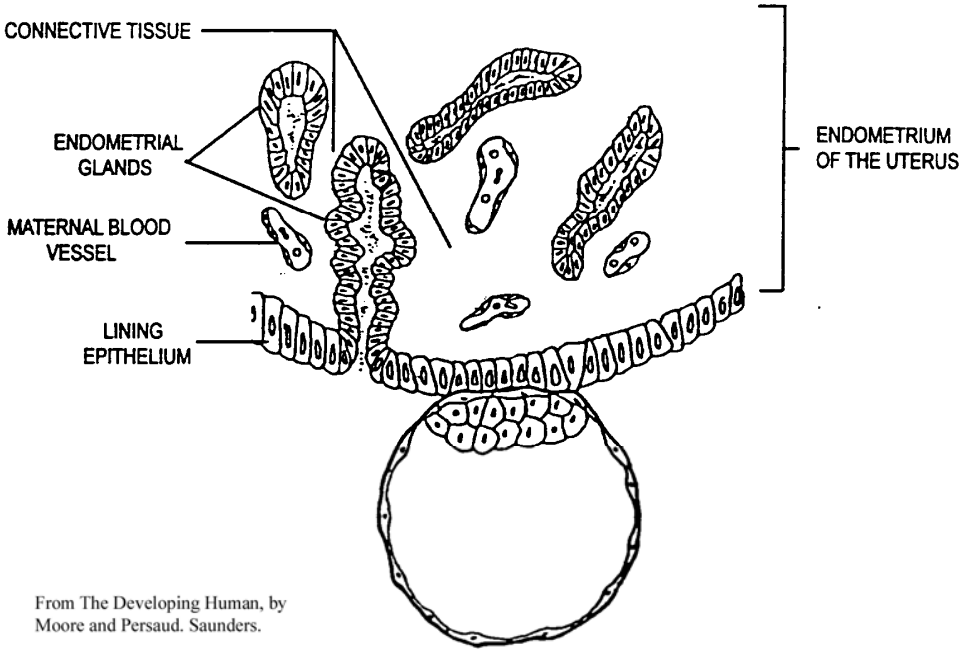
- At 5 or 6 days after fertilization, the trophoblast produces enzymes which begin to disrupt the zona pellucida.

- The embryo breaks out of the zona pellucida ('hatches').
  can then make contact w/ tissues of mother



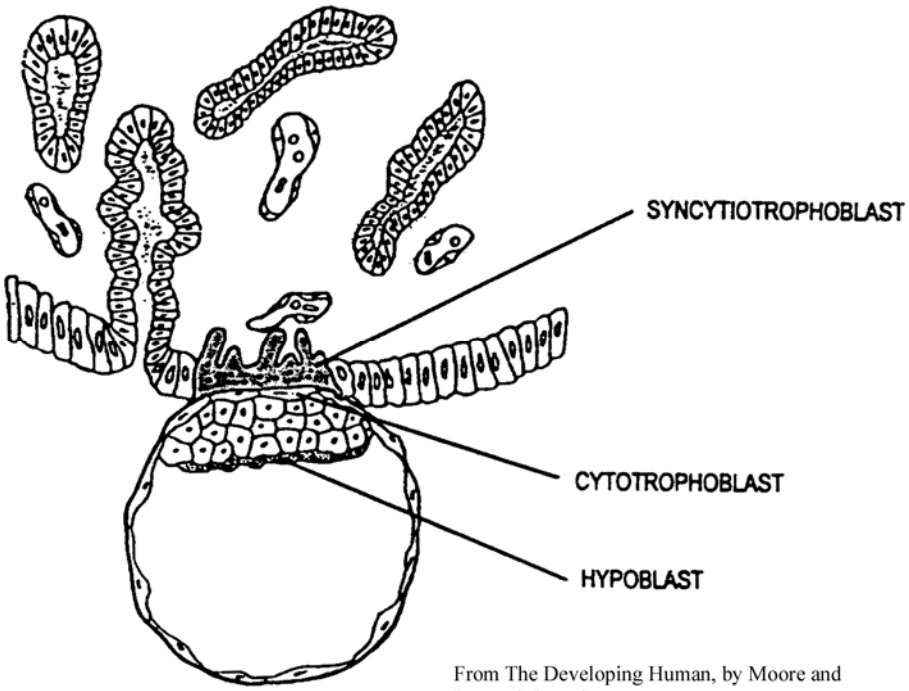


- Contact occurs between the epithelial cells of the uterine lining and the trophoblast. The uterine lining is called the endometrium and includes the layers of epithelium and connective tissue that receive the embryo during implantation, and that are shed during parturition and menstruation.



