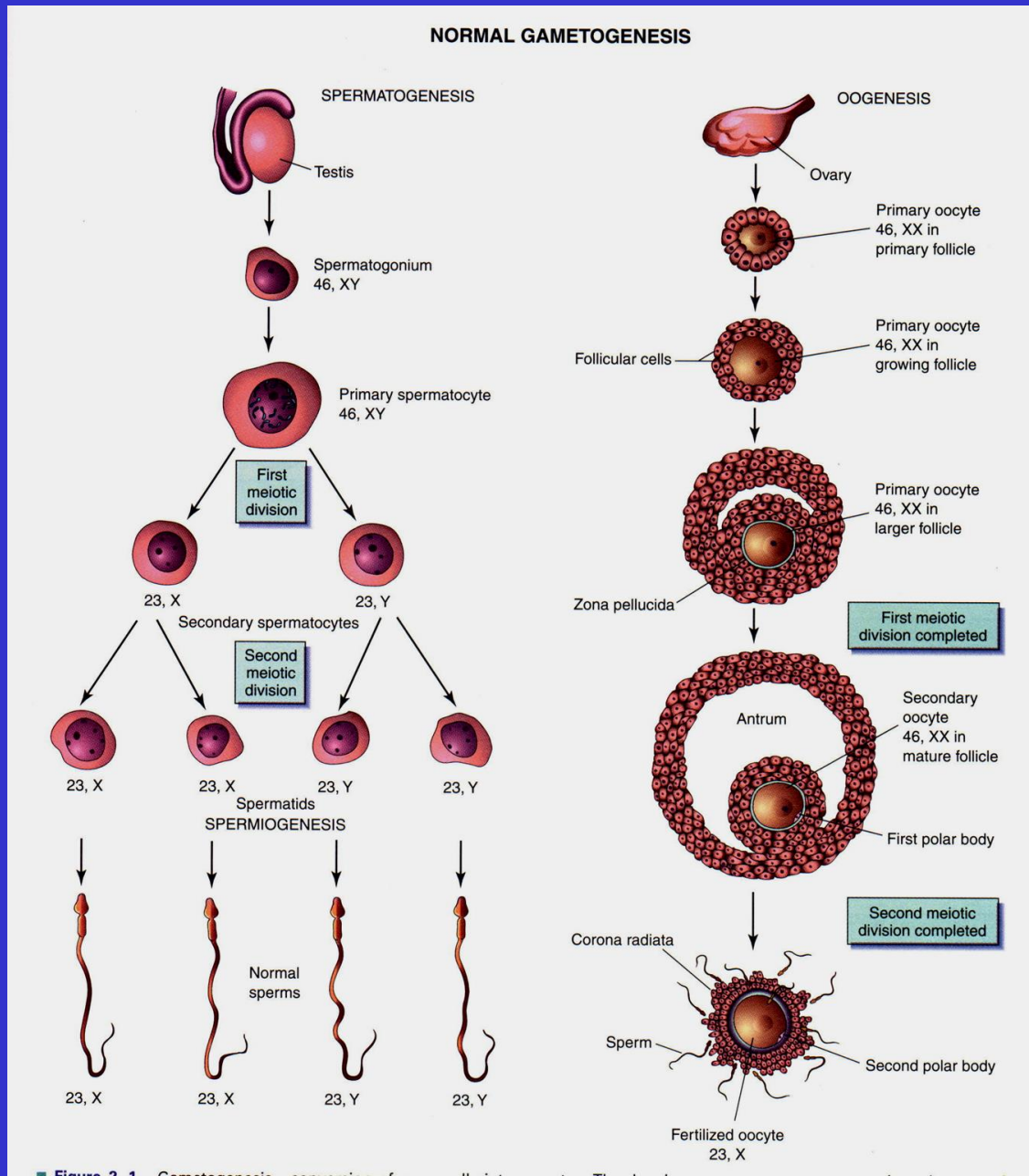
The Beginning of Human Development

C.L. Chien, 2002

Gametogenesis
(gamete formation)
gametes—sperms and oocytes

Spermatogensis
(Sperm formation)

Oogenesis
(Oocyte formation)



Spermatogenesis

Sertoli cells

-- support and nurture the germ cells

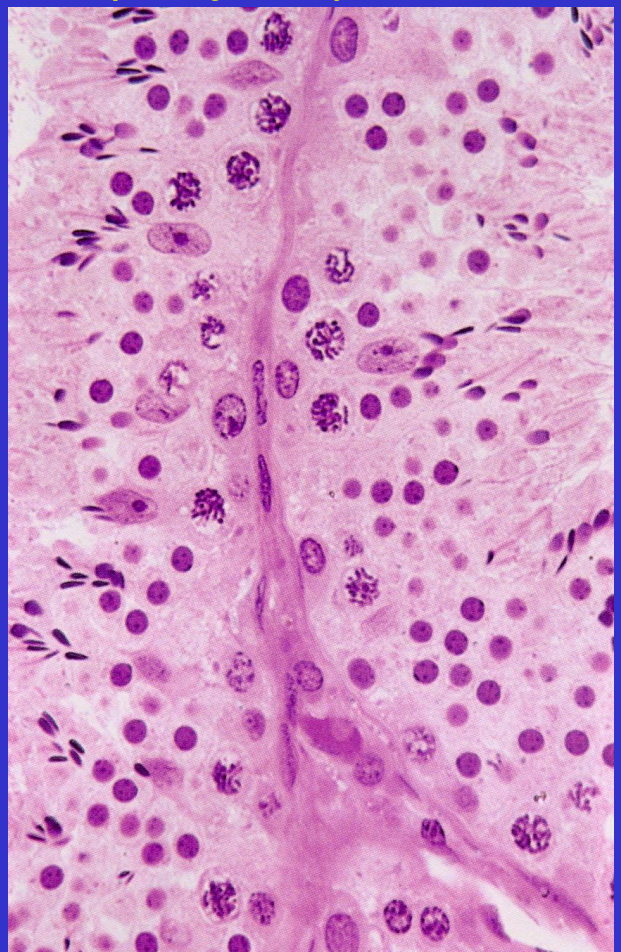
-- regulation of spermatogenesis

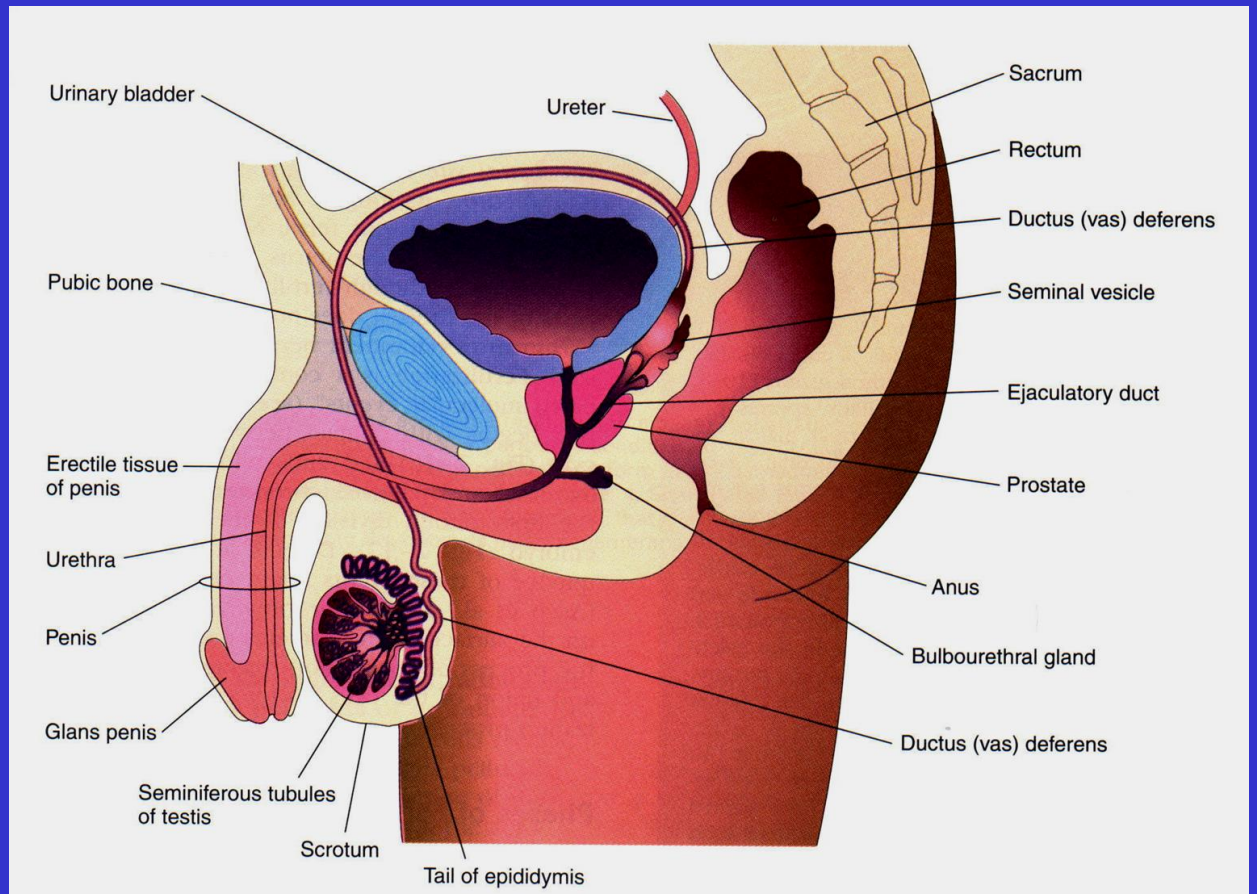
Mature sperm

-- a free-swimming, actively motile cell

-- enter the lumina of seminiferous tubules

-- epididymis (store and become mature) → ductus deferens→ urethra





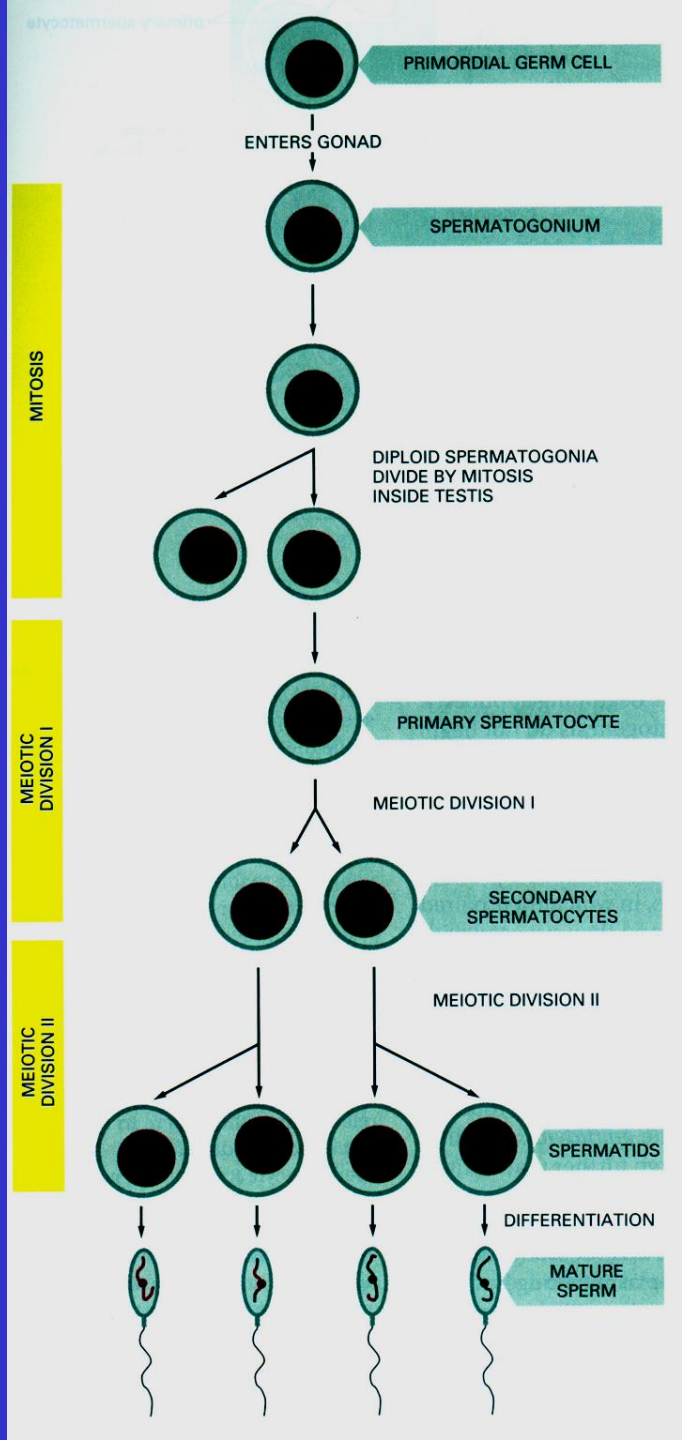
■ **Figure 2–15.** Sagittal section of the male pelvis primarily to show the male reproductive system.

Spermatogenesis

mitosis
spermatogonia -------- primary spermatocyte (46, XY)

meiosis I
---------- secondary spermatocytes (haploid; 23, X; 23, Y)

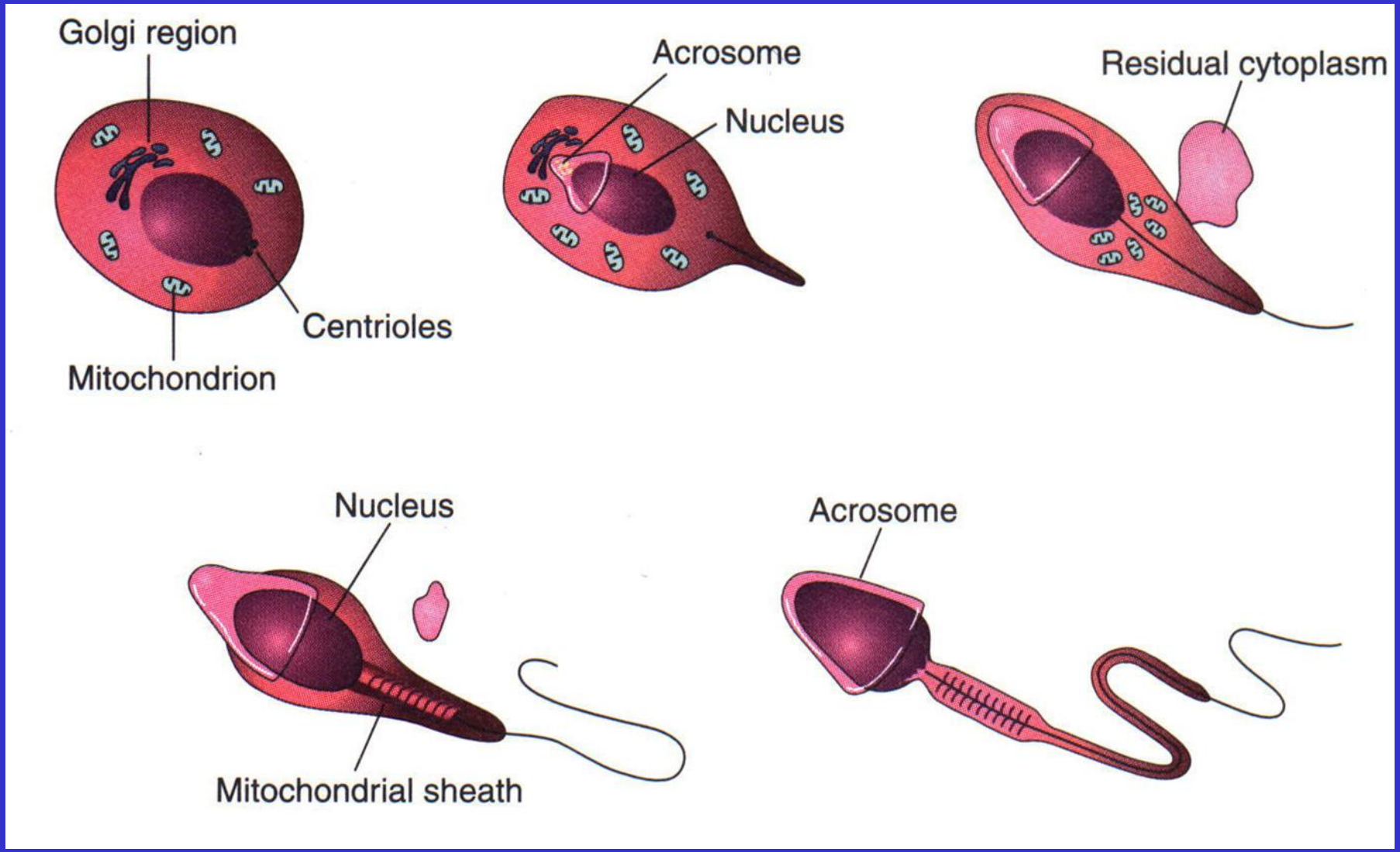
meiosis II
---------- spermatids (haploid, x4) → **spermiogenesis (transformation)** → sperms (x4)



Spermiogenesis (transformation)

Mature sperm

-- a free-swimming, actively motile cell



Structure of mature sperm:

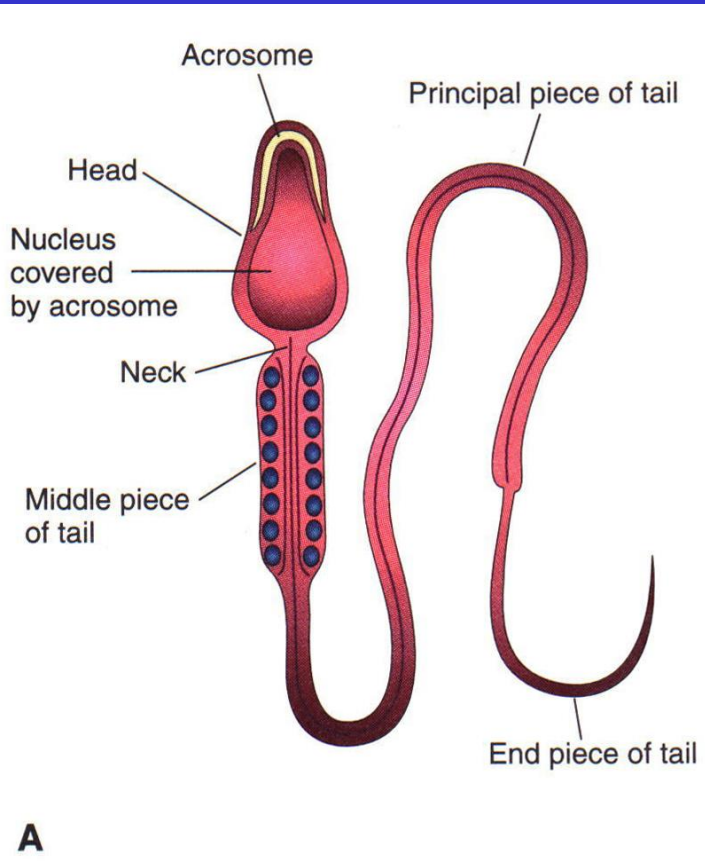
-- a *head* contains haploid nucleus

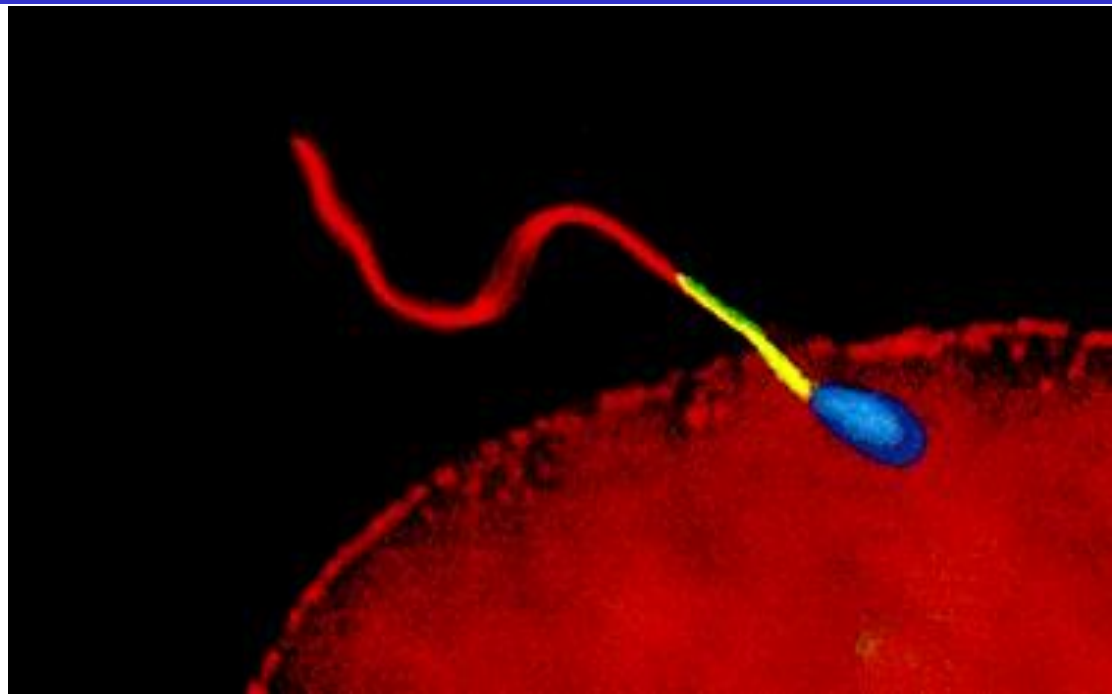
-- acrosome (acrosin, one of the enzymes); produced by Golgi apparatus

-- facilitate sperm penetration of corona radiata and zona pellucida during fertilization

-- a *tail* contains three segments:

1. middle piece (mid-piece): contains mitochondria
2. principle piece
3. end piece: provides the motility of the sperm





