Fertilization in Humans and First Week of Development

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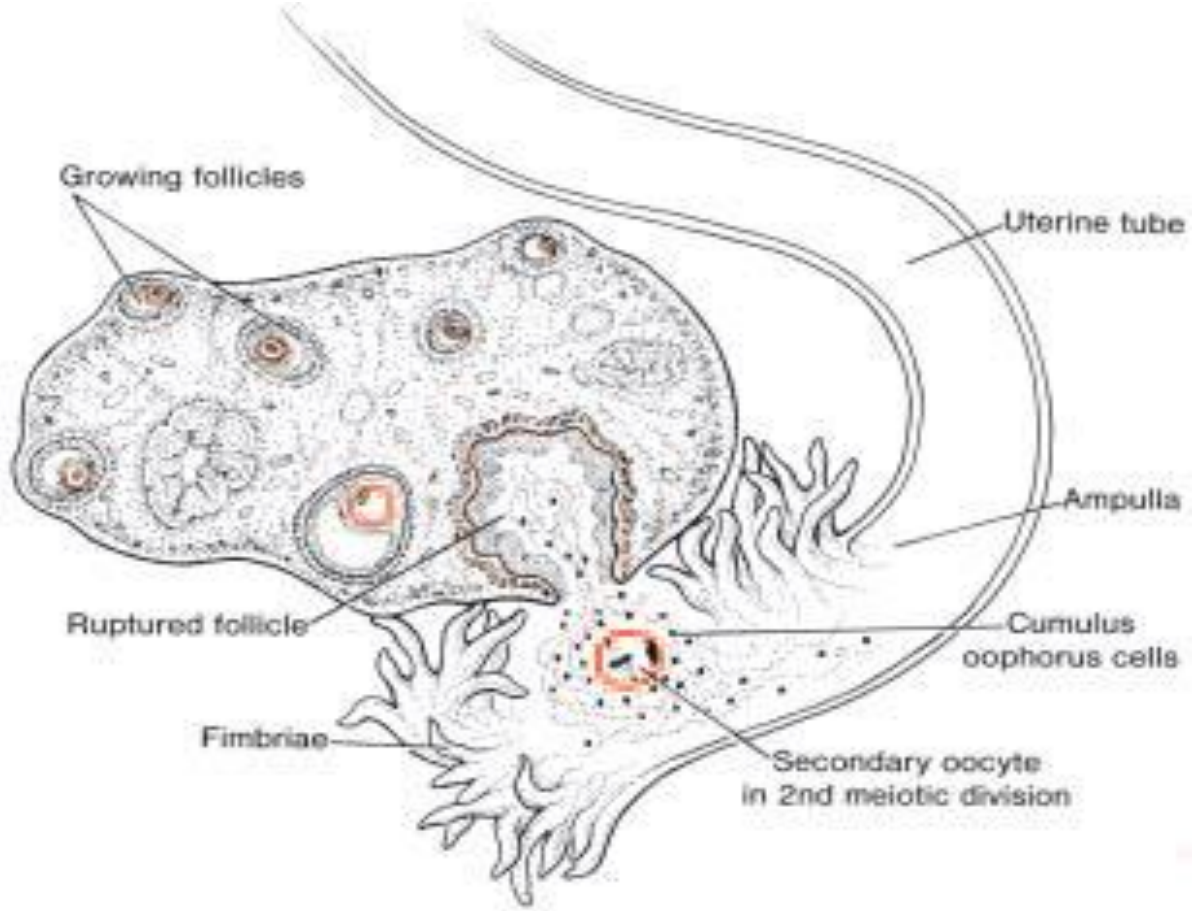
- **TRANSPORTATION OF GAMETES**
- **Oocyte Transport**
- secondary oocyte is expelled at ovulation from the ovarian follicle with the escaping follicular fluid
- During ovulation fimbriated end of the uterine tube becomes closely applied to the ovary
- fingerlike processes of the tube, ***fimbriae***, move back & forth over the ovary
- sweeping action & cilia of the mucosal cells of the fimbriae "sweep" the secondary oocyte into the funnel-shaped infundibulum of the uterine tube
- oocyte passes into the ampulla of the tube

Oocyte transport

- Shortly before ovulation
  - Fimbriae start sweeping
- Cilia in the fallopian tubes



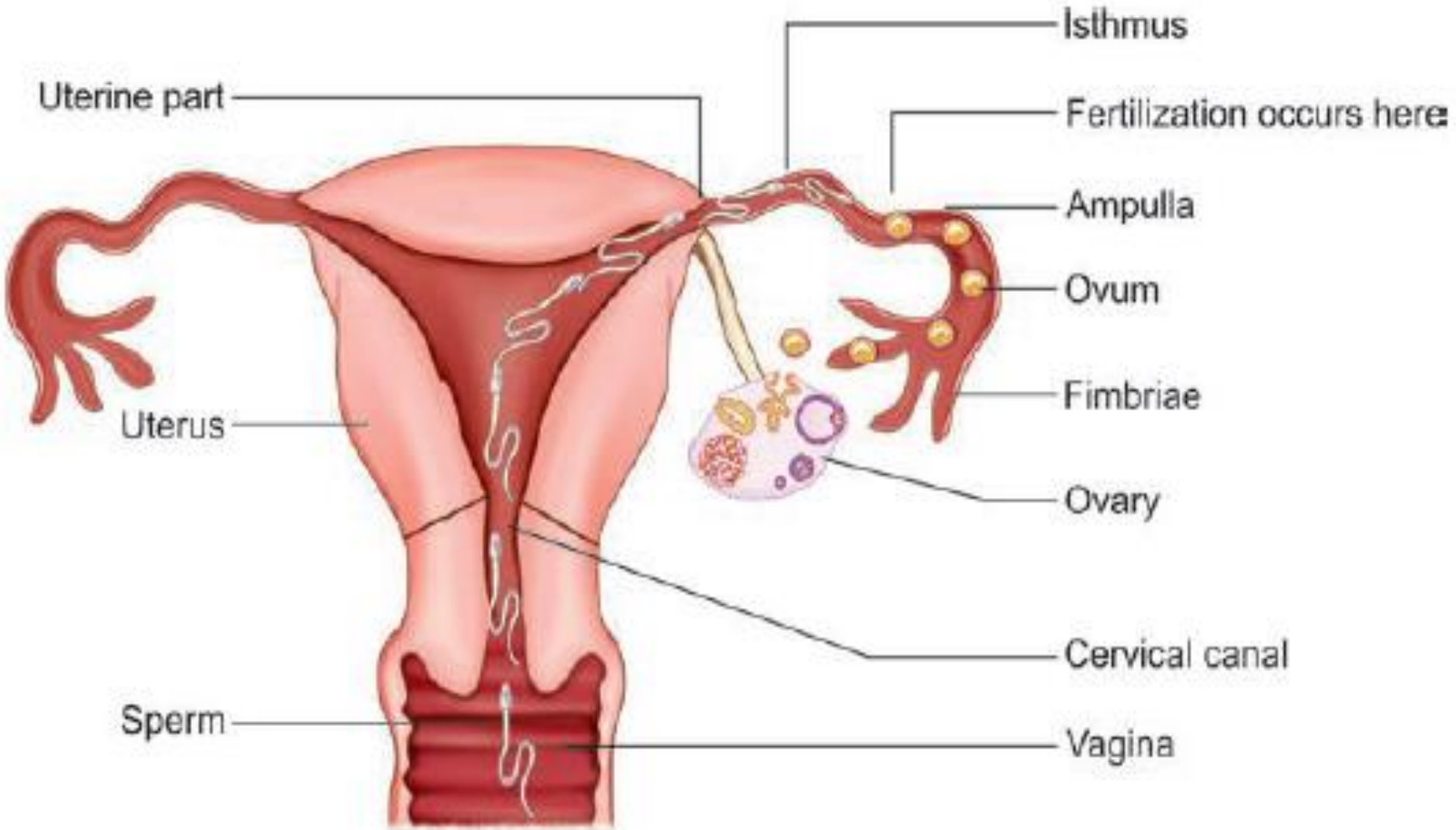
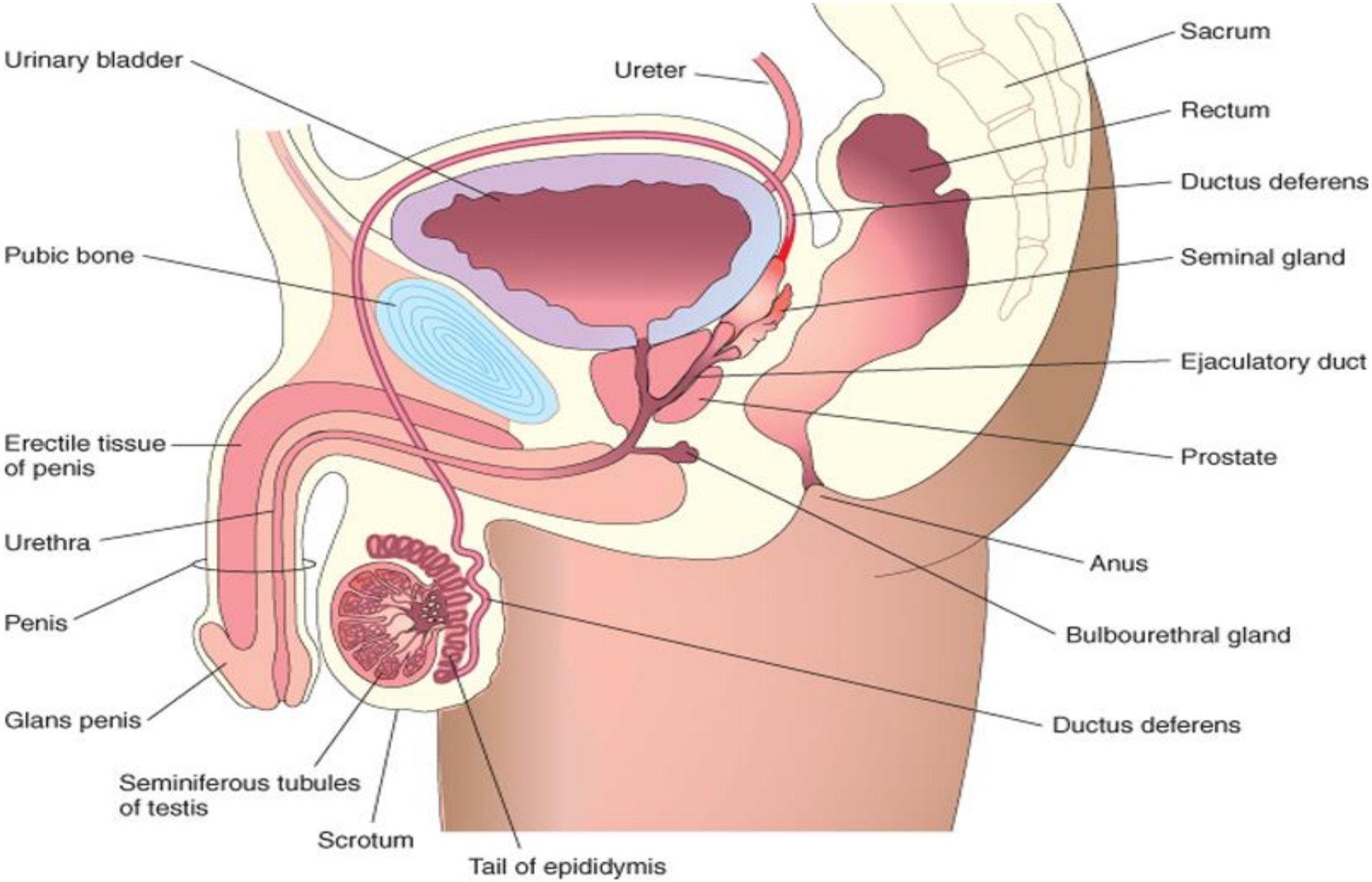


Fig. 4.2: Path taken by the sperm and ovum, for fertilization

- **Sperm Transport**
- From storage site in the epididymis, mainly the tail
- sperms are rapidly transported to the urethra by peristaltic contractions of the thick muscular coat of the ductus deferens
- accessory sex glands-*seminal glands* (vesicles), *prostate & bulbourethral glands*
- produce secretions that are added to the sperm-containing fluid in the ductus deferens & urethra
- **200 to 600 million** sperms are deposited around the external os of the uterus & in the fornix of the vagina during sexual intercourse



- sperms pass slowly through the cervical canal by movements of their tails
- enzyme **vesiculase** produced by the seminal glands
- coagulates some of the semen or ejaculate & forms a vaginal plug that may prevent the backflow of semen into the vagina
- cervical mucus increases in amount & becomes less viscid, making it more favorable for sperm transport
- Passage of sperms through the uterus & uterine tubes results mainly from muscular contractions of the walls of these organs

- ***Prostaglandins*** in the semen stimulate uterine motility at the time of intercourse
- Also assist in the movement of sperms to the site of fertilization in the ampulla of the tube
- volume of **sperm** or **ejaculate** (sperms suspended in secretions from accessory sex glands) averages 3.5 mL, with a range of 2 to 6 mL
- nonmotile during storage in the epididymis, but become motile in the ejaculate
- move slowly in the acid environment of the vagina, but move more rapidly in the alkaline environment of the uterus
- Only approximately 200 sperms reach the fertilization site
- Most sperms degenerate & are resorbed by the female genital tract

- **FERTILISATION**
- Fertilization is a complex sequence of coordinated molecular events that begins with contact between a sperm & an oocyte & ends with the intermingling of maternal & paternal chromosomes at metaphase of the first mitotic division of the zygote, a unicellular embryo
- process by which male & female gametes fuse
- occurs in the **ampullary** region **of the uterine tube**
- normally occurs in the uterine (fallopian) tube within 12 to 24 hours after ovulation
- Chemical signals (*attractants*), secreted by the oocyte & surrounding follicular cells, guide the **capacitated sperms** (*sperm chemotaxis*) to the oocyte
- **carbohydrate binding molecules** & ***gamete-specific proteins*** on the surface of the sperms are involved in *sperm-egg recognition* & their union

- Freshly ejaculated sperms are unable to fertilize oocytes
- Sperm that reach the vicinity of the oocyte within minutes after ejaculation

are not capable of fertilizing it until about 7 hours later

- must undergo
- (1) **capacitation**
- (2)the **acrosome reaction**
- to acquire this capability

1. Capacitation

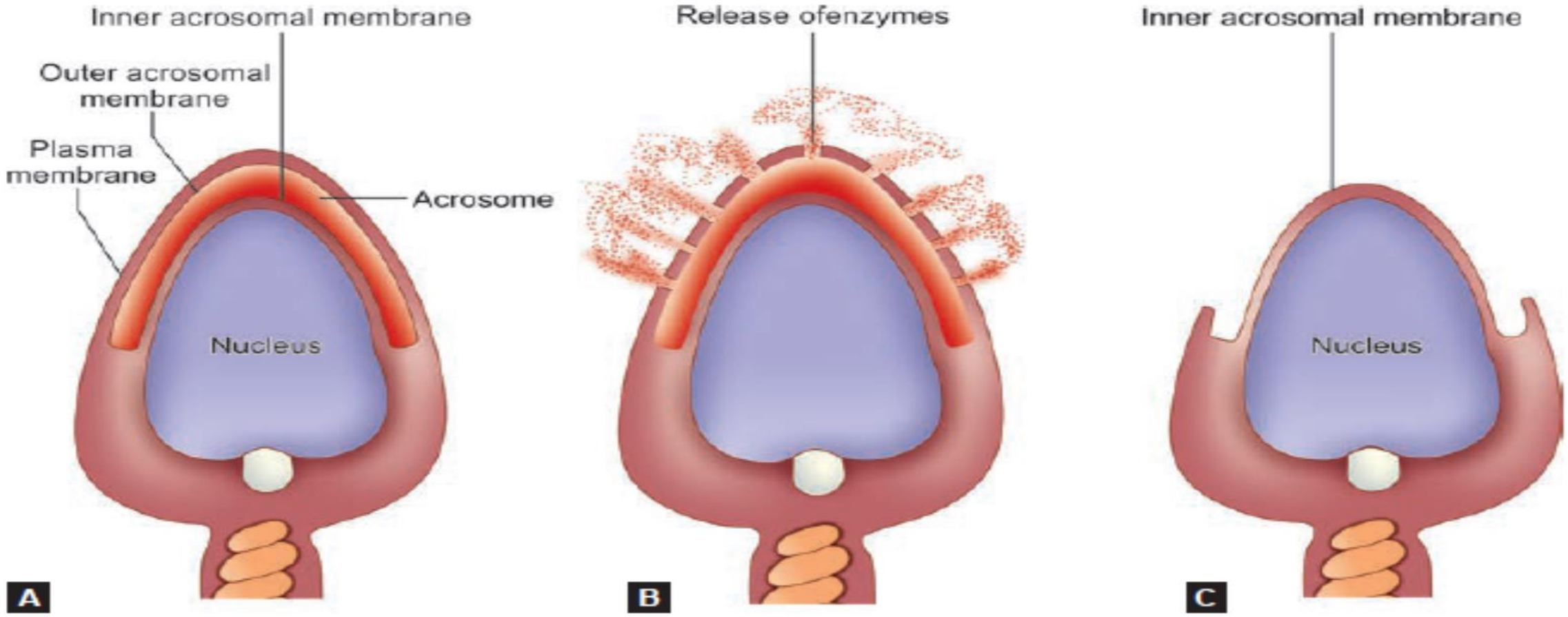
- a series of functional changes that cause the sperm's tail to beat even more vigorously
- prepare its plasma membrane to fuse with the oocyte's plasma membrane.
  - Last about 7 hours
  - Takes place in the uterus/uterine tubes
- secretions in the female reproductive tract remove:
- cholesterol, glycoprotein coat and seminal plasma proteins from the plasma membrane that overlies the acrosomal region of the spermatozoa.