From The Developing Human, by Moore and Persaud. Saunders.

- At the site of contact between the trophoblast and the endometrium, the syncytiotrophoblast begins to form. The cells in the trophoblast proliferate, but the daughter cells fuse together to form a multinucleated mass that is the syncytiotrophoblast. What was formerly called the trophoblast is then called the cytotrophoblast to distinguish it from the syncytiotrophoblast.



From The Developing Human, by Moore and Persaud. Saunders.

- The syncytiotrophoblast is highly invasive and draws the embryo into the endometrium. It is also the source of human chorionic gonadotropin (hCG). hCG is an essential hormone for pregnancy and its detection in the maternal plasma or urine is the basis of the common pregnancy test.

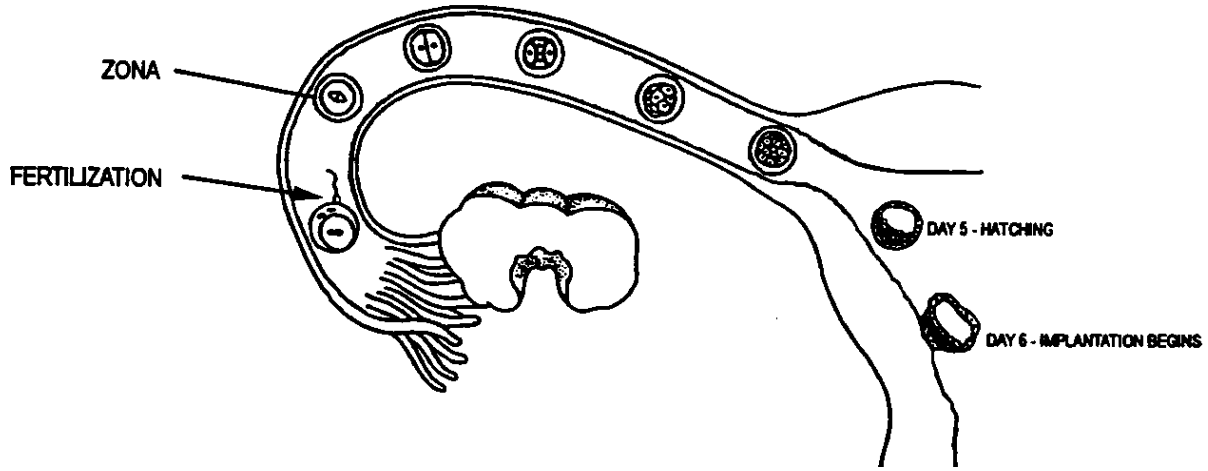
TUBAL TRANSIT

- While cleavage and blastocyst formation take place, the embryo is migrating from the site of fertilization in the ampulla of the uterine tube to the uterus.

- Both the beat of the cilia on the epithelial cells that line the uterine tube, and contractions of the smooth muscle in the muscular layer of the uterine tube wall are thought to contribute to the movement of the embryo from the ampulla to the uterus.

- The trophoblast is opportunistic and will begin the process of implantation in any convenient place (ectopic pregnancy) after the blastula has hatched free of the zona pellucida.

- The zona pellucida normally remains intact during this time, keeping the embryo from implanting in the uterine tube by preventing contact between the trophoblast and the epithelium lining the tube.

