OVULATION, OVUM & SPERM TRANSPORT, FERTILIZATION & EARLY EMBRYONIC DEVELOPMENT

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Lecture objectives

- **At the end of this lecture you should be able to describe:**
  - Mechanism of ovulation, ovum and sperm transport
  - Process of fertilization and implantation
  - Main events durinf 1st and 2nd week of development

Ovulation

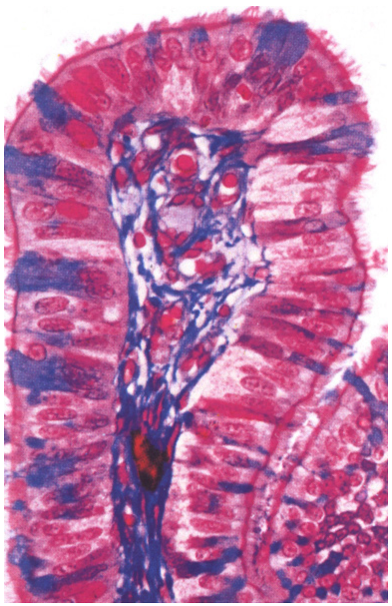
- Review the structure of the ovary
- Recruitment and growth of the follicles
- Events at the time of ovulation
  - Ovulation is a hormone-mediated process resulting in the release of the secondary oocyte
- Factors involved:
  - Increase in the volume and pressure of the follicular fluid
  - Enzymatic proteolysis of the follicular wall by activated plasminogen
  - Hormonally directed deposition of glycosaminoglycans between the oocyte–cumulus complex and the stratum granulosum
  - Contraction of the smooth muscle fibers in the theca externa layer, triggered by prostaglandins

Ovum transport

- The ends of the oviducts come into close contact with the ovary during ovulation.

- Fimbriae of oviduct ends "sweep" the ovulated ovum into the oviduct.

- Peristaltic waves of oviduct musculature bring the ovum into the ampulla of the oviduct.



Sperm capacitation

- Review structure of the testis
- Review process of spermatogenesis
- Sperms **cannot** fertilize oocytes when they are newly ejaculated.
- The process of capacitation takes 5-7 hours.
- Capacitated sperms are more active.

Capacitation..

- Location: capacitation occurs in the uterus and oviducts and is facilitated by substances of the female genital tract.
- Involves removal of seminal plasma proteins and glycoprotein coat from acrosome site
- The acrosomal reaction cannot occur until capacitation has occurred.

Stage 1 of fertilization:

- The acrosome reaction must be completed before the sperm can fuse with the secondary oocyte
  - Perforations develop in the acrosome
  - Point fusions of the sperm plasma membrane and the external acrosomal membrane occur
  - Acrosome enzymes that facilitate fertilization
  - Facilitated by zona proteins
- Passage of sperm through the corona radiata depends on enzyme action:
  - hyaluronidase released from sperm acrosome
  - Tubal mucosal enzymes
- Flagella action also aids corona radiata penetration

Stage 2 of fertilization:

- Penetration of the zona pellucida around the oocyte:
  - Acrosomal enzymes: esterases, acrosin, and neuraminidase cause lysis of the zona pellucida

- Once sperm penetrates zona pellucida, the **zona reaction** occurs:
  - This reaction makes the zona pellucida impermeable to other sperms.
  - When more than one sperm manages to enter the ovum (dispermy = 2; triploidy = 3), the fetus nearly always aborts.

Stages 3 & 4 of fertilization:

- Fusion of plasma membranes of oocyte and sperm
  - Head and tail of a sperm enter the cytoplasm of the oocyte, but the sperm plasma membrane remains behind.

- 2^{nd} meiotic division of oocyte is completed
  - The secondary oocyte was previously arrested in metaphase of the 2^{nd} meiotic division, and now forms the mature ovum and another polar body.

Stage 5 of fertilization:

- Formation of male and female pronuclei:
  - Chromosomal material of the sperm decondensates and enlarges
  - Chromosomal material of the ovum decondensates following the completion of meiosis

- At this stage, the male and female pronuclei are indistinguishable.

- As they grow, the pronuclei replicate their DNA → still 1N (haploid)- 23 chromosomes, each in chromatid pairs

Stage 6 of fertilization:

- Membranes of the pronuclei break down, chromosomes condense and arrange themselves for mitotic cell division
  - On membrane dissolution, there is 1 cell with 46 chromosomes = diploid (2N)
  - The first cleavage follows shortly, leaving 2 cells, each with 46 chromosomes.
    - Mitosis in the new zygote uses centrioles derived from the sperm. The oocyte has no centrioles.

- **Cleavage**
- It is a repeated mitotic divisions of the zygote in order to increase the number of embryonic cells (***blastomeres***).

- **Site:** cleavage occurs as the zygote travels along the fallopian tube.

- **Duration and steps:**
  - 30 hours after fertilization the zygote divides into 2-cell stage.
  - 40 hours - 4-cell stage
  - 72hrs - 16-cell stage (**morula**).
  - NB: Blastomeres increase in number but their sizes are reduced.
- ***Compaction***:
  - After the 8-cell stage, blastomeres are tightly aligned to form compact ball of cells.
  - Mediated by cell surface adhesion glycoprotein.
  - Compaction is essential for cellular interaction and for segregation of cells that form inner cell mass from outer cell mass.

Blastocyst Formation

- Morula (16-32 cells) enters the uterine cavity on day 4.
- Secretions from the uterine glands diffuse through zona pellucida between its cells to form fluid space, ***blastocele***.
- As fluid increases, it separates the blastomeres into two parts: trophoblast and embryoblast
- During this stage the conceptus is called **blastocyst or blastula**.
- ***Degeneration of the blastocyst is termed as Hatching***
- The blastula derives its nutrition from secretions of the uterine glands.
- This hatching allows the blastula to increase rapidly in size and start the process of implantation.