2. **Acrosome reaction (ZP3 Zona protein)**

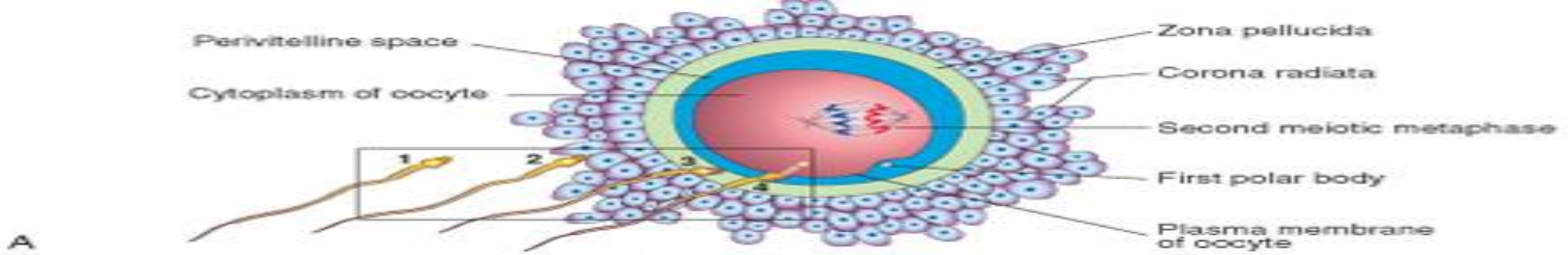
- **acrosome** a structure that covers the head of a sperm , contains several enzymes
- One of the glycoproteins in the zona pellucida acts as a sperm receptor
- binds to specific membrane proteins on the acrosome to trigger the **acrosomal reaction**
- intact acrosome of the sperm binds to a ***glycoprotein (ZP3)*** on the zona pellucida

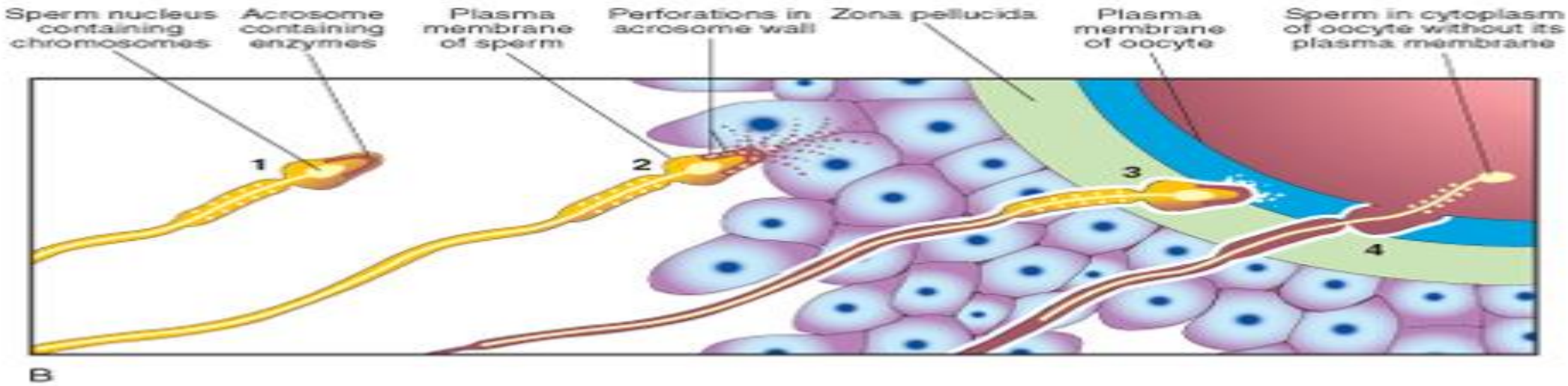
- sperm plasma membrane, calcium ions, prostaglandins & progesterone play a critical role in the acrosome reaction
- **acrosome reaction** of sperms must be completed before the sperm can fuse with the oocyte
- When **capacitated sperms** come into contact with the corona radiata surrounding a secondary oocyte
- They undergo complex ***molecular changes*** that result in the development of perforations in the acrosome

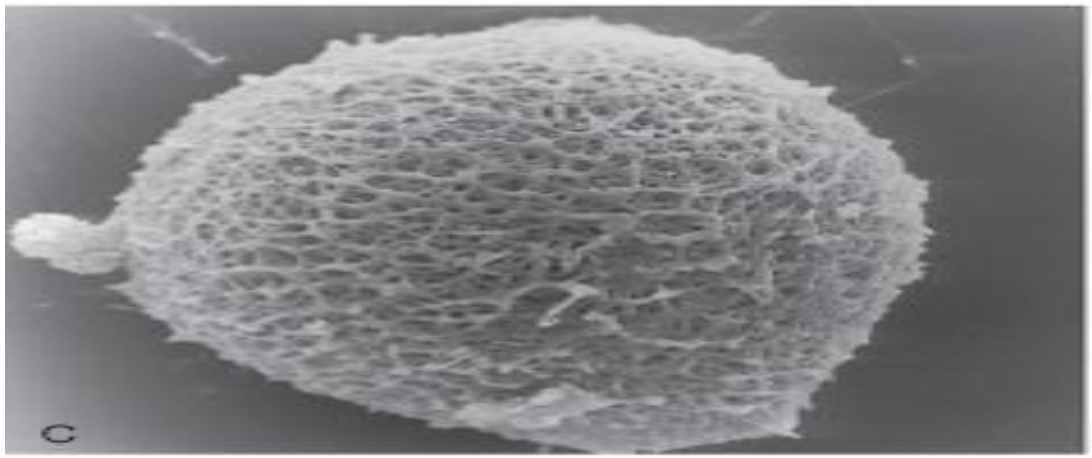
- Multiple point fusions of the plasma membrane of the sperm & the external acrosomal membrane occur
- Breakdown of the membranes at these sites produces apertures
- changes induced by the acrosome reaction release enzymes ***hyaluronidase*** & ***acrosin***, from the acrosome that facilitate fertilization

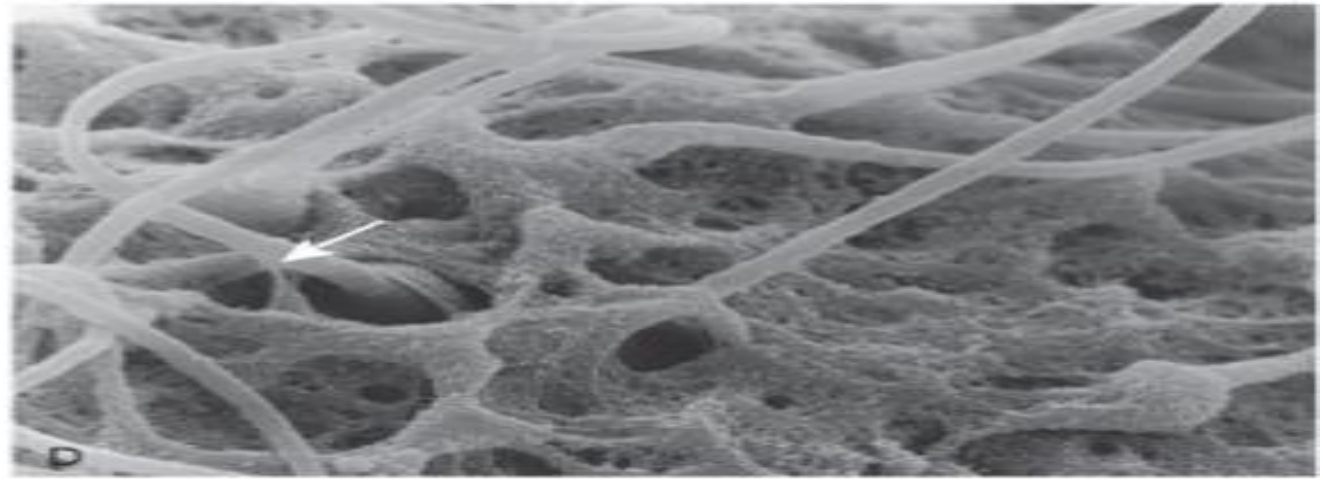


Figs 4.5A to C: Covering of acrosome and release of acrosomal enzymes



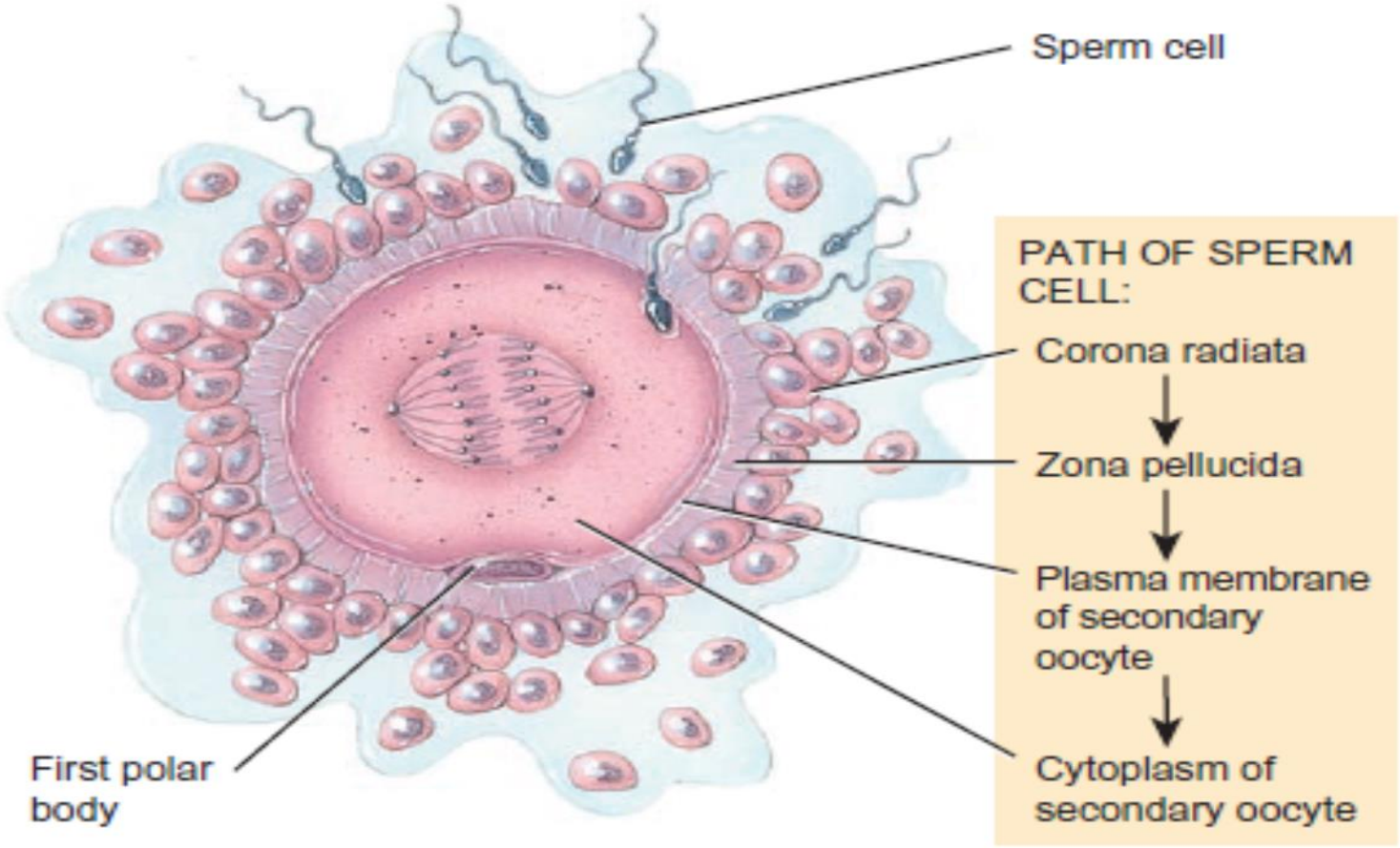






PHASES OF FERTILIZATION

- penetration of the corona radiata
- penetration of the zona pellucida
- fusion of the oocyte and sperm cell membranes