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Xenogenic spermatogenesis

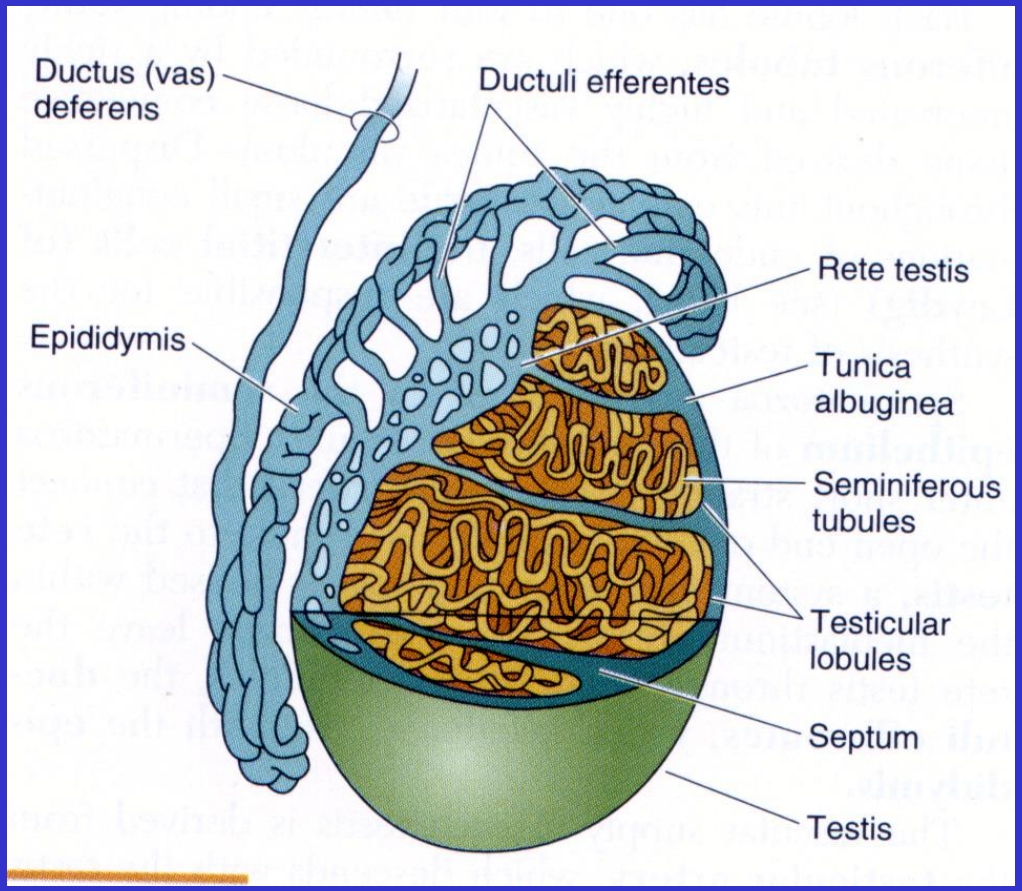
referred to the possibility of transplantation of spermatogonial stem cells across species

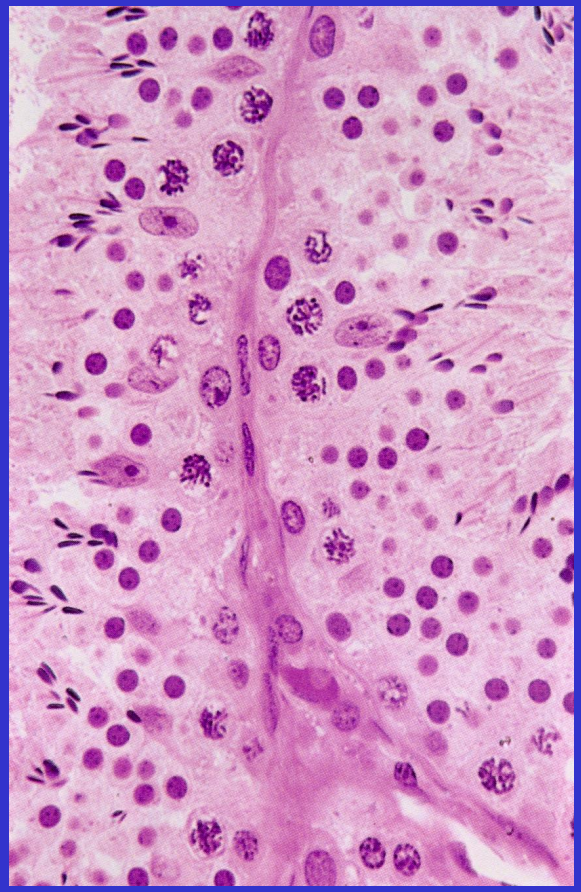
e.g: rat to mouse

ENU mice

Mutagenesis of mouse spermatogonia by injection of **N-ethyl-N-nitrosourea (ENU)**

Mouse models for human diseases



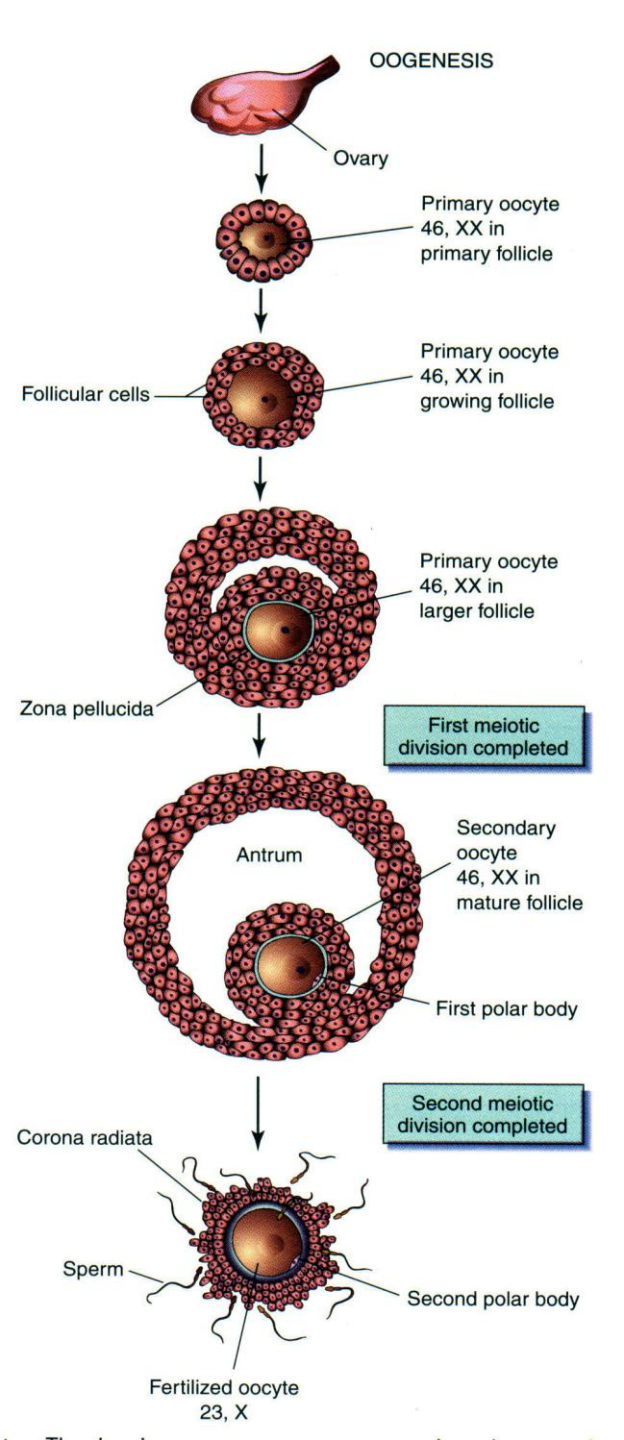


Oogenesis: Oogonia → mature oocytes

Prenatal maturation of oocytes

mitosis meiosis I
oogonia ---------- primary oocytes (before birth) ----------

----- primordial follicle (primary oocyte + follicular epithelial cell)
→ primary follicle (primary oocyte + cuboidal to collumnar epithelial cells)
→ secondary follicle [primary oocyte surrounding by zona pellucida + cuboidal follicular cells (> 1 layer)]
→ oocyte maturation inhibitor ***(OMI)***; to keep the meiosis arrested)
→primary oocytes remain in suspended prophase I (dictyotene核網期)



Postnatal maturation of oocyte

-- no primary oocytes form after birth

-- long duration of meiosis I → nondisjunction may occur (in older ladies)

Primary oocyte (46+ XX)

1. increase in size (in primary follicle → growing f. → larger f. → mature f.)
2. completes the 1st meiotic division, shortly before ovulation
3. about 2 million in newborn female infant

Secondary oocyte (23+ X)

-- begins the 2nd meiotic division (metaphase)

-- 1st polar body (later on, degeneration)

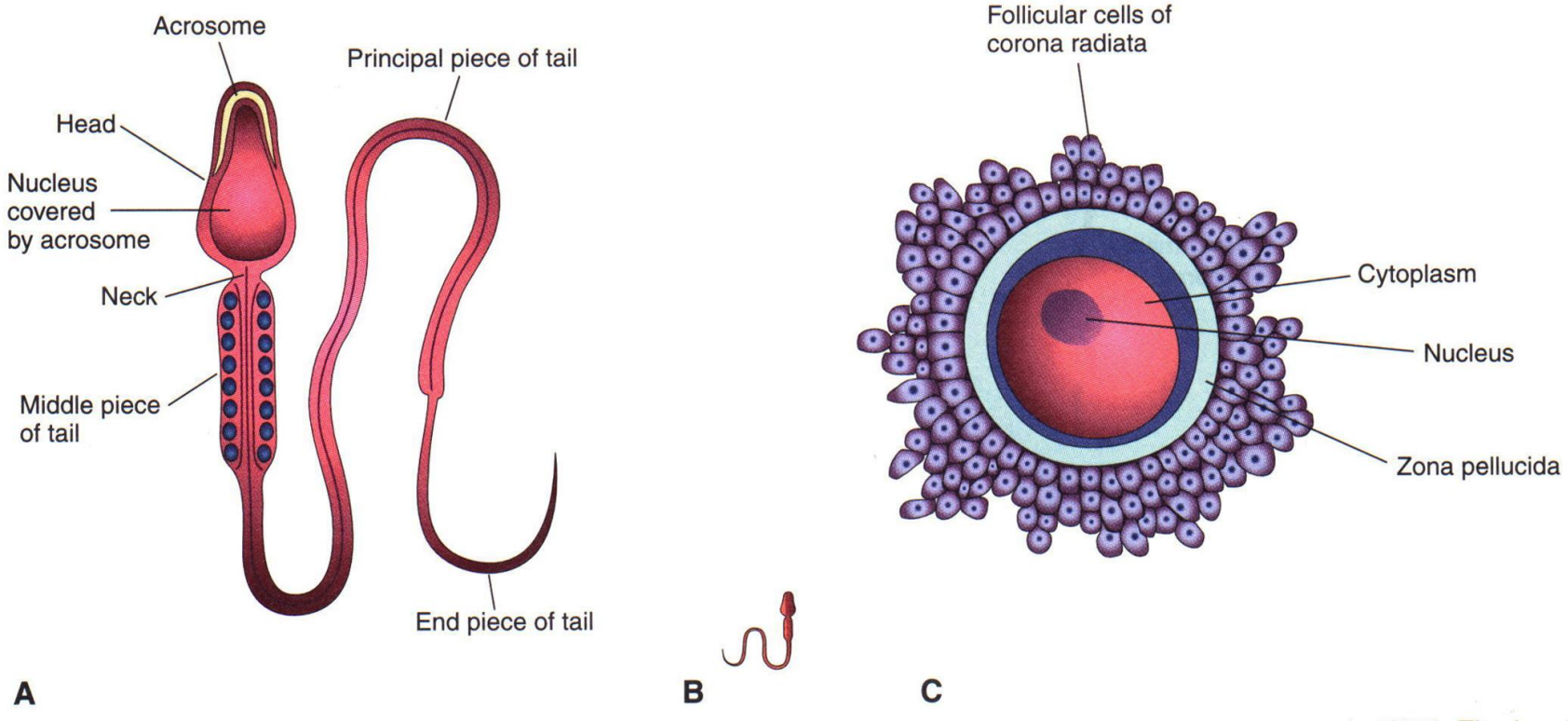
-- *if fertilization occurs*, the 2nd meiotic division is completed ⇒ fertilized oocyte; the 2nd polar body (later on, degeneration)

The number of primary oocyte*:*

1. newborn female infant: about 2 million
2. adolescence: less than 40 thousand
3. about 400 → secondary oocytes (expelled at ovulation)

Comparison of male and female gametes

	Sperm	Secondary oocyte
Size	small	large
Motility	motile	immotile
Cytoplasm	sparse	abundant
Chromosome	23+X; 23+Y	23+X
Unique characteristics	head and tail	corona radiata, zona pellucida

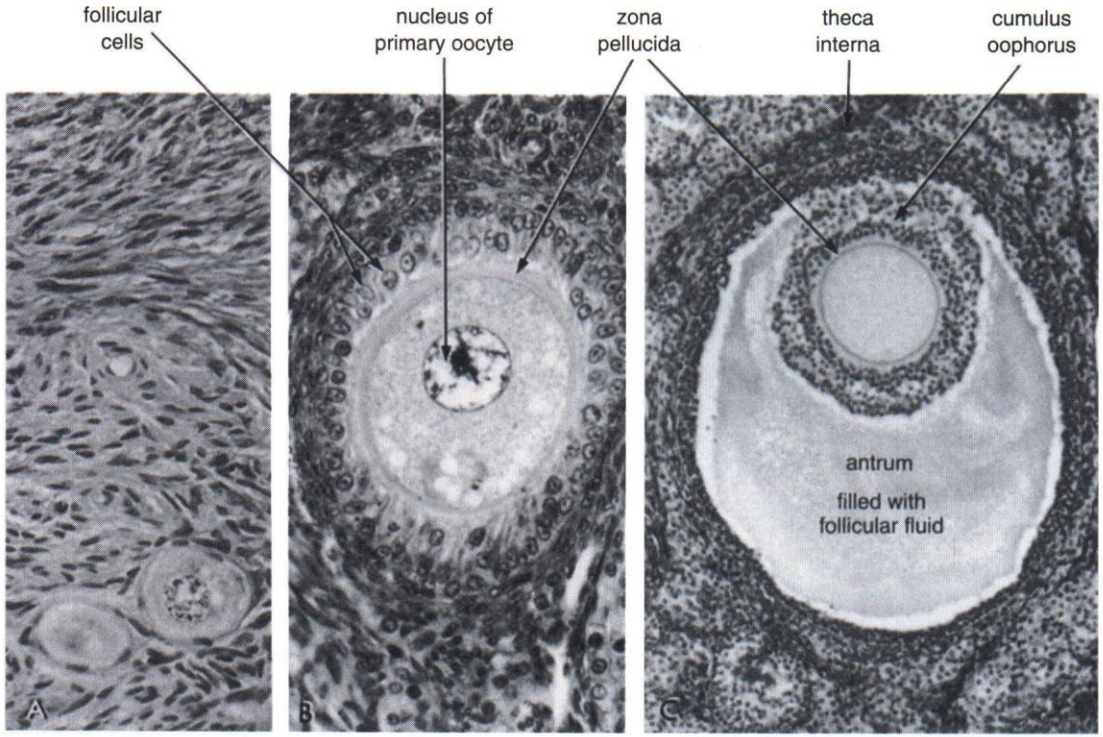


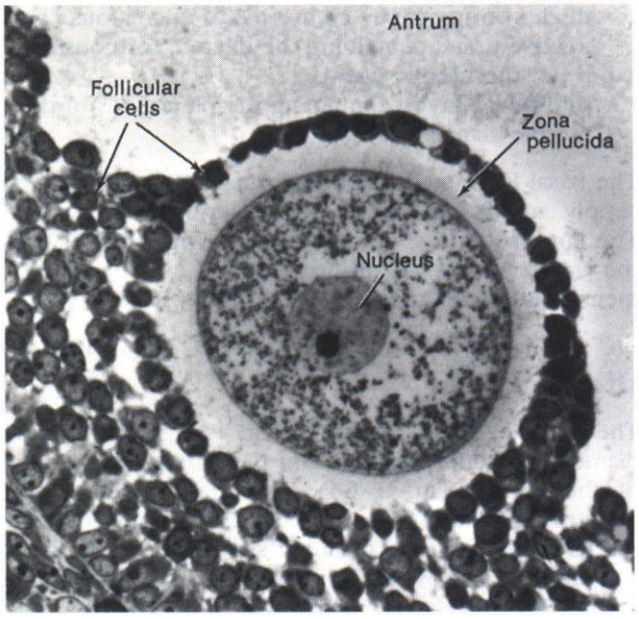
Female reproductive cycles: Ovarian cycle

Development of the ovarian follicle

1. growth and differentiation of a primary oocyte
2. proliferation of follicular cells
3. formation of zona pellucida
4. development of the theca folliculi (Gr. *Theke,* box)
   - 4-1. theca interna: vascular and glandular layer
   - 4-2. theca externa: connective tissue
5. formation of antrum (filled with follicular fluid)

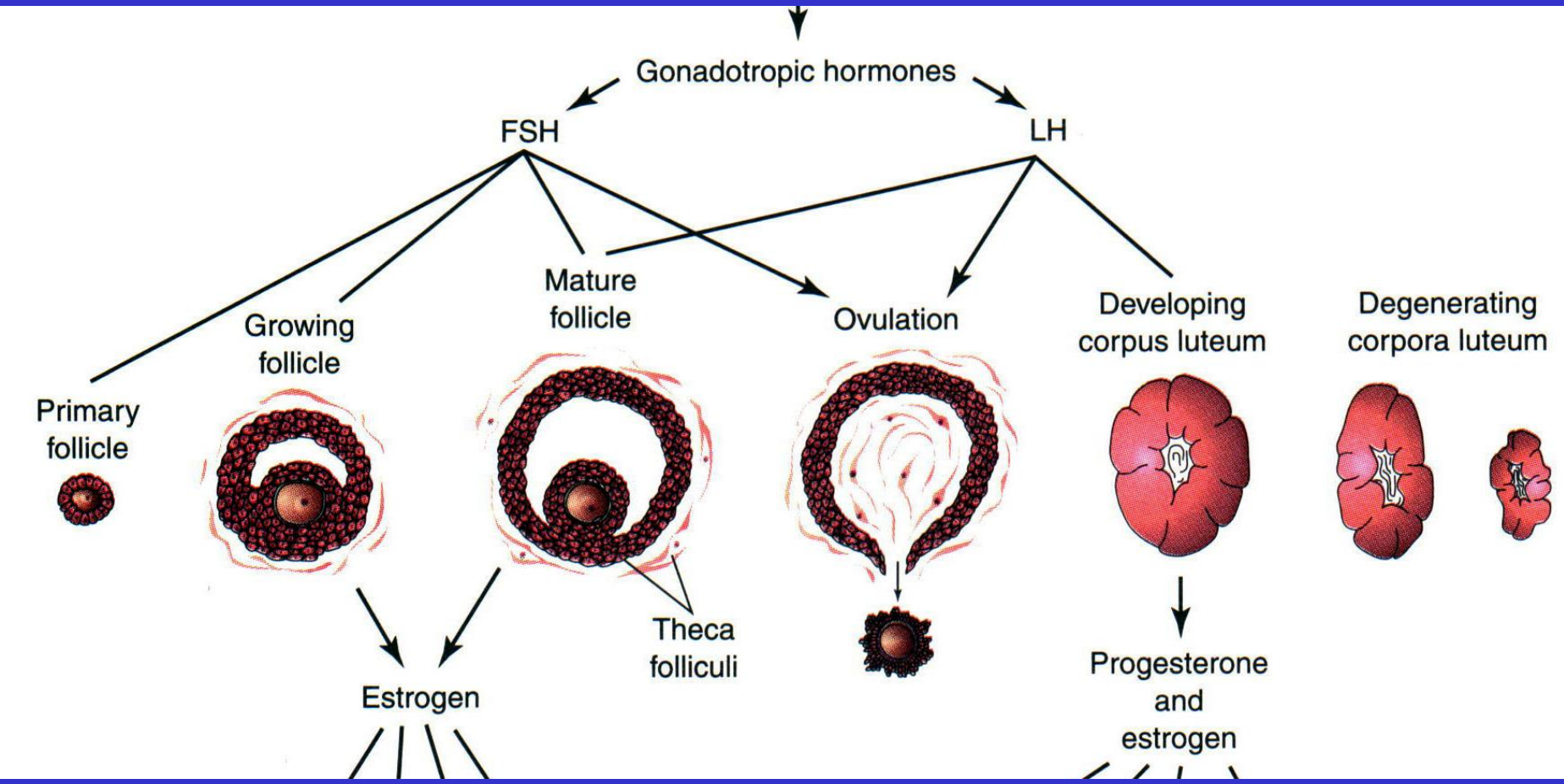
   secondary follicle → mature follicle (with cumulus oophorus)





gure 2–8. Photomicrographs of sections from adult human ovaries. *A,* Ovarian cortex showing two primordial follicles

FSH: stimulates the development of ovarian follicles
FSH + LH: maturation of follicular cells
Estrogen: produce by growing follicles and theca interna & interstitial glands of ovary



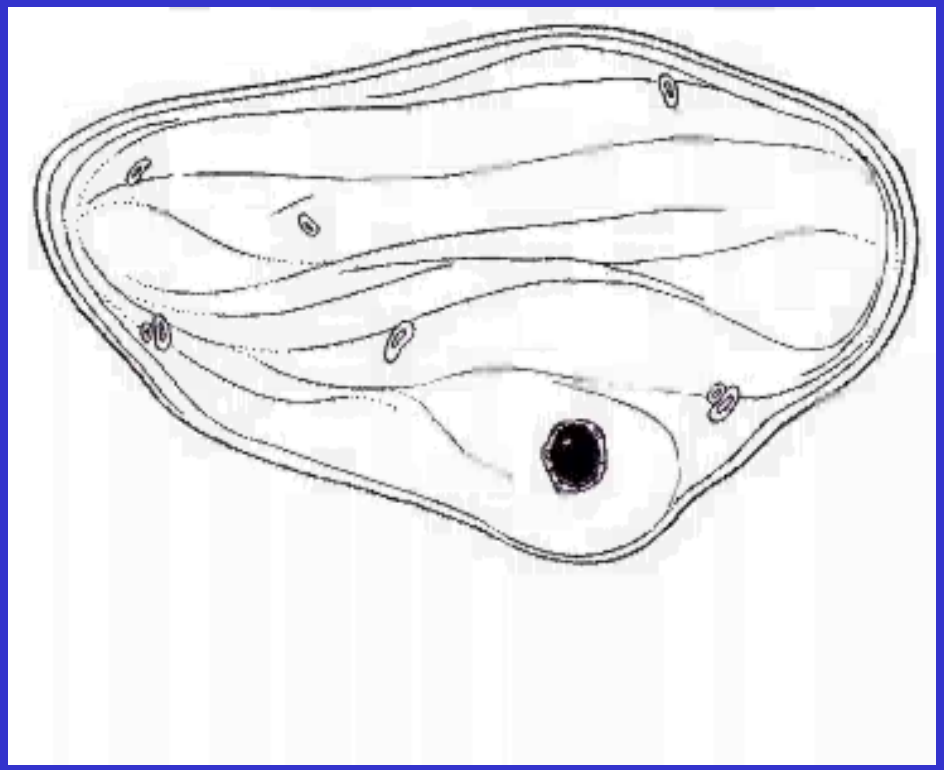
Ovulation

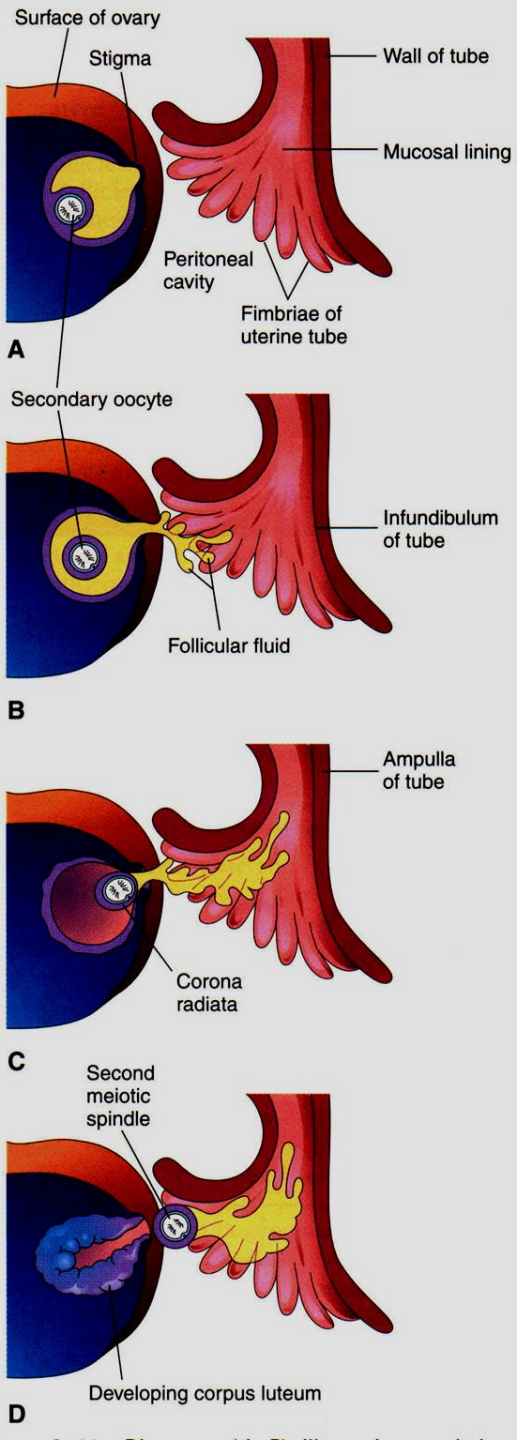
is triggered by a surge of LH production: **LH surge**

stigma – avascular spot on the surface of ovarian follicle; secondary oocyte expells from this spot under the LH surge