Implantation

- **Definition:** Implantation is adherence and embedding of the blastocyst in the endometrium.
- **Proper site of implantation** fundo-posterior/ fundo-anterior aspect of the uterus.
- **Duration:** from day 6 to day 12 after fertilization.
- Meanwhile the embryoblast produces ***bilaminar embryonic disc*** composed of **epiblast** and **hypoblast**. The trophoblast differentiates into:
  - Cyto/syncytiotrophoblast
- Extraembryonic structures forming during the second week are the ***amnion, yolk sac, connecting stalk, and chorionic sac.***

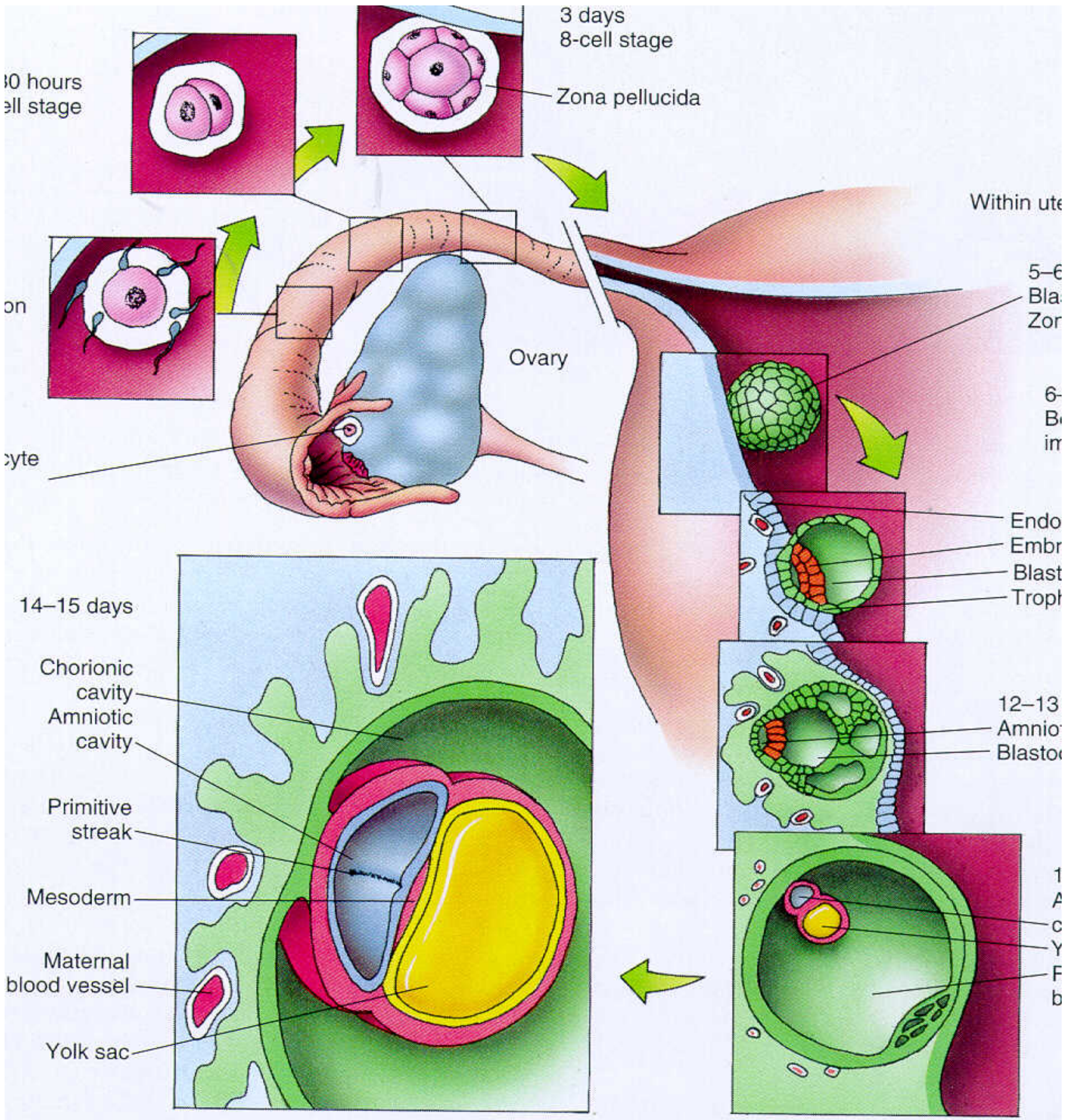
Steps of implantation:

At day 6 after fertilization, the blastocyst attaches to the endometrial epithelium by its embryonic pole. Trophoblast proliferates and differentiates into two layers:

-an inner cellular layer of ***cytotrophoblast***.

-an outer ***syncytiotrophoblast*** multinucleated protoplasmic mass in which no cell boundaries can be observed.

Cells in cytotrophoblast divide and then migrate into the syncytiotrophoblast. At the embryonic pole syncytiotrophoblast show finger-like processes that expand to invade the endometrium.



3 days
8-cell stage
0 hours
ell stage
Zona pellucida
Within ute
on
5–6
Bla
Zor
Ovary
6-
B
im
cyte
Endo
Embr
Blast
Troph
14–15 days
Chorionic cavity
Amniotic cavity
12–13
Amnio
Blasto
Primitive streak
Mesoderm
Maternal blood vessel
Yolk sac

Factors assisting invasion of syncytiotrophoblast into endometrium

1-Syncytiotrophoblast produces ***proteolytic enzymes***

2-Syncytiotrophoblast displaces endometrial cells in the central part of the implantation site.

3-***Molecular events*** related to human implantation involves **proteolytic enzymes, prostacyclins, cytokines, steroid hormones and growth factors.**

4-Connective tissue cells of endometrium accumulate glycogen and lipids and assume polyhedral appearance – ***decidua reaction***.