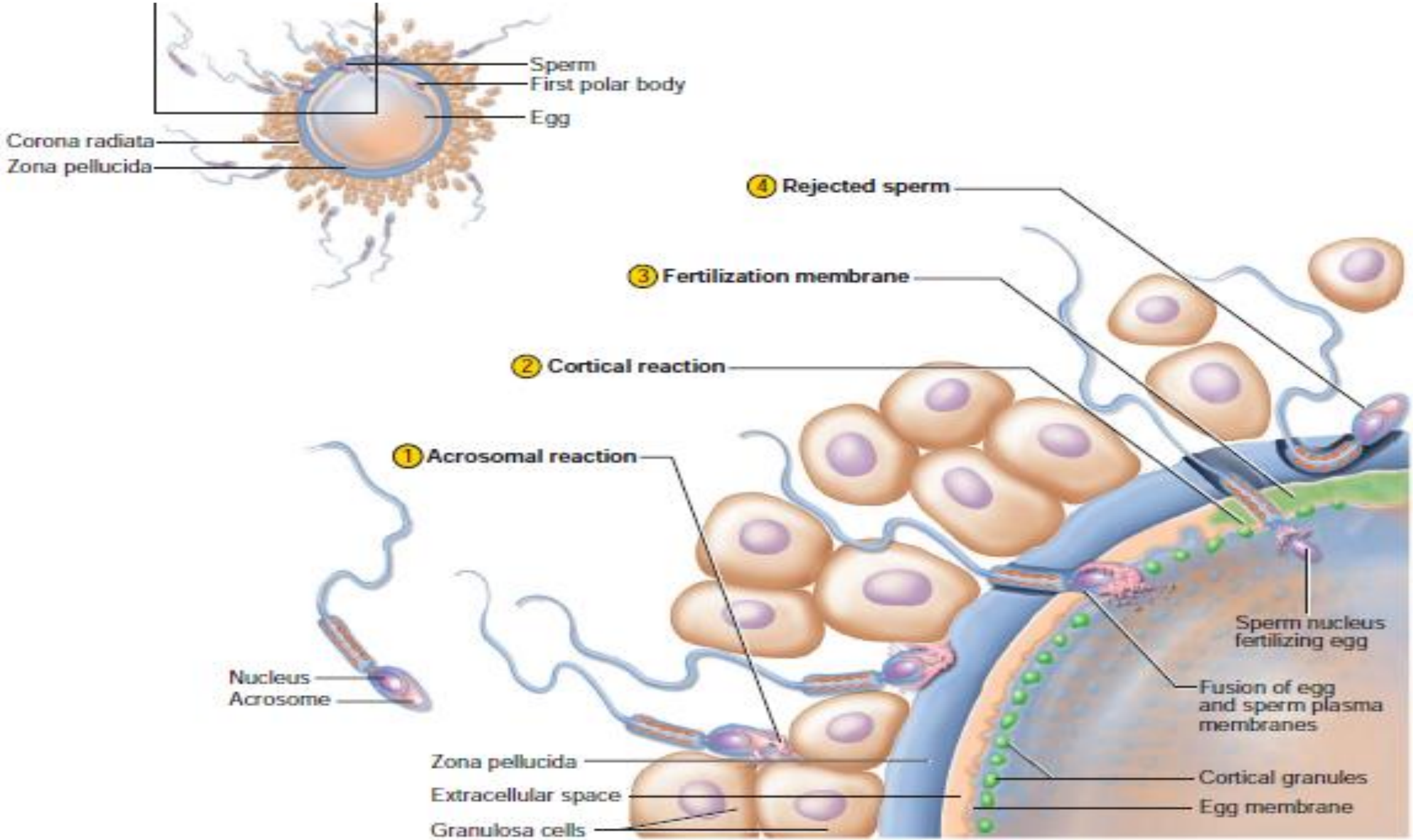
(a) Sperm cell penetrating a secondary oocyte

1. Penetration of the Sperm through the Corona radiata

- Only 300 – 500 out of 200 - 300 million ejaculated sperms reach fertilization site
- Only capacitated sperms pass freely through corona radiata
- Only one fertilizes the egg
- Dispersal of the follicular cells appears to result mainly from the action of the enzyme *hyaluronidase* released from the acrosome of the sperm
- *Tubal mucosal enzymes* also appear to assist the dispersal
- Movements of the tail of the sperm are also important in its penetration of the corona radiata

2. PENETRATION OF THE ZONA PELLUCIDA

- Zona pellucida is a glycoprotein surrounding oocyte & induces acrosome reaction
- Acrosome reaction mediated by zona protein ZP3
- Enzymes i.e. *esterases, acrosin & neuraminidase* lyse Zona pellucida
- allows sperm to penetrate the zona, thereby coming in contact with the plasma membrane of the oocyte
- Contact changes permeability of the zona pellucida


Sperm
First polar body
Egg
Corona radiata
Zona pellucida
4 Rejected sperm
3 Fertilization membrane
2 Cortical reaction
1 Acrosomal reaction
Nucleus
Acrosome
Zona pellucida
Extracellular space
Granulosa cells
Sperm nucleus fertilizing egg
Fusion of egg and sperm plasma membranes
Cortical granules
Egg membrane

- **Cortical and zona reactions**
  - This prevents polyspermy
- release of lysosomal enzymes from **cortical granules** lining the plasma membrane of the oocyte
- contents of these granules are released into the perivitelline space
- these enzymes alter properties of the zona pellucida **(zona reaction)** to prevent sperm penetration
- cause changes in the plasma membrane that make it impermeable to other sperms
- inactivate **species-specific** receptor sites for spermatozoa on the zona surface
- zona reaction prevent more sperms to enter the oocyte.

3. FUSION OF THE OOCYTE AND SPERM CELL MEMBRANES

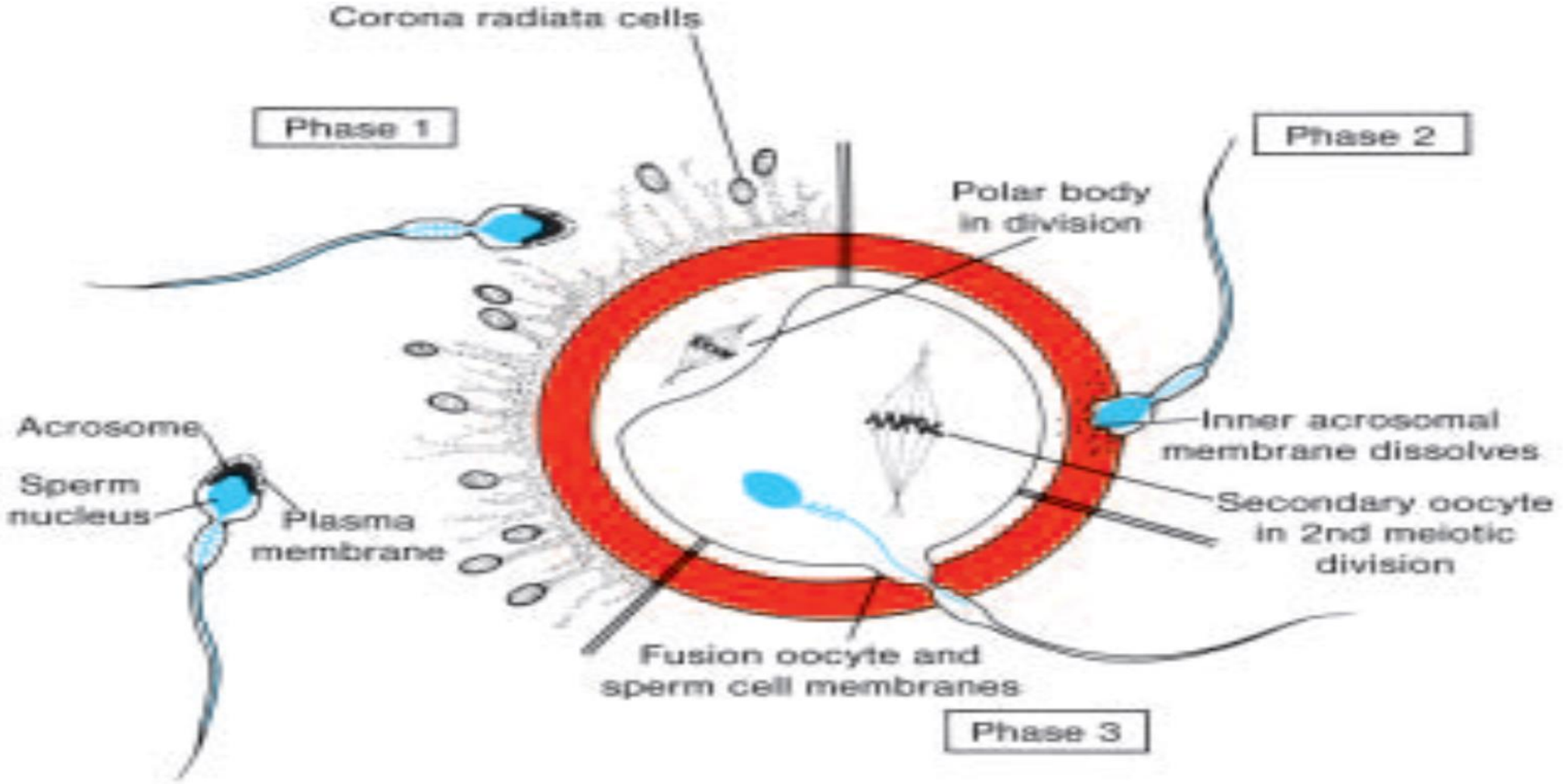
plasma membranes of the sperm & egg fuse

NB: In the human, both the head & tail of the spermatozoon enter the cytoplasm of the oocyte, but the plasma membrane is left behind on the oocyte surface.

When sperm has entered the oocyte, the egg responds in 3 ways

1. **Cortical and zona reactions**
2. **Resumption of the second meiotic division**
   - oocyte finishes its second meiotic division
   - Mature oocyte formation and 2nd polar body
   - Chromosomes(22 plus x) arranged in vesicular nucleus called the **female pronucleus**

- **3.Metabolic activation of the egg**
- Activating factor is carried by the sperm
- Activation encompasses the initial cellular and molecular events associated with early embryogenesis



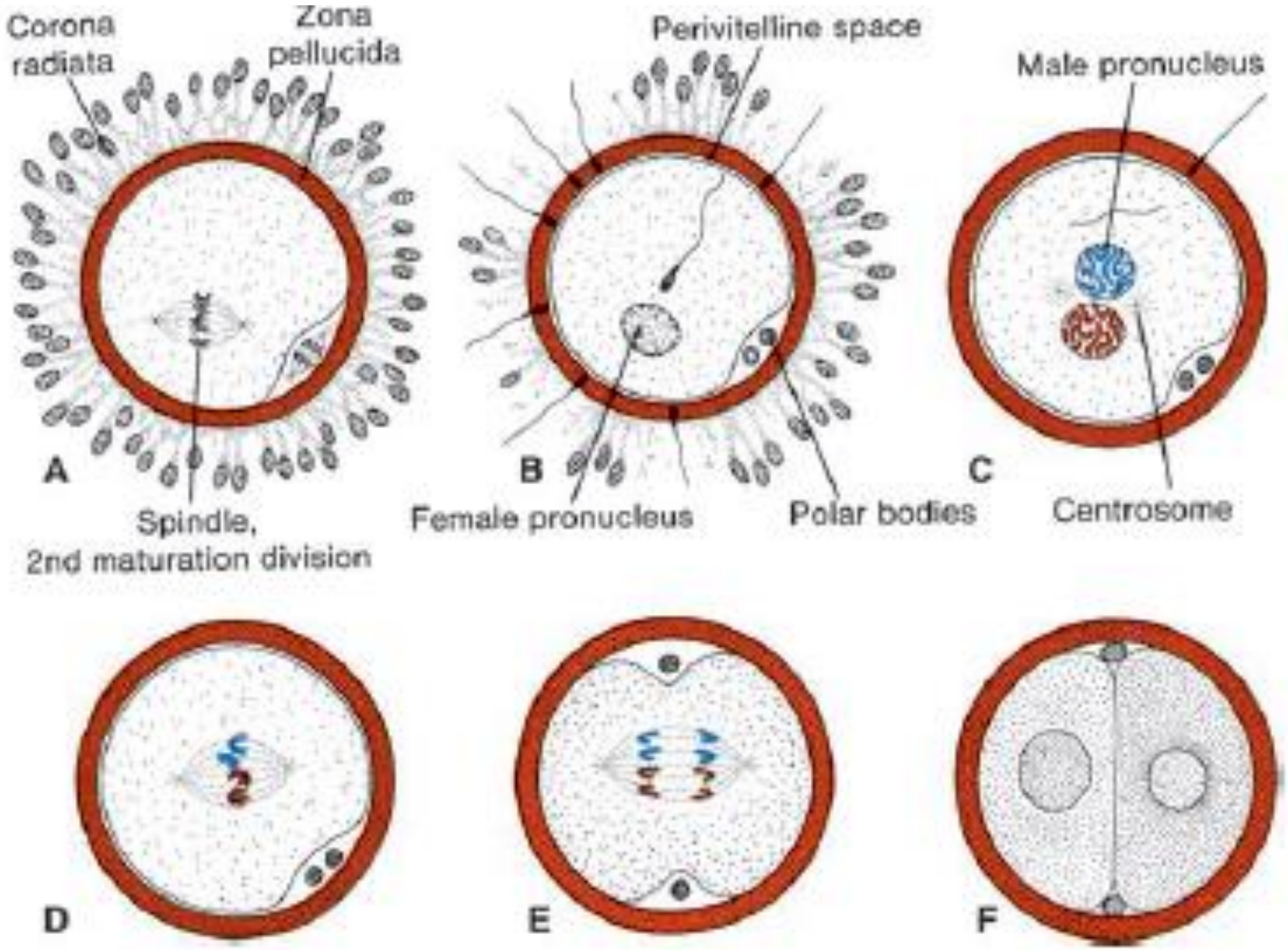


Figure 3.6 **A.** Oocyte immediately after ovulation, showing the spindle of the second meiotic division. **B.** A spermatozoon has penetrated the oocyte, which has finished its second meiotic division. Chromosomes of the oocyte are arranged in a vesicular nucleus, the female pronucleus. Heads of several sperm are stuck in the zona pellucida. **C.** Male and female pronuclei. **D,E.** Chromosomes become arranged on the spindle, split longitudinally, and move to opposite poles. **F.** Two-cell stage.

- Formation of **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
- Morphologically, the male & female pronuclei are indistinguishable
- oocyte containing 2 haploid pronuclei is called an ***ootid***.