Cause of expulsion of oocyte:

1. intrafollicular pressure
2. contraction of smooth muscle in the theca externa
3. enzymatic digestion of follicular wall

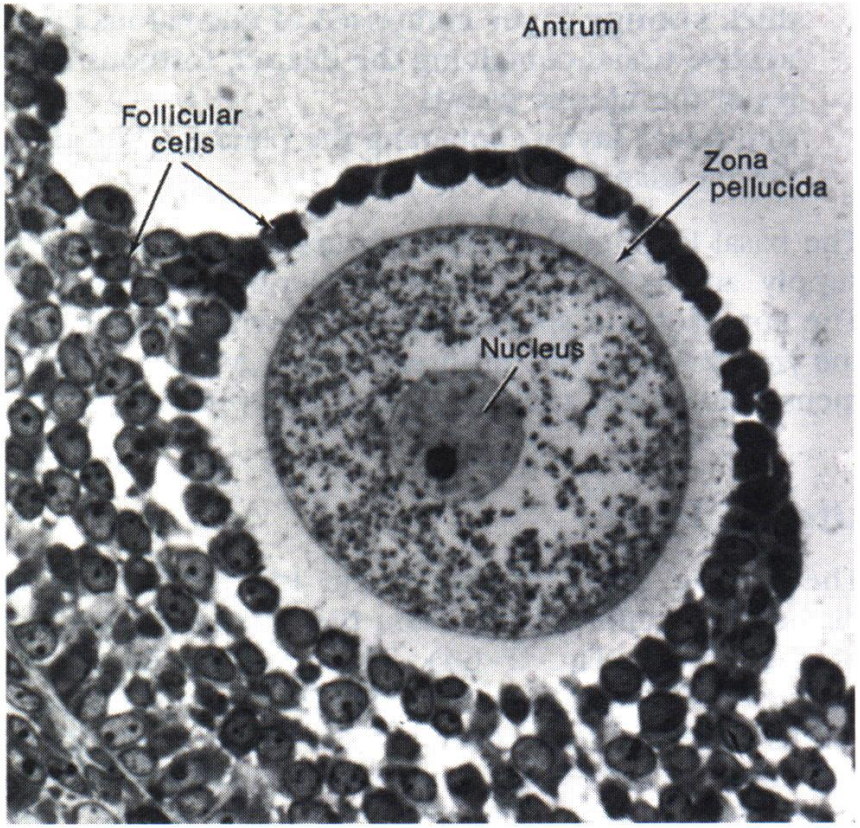


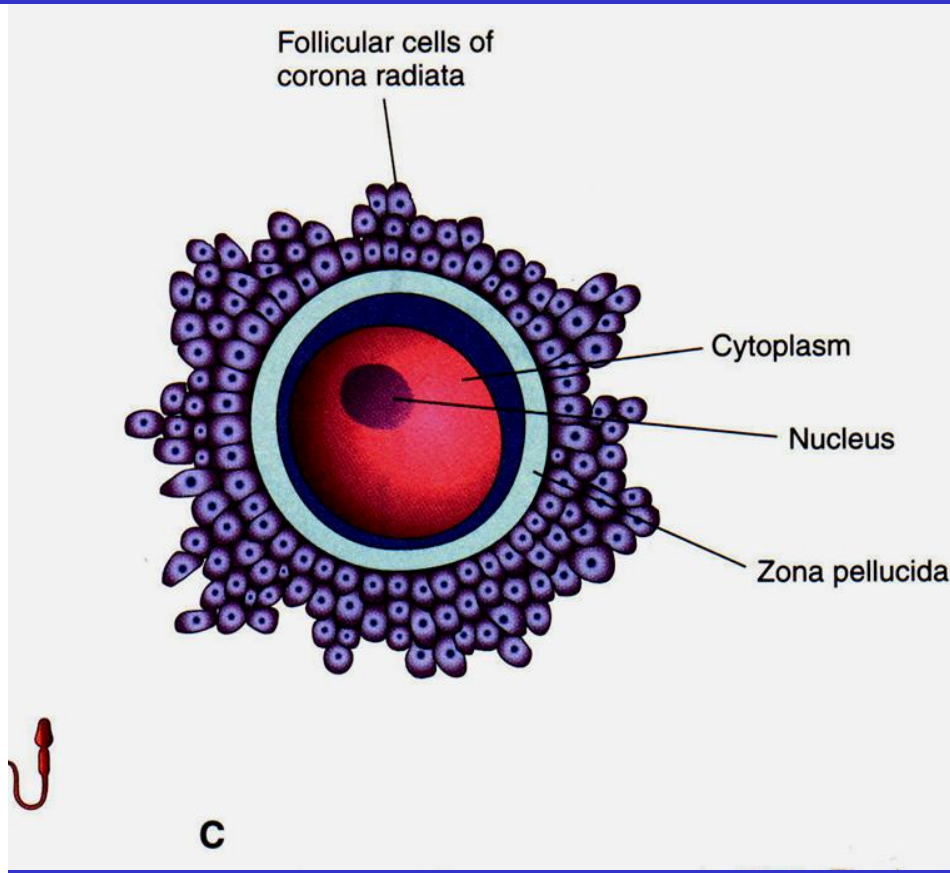


Structure of expelled secondary oocyte:

surrounding by:

1. zona pellucida
2. corona radiata
3. cumulus oophorus





Corpus luteum

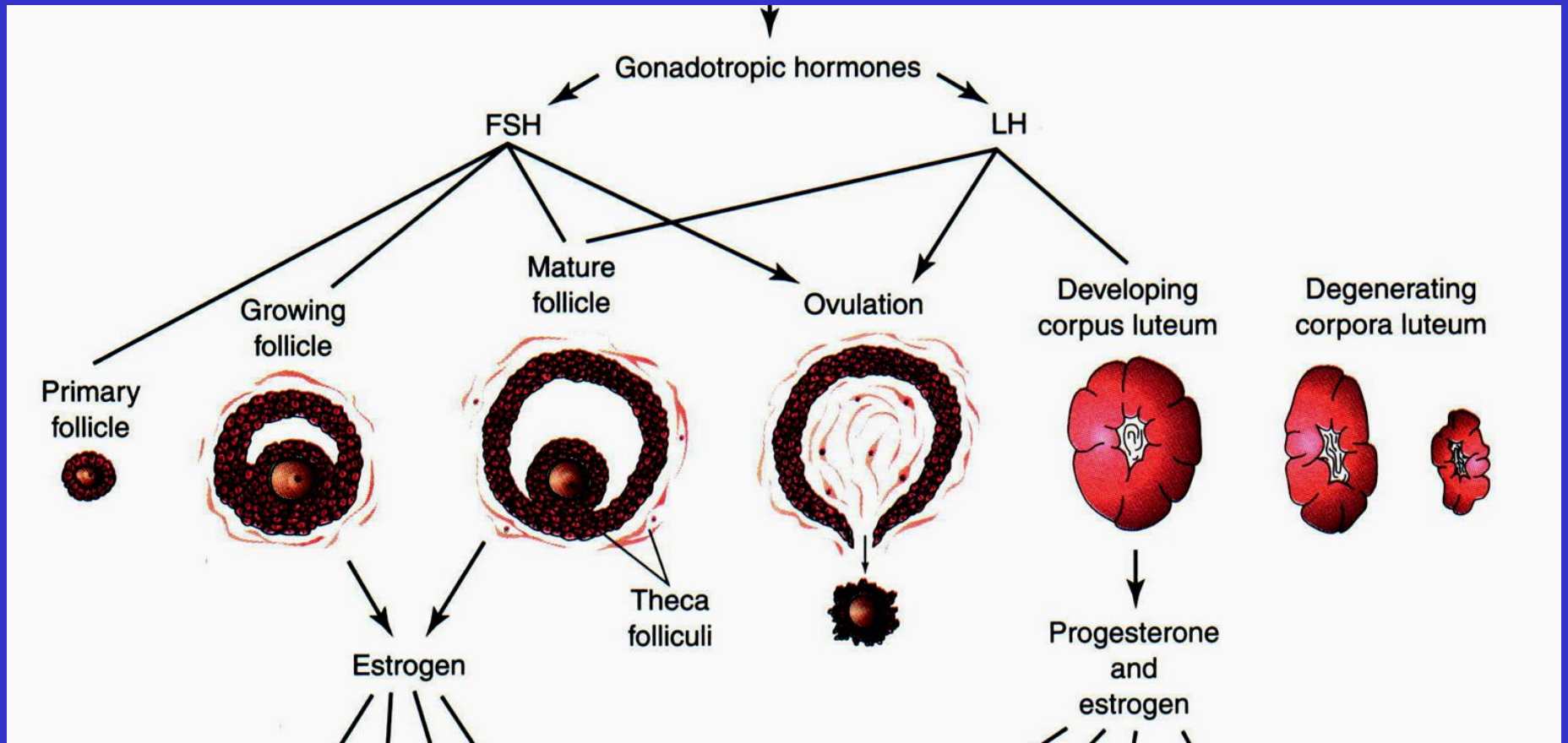
after ovulation: ovarian follicle ⇒ corpus luteum → progesterone (mainly) & estrogen → prepare the endometrium for implantation of blastocyst

***fertilized:* corpus luteum**

⇒ corpus luteum of pregnancy (for the first 20 week of pregnancy)

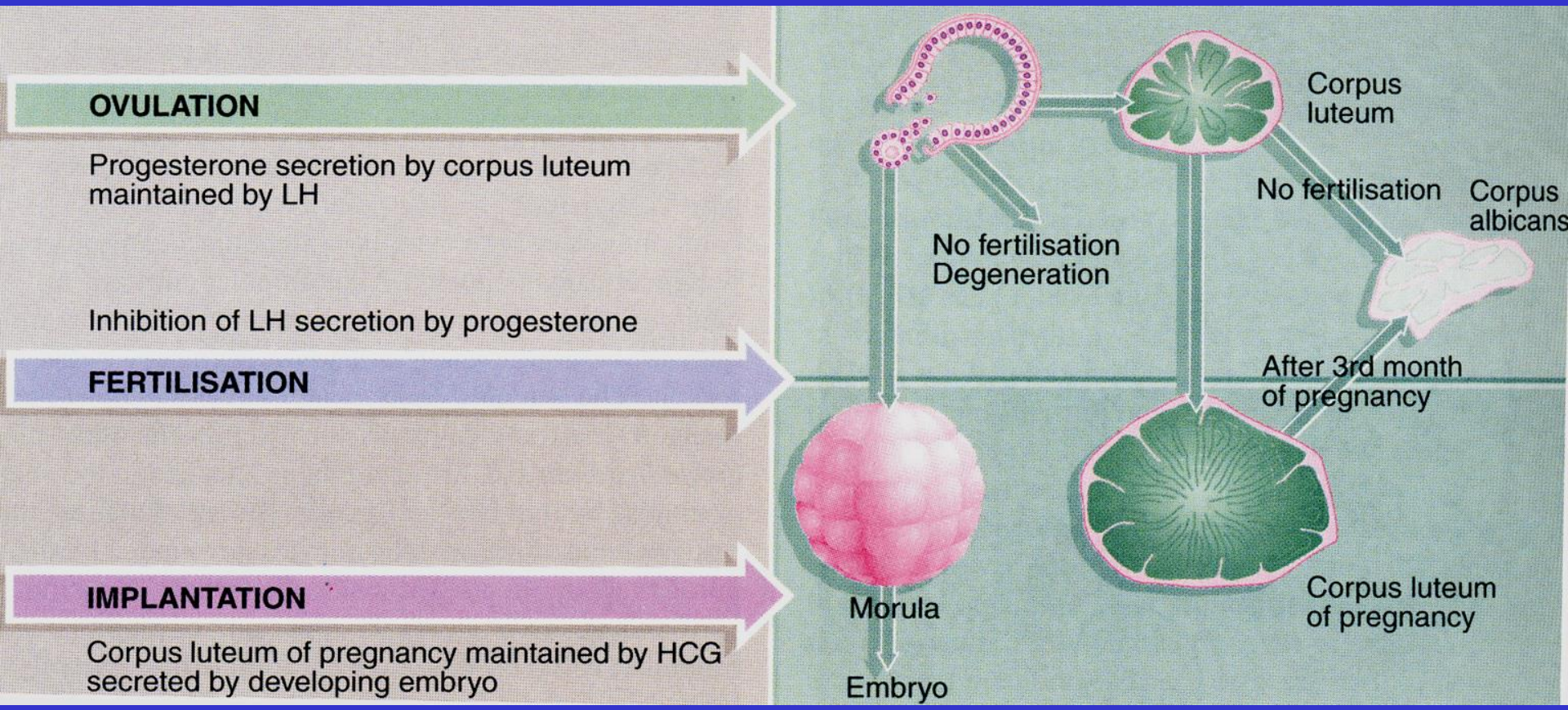
hCG -- secreted by syncytiotrophoblast of chorion

-- can prevent the degeneration of corpus luteum of pregnancy



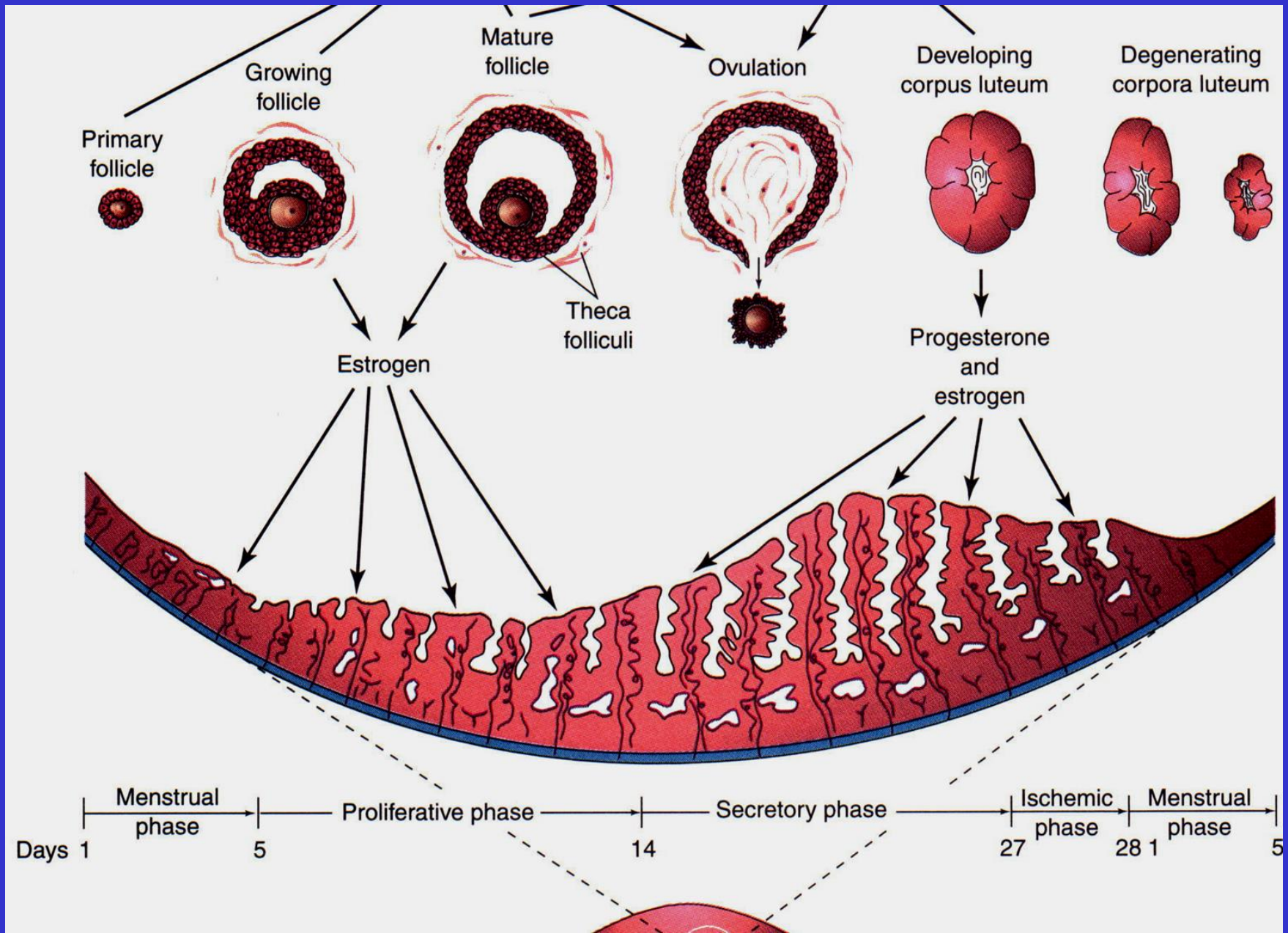
Not fertilized:

corpus luteum → involute degenerate (about 10 ~ 12 days after ovulation) → corpus luteum of menstruation → transformed (white scar) → corpus albicans (atretic corpus luteum)

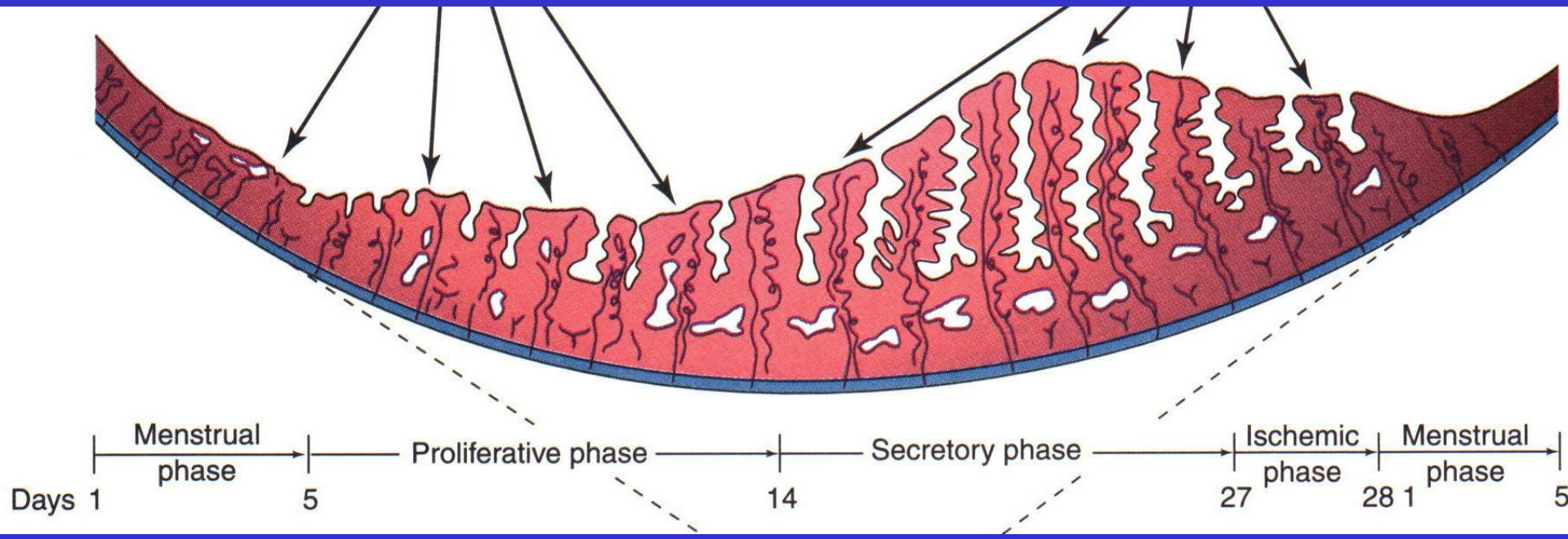


Menstrual cycle

-- the length of menstrual cycle: 23– 35 days, average 28 days



Menstrual cycle



Phases of menstrual cycle:

Menstrual phase -- lasts 4 – 5 days

-- functional layer of uterine wall → sloughed off and discarded with menstrual flow