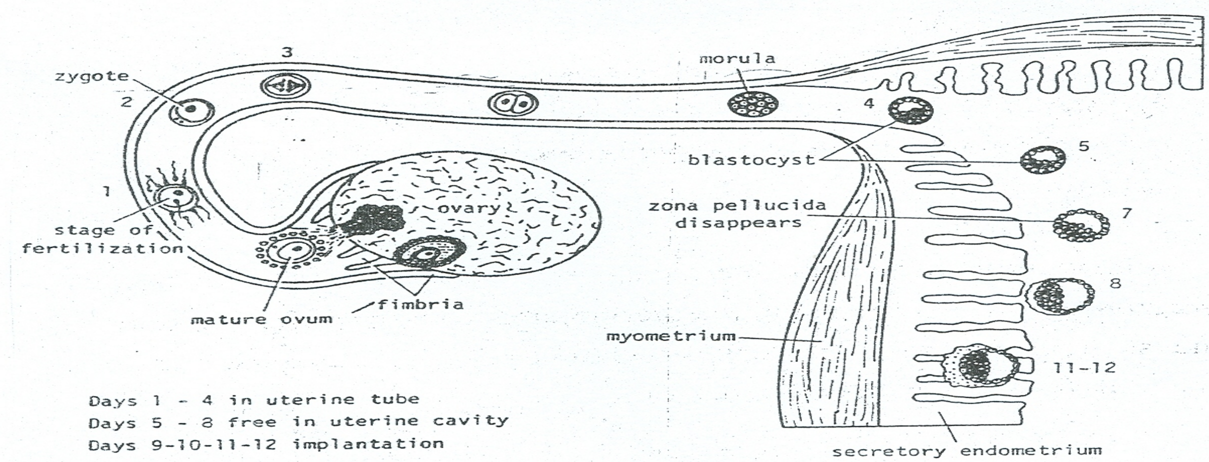
Sources of nutrition of implanted blastocyst

- Embryotroph -.

- Syncytiotrophoblast engulfs degenerated ***decidual cells*** providing rich nutrition to the embryo.

- Communication of the eroded capillaries with the syncytial lacunae establishes the ***primordial utero-placental circulation*** as early as day 11 or 12.

Changes which take place in the zygote while passing the uterine tube and entering uterine cavity to be implanted in endometrium.



Clinical Notes

-**Implantation bleeding** may occur as a result of increased blood flow into lacunar spaces. Since this bleeding which is usually slight (spotting) occurs around day 28 of the cycle, it may be confused with a normal menstrual bleeding and so cause inaccuracy in determining the expected date of delivery.

-Syncytiotrophoblast secretes human chorionic gonadotrophins (hCG) which enters the maternal blood in the syncytial lacunae. hCG maintains the hormonal activity of the corpus luteum up to the middle of pregnancy and forms the basis of **pregnancy test** as early as the end of the second week.

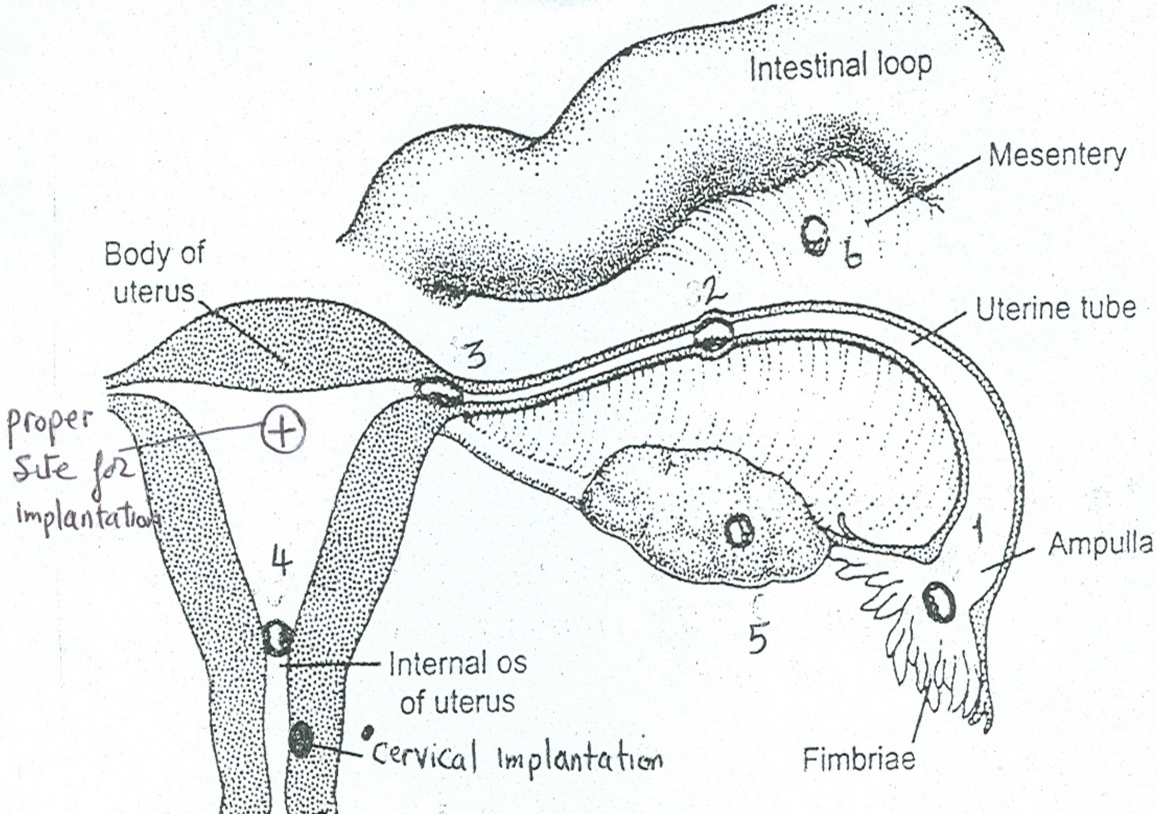
Because the implanted embryo represents a foreign body (50% of its genes are paternally derived) it should be rejected by maternal immune system. However, syncytiotrophoblast resists killer cells and antigens.