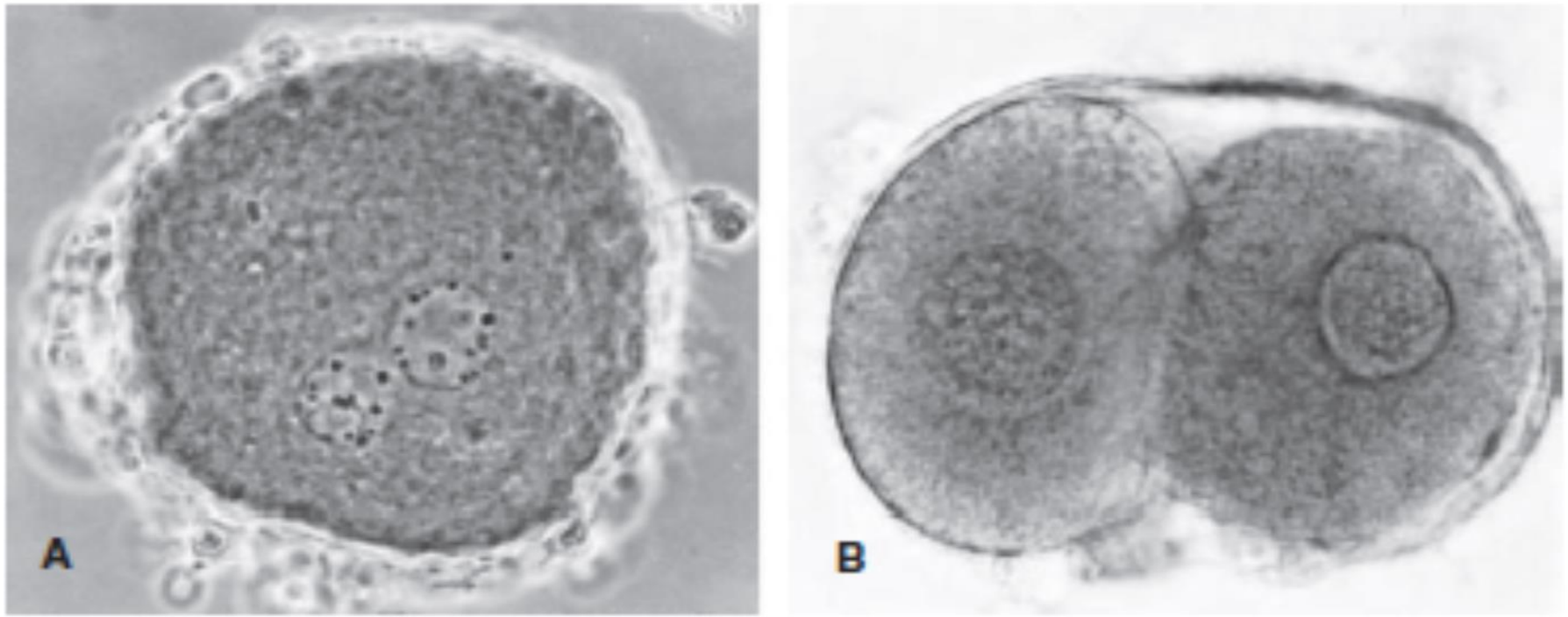
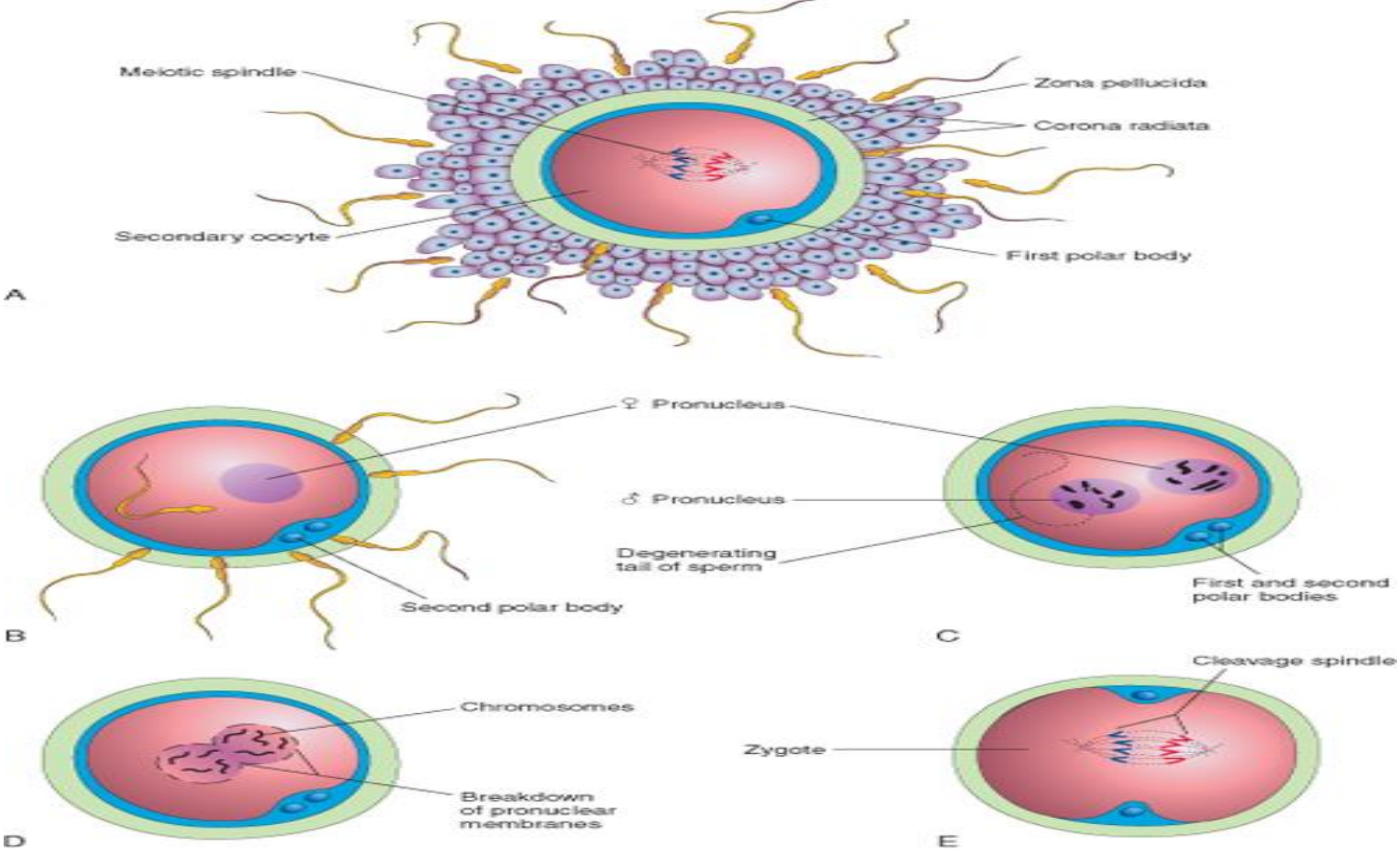


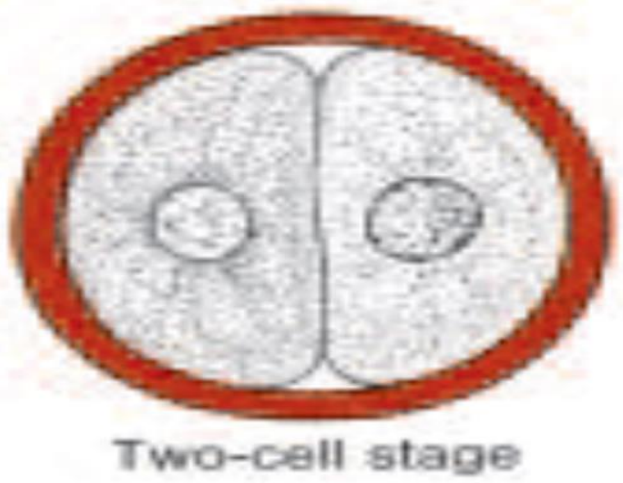
Figure 3.7 A. Phase contrast view of the pronuclear stage of a fertilized human oocyte with male and female pronuclei. B. Two-cell stage of human zygote.

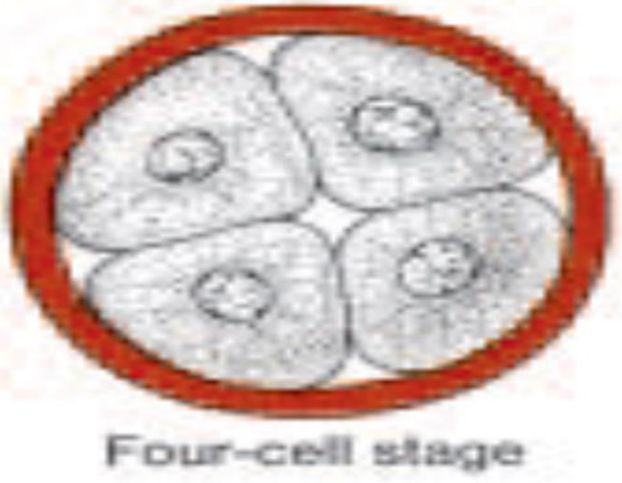
- Replication of DNA in both pronuclei
- the pronuclei fuse into a single diploid aggregation of chromosomes, the ootid becomes a **zygote**
- Mingling of the maternal & paternal chromosomes is called **amphimixis**
- The 23 maternal & 23 paternal (double) chromosomes split longitudinally at the centromere, and sister chromatids move to opposite poles,
- chromosomes in the zygote become arranged on a **cleavage spindle** in preparation for cleavage of the zygote



- **Results of Fertilization**
- 1. restoration of the diploid number of chromosomes (46) in the zygote
- 2. Results in variation of the human species through mingling of maternal and paternal chromosomes
- 3. determination of the sex
- 4. Causes metabolic activation of the ootid & initiates cleavage (cell division) of the zygote

- **Cleavage**
- process of repeated mitotic divisions of zygote within zona pellucida in rapid succession, giving rise to increasing number of smaller cells called ***blastomeres***
- start immediately after fertilization & continue as the zygote is passing through the uterine tube toward uterus
- cleaving zygote is covered by zona pellucida
- journey is facilitated by ciliary beats of uterine epithelium & contraction of uterine tube musculature
- cleavage division lasts for 6 days, i.e. up to 7th day after fertilization