-- eroded endometrium ⇒ thin

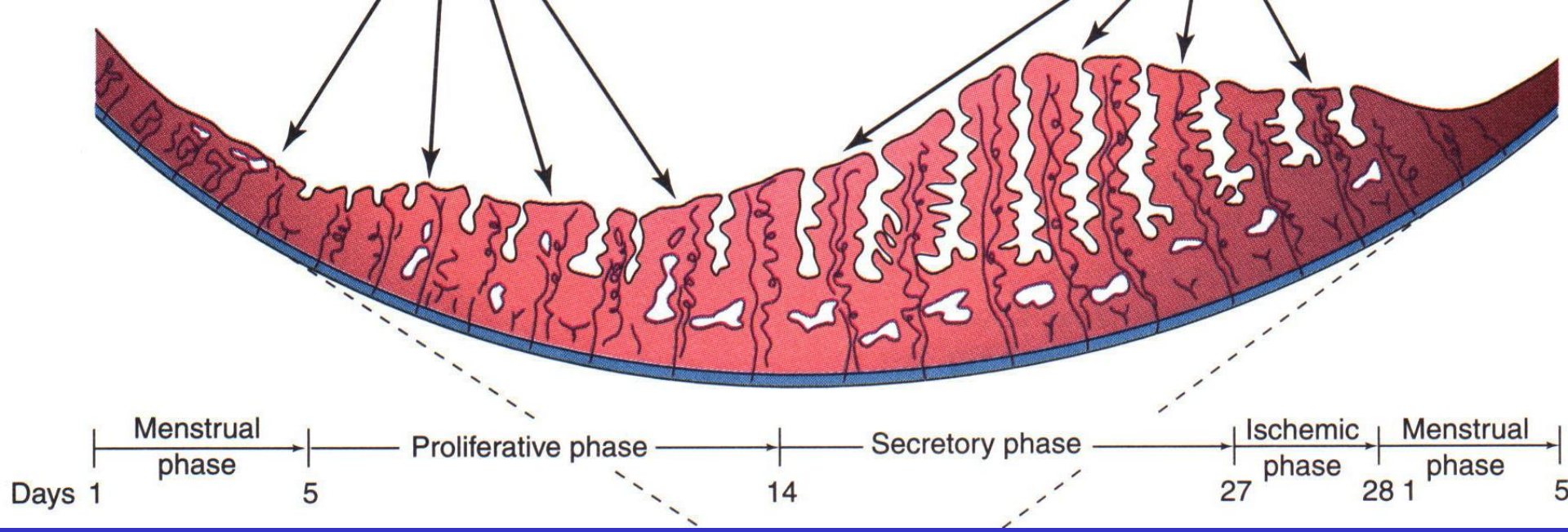
Proliferative (estrogenic) phase -- lasts about 9 days

-- conincides with growth of ovarian follicles

-- controlled by estrogen

-- endometrium ⇒ thick;

glands increase in number and length;spiral arteries. → elongates



Secretory (progestational) phase -- lasts 13 days
-- conincides with the formation and growth of :
-- *corpus luteum* → progesterone & estrogen
-- endometrium → thick
-- spiral a.a. → reached to the superficial layer, coiled
-- venous network → lacunae ⇒ *direct arteriovenous anastomoses*

If fertilization and pregnancy do not occur: ischemic phase
-- corpus luteum degenerates
-- estrogen and progesterone decrease; secretory endometrium → ischemic phase
-- menstruation occurs
-- reproductive cycles normally continue until menopause

If fertilization and pregnancy occur:

-- cleavage of zygote and blastogenesis occur

-- implantation of blastocyte

→ endometrium (about 6th day of the secretory phase; day 20 of a 28-day cycle)

-- syncytiotrophoblast → *hCG*

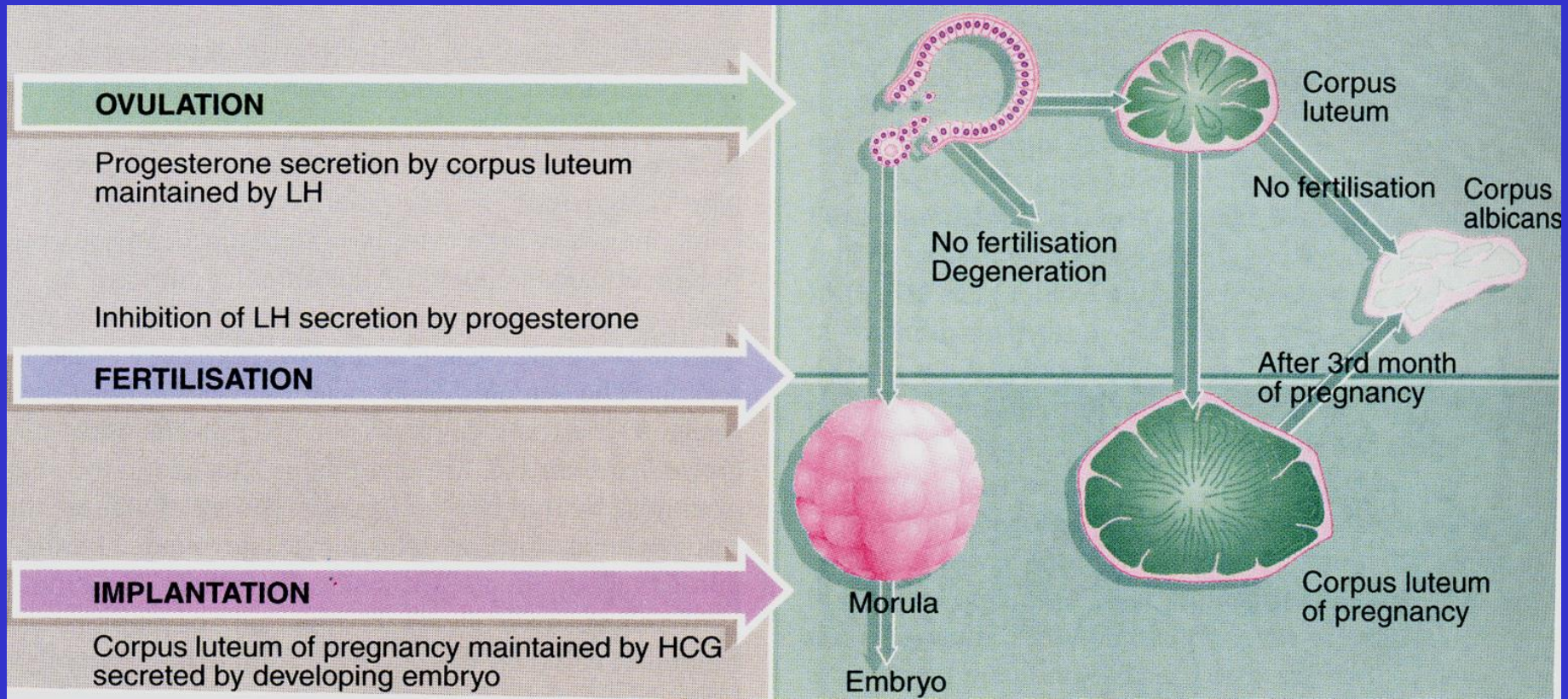
→ corpus luteum of pregnancy → progesterone and estrogens

-- secretory phase continues and menstruation does not occur

-- endometrium → pregnancy phase

** with termination of pregnancy

→ the ovavian and menstrual cycles resume (usually 6-10 weeks)



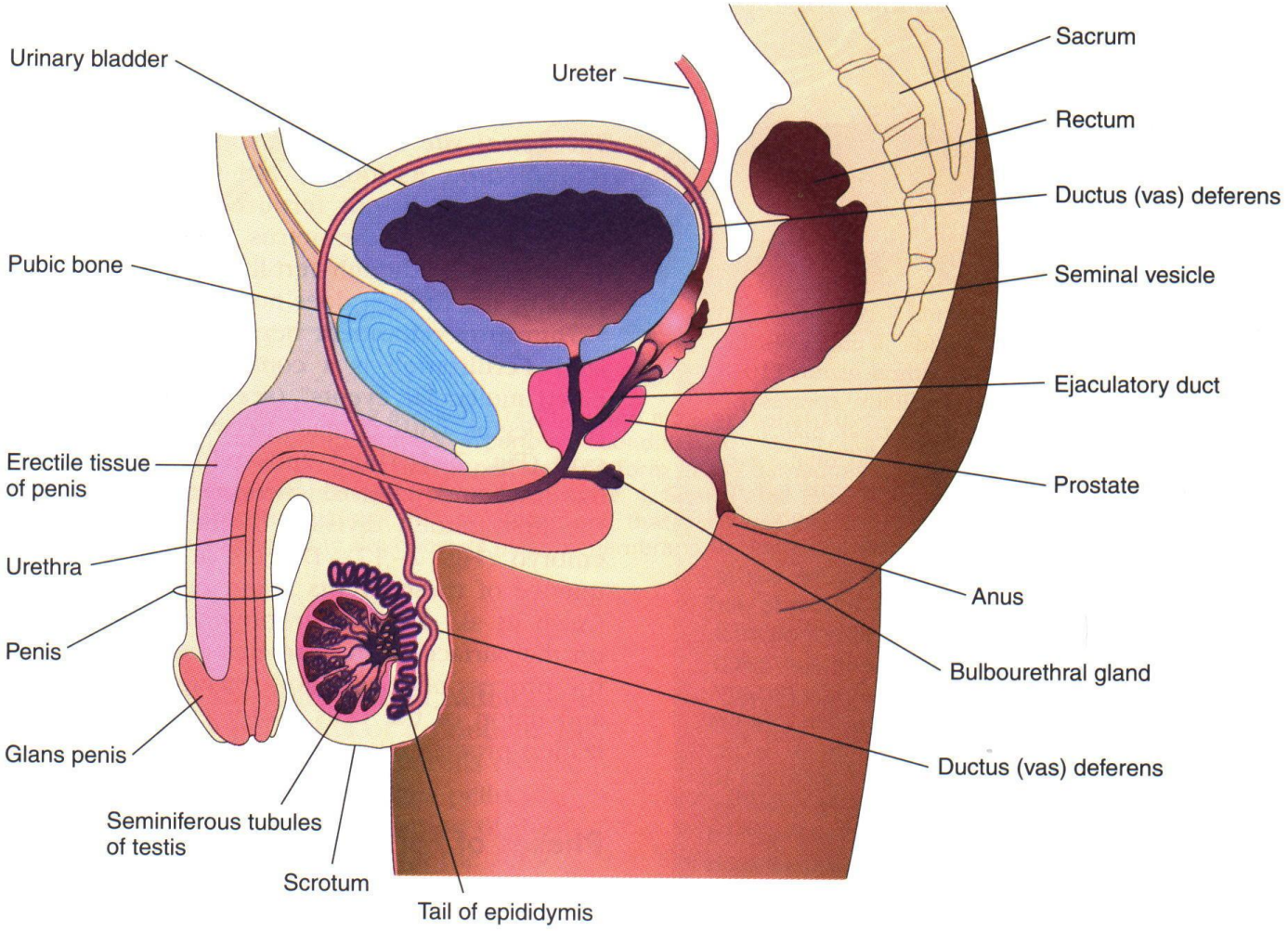
Transportation of gametes

Mature sperm

-- a free-swimming, actively motile cell

-- enter the lumina of seminiferous tubules

-- epididymis (store and become mature) → ductus deferens→ urethra



■ **Figure 2–15.** Sagittal section of the male pelvis primarily to show the male reproductive system.

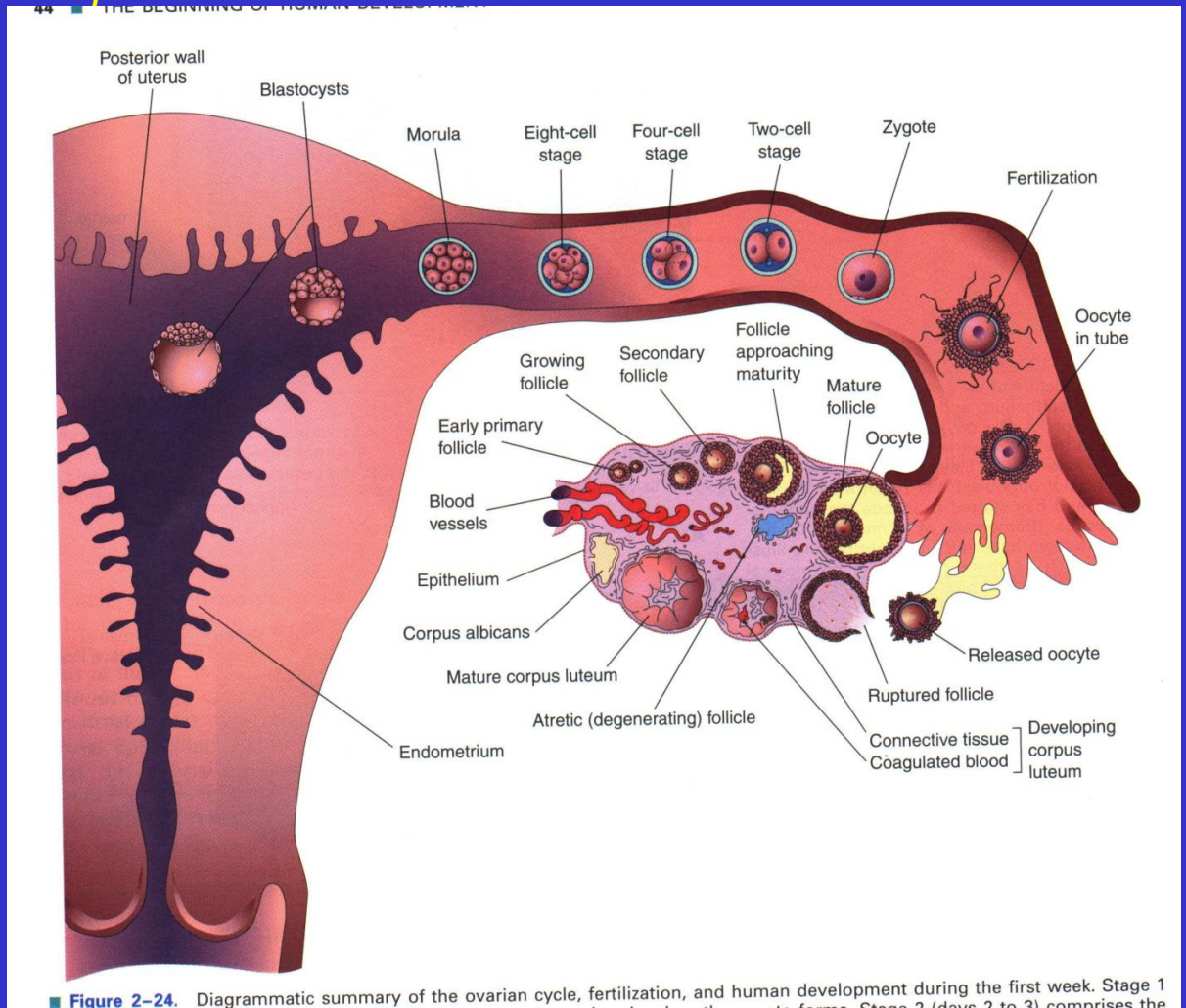
Maturation of sperms

***Capacitation**:* a period of conditioning in the female reproductive tract, lasting ~7 hr

Major events occur during capacitation:

1. a glycoprotein coat and seminal proteins are removed from the surface of the sperm's acrosome
2. the membrane cholesterol/phospholipids ratio and membrane potential →altered

* *Capacitated sperms:* more active



■ Figure 2–24. Diagrammatic summary of the ovarian cycle, fertilization, and human development during the first week. Stage 1

Completion of capacitation ⇒ acrosome reaction occurs

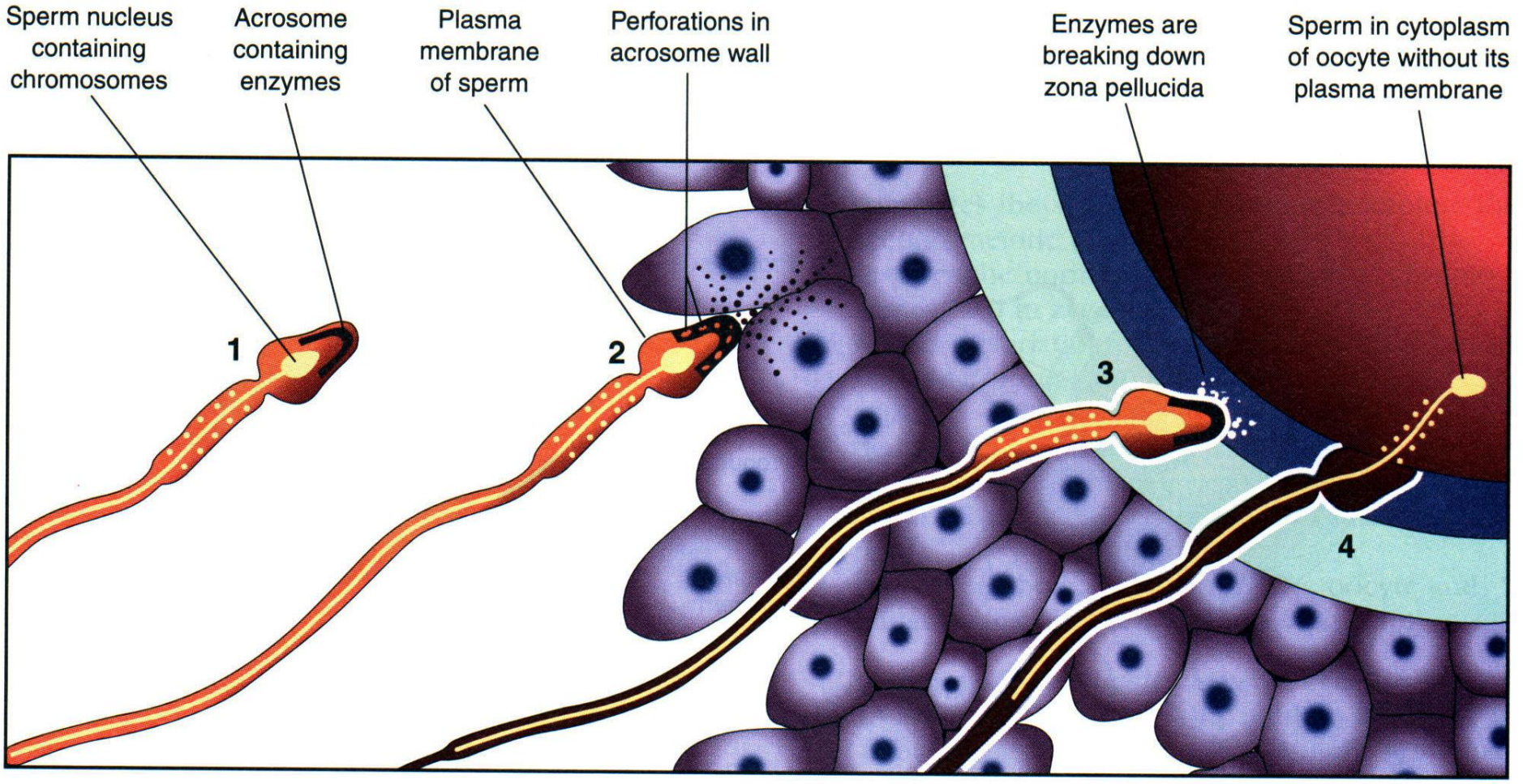
Angiotensin converting enzyme (ACE)

-- present in the acrosome of the sperm

-- involved in inducing acrosome reaction and fertilization process

-- acrosome reaction must be completed before the sperm can fuse with the oocyte

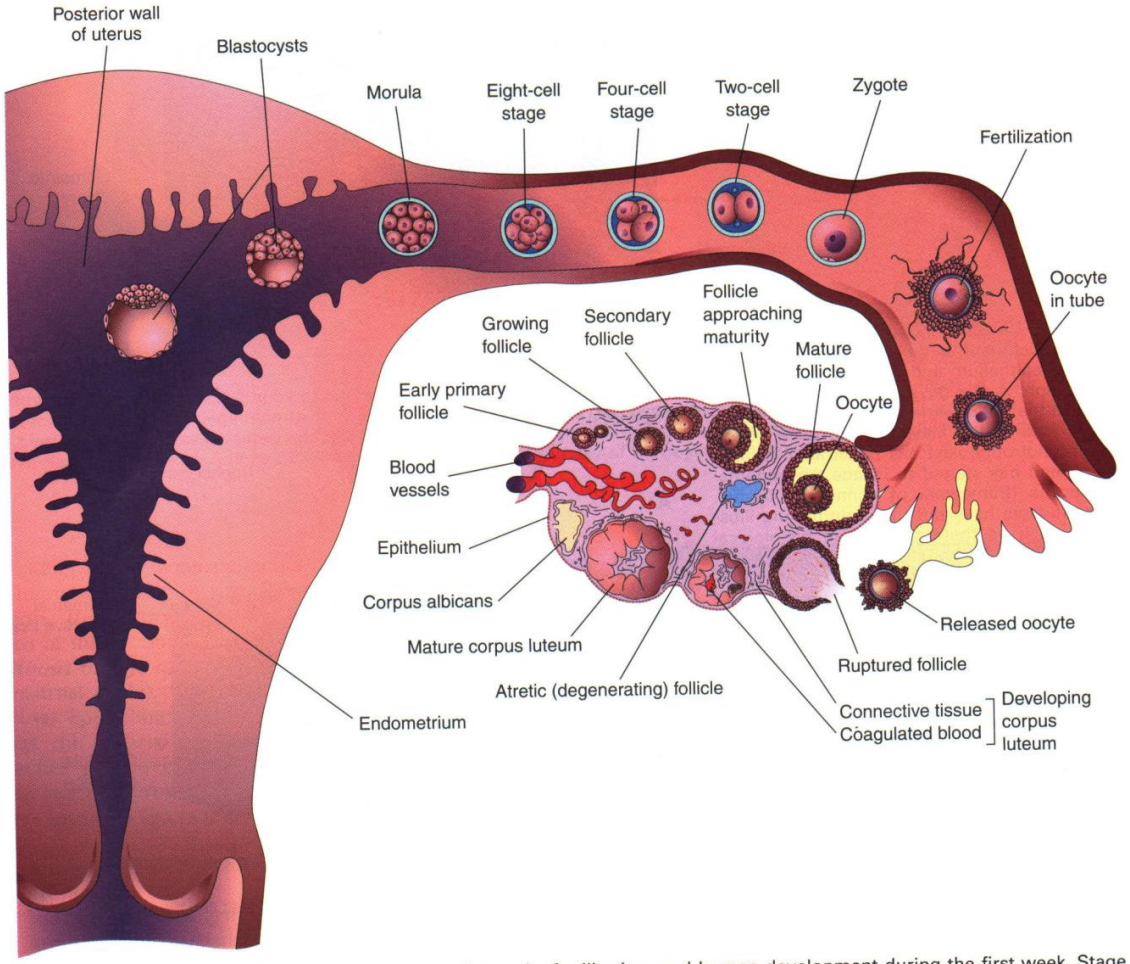
-- enzyme associated with the acrosome reaction: hyaluronidase and acrosin



Viability of gametes

human oocyte: retain the ability for fertilization within 12 hr after ovulation
in vitro study: within 24 hr

human sperms: do not survive for more than 48hr in female genital tract
frozen semen: many years