-**Inhibition of implantation** by:

a) Large doses of estrogen for several days shortly after unprotected sex, - 'morning after pill'.

b) Abortion pill RU 486 disturbs the hormonal balance.

c) IUD causes local inflammation which interferes with implantation.



Abnormal implantation.

Abnormal implantation

a) **Failure of implantation** occurs in about 25% of cases due to: embryonic genetic disorder, lethal chromosome, poorly developed endometrium or inadequate hormones by corpus luteum.

b) **Ectopic pregnancy** is implantation outside the uterus

i- In 95% of cases implantation occurs in the fallopian tube:

Adhesions of uterine tubes are the commonest causes.

ii-Abdominal implantation is rare. Rarely an abdominal pregnancy may continue to full term.

iii-Very uncommon, the blastocyst develops in the ovary proper, causing primary ovarian pregnancy that usually results in intra-abdominal bleeding.

c) Cervical implantation is unusual; some of these pregnancies are not recognized because the conceptus is aborted during early gestation.

d) Implantation in the lower segment of the uterus results in placenta previa.

Abnormal blastocyst and spontaneous abortions:

As many as 50% of all pregnancies end in spontaneous abortions and half of these losses are due to chromosomal abnormalities. These abortions serve as natural screening of embryos for defects.

Abnormal blastocyst may show:

-Trphoblast hypoplasia: absent embryoblast. Conceptus is aborted unnoticed.

-Trophoblast develops with little or no embryonic tissue, a condition known as **vesicular mole or hydatidiform mole**. Moles secrete high levels of hCG and may

- produce benign or malignant (invasive mole, choriocarcinoma) tumors. Moles arise from fertilization of an oocyte lacking a nucleus followed by duplication of male chromosomes.

Bilaminar Germ Disc (Second Week of Development)

At day 8, cells of the inner cells mass differentiate into two layers:

a) **Epiblast**: a layer of highly columnar cells.

b) **Hypoblast**: a layer of small cuboidal cells adjacent to the blastocele.

Cells of each germ layer form a flat disc and together are known as the bilaminar germ disc. At the same time a cavity appears within the epiblast known as the **amniotic cavity**. Epiblast cells which line the amniotic cavity are called **amnioblasts**.

- At day 9, flattened cells from hypoblast form a thin membrane known as exocelomic membrane which together with the hypoblast form the lining of the **exocelomic cavity (primitive yolk sac).**

Day 11 a new population of cells derived from the wall of the yolk sac form loose connective tissue, **extraembryonic (primary) mesoderm** which fills the space between trophoblast externally and the amnion and exocelomic membrane internally.

Soon large cavites develop in the extraembryonic mesoderm and when they fuse a new space known as **extraembryonic celom (chorionic cavity)** is formed.

The space surrounds the amniotic cavity and primitive yolk sac except when the germ disc is connected to the trophoblast by **connecting stalk**. The extraembryonic coelom separates the extraembryonic mesoderm into two layers; outer somatic (***somatopleuric***) mesoderm and inner splanchnic (***splanchnopleuric***) mesoderm.