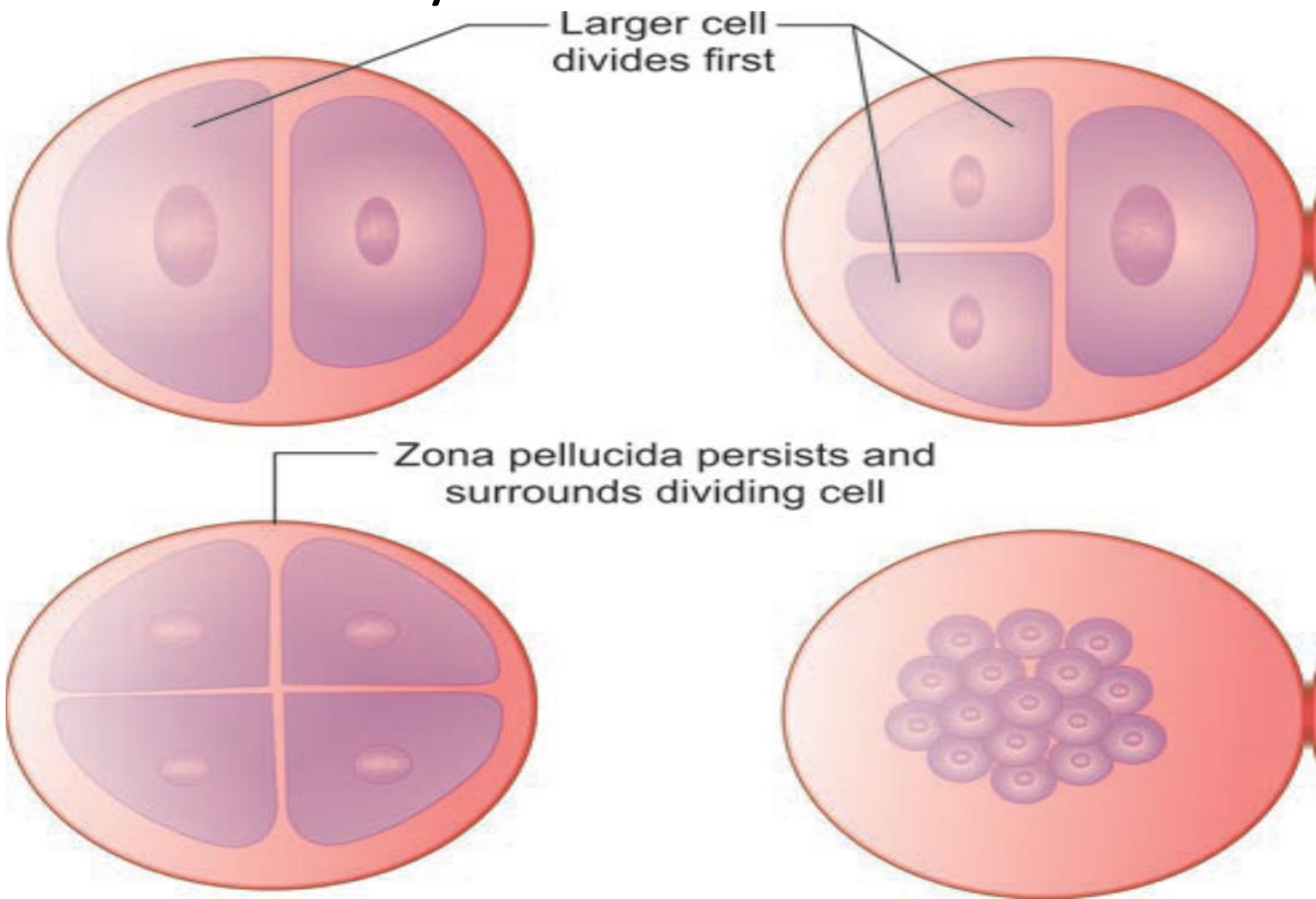

Two-cell stage


Four-cell stage

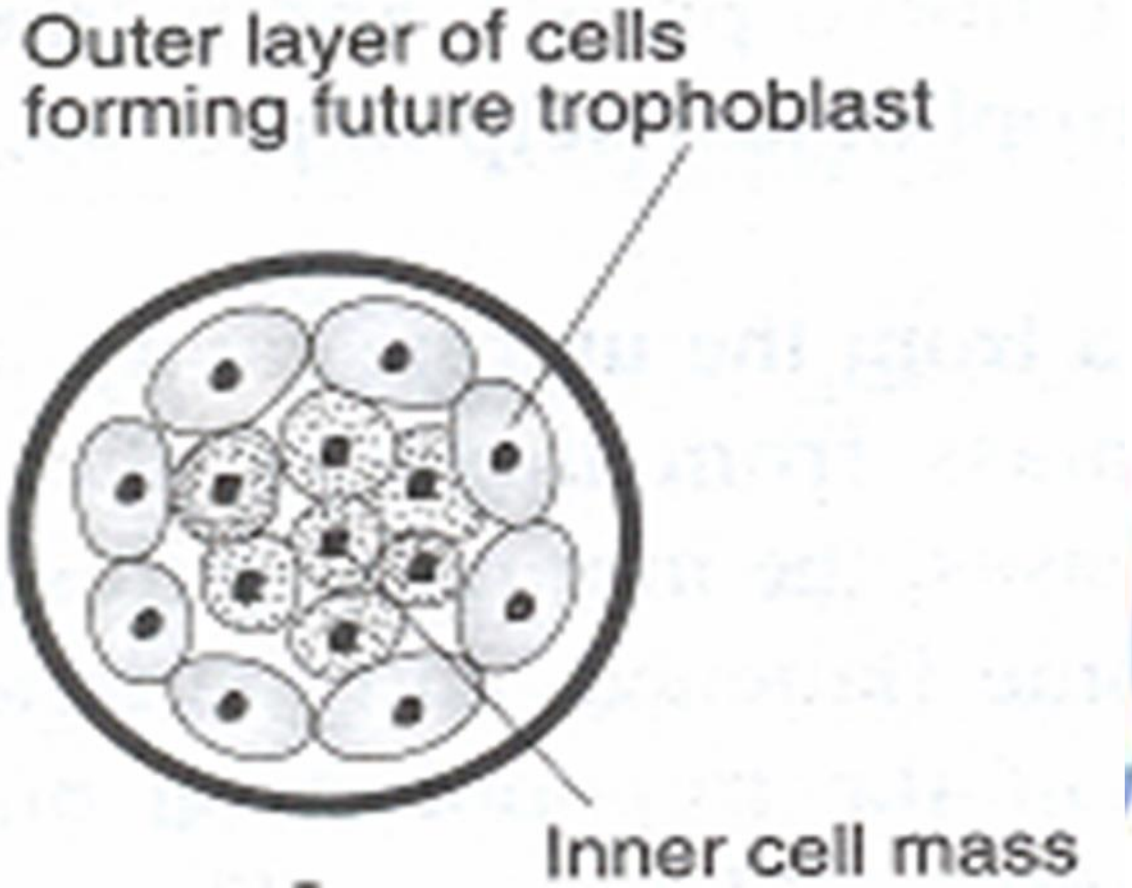

Morula

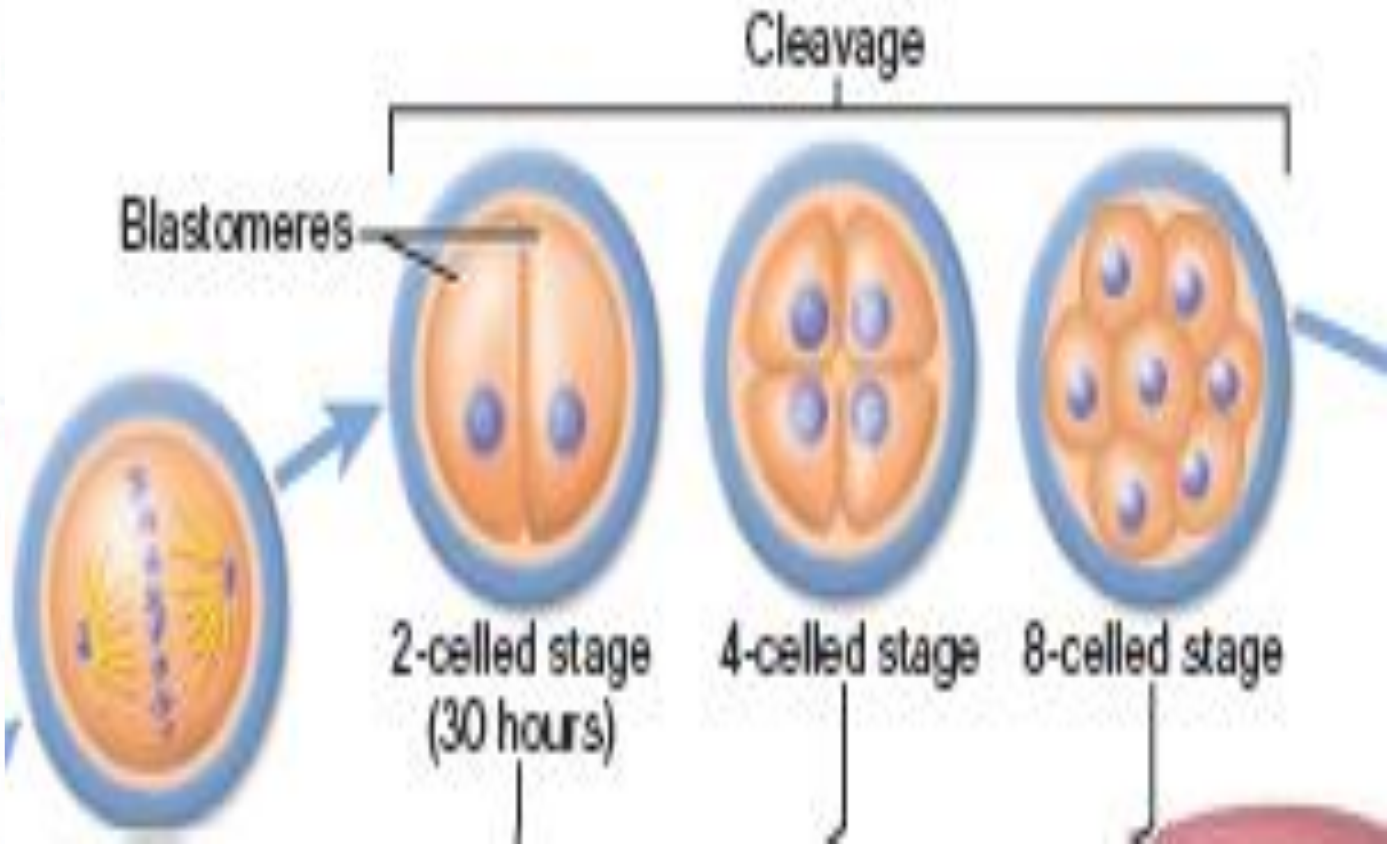
- ***Stages of cleaving egg:***
- During its journey through uterine tube, the cleaving egg passes through the following stages
- 1st cleavage division the zygote forms one large cell & one small cell
- next cleavage the larger cell divides first followed by smaller one

- ***Subdivisions of cleavage:***
- There are 3 subdivisions in cleavage
- They are:
- 1. Stage of compaction
- 2. Morula
- 3. Blastocyst

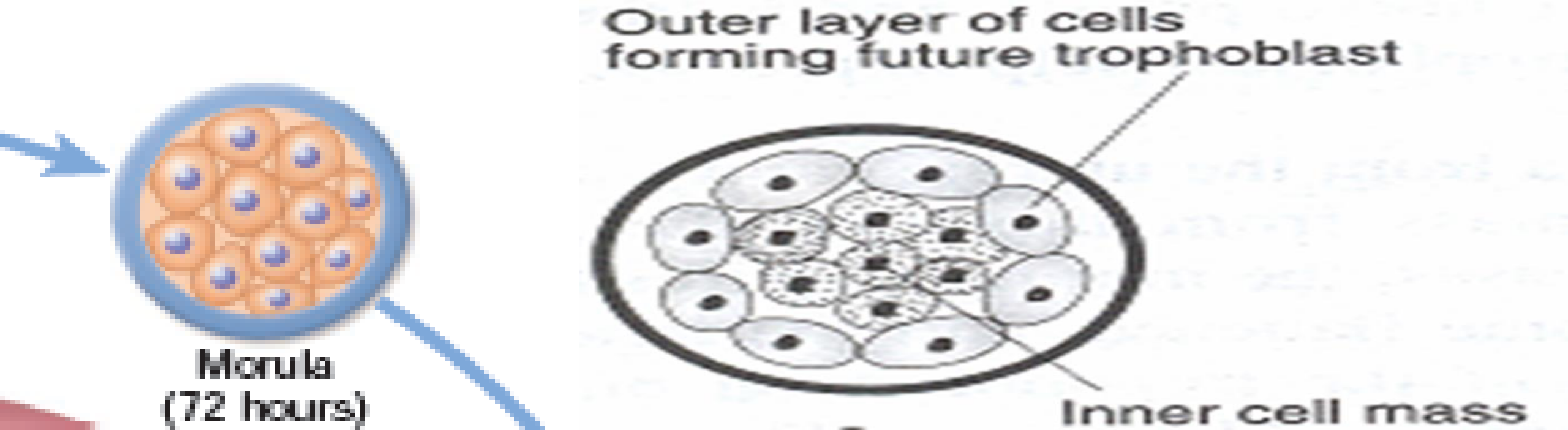


- **Stage of Compaction:**
- 3rd division 8-cell stage
- blastomeres change their shape & maximize their contact with each other to form a compact ball of cells held together by tight junctions.
- Segregates inner cells ,which communicates by gap junctions from outer cells.

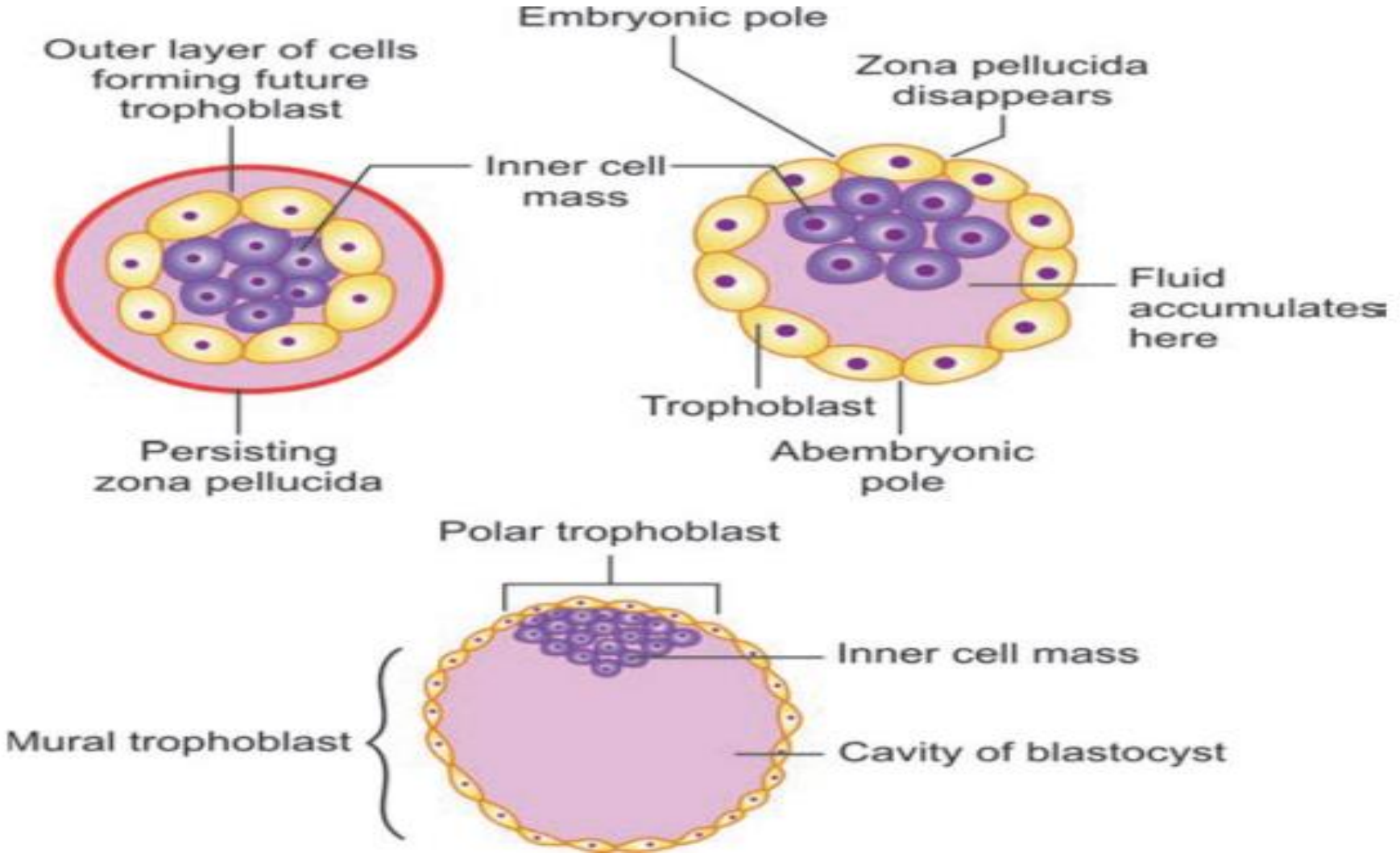




- **Morula**
- have 16 cells- 4th division
- looks like a mulberry
- 12 to 32 blastomeres is called a **morula**
- morula forms 3 or 4 days after fertilization & enters the uterus
- Still surrounded by the zona pellucida
- consists of an inner cell mass completely surrounded by an outer cell mass



- **Outer cell mass** give rise to a structure called ***trophoblast*** that forms the coverings of the embryo
- **inner cell mass** gives rise to the embryo proper called ***embryoblast***
- cells of the trophoblast help to provide nutrition to the embryo



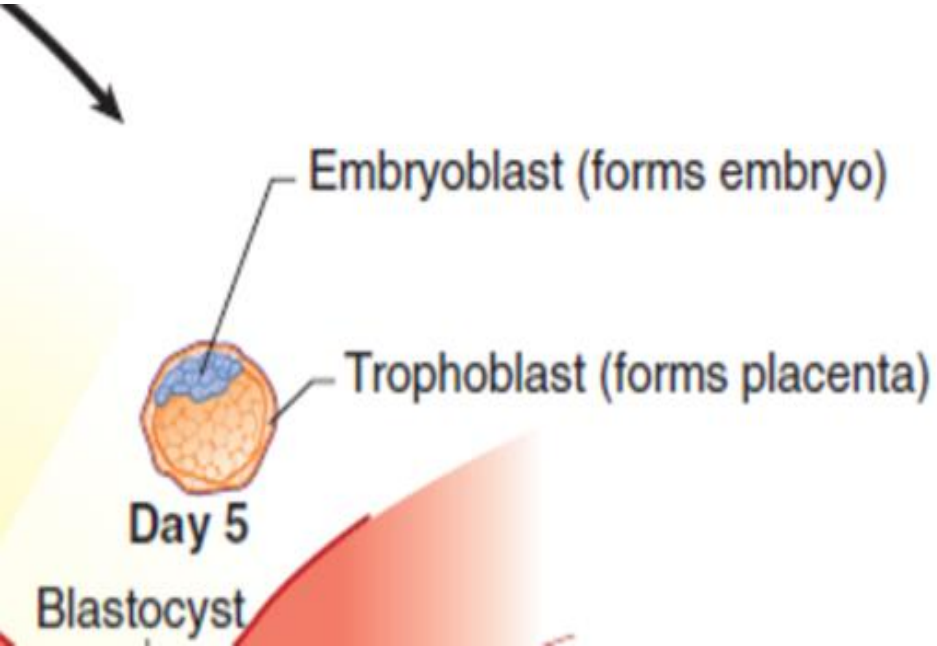
Blastocyst Formation

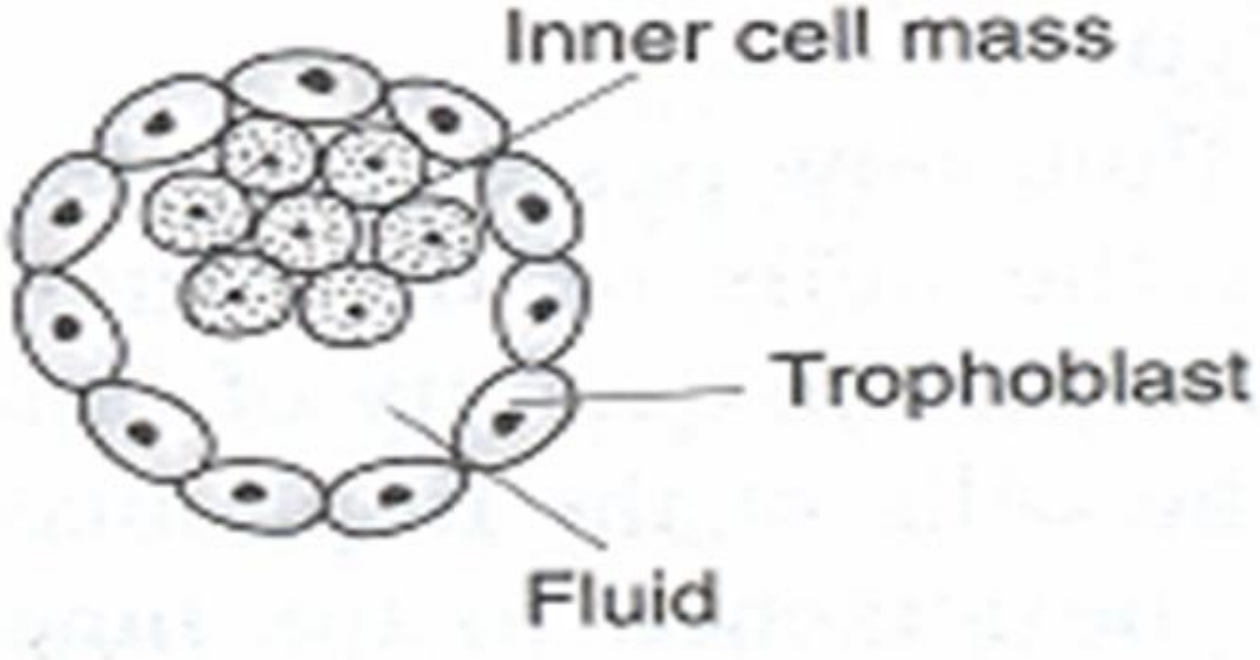
- Between 4th & 5th day & 32–64 cells stage

➢Morula still surrounded by Zona pellucida enter uterus

- fluid begins to penetrate through the zona pellucida into intercellular space of the inner cell mass
- a glycogen-rich secretion from the glands of the endometrium passes into the uterine cavity & enters the morula through the zona pellucida
- partially separates the cells of the inner cell mass from those of the trophoblast
- quantity of fluid increases, the morula acquires the shape of a cyst

- cells of the trophoblast become flattened & the inner cell mass gets attached to the inner side of the trophoblast on one side only
- **Blastocele;** single cavity form inside morula
- **Blastocyst;** embryo consisting blastocele
- **Embryoblast:** Cells of the inner cell mass which gives rise to the embryo
- **Trophoblast:** cell of outer cell mass which gives rise to the embryonic part of the placenta