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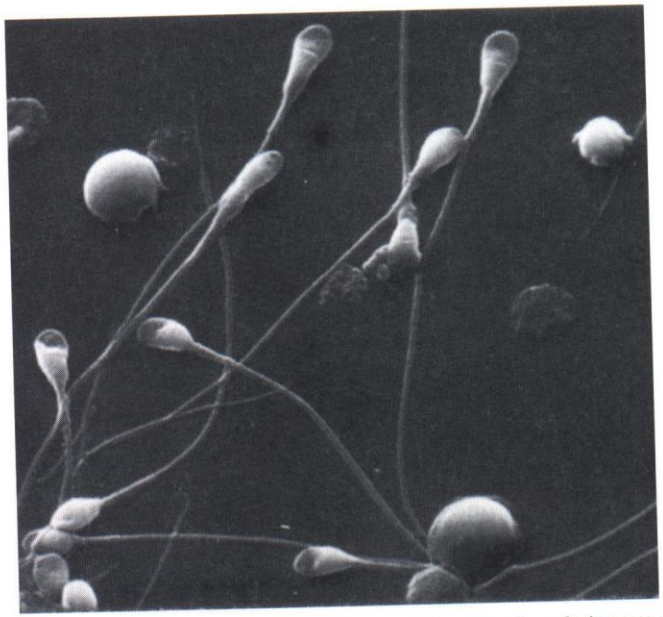
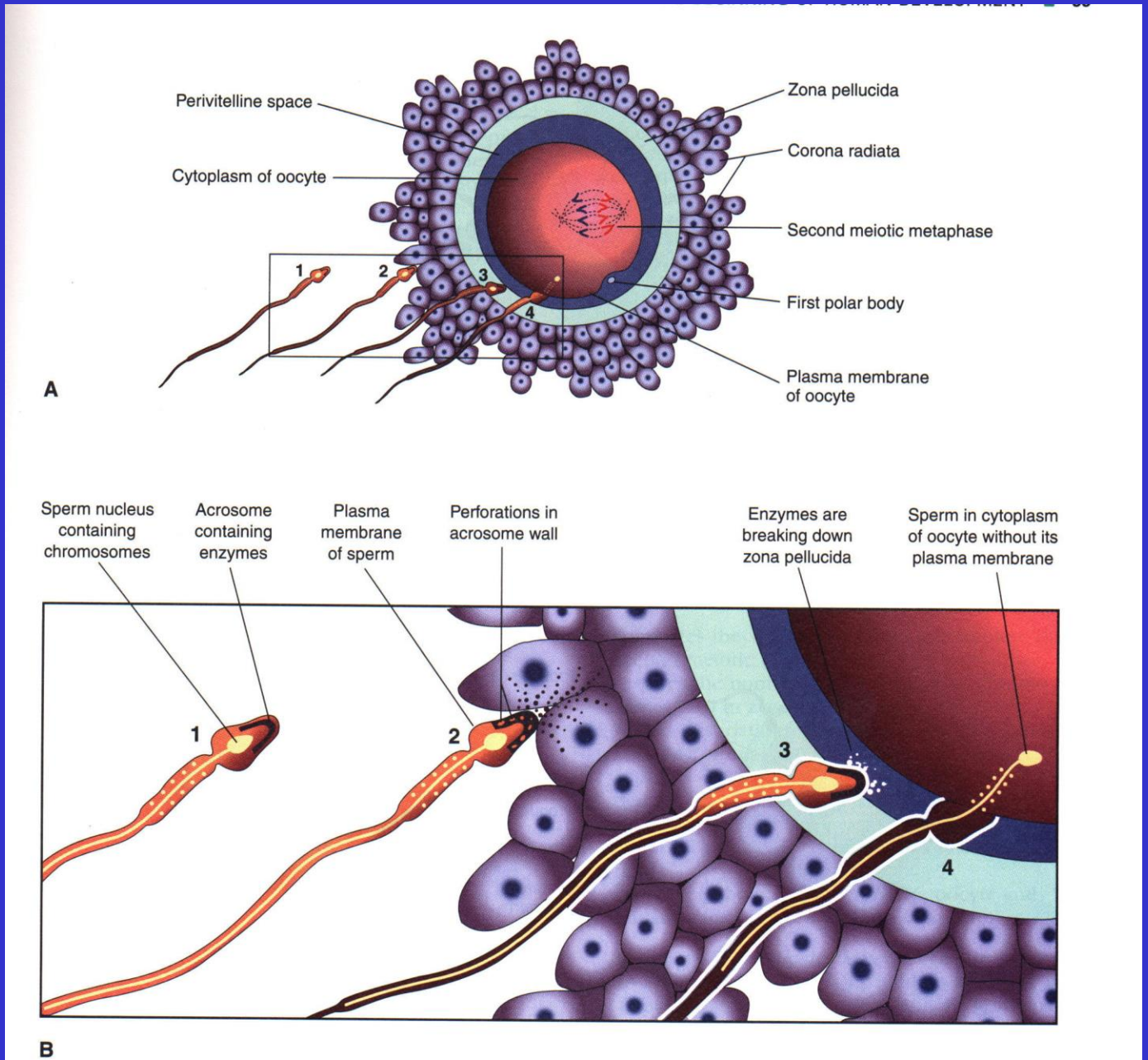


■ Figure 2–14. Scanning electron micrograph of human

Fertilization: (usual site: at the ampulla of uterine tube)

Passage of sperm: through corona radiata surrounding the zona pellucida of an oocyte



Possible mechanisms:

1. by hyaluronidase and tubal mucosal enzymes
2. movement of tail of the sperm

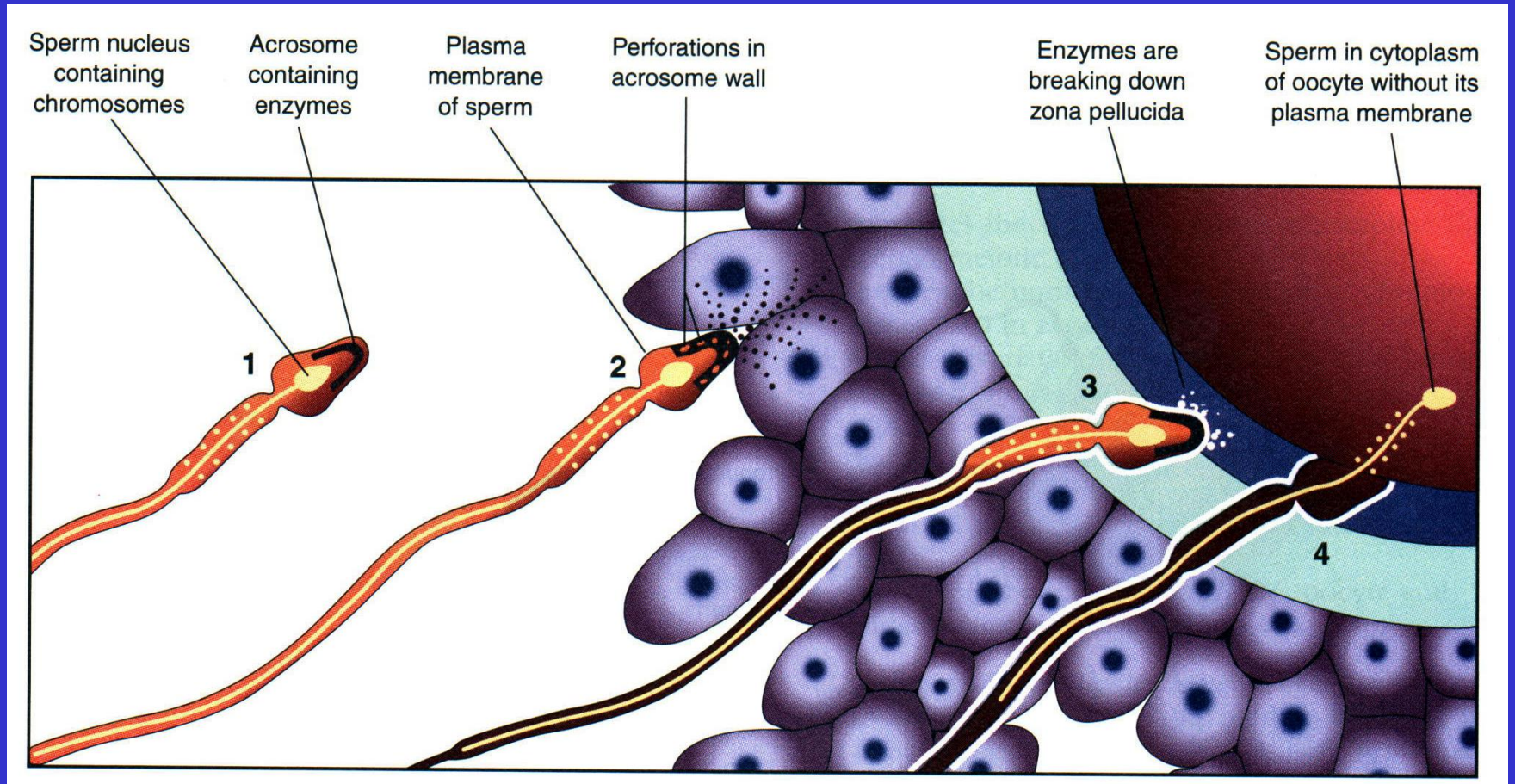
Penetration of zona pellucida surrounding the oocyte

-- enzymes associated with lysis of zona pellucida:

-- acrosin (mainly), esterases and neuraminidase

$\rightarrow$ forming a path for the sperm $\rightarrow$ enter the oocyte

-- in human: normally only a single sperm is allowed to penetrate the oocyte

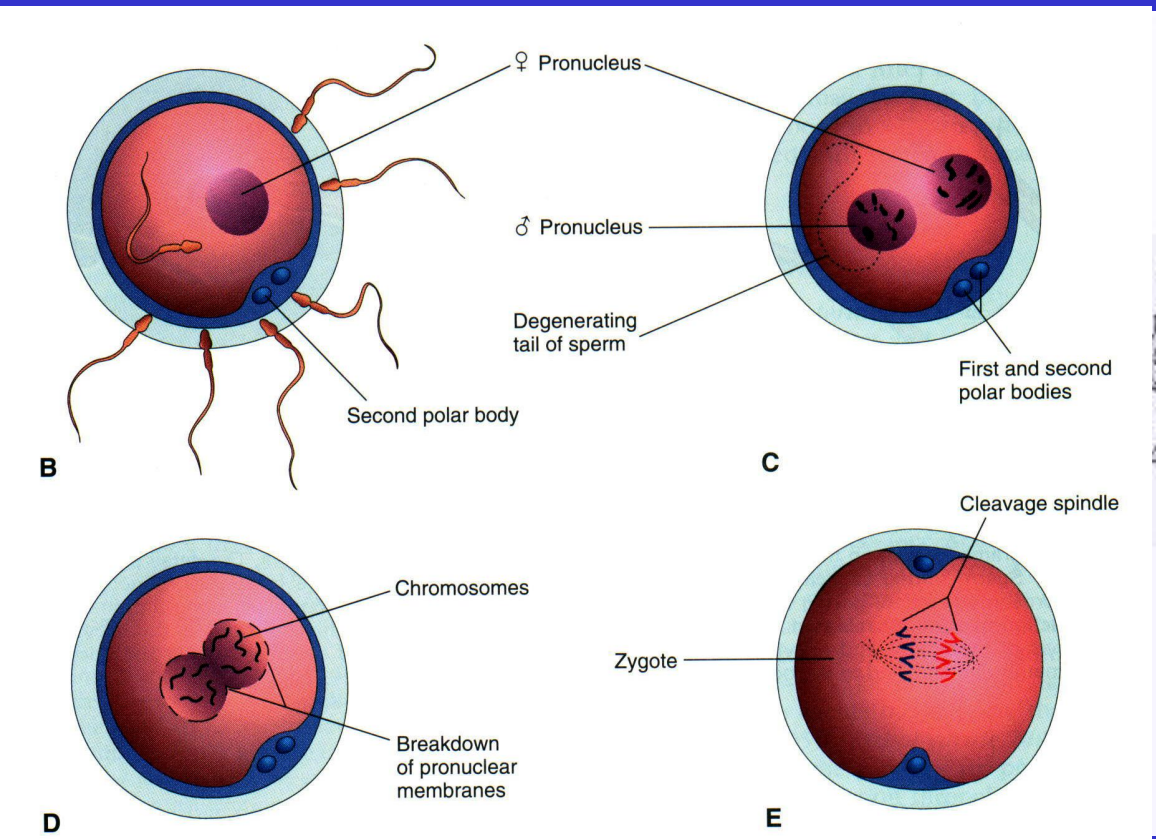


zona reaction:

-- following the penetration of the sperm in the zona pellucida, an amorphous (glycoprotein) layer forms in this zone prevent the entry of other sperms

-- this reaction is believed to result from the action of lysosomal enzymes released by *cortical granules* near the plasma membrane of oocytes

-- the contents of these granules also cause changes in the plasma membrane of oocyte that make it impermeable to sperms



Fusing of plasma membranes of the oocyte and sperm

Completion of 2nd meiotic division of oocyte and formation of female pronucleus

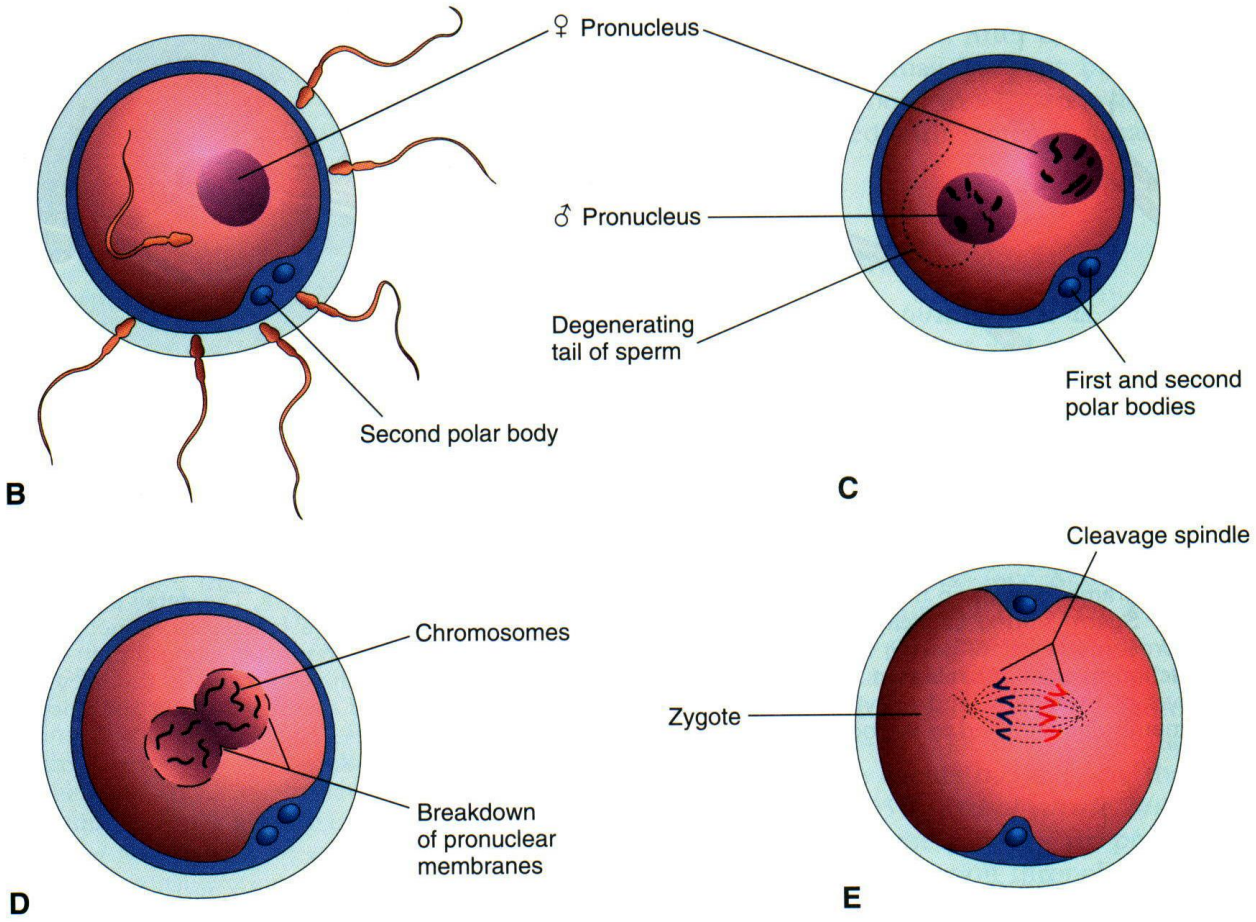
Formation of male pronucleus (from sperm)

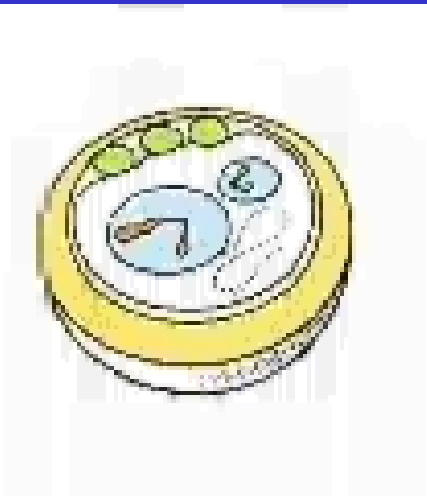
Early pregnancy factor:

-- appears in the maternal serum within 24 to 48 hr after fertilization

-- used for pregnancy test during the first 10 days of development

-- is an immunosuppressent protein and secreted by the trophoblastic cells



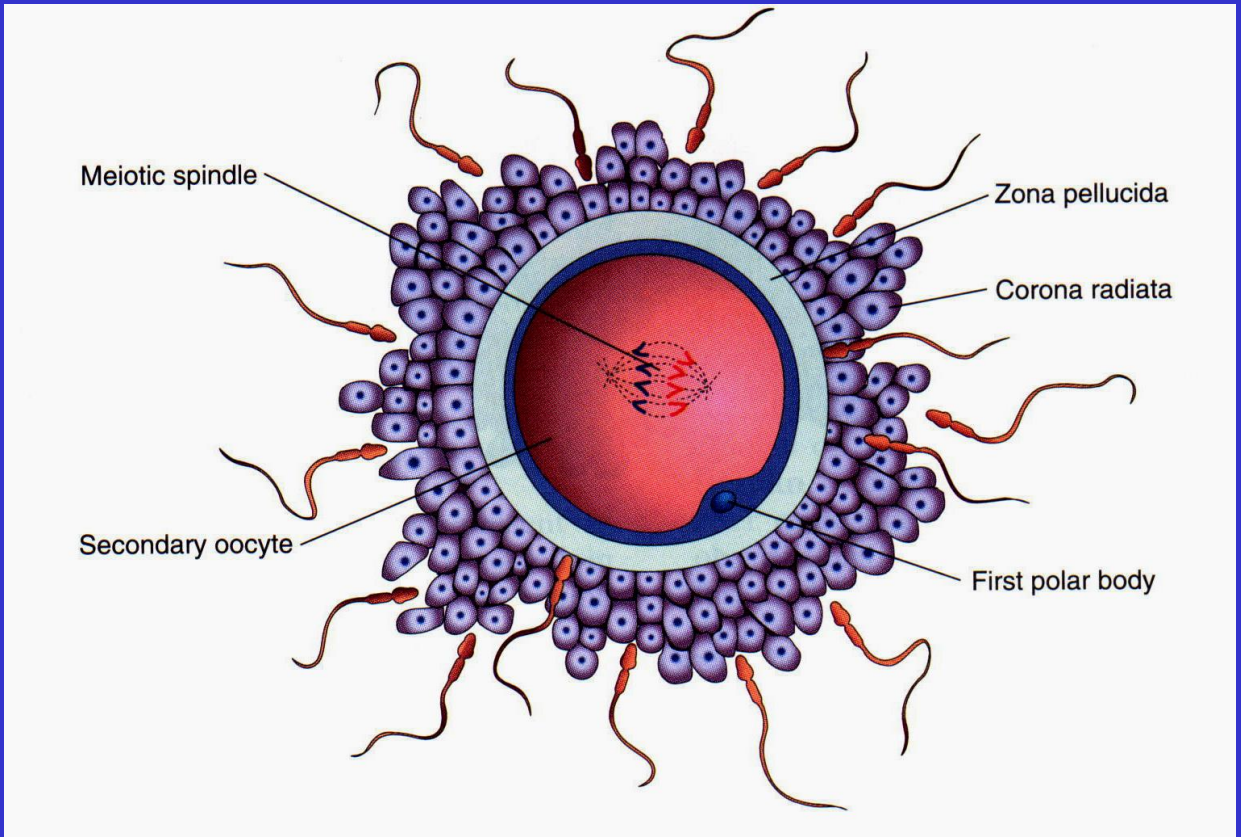


Results of fertilization

Fertilization:

-- stimulates the secondary oocyte → complete 2nd meiotic division
-- restores the normal diploid number of chromosomes in zygote
-- results in variation of human species
-- determines chromosomal sex of embryo
-- causes metabolic activation of the oocyte and initiates cleavage

Zygote is genetically unique:
-- a recombination of chromosomes from cells of either of the parents

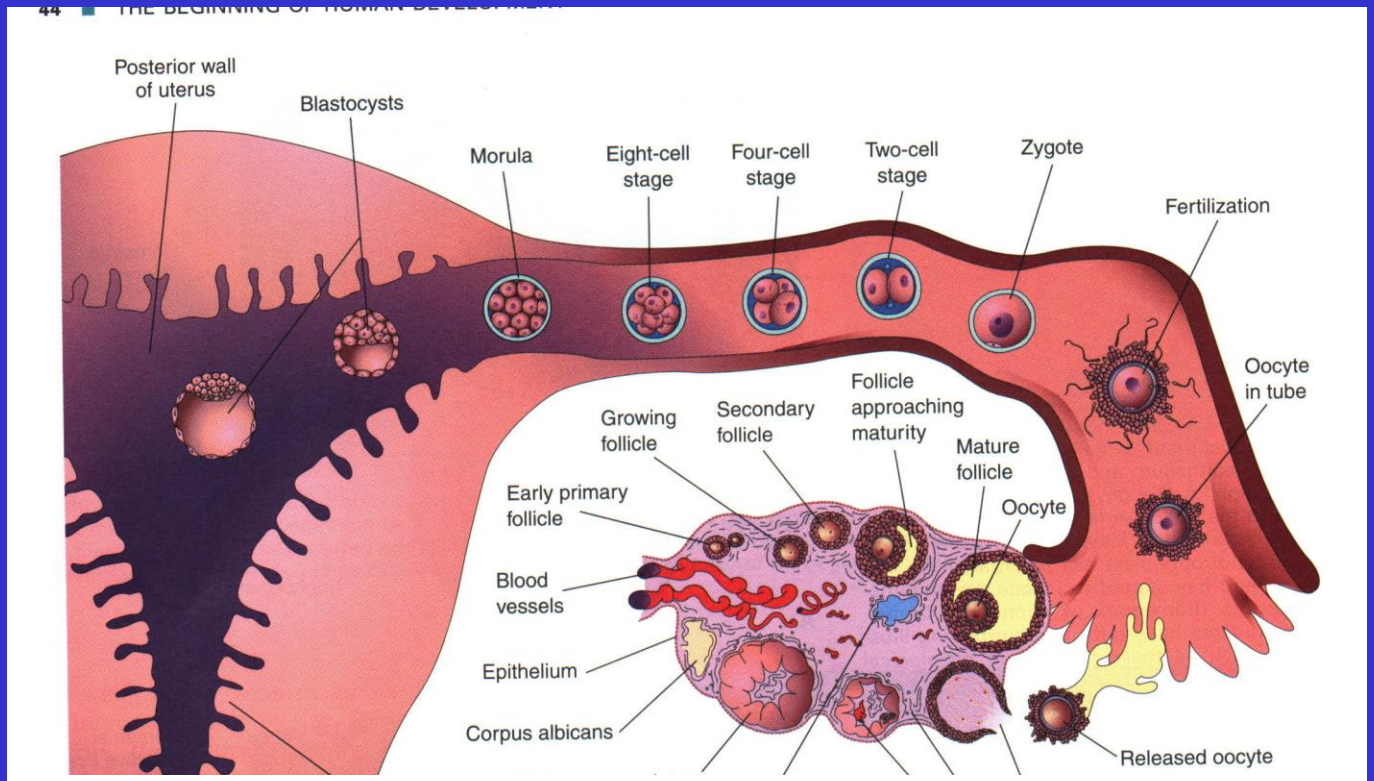


Cleavage of zygote

-- begins with repeated mitotic divisions of the zygote and ends with a blastocyst