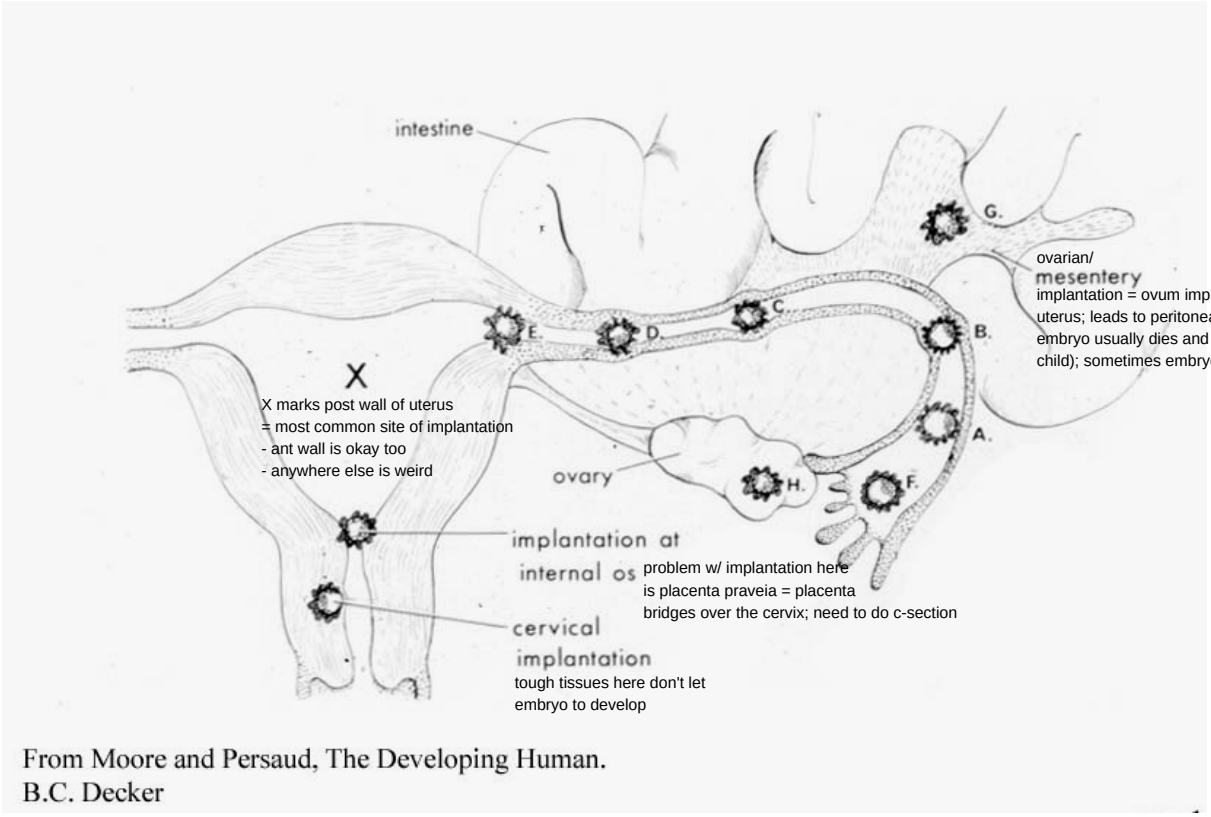


TUBAL PREGNANCY

- Ectopic pregnancy occurs when the embryo implants in a place other than the body of the uterus. Approximately 1 in every 100 pregnancies is ectopic and more than 90 % of these are tubal.

- The uterine tube is unable to enlarge sufficiently to accommodate the rapidly growing embryo and will rupture.

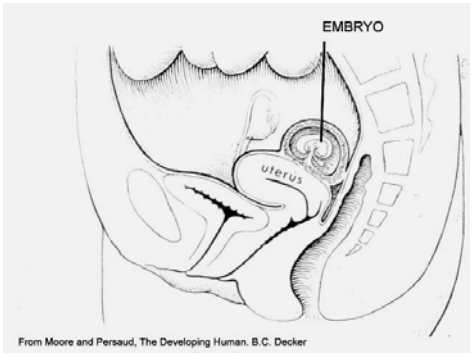
- Abnormalities of the uterine tube (eg. scars which develop as a result of a previous infection in the tube) may lead to delayed passage with the result that the zona pellucida comes off while the embryo is still in the tube. Ectopic pregnancy often (but not always) is associated with a previous history of tubal inflammation.