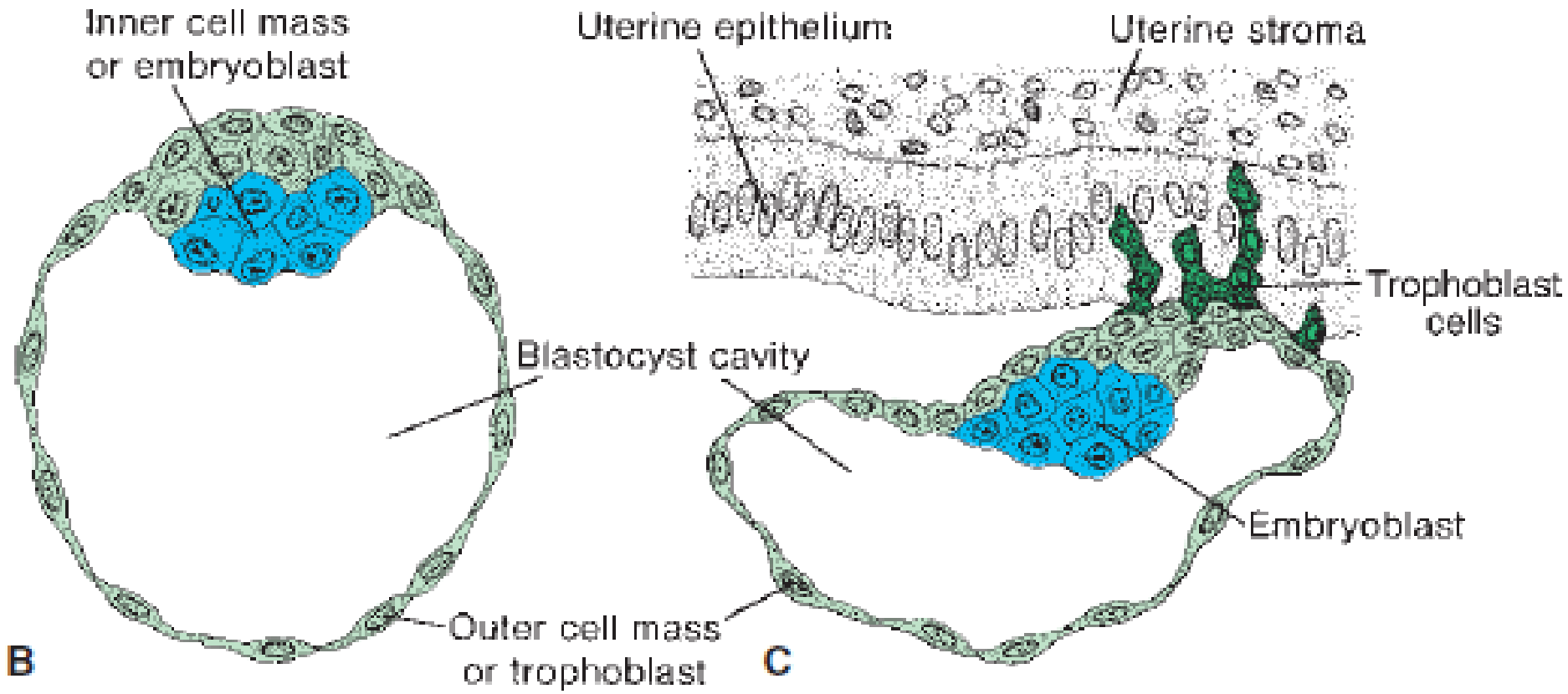
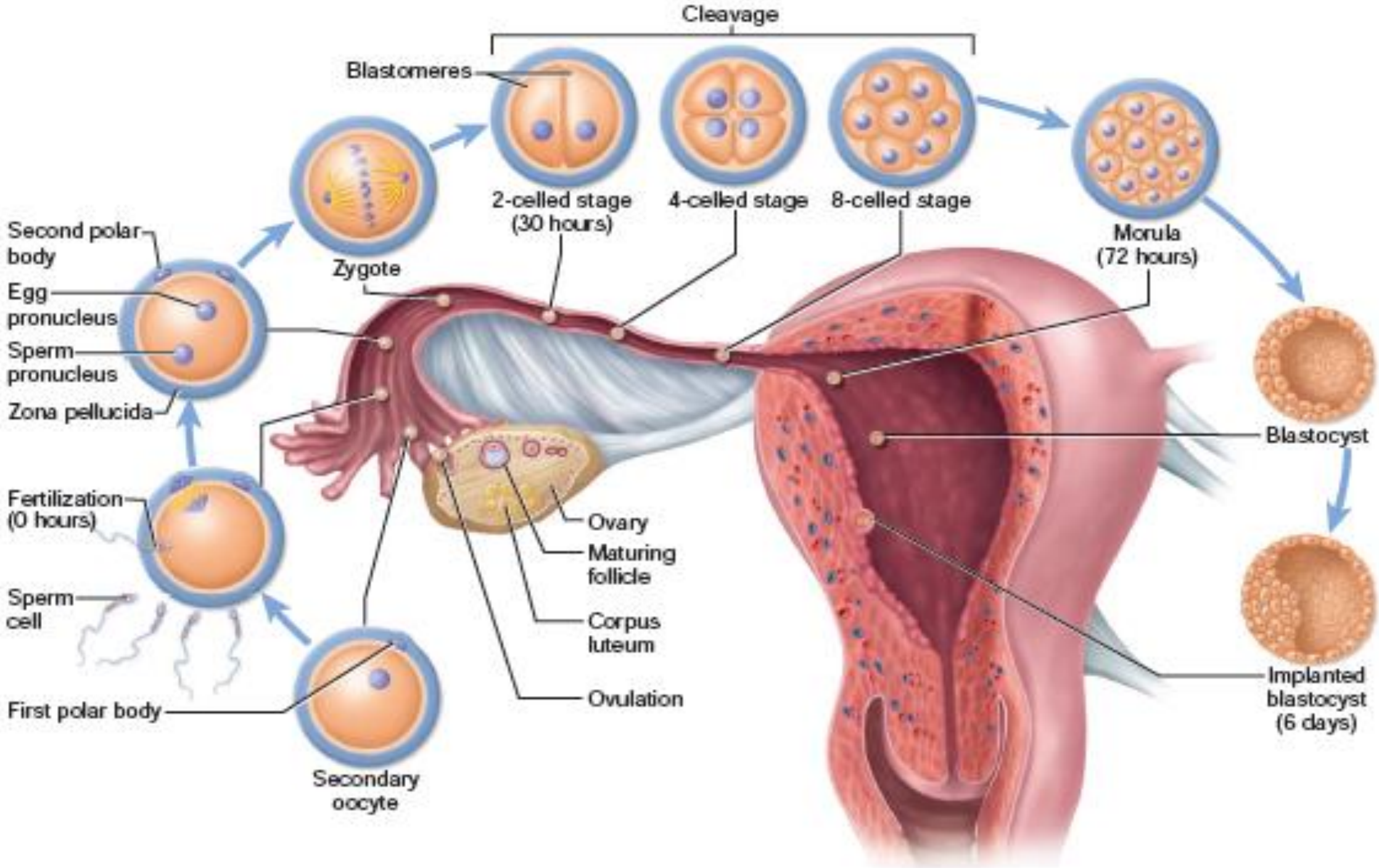
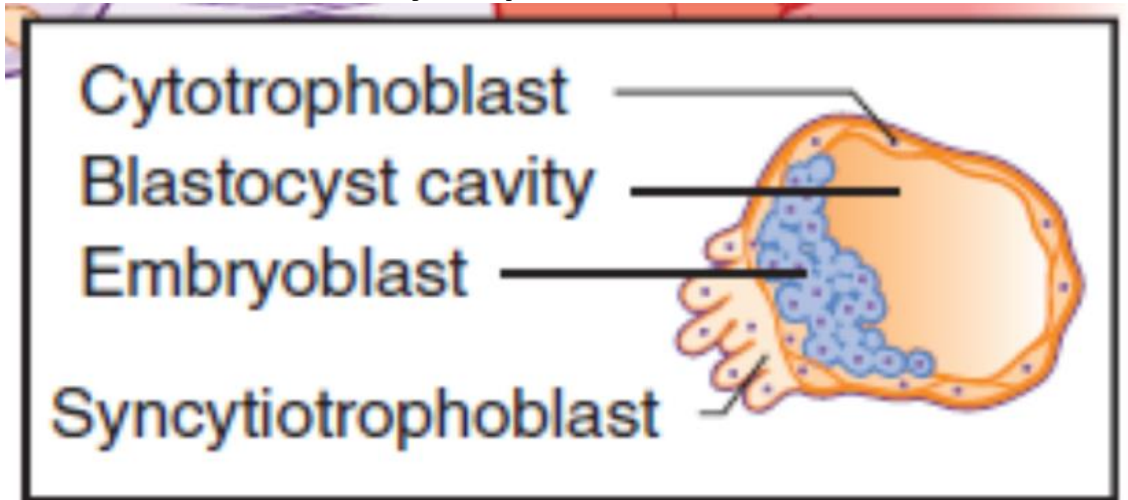


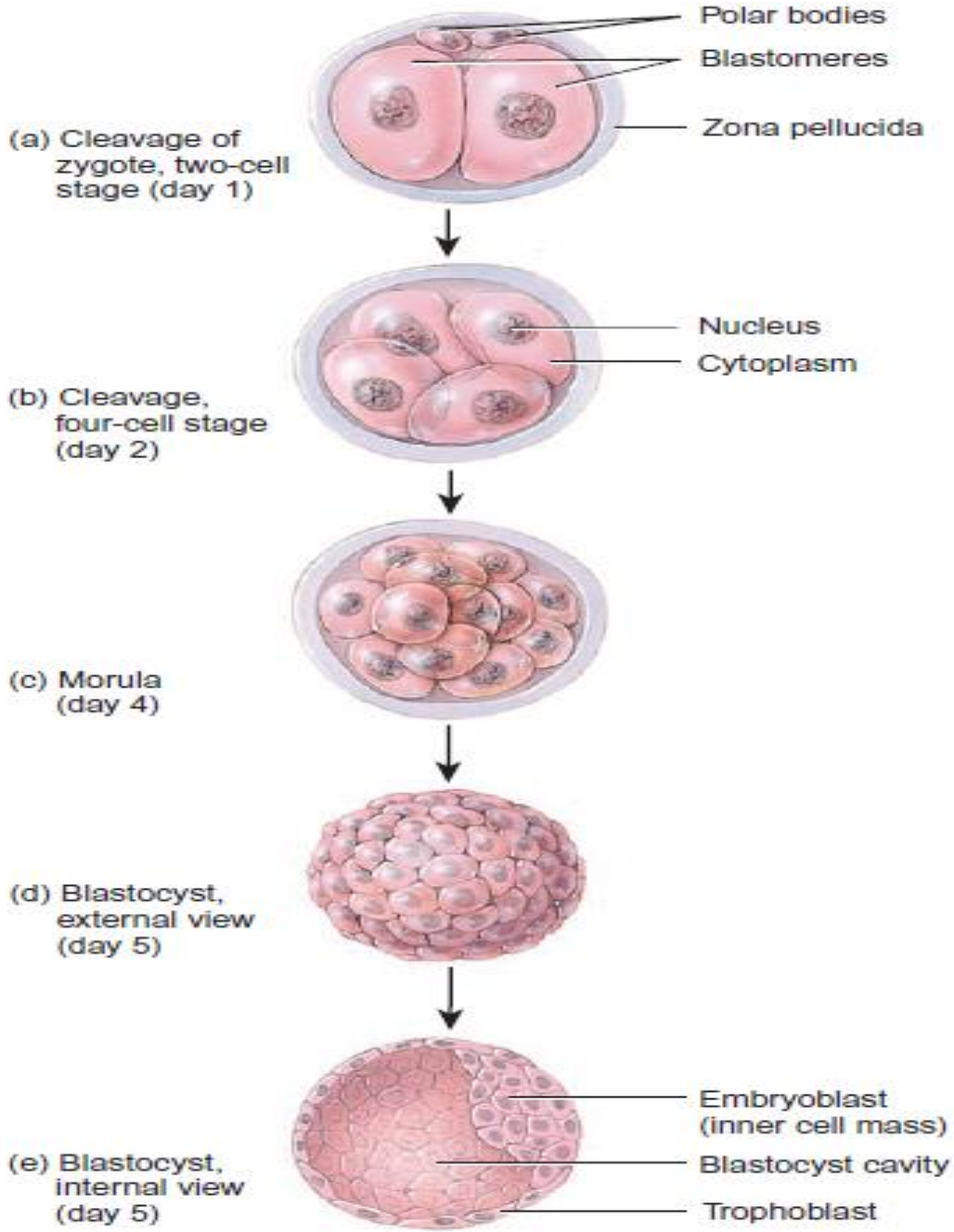
Figure 3.10 **A.** Section of a 107-cell human blastocyst showing inner cell mass and trophoblast cells. **B.** Schematic representation of a human blastocyst recovered from the uterine cavity at approximately 4.5 days. *Blue*, inner cell mass or embryoblast; *green*, trophoblast. **C.** Schematic representation of a blastocyst at the sixth day of development showing trophoblast cells at the embryonic pole of the blastocyst penetrating the uterine mucosa. The human blastocyst begins to penetrate the uterine mucosa by the sixth day of development.


Cleavage
Blastomeres
2-celled stage
(30 hours)
4-celled stage
8-celled stage
Morula
(72 hours)
Zygote
Second polar body
Egg pronucleus
Sperm pronucleus
Zona pellucida
Fertilization
(0 hours)
Sperm cell
First polar body
Secondary oocyte
Ovary
Maturing follicle
Corpus luteum
Ovulation
Blastocyst
Implanted blastocyst
(6 days)

- **Embryoblast** now projects into the **blastocystic cavity &** the **trophoblast** forms the wall of the **blastocyst**
- **zona pellucida** gradually degenerates & disappears on the 5th day
- Shedding of the zona pellucida permits the blastocyst to increase rapidly in size
- the blastocyst attaches to the endometrial epithelium
- Trophoblast starts to proliferate rapidly & differentiates into 2 layers

1. An inner layer of **cytotrophoblast**
2. An outer layer of **syncytiotrophoblast** (multinucleated cytoplasmic mass in which no cell boundaries can be observed)




Polar bodies
Blastomeres
Zona pellucida
(a) Cleavage of zygote, two-cell stage (day 1)
Nucleus
Cytoplasm
(b) Cleavage, four-cell stage (day 2)
(c) Morula (day 4)
(d) Blastocyst, external view (day 5)
Embryoblast (inner cell mass)
Blastocyst cavity
Trophoblast
(e) Blastocyst, internal view (day 5)

- **Function of the Zona Pellucida**
- zona pellucida is a specialized extracellular matrix surrounding the developing oocyte
- It is formed by secretions from the oocyte and the granulosa cells

1) function of the zona pellucida is to prevent implantation of the blastocyst at an abnormal site
2) Zona pellucida holds the blastomeres of the early embryo together. The developing embryo is genetically different from the mother.