-- site of occurance of cleavage: from uterine tube toward the uterus

-- about 30 hr after fertilization: zygote → blastomeres



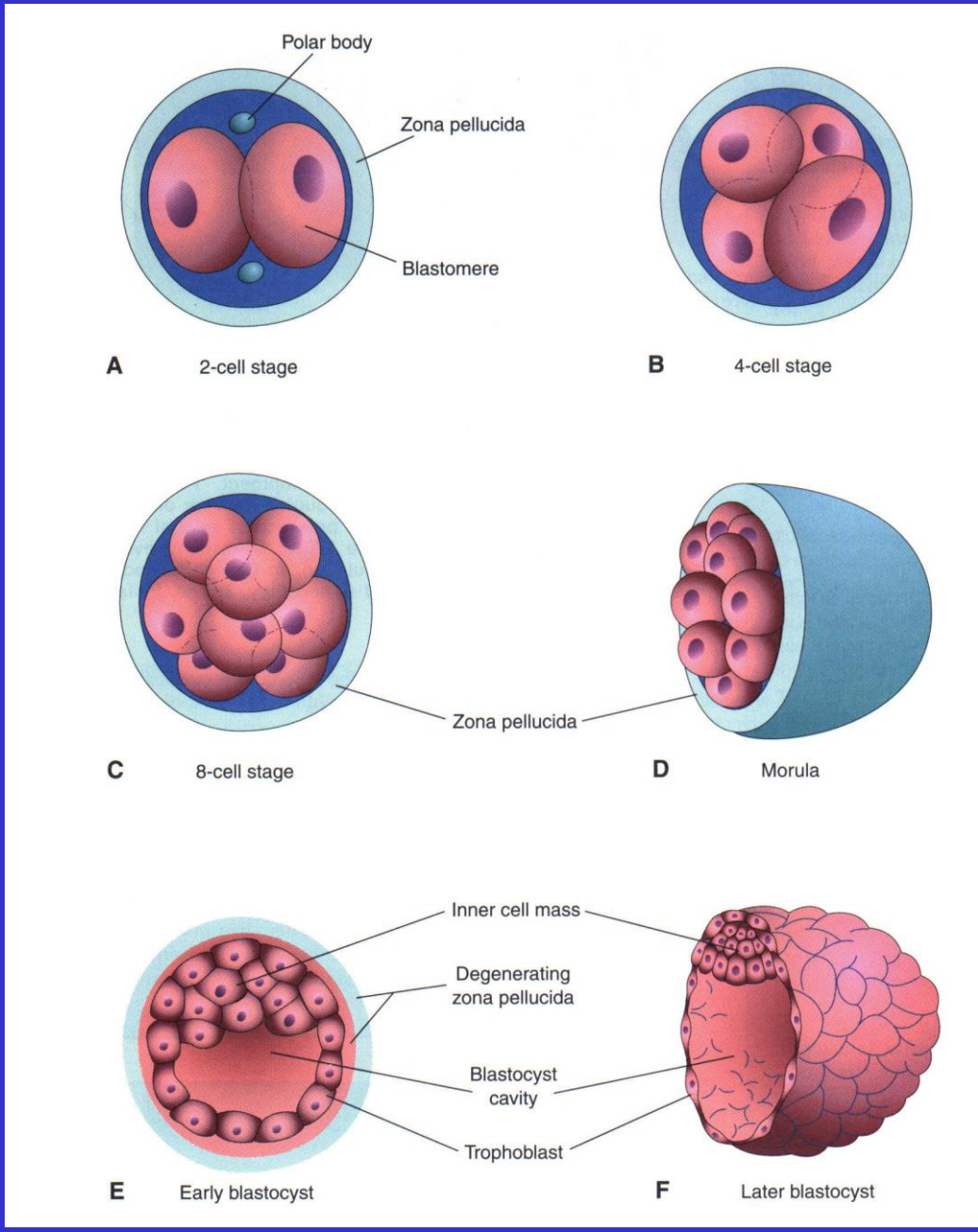
Compactation:

-- after nine-cell stage: blastomeres → shape changed → tightly align themselves against each other ⇒ compact ball

-- probably mediated by cell surface adhesion glycoproteins

-- permits greater cell-cell interaction and is a prerequisite for segregation of the internal cells ⇒ **inner cell mass (embryoblast)**

morula — when the blastomeres up to 12-15 in number

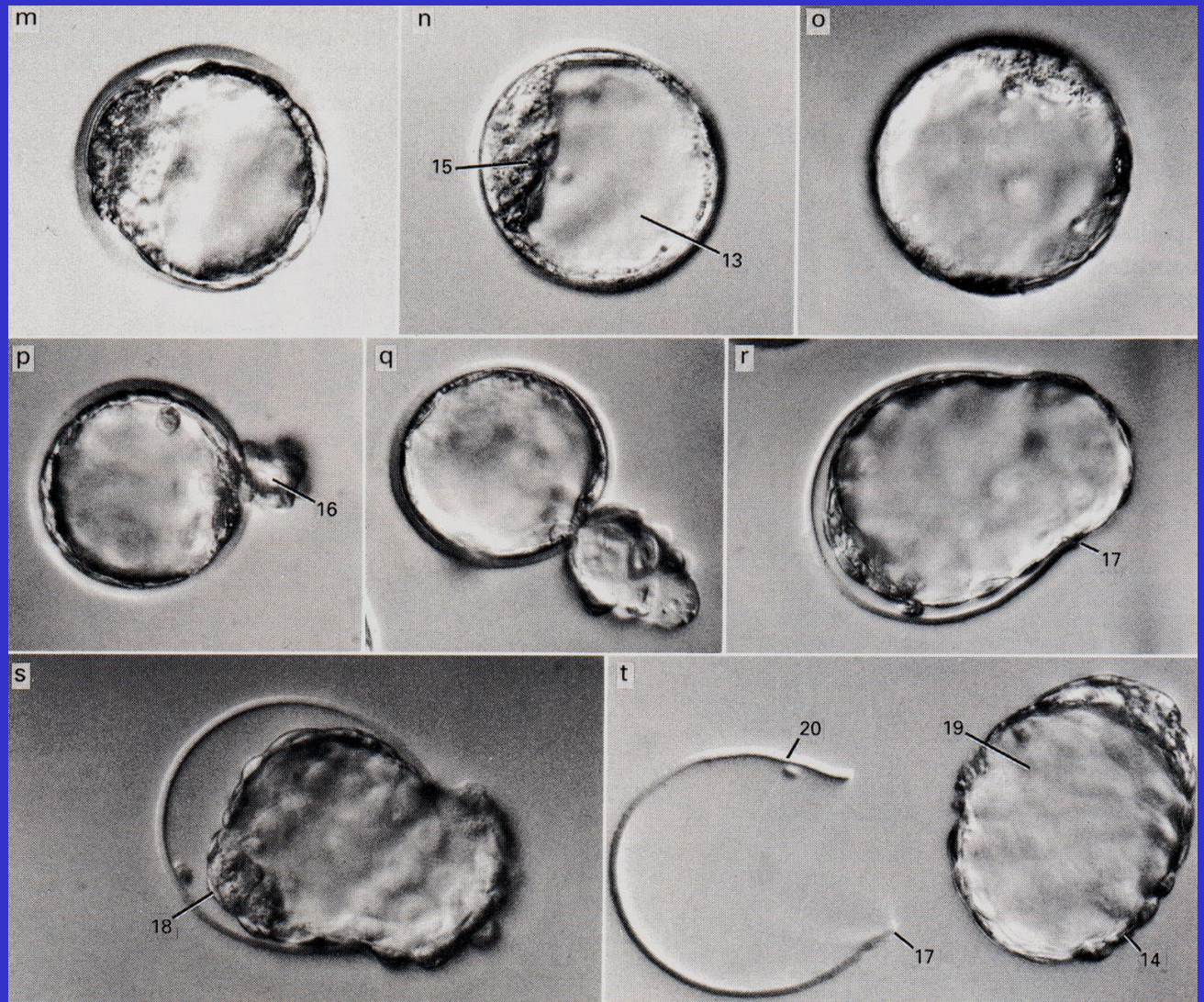


Formation of blastocyst (Blastogenesis)

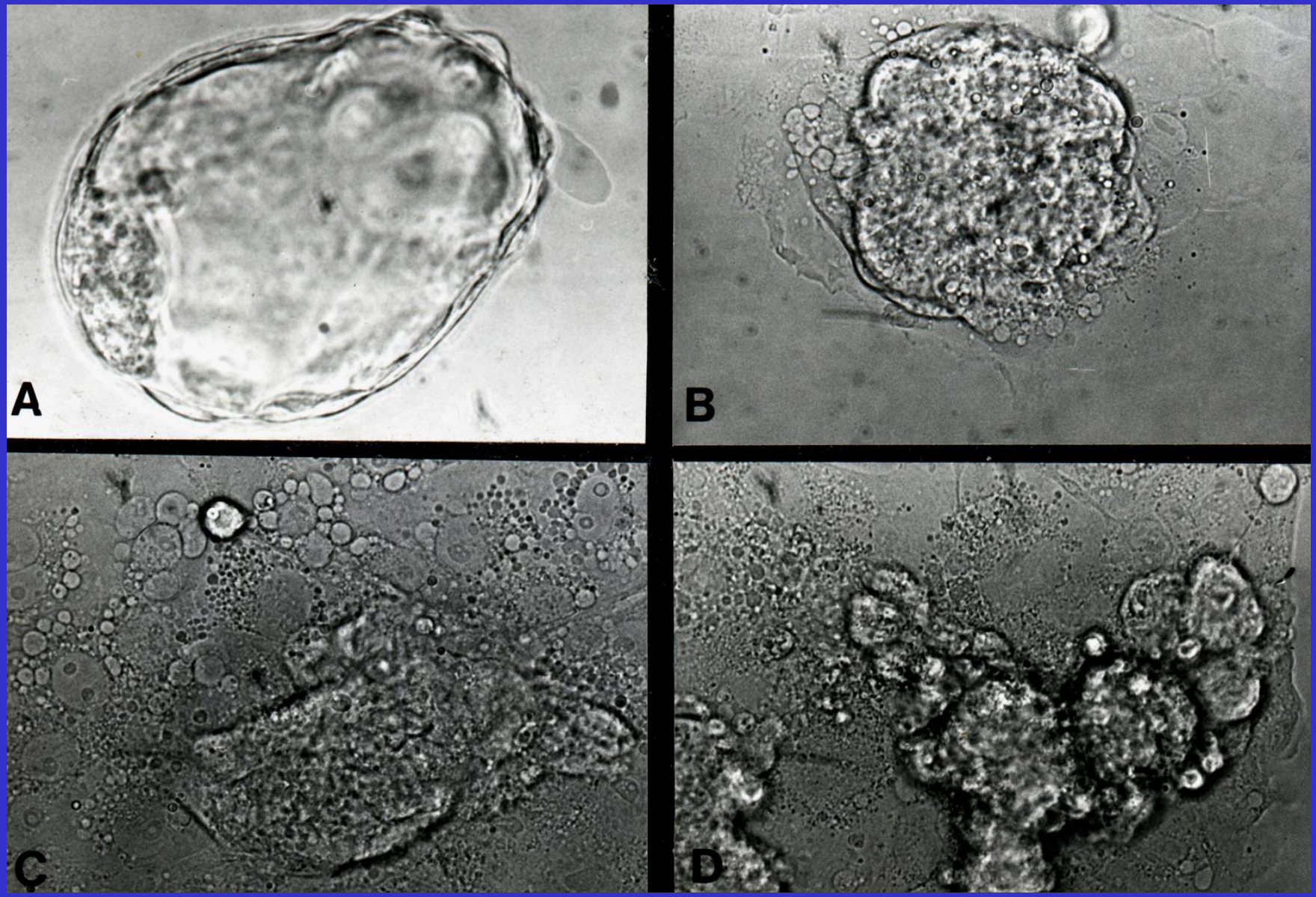
*morula → uterus (about 4 days after fertilization) → formation of blastocyst cavity (blastocele)

-- outer layer → trophoblast → embryonic part of placenta

-- inner layer → **inner cell mass (or embryoblast)** → embryo



Blastocyst hatching out and implantation



Human Embryonic Stem (ES) cells: derived from **inner cell mass**

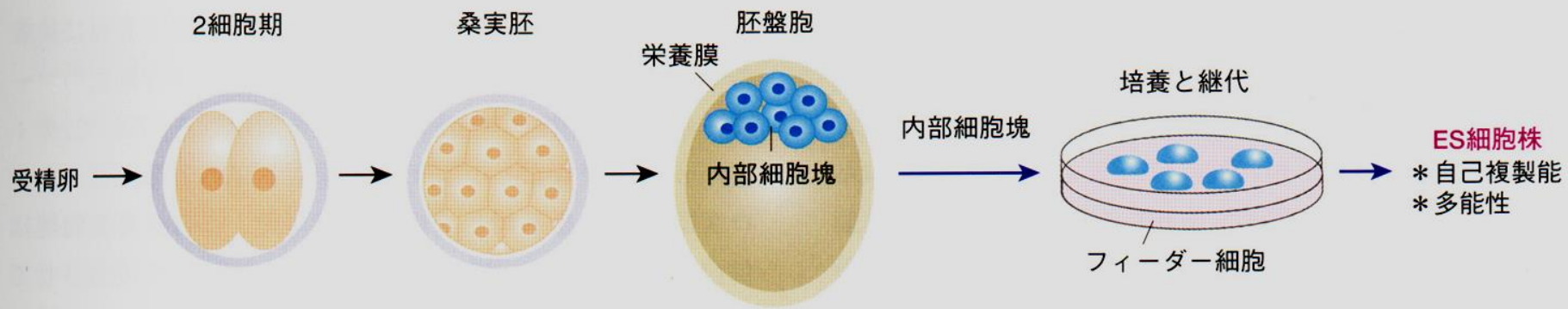
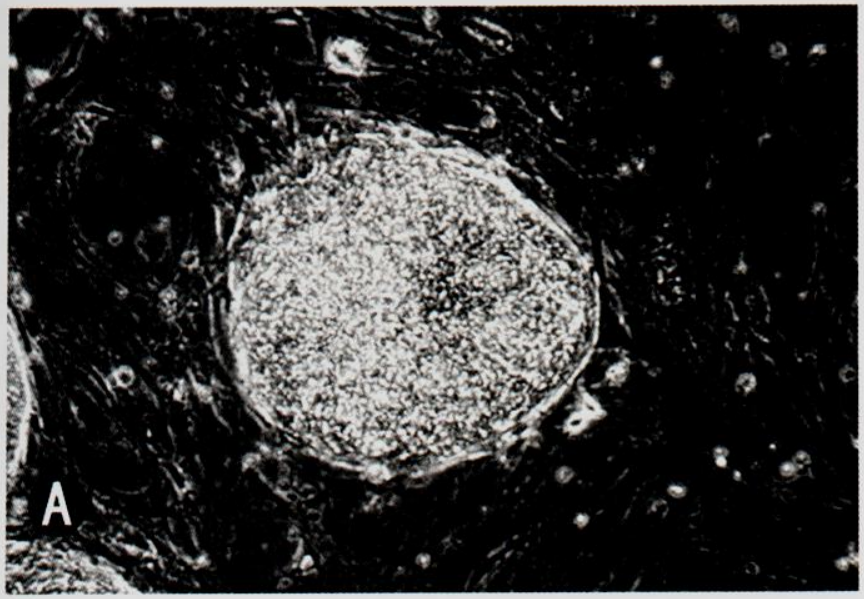
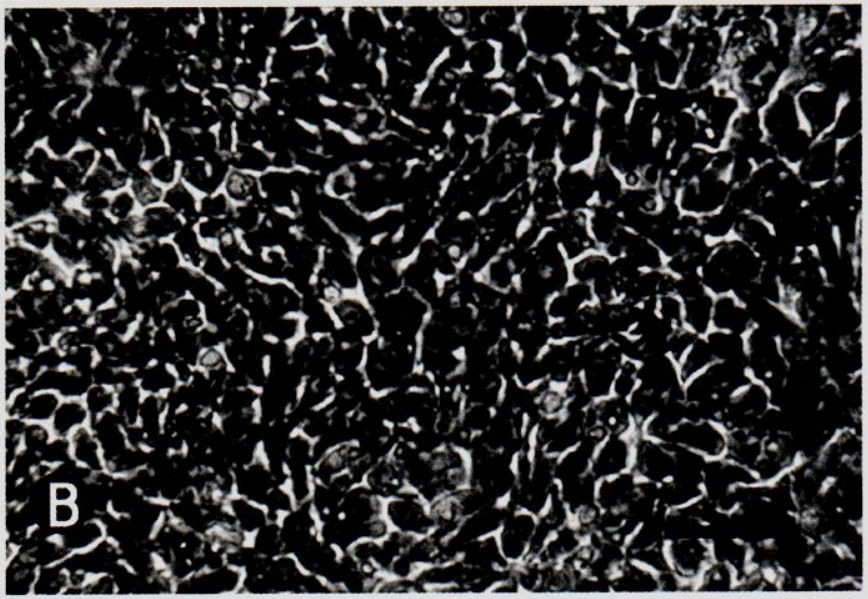
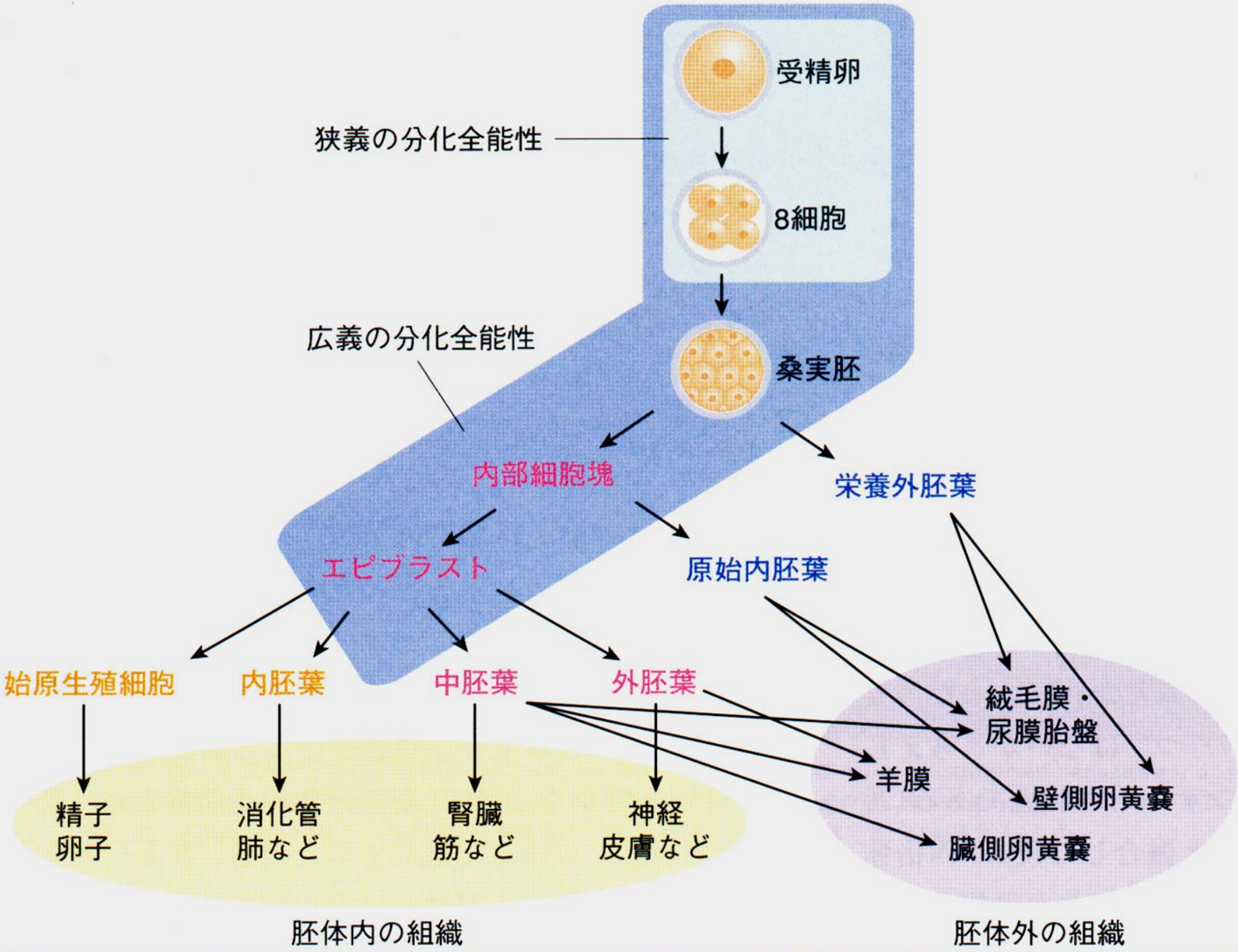