From Moore and Persaud, The Developing Human.
B.C. Decker

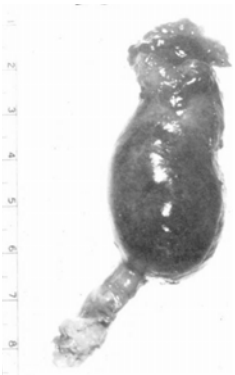
Placenta Praevia

Peritoneal Pregnancy



- demonstrates that uterus is not special place for implantation
- trophoblast doesn't care where it gets its blood supply to develop the embryo (opportunistic; anywhere it can attach and get blood)

Tubal Pregnancy



- More than 90% of ectopics are tubal.
- Risk of rupture and life-threatening hemorrhage.
- Treatment options: medical and surgical
- Salpingostomy vs. salpingectomy

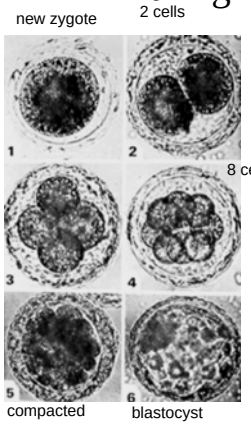
since uterine tube has rich blood supply

- medical option if danger is not immediate; problem is patient may not follow up on treatment

salpingostomy = try to preserve tube (open tube and hope for no scarring)
- problem: might miss embryonic material behind

salpingectomy = take whole tube out (no liability issues)

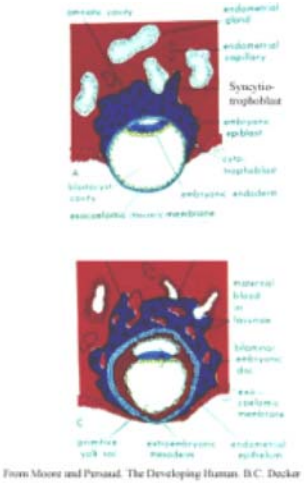
During Tubal Transit



- Increasing cell numbers
- Increasing complexity
- No net growth in size
- Retained within the zona pellucida

if embryo's travel time gets delayed, stays in tube and implants there
- e.g. rougher road/tube (scarring) would delay travel time
- any kind of tube anatomy abnormality really
- abnormalities can be congenital (bends/twists) or acquired (from surgery; pelvic inflammatory disease = infection -> inflammation -> scarring -> distorts normal anatomy; or endometriosis -> inflammation -> scarring)

The embryo continues to develop as implantation proceeds



- Amniotic cavity and amniotic membrane
- Yolk sac and yolk sac membrane
- Bilaminar Embryonic disc … the epiblast and the hypoblast