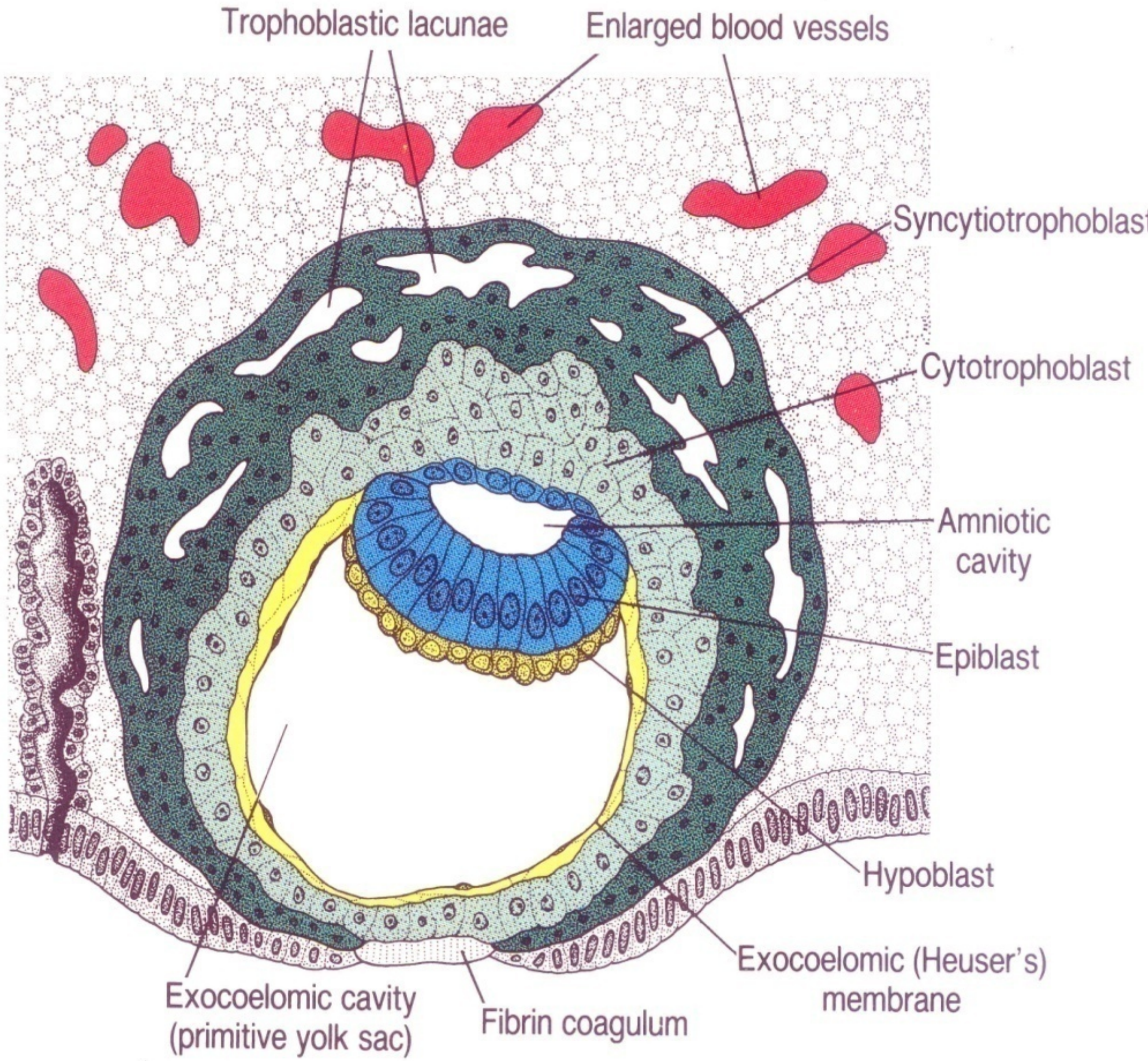


Figure 3.3 A 9-day human blastocyst. The syncytiotrophoblast shows a large number of lacunae. Flat cells form the exocoelomic membrane. The bilaminar disc consists of a layer of columnar epiblast cells and a layer of cuboidal hypoblast cells. The original surface defect is closed by a fibrin coagulum.

At day 13, the trophoblast is characterized by villous structures. Cells of the cytotrophoblast proliferate and penetrate into the syncytiotrophoblast, thus forming cellular columns surrounded by syncytium, **primary chorionic villi**.

Meanwhile the hypoblast produces additional cells that migrate along the inside of the exocelomic membrane to enclose a new cavity, -secondary or **definitive yolk sac** within the exocelomic cavity.

By mid week two embryo, extraembryonic coelom expands and forms the **chorionic cavity**. The trophoblast and its lining of somatic mesoderm are known as the **chorion (chorionic plate**). The whole vesicle of the two-week embryo, about 2-3mm, is known as the **chorionic vesicle**. The germ (embryonic) disc is 0.2mm.

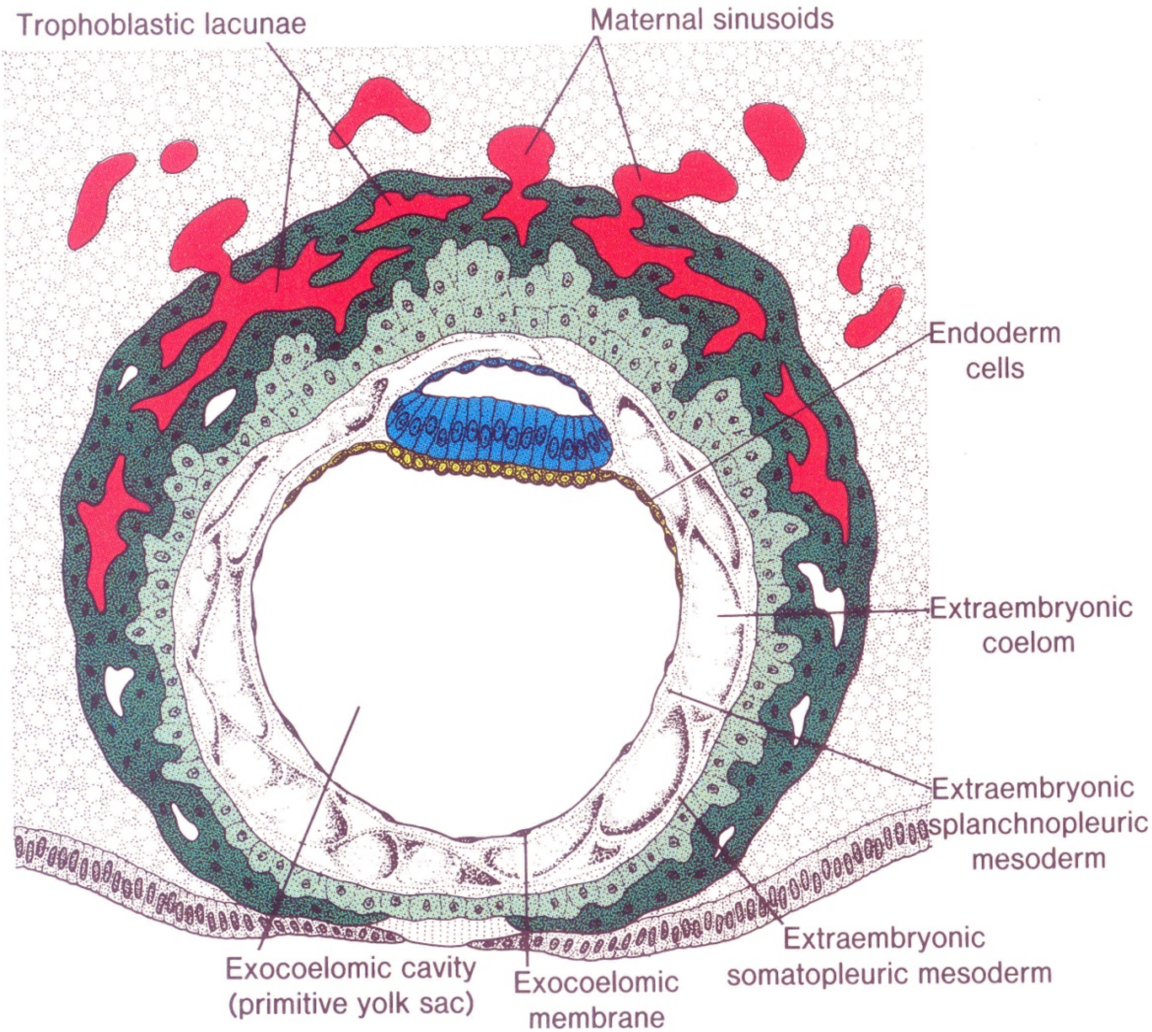
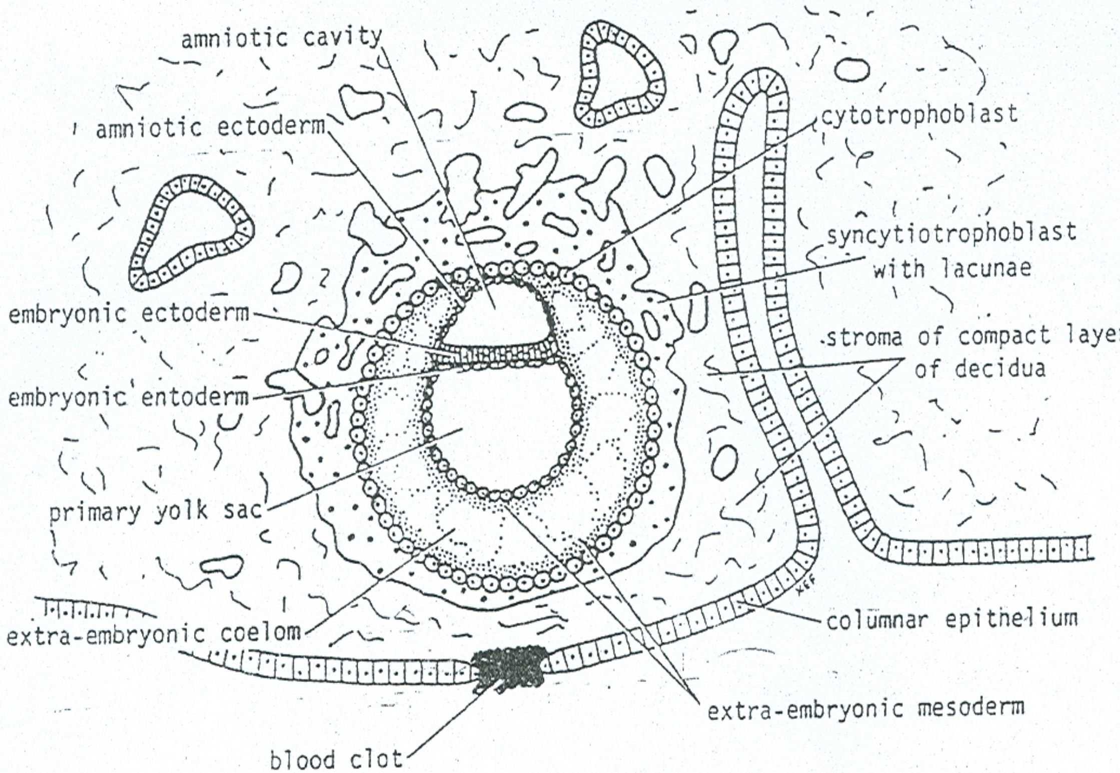