- This may evoke immunological reactions if embryonic and maternal tissues come in contact

3) Zona pellucida is responsible for the zona reaction that prevents any additional spermatozoa from entering the fertilized ovum (zygote)

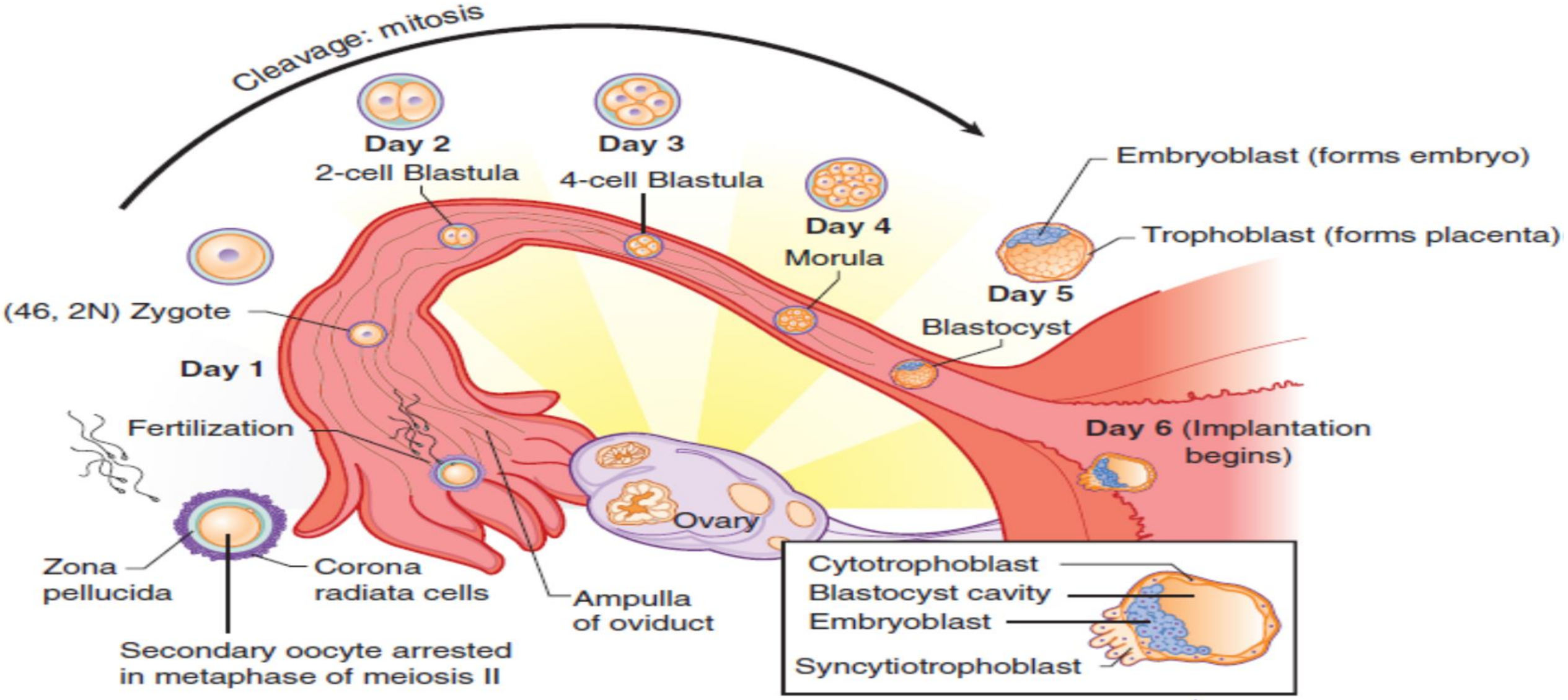
4) The glycoprotein of the zona pellucida is responsible for induction of the acrosomal reaction

- The zona pellucida allows only a sperm of the same species to fertilize the oocyte

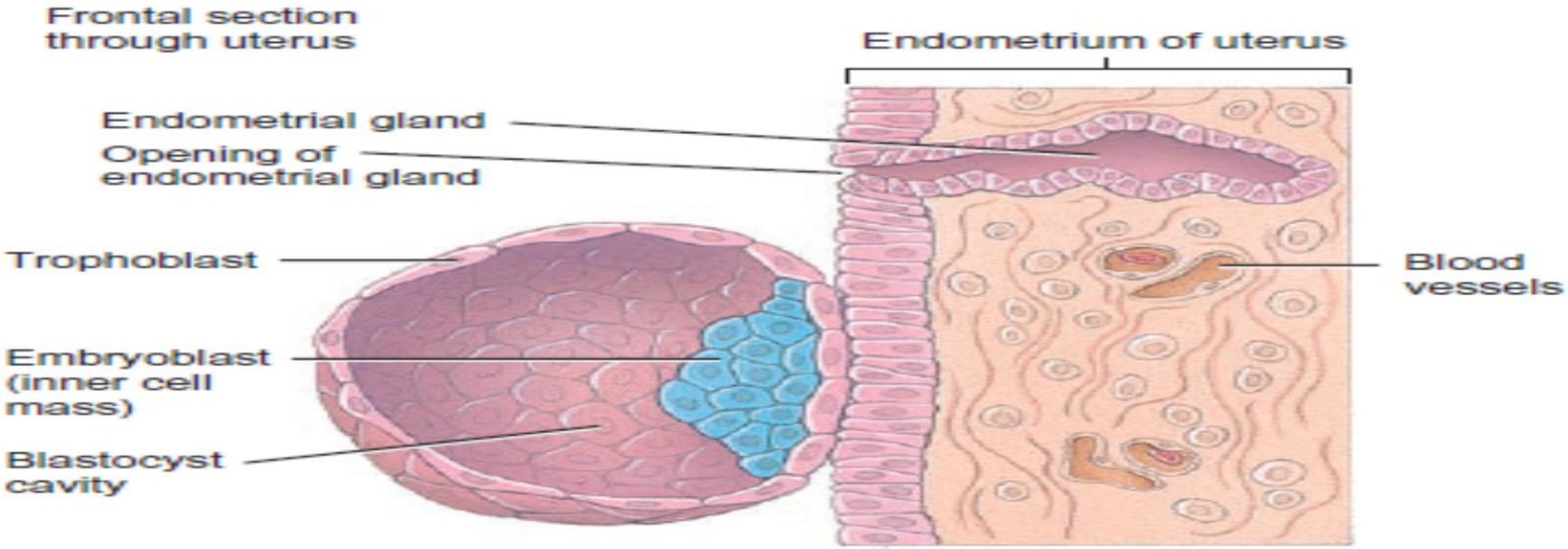
5) Sperms of other species cannot pass through the zona pellucida

- There are four types of zona pellucida glycoproteins ZP1, ZP2, ZP3 and ZP4 which have different roles in different phases of fertilization
- ZP2 plays an important role in sperm binding, gamete recognition, penetration and prevention of polyspermy

- 6. During early stages of cleavage it acts as a porous filter through which certain substances secreted by the uterine tube can reach the embryo
- 7. It facilitates differentiation of trophoblastic cells



- **Implantation**
- It is the process of attachment of blastocyst to uterine endometrium & subsequent invasion (embedding) of blastocyst (conceptus) into the uterine endometrium in placental animals
- mucosa of the uterus is in the secretory phase
- Usually happen on day 6 after fertilization
- Blastocyst loosely attaches to the endometrium of the uterus
- 7th day the blastocyst attaches to the endometrium more firmly
- endometrial glands in the vicinity enlarge, & the endometrium becomes more vascular by forming new blood



(b) Frontal section through endometrium of uterus and blastocyst, about 6 days after fertilization

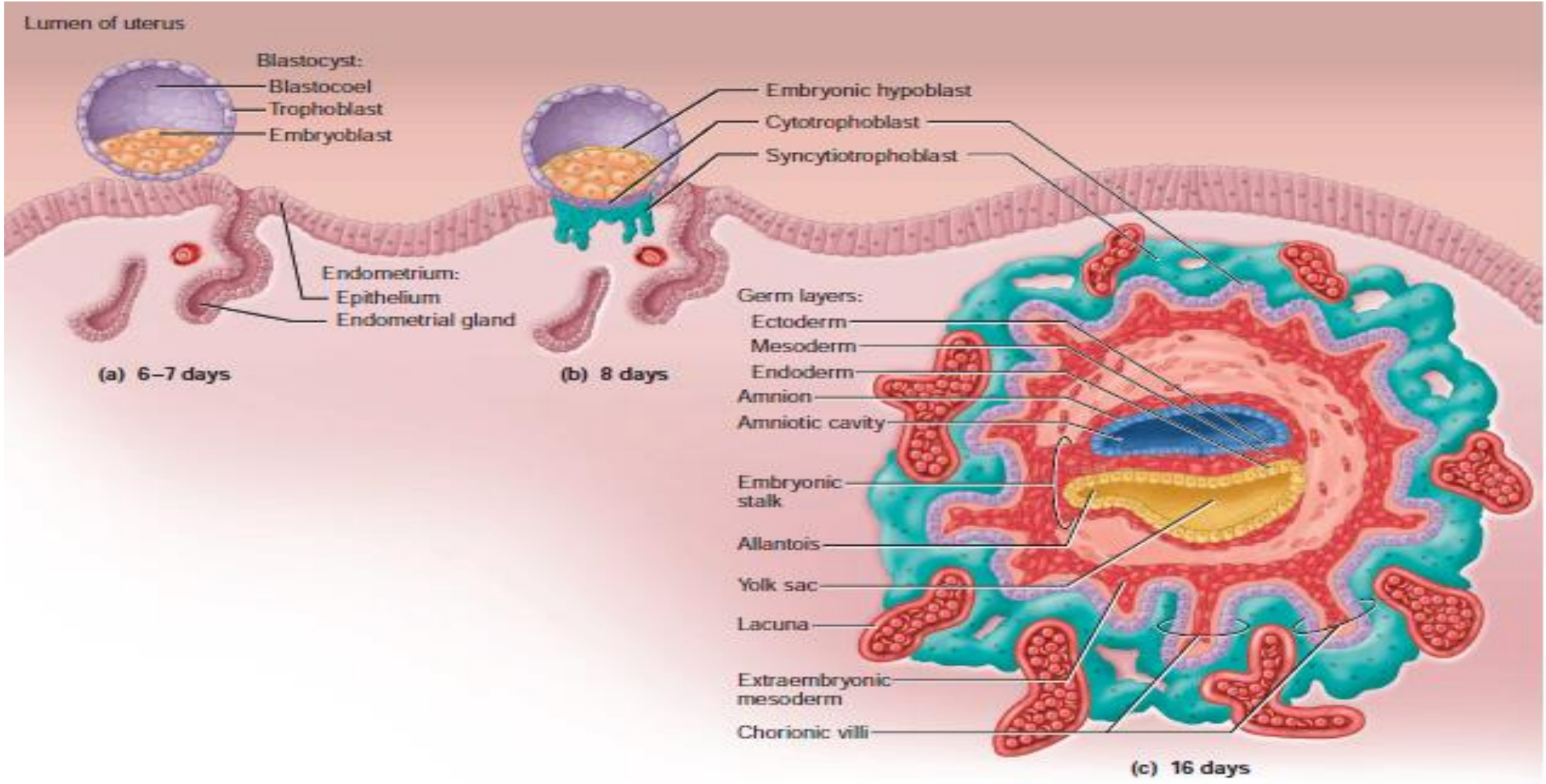
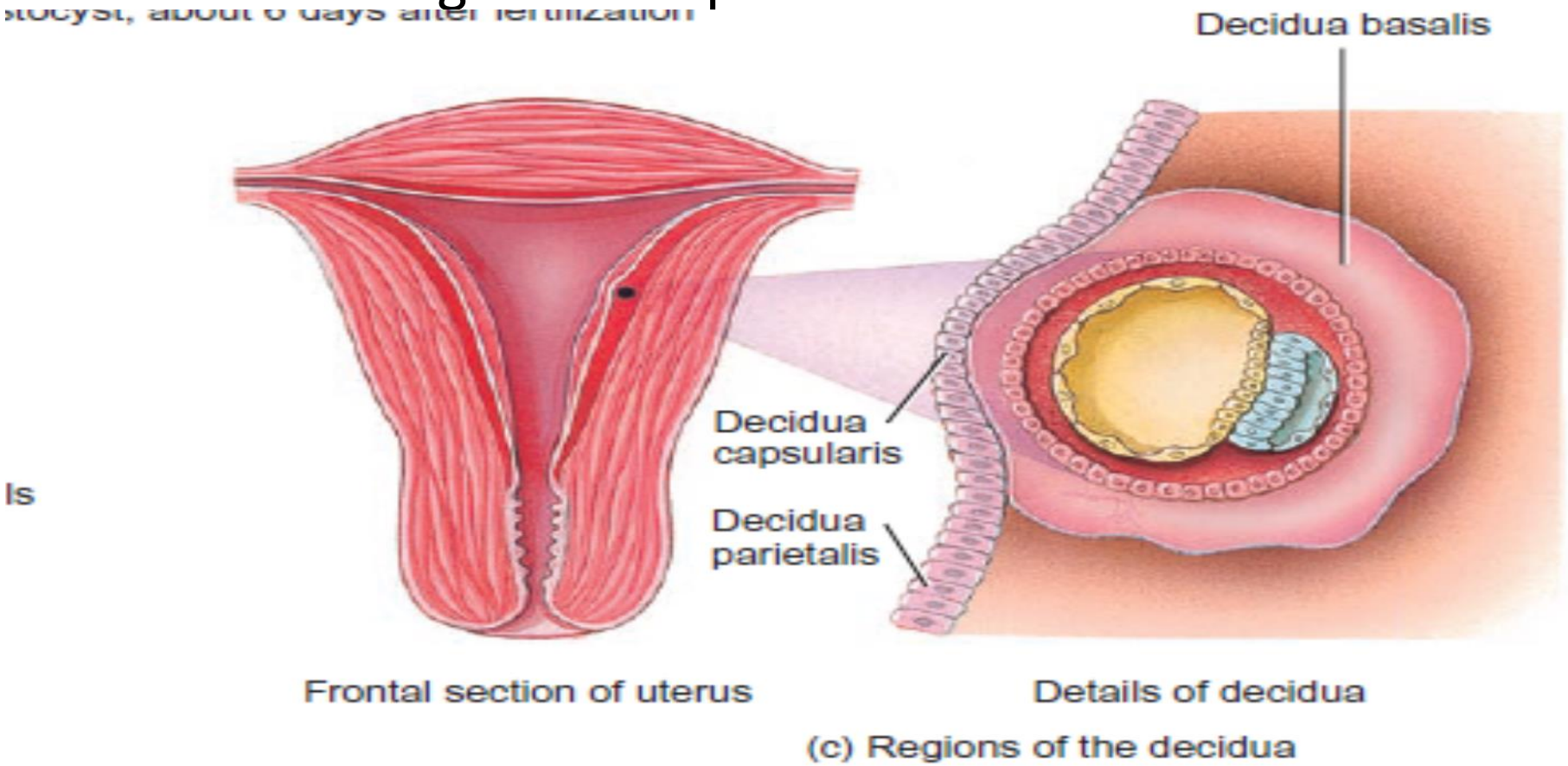
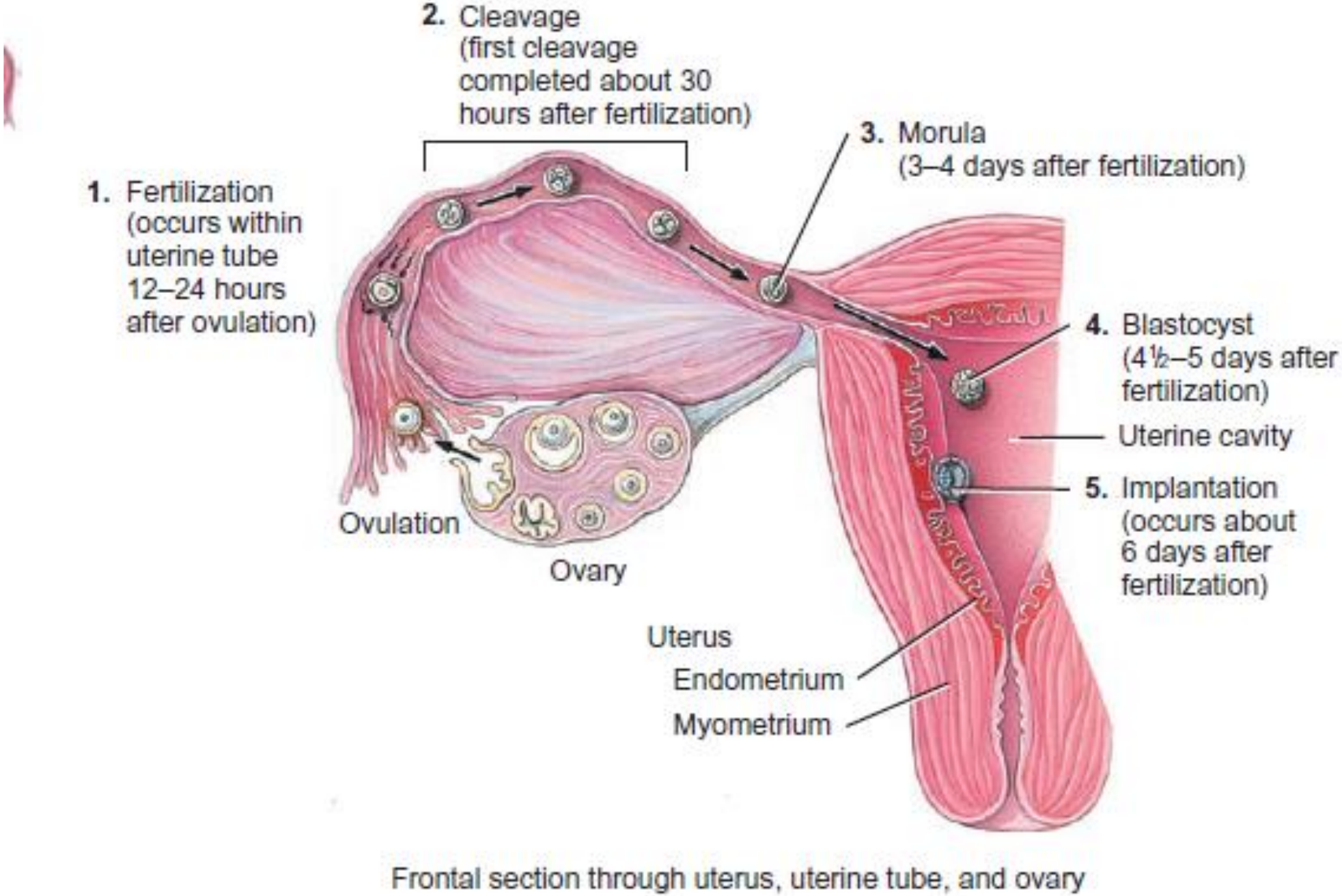