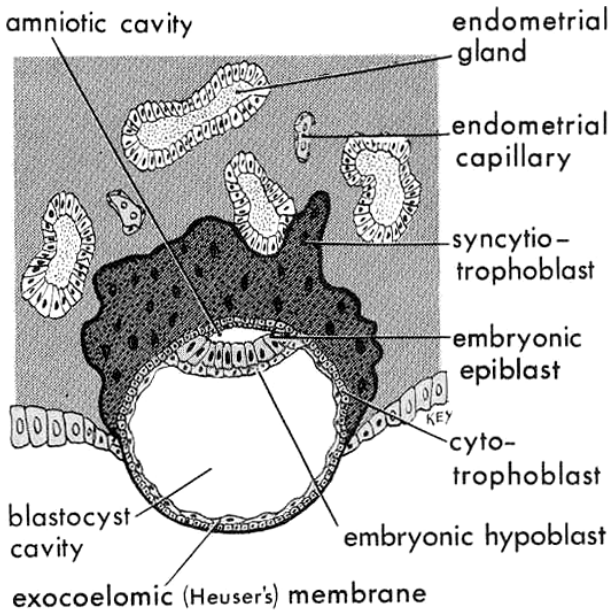
FORMATION OF THE BILAMINAR EMBRYO

embryo on its way into endometrium

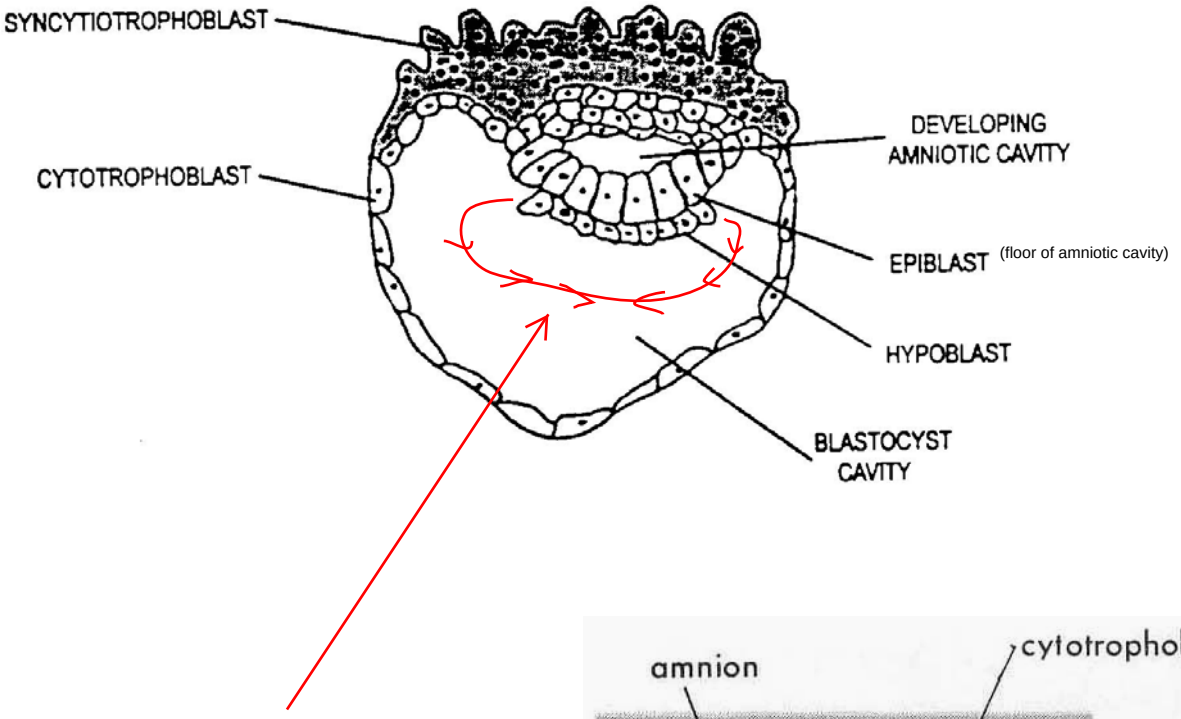
- Simultaneously with the process of implantation, the inner cell mass is converted into a two layered structure called the bilaminar embryonic disc.

- The cells of the inner cell mass which face the blastocyst cavity form a single layer of primitive cells collectively called the hypoblast.

- Cavities appear in the remainder of the inner cell mass, which coalesce to form the amniotic cavity. The floor of the amniotic cavity is formed of a single layer of cells that are collectively referred to as the epiblast. The cells of the amniotic membrane are also derived from the inner cell mass.