Figure 3.4 Human blastocyst of approximately 12 days. The trophoblastic lacunae at the embryonic pole are in open connection with maternal sinusoids in the endometrial stroma. Extraembryonic mesoderm proliferates and fills the space between the exocoelomic membrane and the inner aspect of the trophoblast.



11 day blastocyst showing formation of two germ layers. Also shown are the amniotic cavity, primary yolk sac, and extraembryonic mesoderm.

Formation of Chorionic Cavity

56 *Part One: General Embryology*

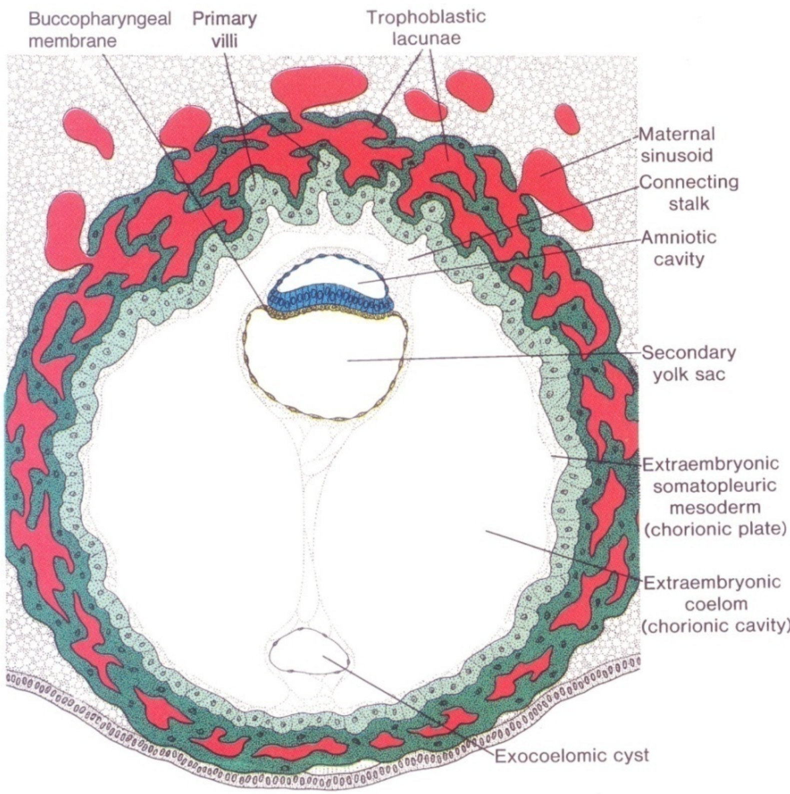


Figure 3.6 A 13-day human blastocyst. Trophoblastic lacunae are present at the embryonic as well as the abembryonic pole, and the uteroplacental circulation has begun. Note the primary villi and the extraembryonic coelom or **chorionic cavity.** The secondary yolk sac is entirely lined with endoderm.

area immediately surrounding the implantation site but soon occur throughout the endometrium.