Figure 4.3 Implantation. (a) Structure of the blastocyst 6 to 7 days after ovulation, when it first adheres to the uterine wall. (b) The progress of implantation about 1 day later. The syncytiotrophoblast has begun growing rootlets, which penetrate the endometrium. (c) By 16 days, the conceptus is completely covered by endometrial tissue. The embryo is now flanked by a yolk sac and amnion and is composed of three primary germ layers.

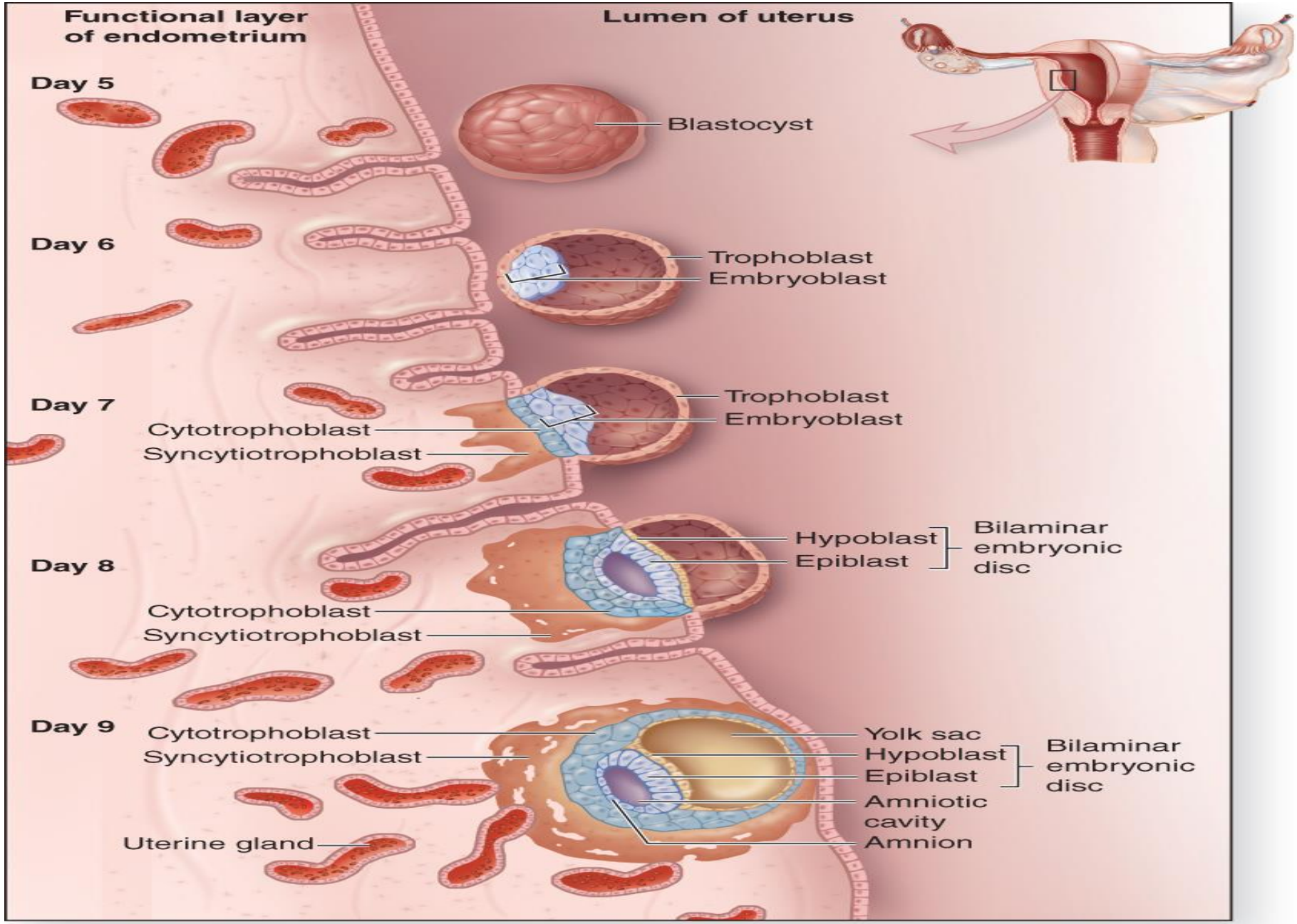
- After implantation, the functional layer of the endometrium is known as the **decidua**
- Different regions of the decidua have different names based on their positions relative to the site of the implanted blastocyst
- **Decidua basalis**
- is the portion of the endometrium beneath the implanting embryo
- It provides large amounts of glycogen and lipids for the developing embryo and fetus and later becomes the maternal part of the placenta
- **decidua capsularis**
- is the portion of the endometrium that will cover the embryo after it implants in the endometrium

- **decidua parietalis**
- is the remaining modified endometrium that lines the noninvolved areas of the rest of the uterus
- As embryo and later fetus enlarges and pushes into the uterine cavity becomes thin and disappears as the enlarged fetus fills the uterine cavity and pushes against the surrounding decidua parietalis





Frontal section through uterus, uterine tube, and ovary


Functional layer of endometrium
Lumen of uterus
Day 5
Blastocyst
Day 6
Trophoblast
Embryoblast
Day 7
Trophoblast
Embryoblast
Cytotrophoblast
Syncytiotrophoblast
Day 8
Hypoblast
Epiblast
Bilaminar embryonic disc
Cytotrophoblast
Syncytiotrophoblast
Day 9
Cytotrophoblast
Syncytiotrophoblast
Yolk sac
Hypoblast
Epiblast
Bilaminar embryonic disc
Amniotic cavity
Amnion
Uterine gland

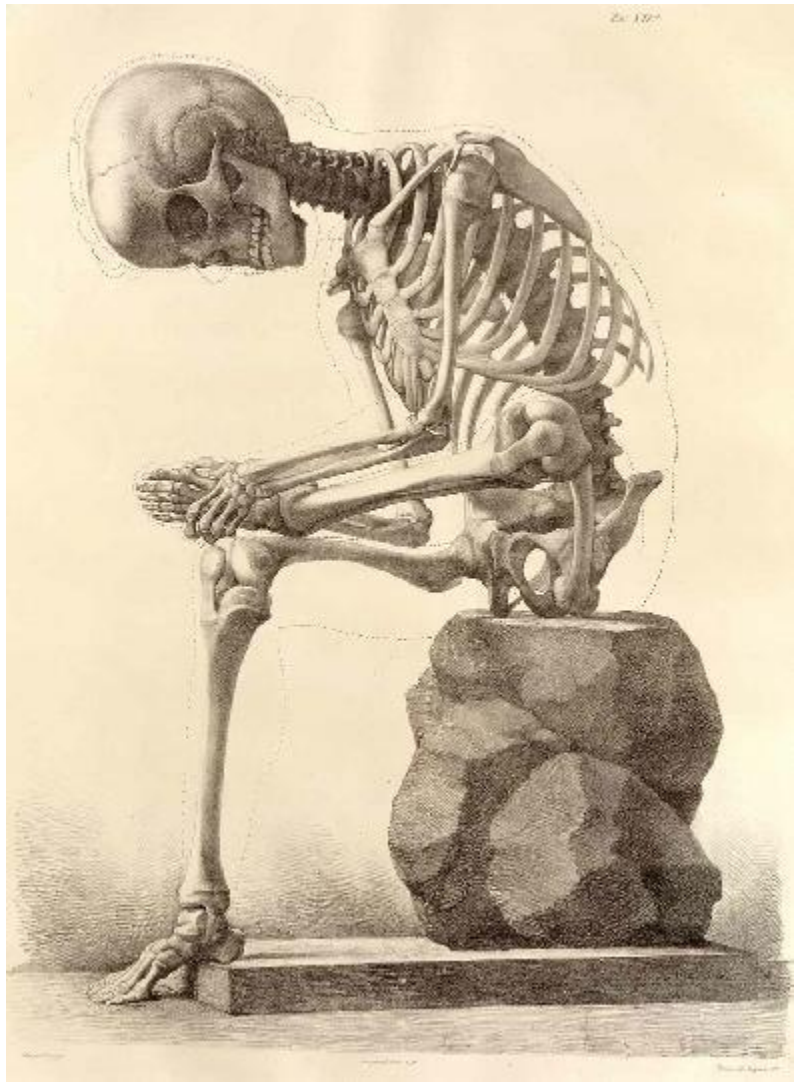
- **CLINICAL Application**
- **Ectopic Pregnancy**
- development of an embryo or fetus outside the uterine cavity occurs when movement of the fertilized ovum through the uterine tube is impaired by :
- scarring due to prior tubal infection,
- decreased movement of uterine tube smooth muscle,
- abnormal tubal anatomy
- common site is the uterine tube, may also occur in the ovary, abdominal cavity, or uterine cervix
- Premature hatching of the zona pellucida

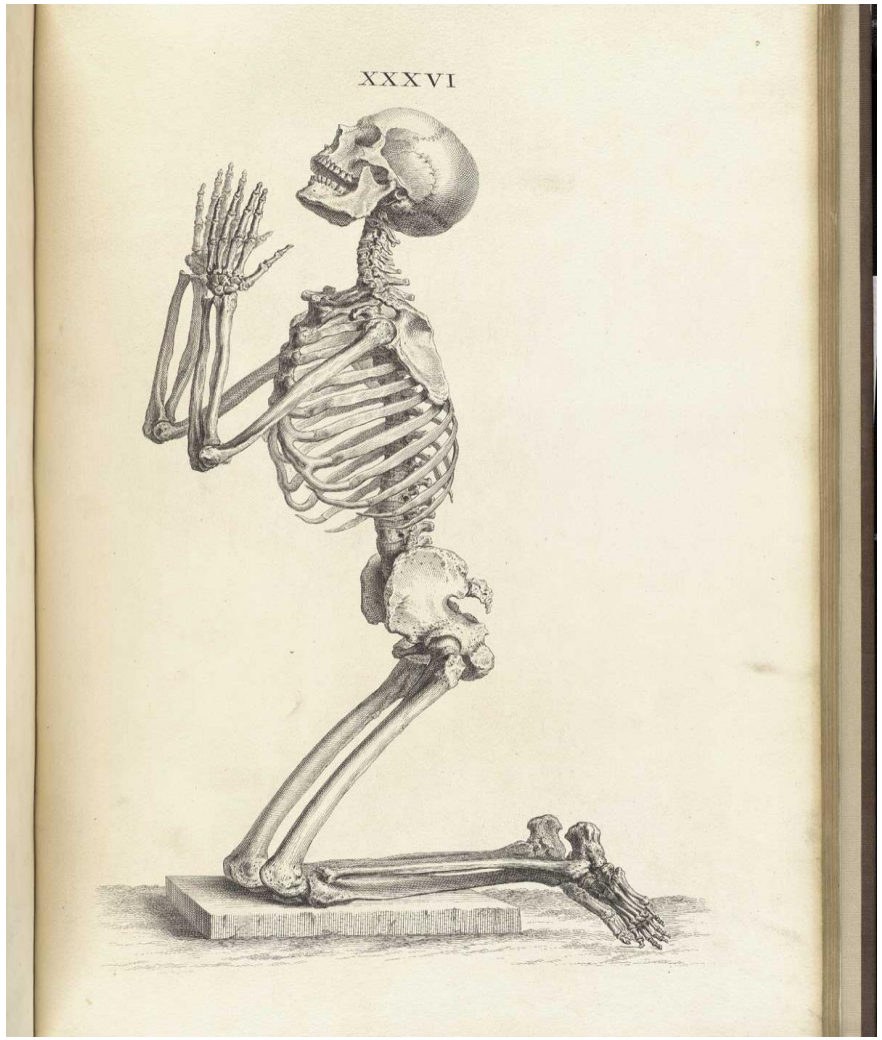
- In vitro fertilization (IVF) of oocytes and transfer of the cleaving zygotes into the uterus have provided an opportunity for many women who are sterile (e.g., owing to tubal occlusion) to bear children
- The first of these in vitro fertilization babies was born in 1978. Since then, *approximately* two million children have been born after an in vitro fertilization procedure

- ***Hydatidiform mole:***
- It is a form of abnormal blastocyst that resulted from development of trophoblast/outer cell mass that forms the placenta
- There will be little or no embryonic tissue.
- The moles secrete high levels of HCG and can produce benign (invasive mole) or malignant (*Choriocarcinoma*) tumors
- Genetic analysis of moles indicates diploid chromosomes of paternal origin.
- This results from fertilization of an oocyte without nucleus and duplication of paternal chromosomes to maintain diploid state.
- Paternal genes regulate the development of trophoblast

- Nondisjunction of chromosomes 13 and 18 results in **Patau syndrome (trisomy-13)** and **Edward syndrome (trisomy-18),** respectively
- Affected individuals have three copies of the respective chromosome
- Nearly all foetuses with these trisomies die before birth
- Live-born infants with these syndromes are severely deformed & fewer than 5% survive for one year

- Quiz
- 1. what is the importance of the inner cell mass of the cleaving embryo
- 2. What is the cellular origin of the syncytiotrophoblast of the implanting embryo?
- 3. What is fertilization? Give steps and results of the Fertilization
- 4. Define implantation and its stages
- 5. How is the blastocyst formed and what is the fate of the parts of the blastocyst?




XXXVI