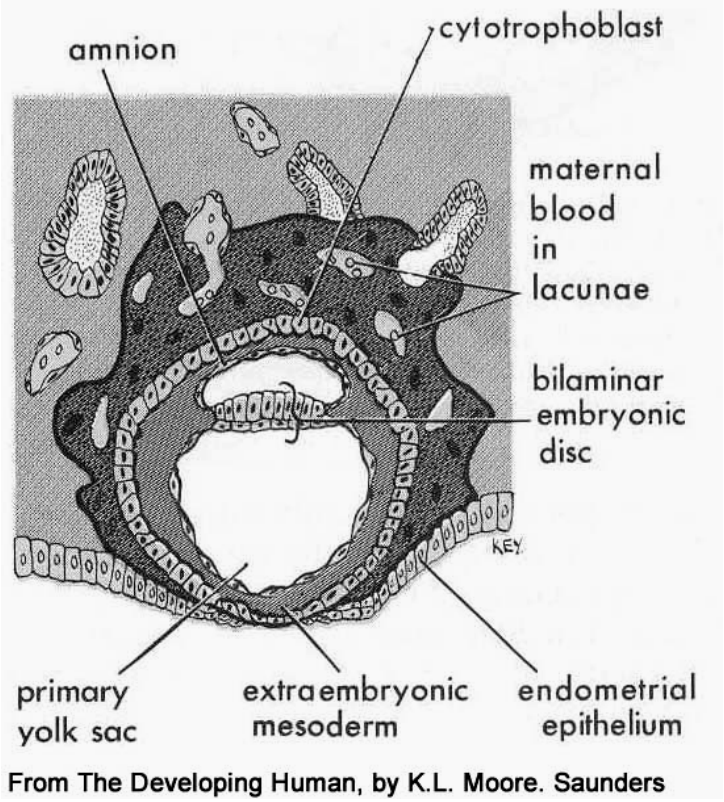


From The Developing Human, by K.L Moore. Saunders

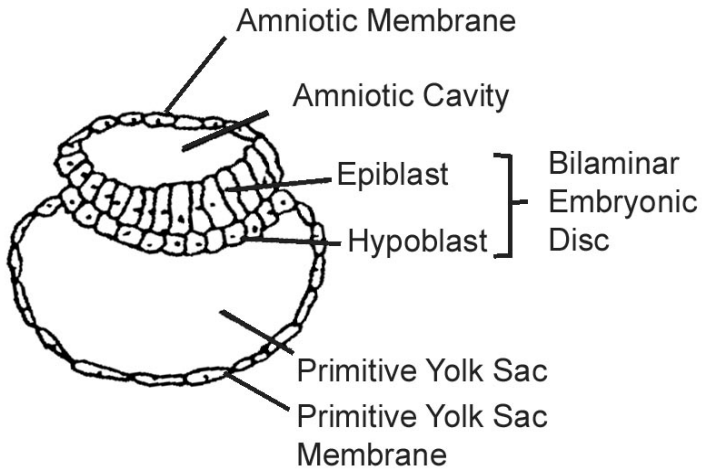


- The cells at the margins of the hypoblast begin to multiply and spread, with the margins of the hypoblast growing together to create a sac, called the primitive yolk sac. Later, during gastrulation, the hypoblast is replaced by endoderm. Consequently the hypoblast makes no direct tissue contribution to the fetus.

- At this stage the embryo consists of two layers, the epiblast and the hypoblast. These two layers are sandwiched between two balloons, the primitive yolk sac and the amniotic cavity.

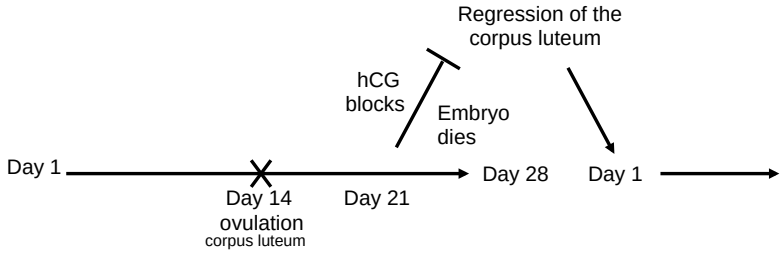


From The Developing Human, by K.L. Moore. Saunders



Early Pregnancy Loss

- 70% of zygotes die w/o woman knowing she was ever preggo
- she thinks she's "late" when she was actually preggo



corpus luteum
forms; many fates:
1. no implantation -> CL regresses -> menstruation at day 28
2. fertilization;
- implantation at 21; hCG secreted -> blocks CL regression -> no menstruation on day 28
- if embryo dies -> hCG decrease -> CL regresses later than normal -> next menstrual period begins, but a bit *later* than normal

what killed the embyro?
- chromosomally abnormal; x-rays; radiation; physical factors
- but probably not chemical factors since there's no exchange of substances b/w embryo and maternal circulatory sys at this point
- improper implantation
- immune response
- heat (mother has fever for e.g.)