- Large cavities develop in the extraembryonic mesoderm
- Cavities become confluent to form extraembryonic coelomic cavity (chorionic cavity)
- Chorionic cavity surrounds amniotic cavity and primitive yolk sac except at the region of connecting stalk

Changes in the TROPHOBLAST 8th Day

CYTOTROPHOBLAST-

- Inner layer of mononucleated cells
- mitotic figures present

SYNCYTIOTROPHOBLAST-

outer multinucleated layer with no distinct cell boundaries

produces hormone

(human chorionic gonadotrophin,HCG)

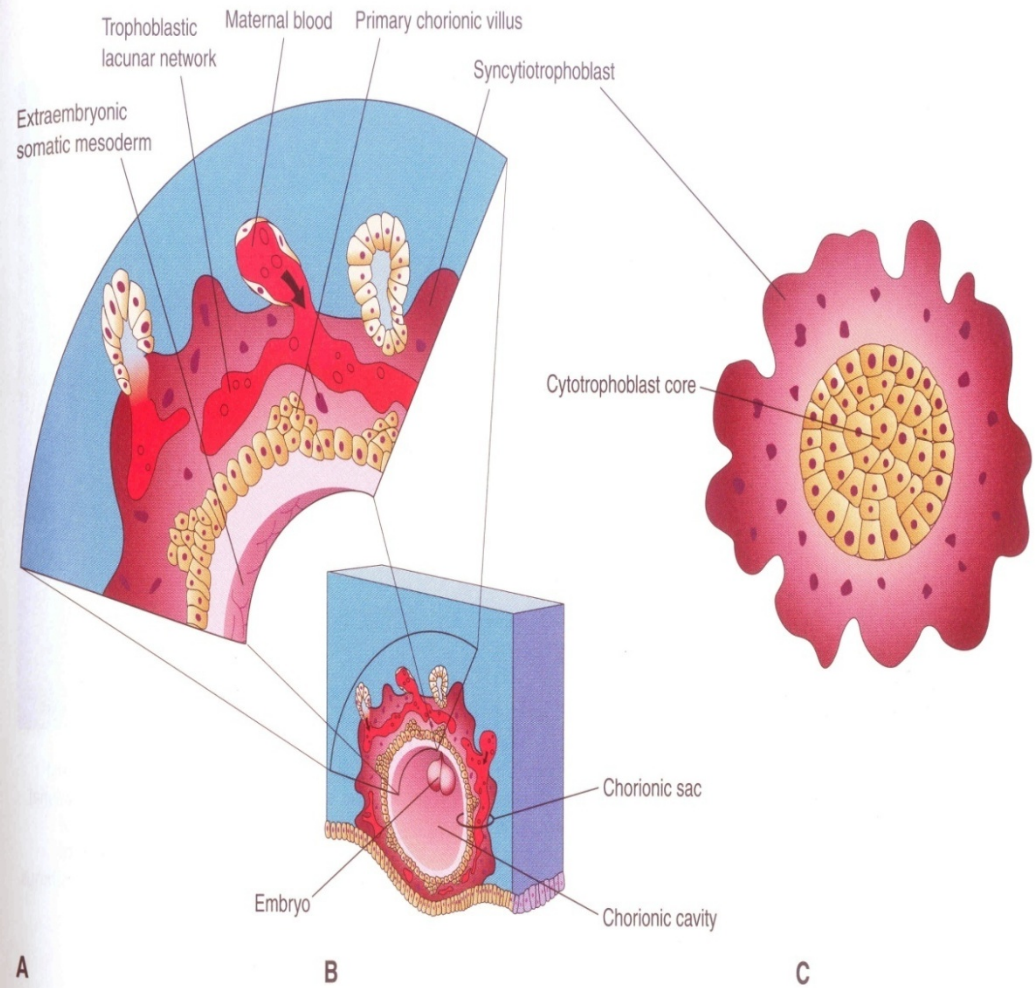
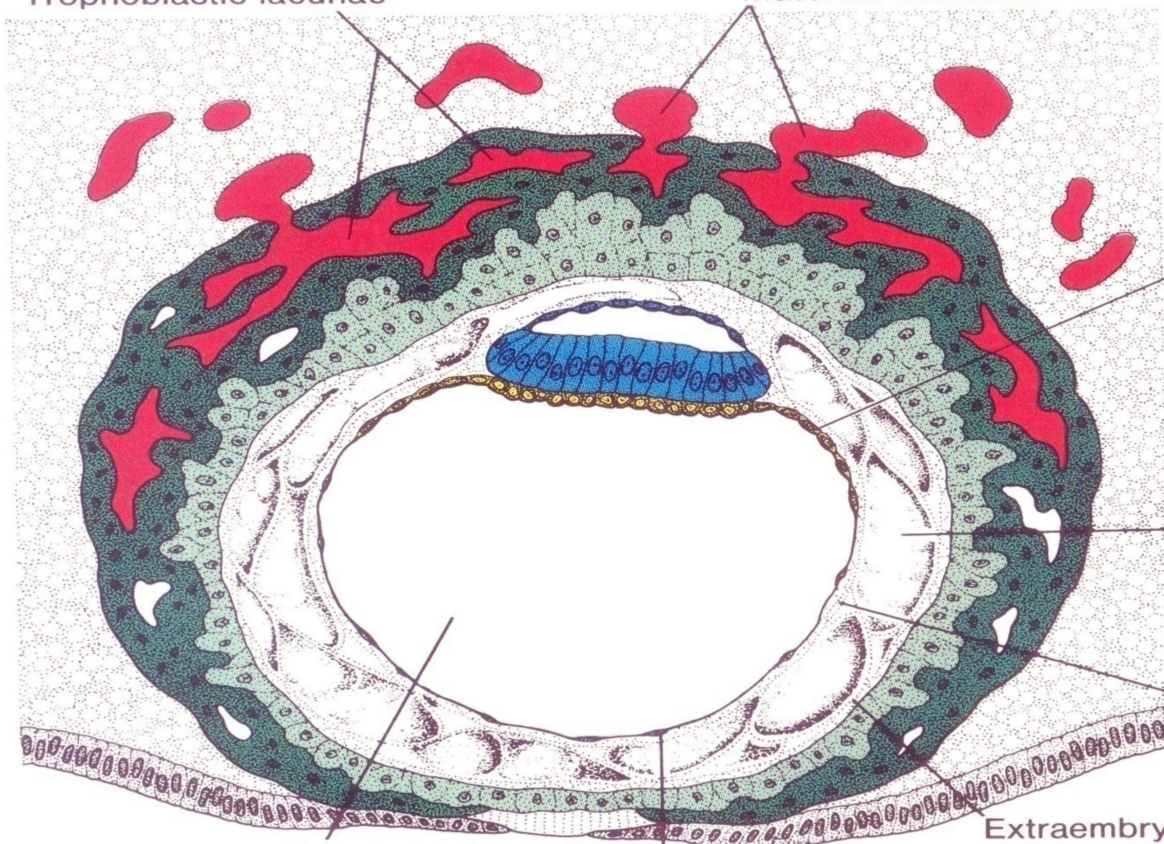


Figure 3-7. A, Detail of the section (outlined in B) of the wall of the chorionic sac. B, Sketch of a 14-day conceptus illustrating the chorionic sac and the shaggy appearance of it created by the primary chorionic villi (× 6). C, Drawing of a transverse section through a primary chorionic villus (× 400).

Establishment of Uteroplacental Circulation



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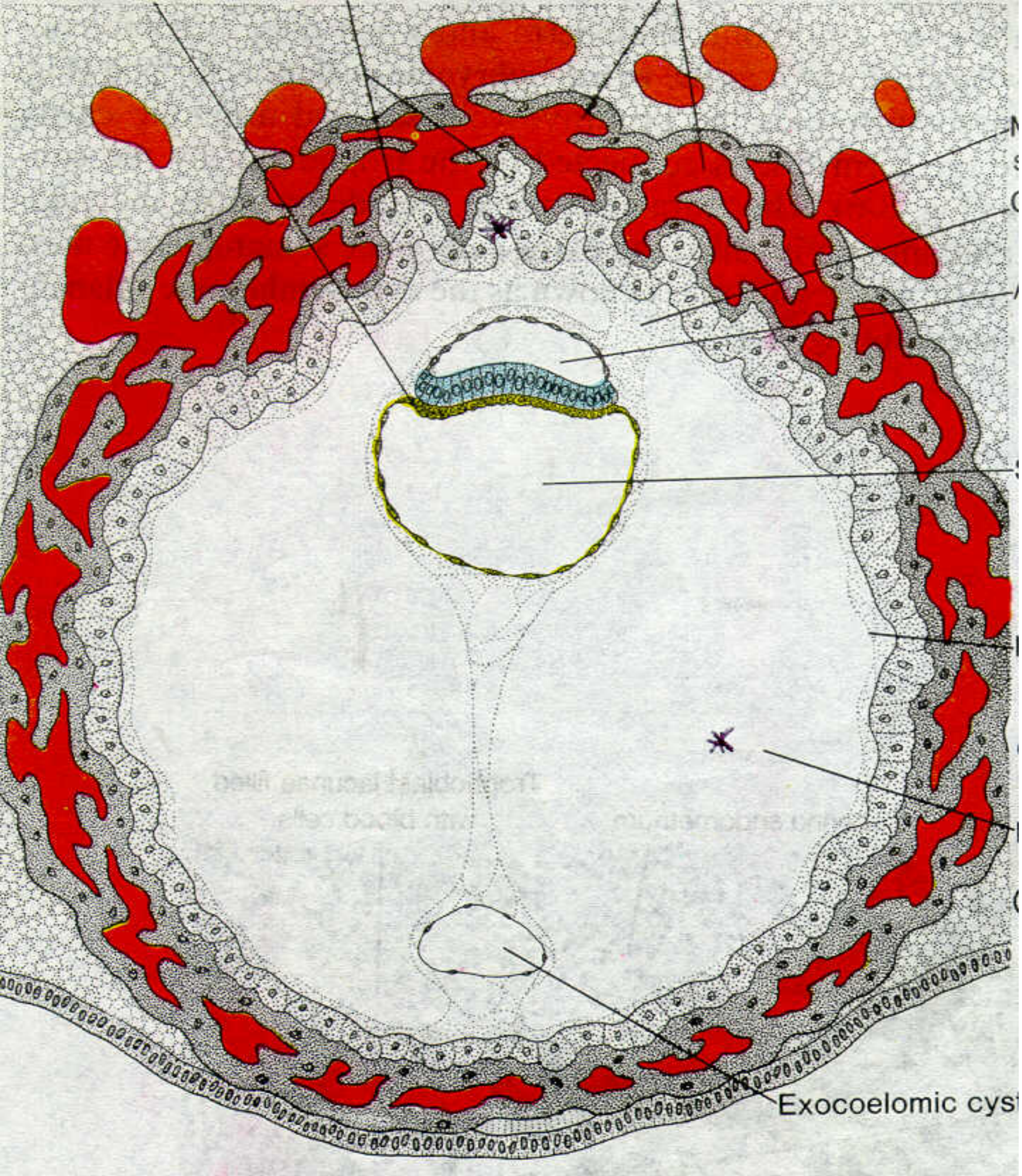
HIGHLIGHTS of 2nd Week of Development

- Complete implantation of blastocyst- anterior or posterior wall of uterine corpus
- Embryoblast has 2 layers(bilaminar germ disc)- epiblast, hypoblast
- Trophoblast consists of 2 layers-cytotrophoblast, syncytiotrophoblast
- 3 cavities formed-amniotic cavity, yolk sac, extraembryonic coelomic cavity (chorionic cavity)
- 2 layers of extraembryonic mesoderm-somatopleure,splanchnopleure
- Start of uteroplacental circulation

At the end of the second week

- Primitive yolk sac replaced by secondary yolk sac
- Formation of chorionic cavity or extraembryonic cavity
- Development of connecting stalk(umbilical cord)
- Trophoblast forms villous structures-PRIMARY VILLI
- Prechordal plate develop as thickening of hypoblast-future cranial region



Bilaminar Germ Disc at end of 2nd week