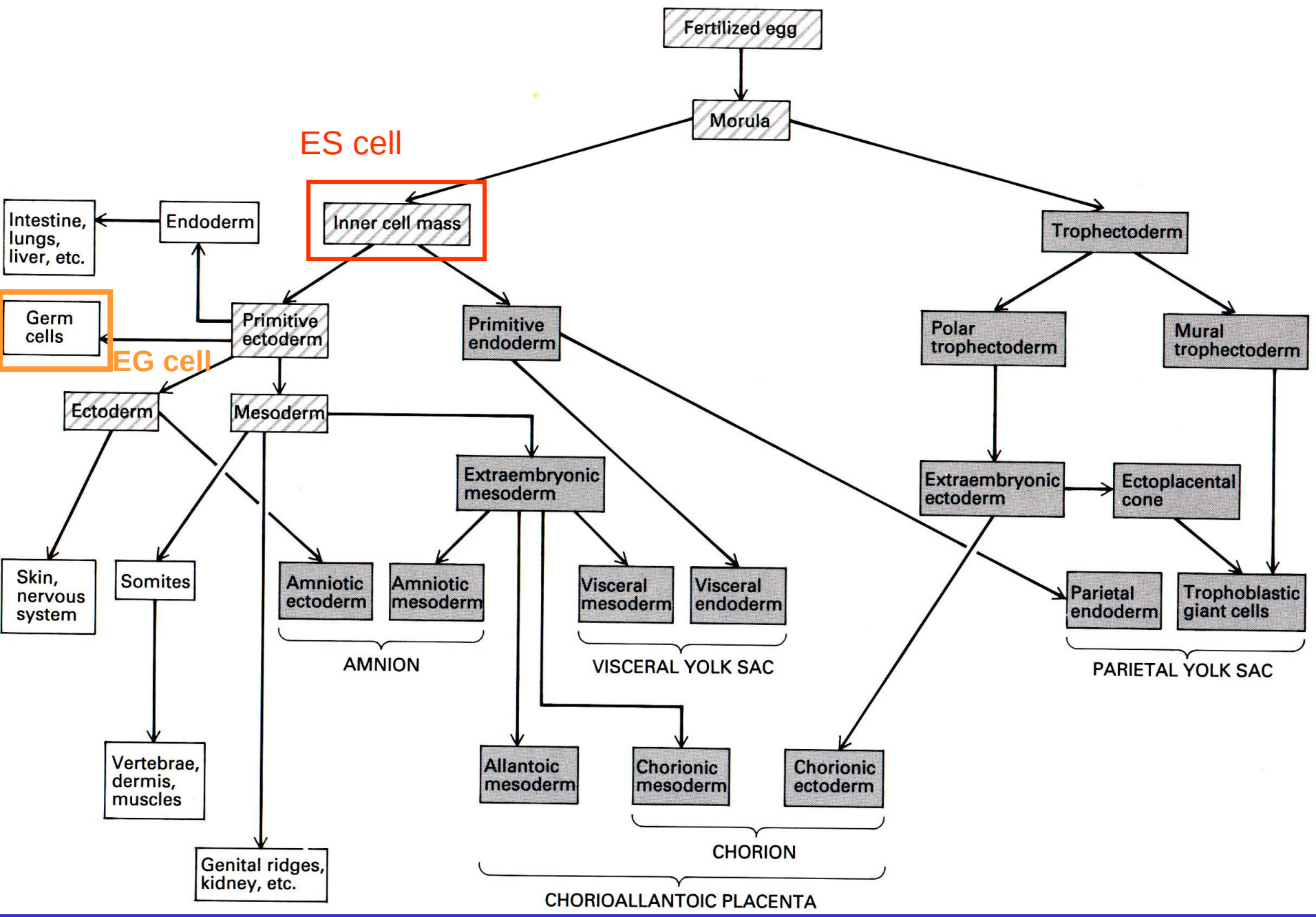
図1. ヒトES細胞の樹立法

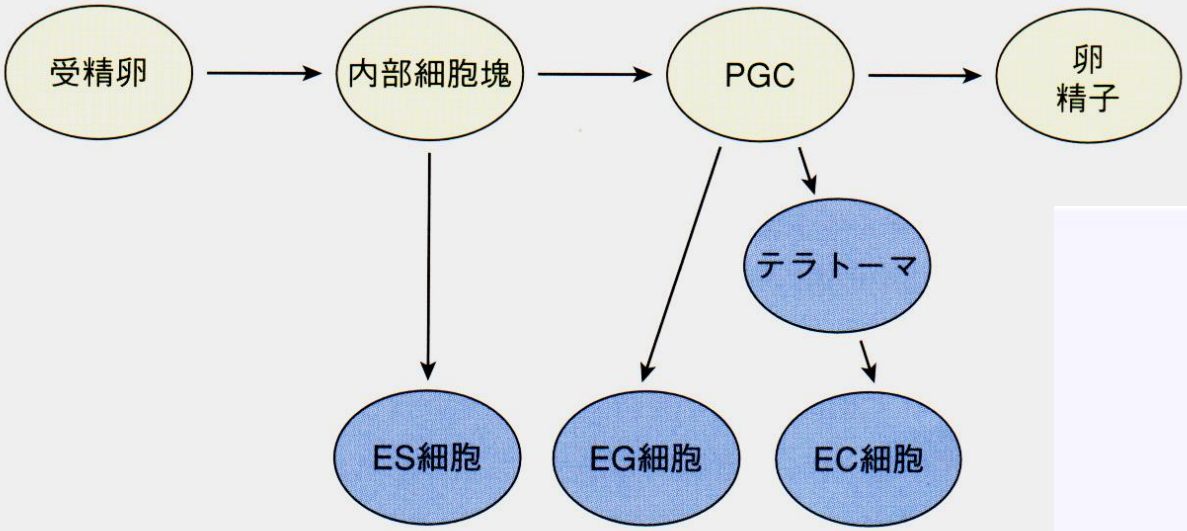
胚盤胞から分離した内部細胞塊をフィーダー細胞（マイトマイシンCやγ線などで不活性化したマウス胎仔線維芽細胞）上で培養し，未分化細胞を選抜しながらフィーダー細胞上で継代培養を続けることにより樹立される．

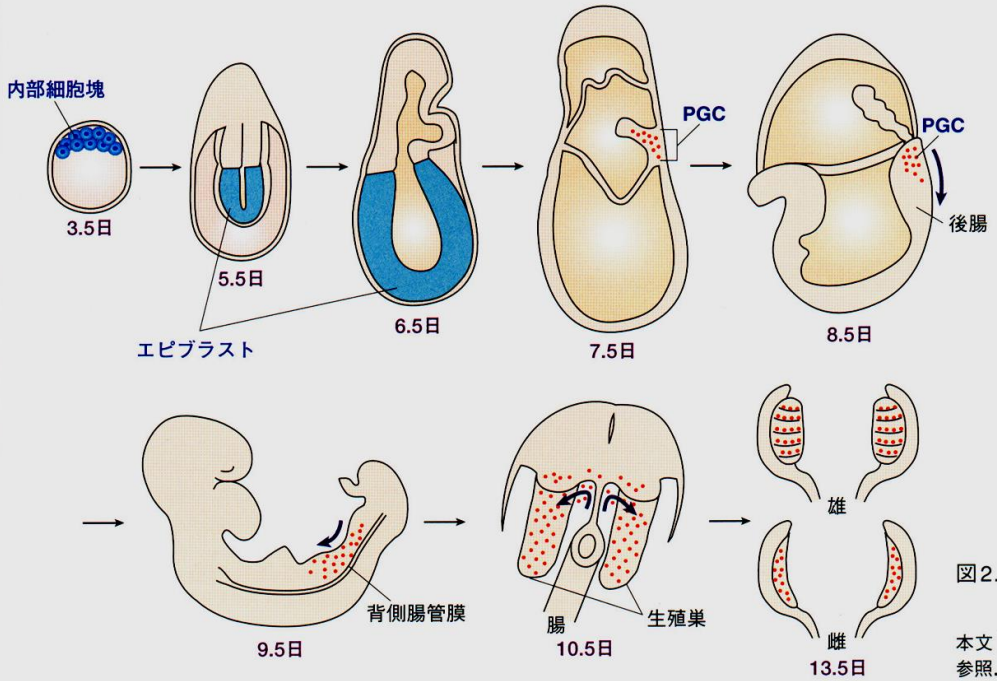





受精卵
狭義の分化全能性
8細胞
広義の分化全能性
桑実胚
内部細胞塊
栄養外胚葉
エピブラスト
原始内胚葉
始原生殖細胞
内胚葉
中胚葉
外胚葉
絨毛膜・
尿膜胎盤
羊膜
壁側卵黄嚢
臓側卵黄嚢
精子
卵子
消化管
肺など
腎臓
筋など
神経
皮膚など
胚体内の組織
胚体外の組織


Fertilized egg
Morula
ES cell
Inner cell mass
Trophectoderm
Endoderm
Intestine, lungs, liver, etc.
Germ cells
EG cell
Primitive ectoderm
Primitive endoderm
Polar trophectoderm
Mural trophectoderm
Ectoderm
Mesoderm
Extraembryonic mesoderm
Extraembryonic ectoderm
Ectoplacental cone
Skin, nervous system
Somites
Amniotic ectoderm
Amniotic mesoderm
Visceral mesoderm
Visceral endoderm
Parietal endoderm
Trophoblastic giant cells
AMNION
VISCERAL YOLK SAC
PARIETAL YOLK SAC
Vertebrae, dermis, muscles
Allantoic mesoderm
Chorionic mesoderm
Chorionic ectoderm
CHORION
Genital ridges, kidney, etc.
CHORIOALLANTOIC PLACENTA





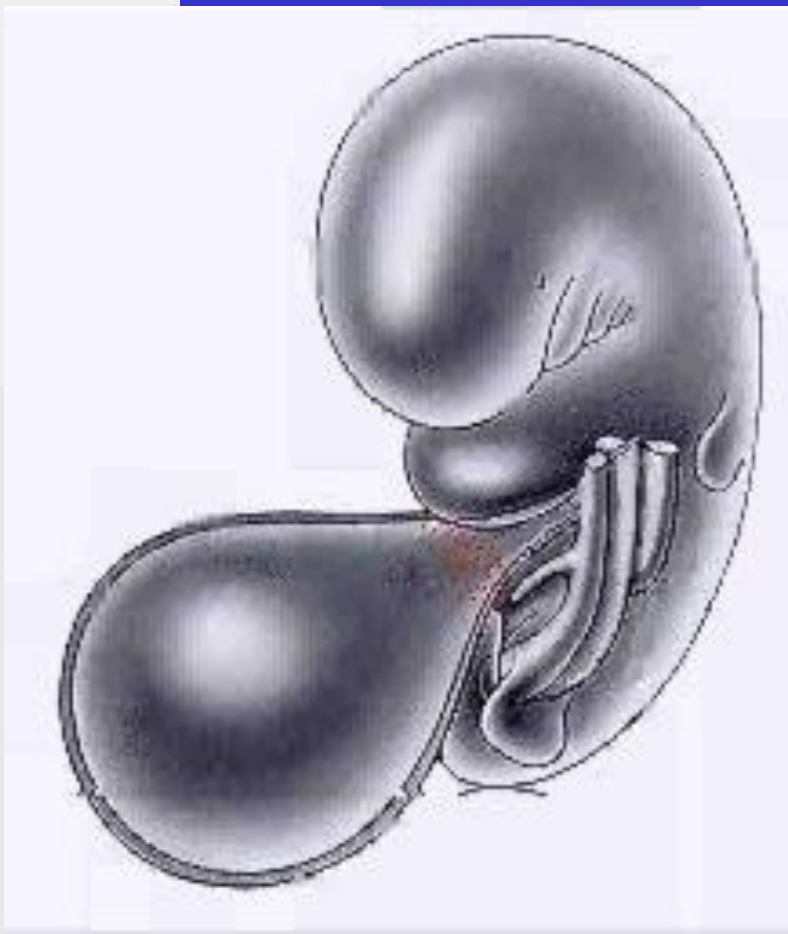
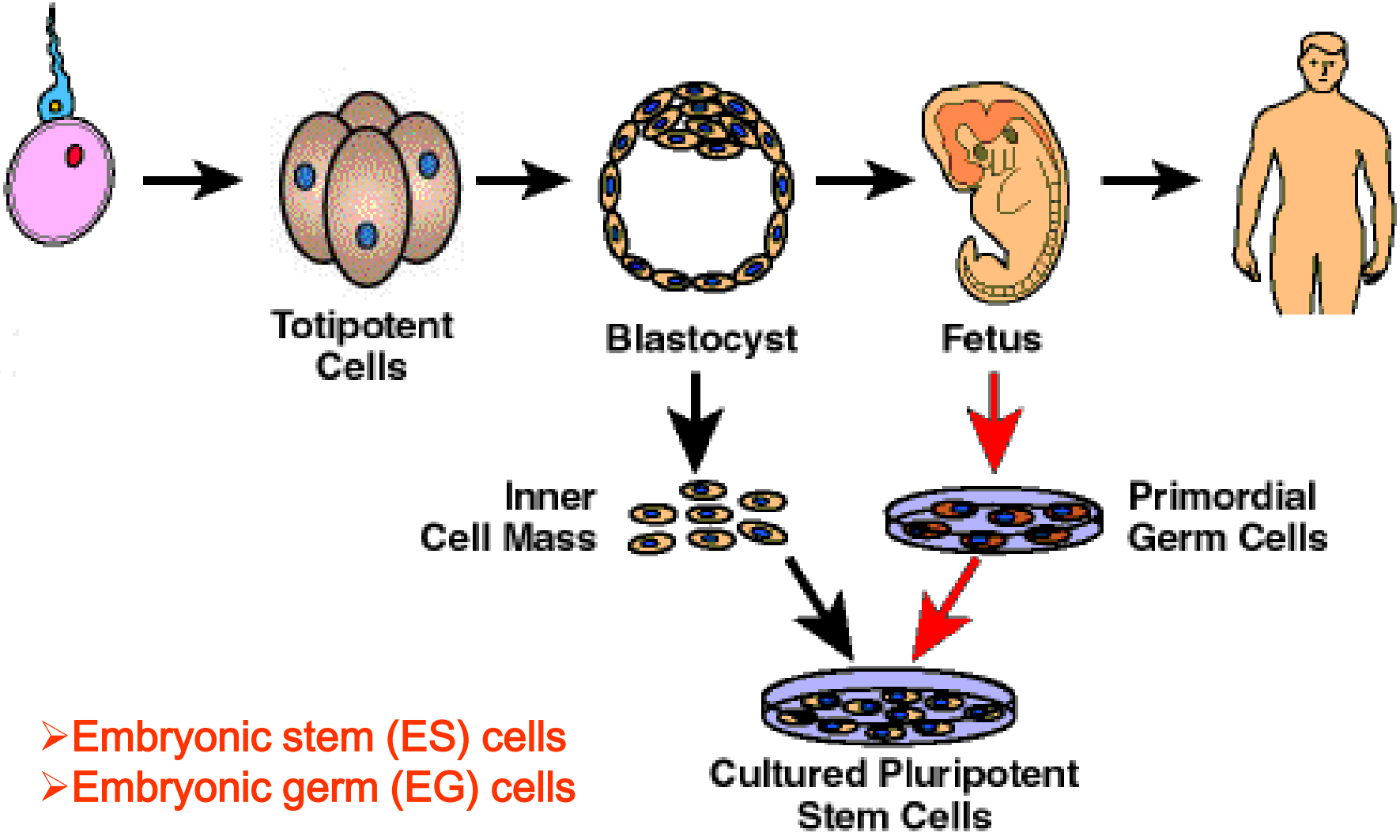


図2. マウス発生における分化多能性細胞とPGC

本文「Ⅰ. 始原生殖細胞（PGC）」参照.

動し，そこで雌雄それぞれの生殖細胞として分化を開始する.

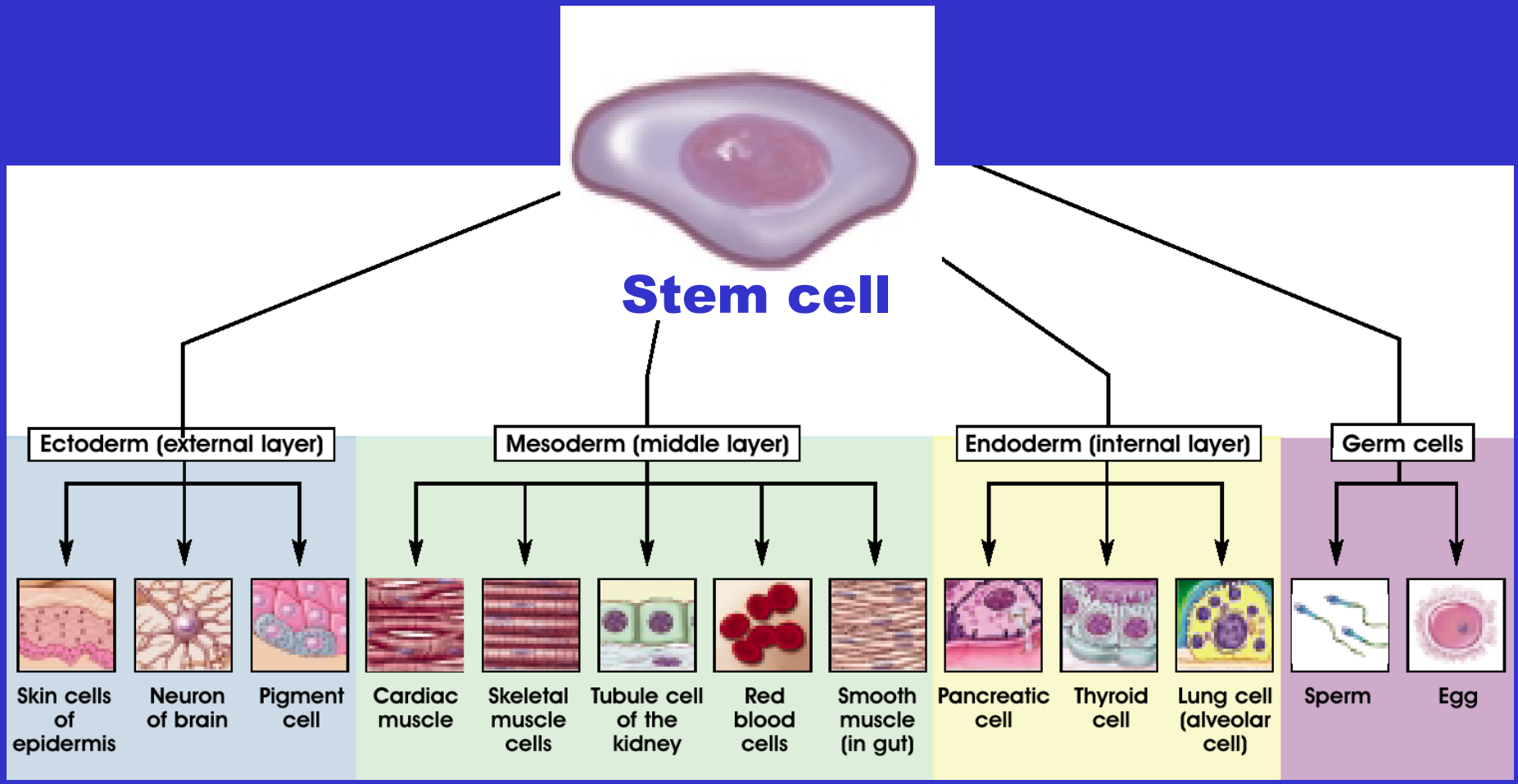
Pluripotent stem cells



- Embryonic stem (ES) cells
- Embryonic germ (EG) cells

Cell differerentiation

With combination of growth differentiation factors

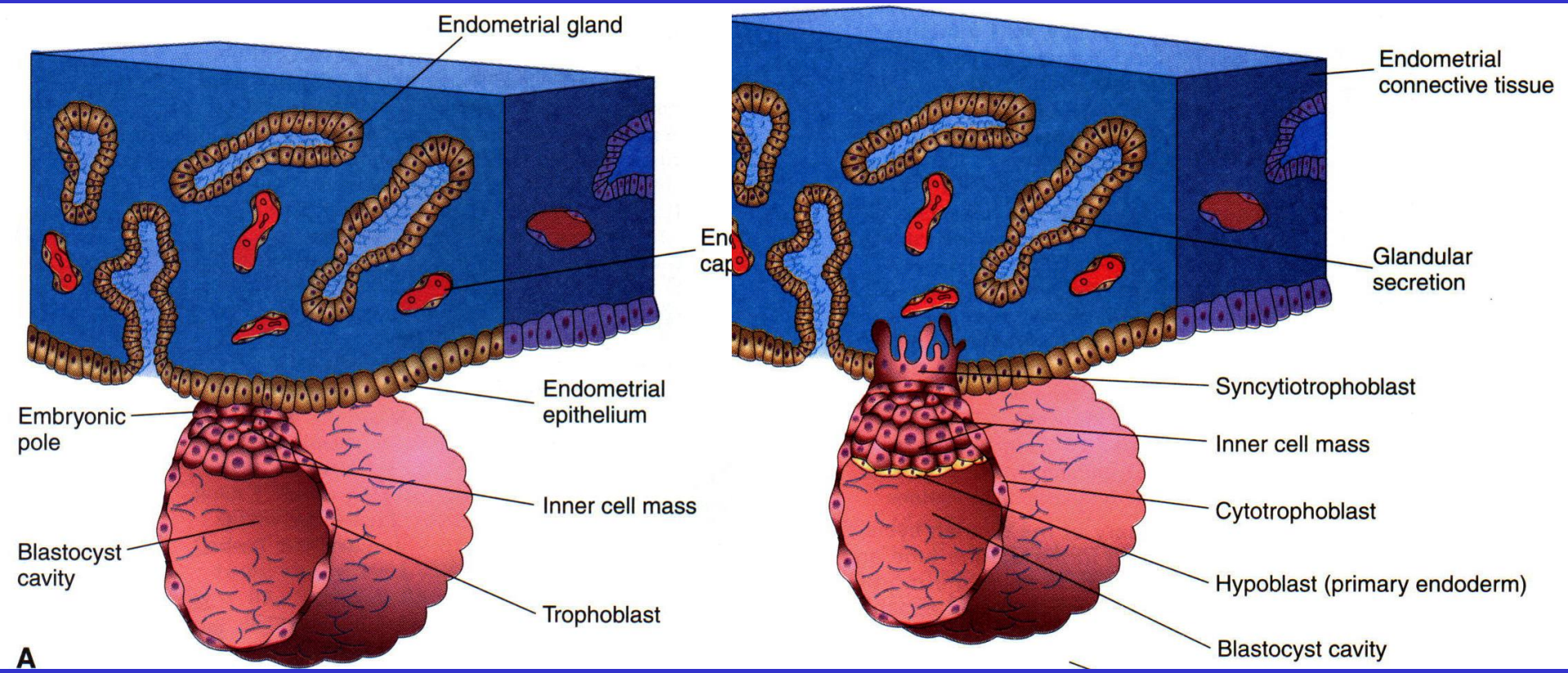


Implantation:

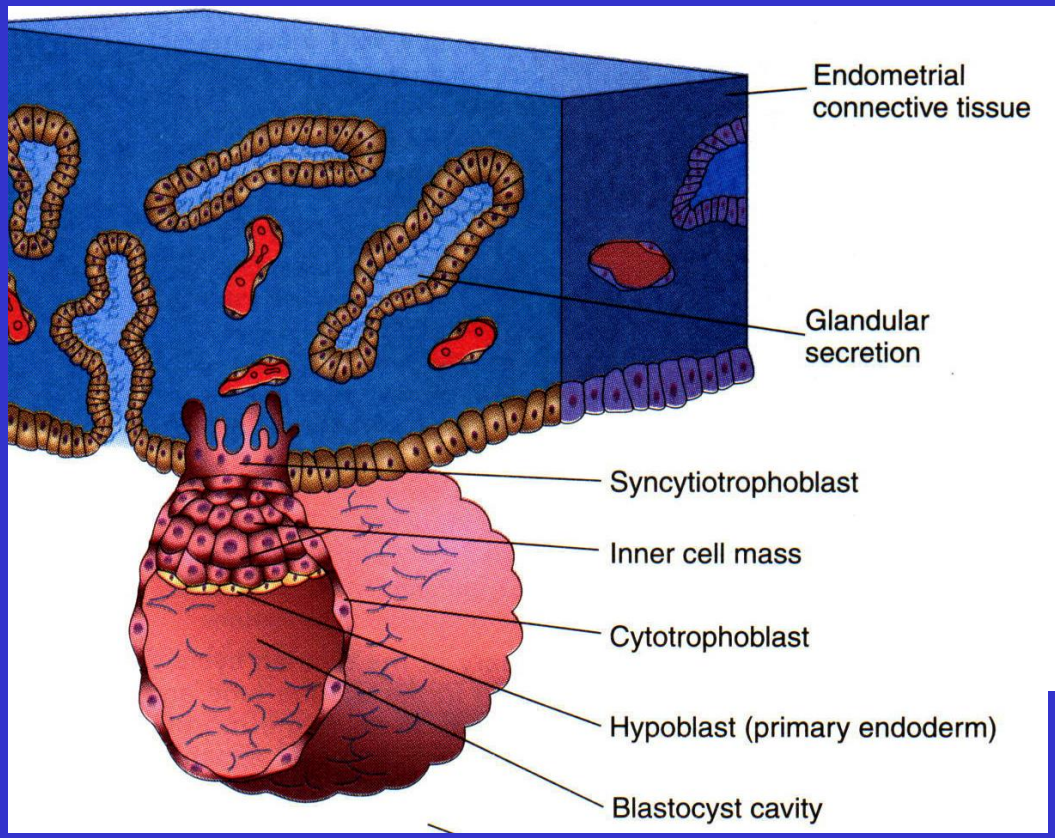
-- begins about 6 days after fertilization and is completed by end of the 2nd wk
blastocyst → embedded in endometrium (embryonic pole)

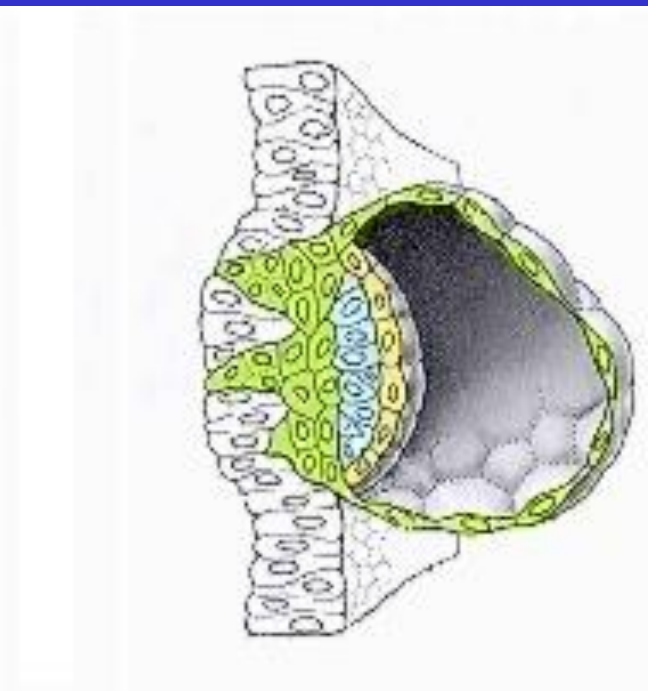
-- *cytotrophoblast* (inner layer)

-- syncytiotrophoblast (outer layer) ⇒ proteolytic enzymes → proteolysis



-- stromal cells surrounding the implantation site ⇒ *decidual cells*
-- *hypoblast:* appears on the surface of the inner cell mass facing the *blastocyst cavity*
-- hormone *(hCG):* -- produced from the syncytiotrophoblast
-- maintains the activity of corpus luteum during pregnancy and forms the basis for pregnancy test

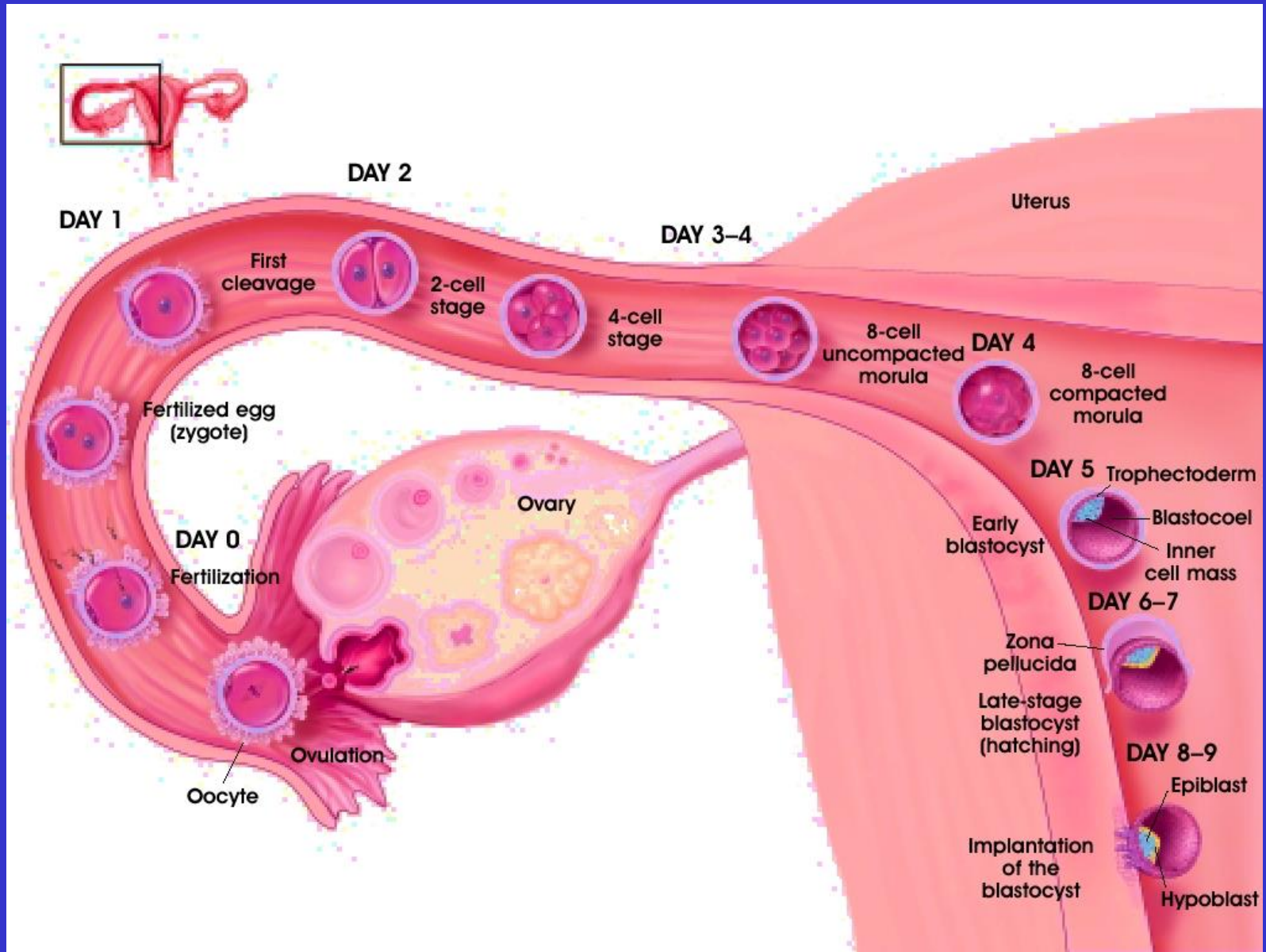




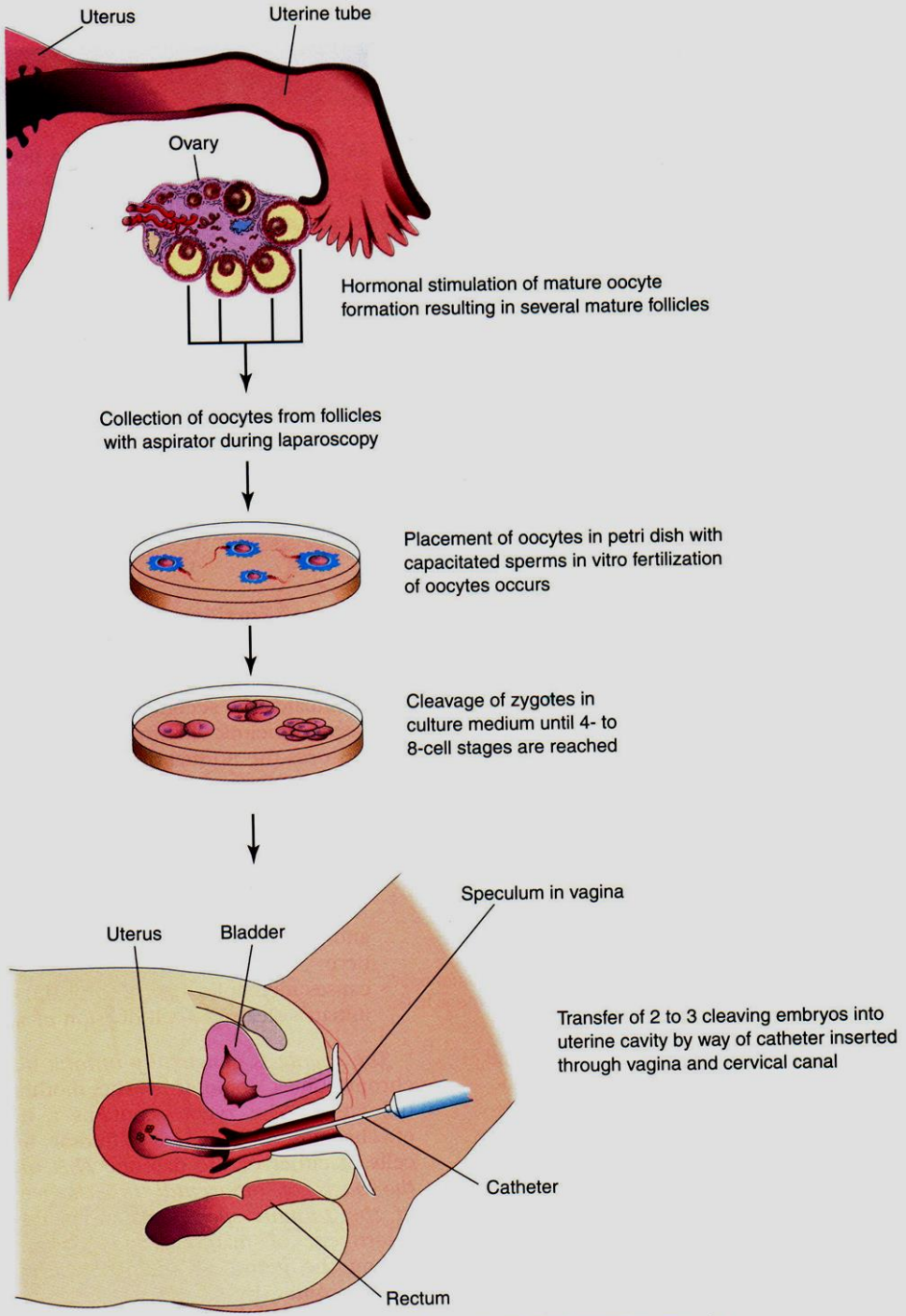
Site of implantation

Upper part of posterior or anterior wall of body of the uterus

* Abnormal implantation sites: Extra-uterine or ectopic pregnancy

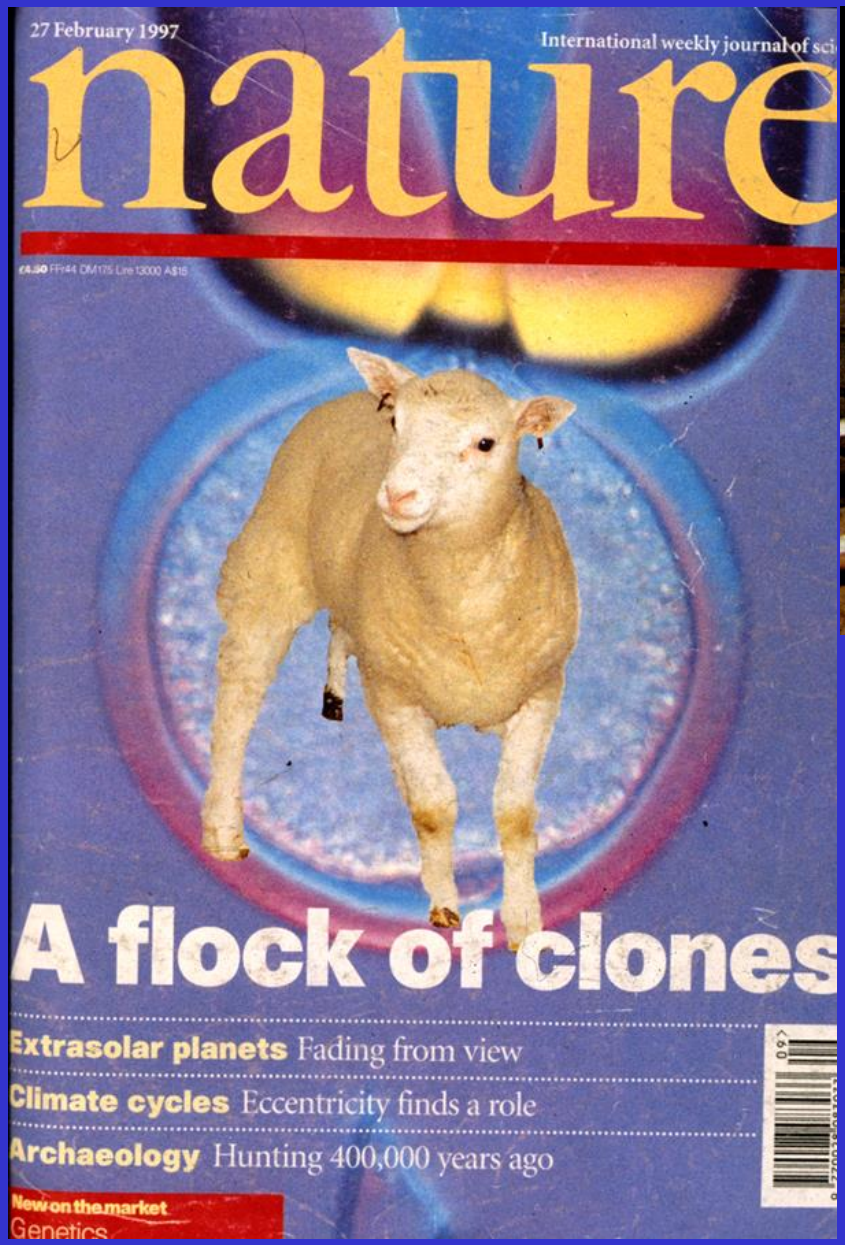


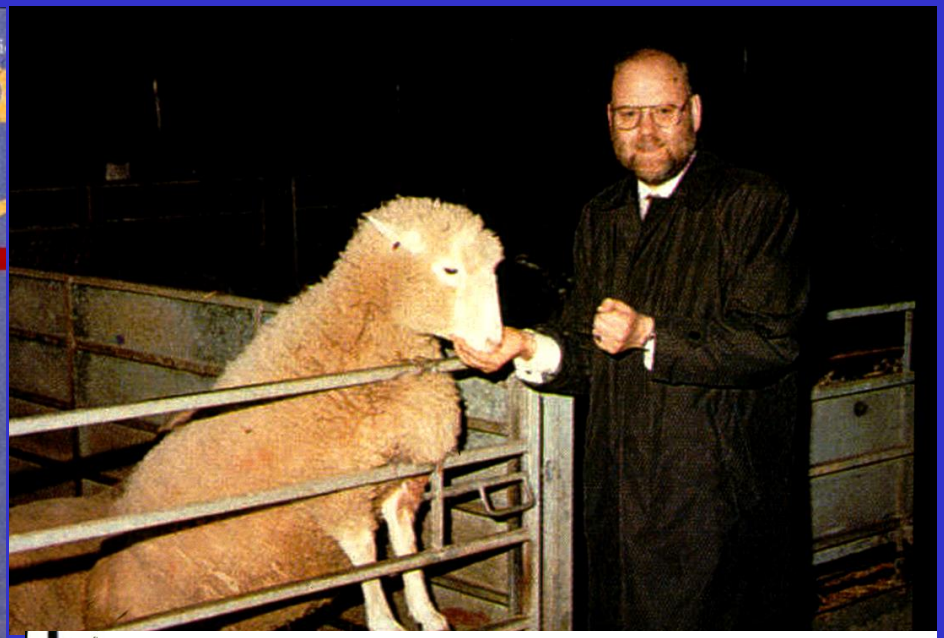
IVF (In Vitro Fertilization) and Embryo transfer

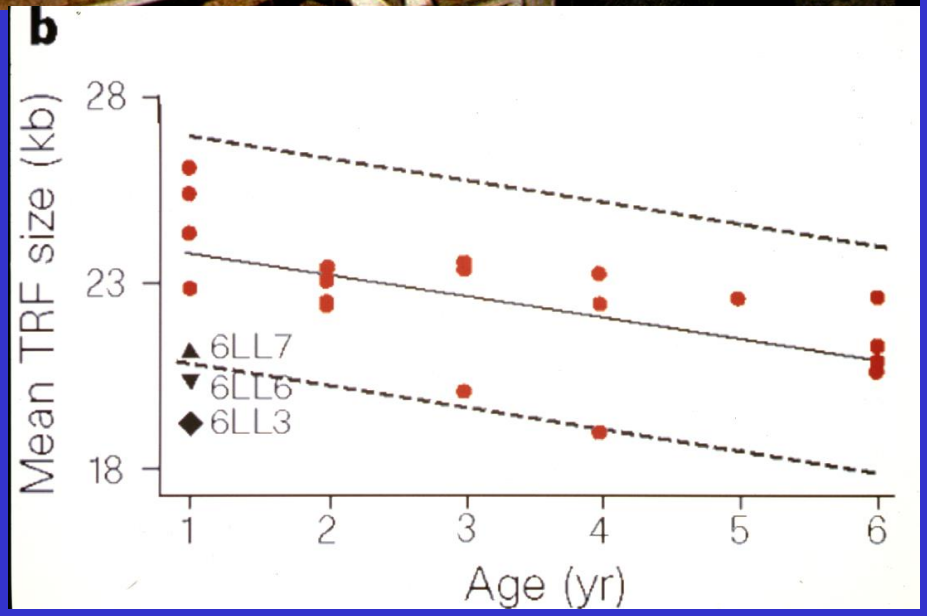


■ **Figure 2–18.** In vitro fertilization and embryo transfer procedures.

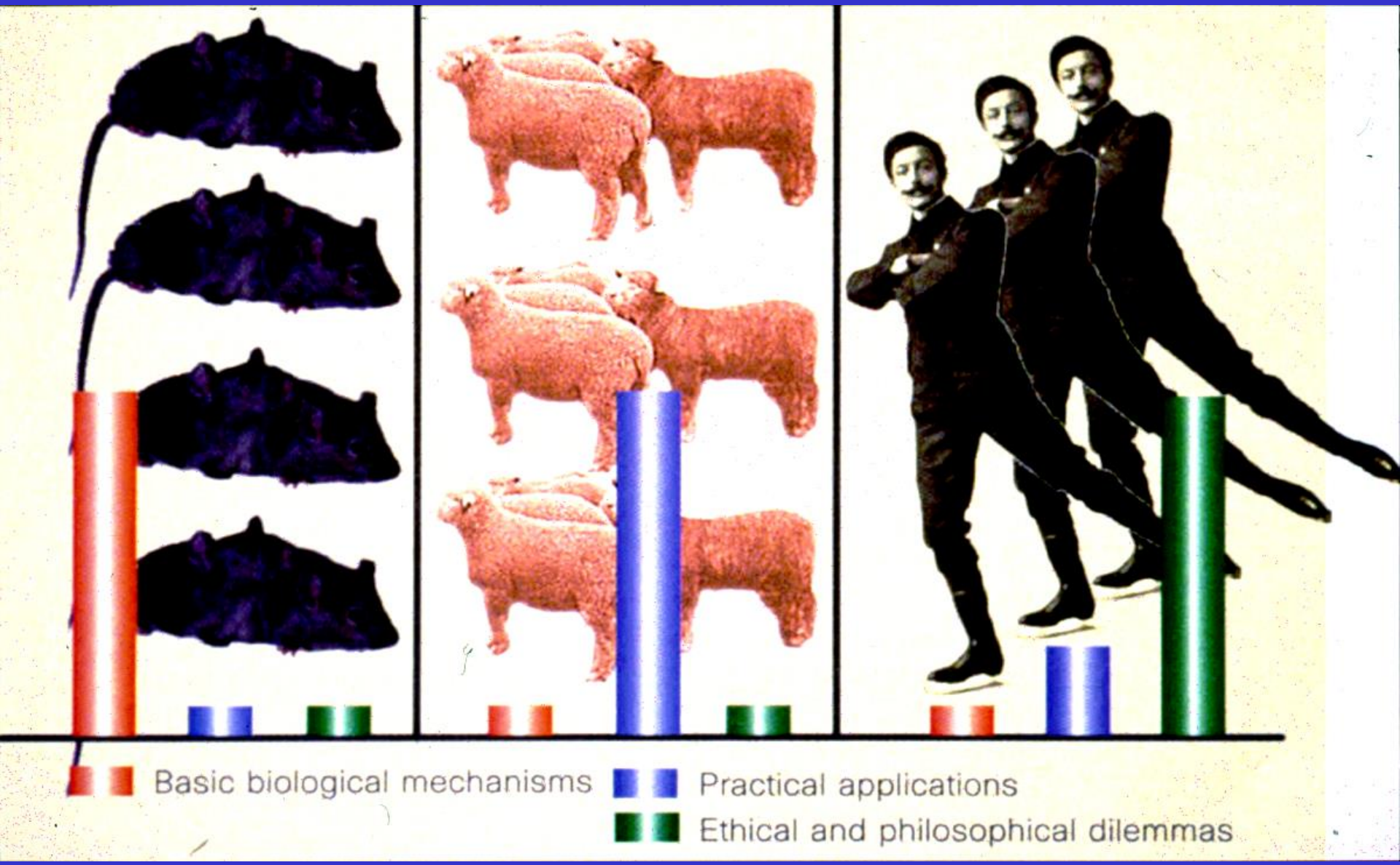
複製羊 "桃莉" (Nature 385: 810-813, 1997)



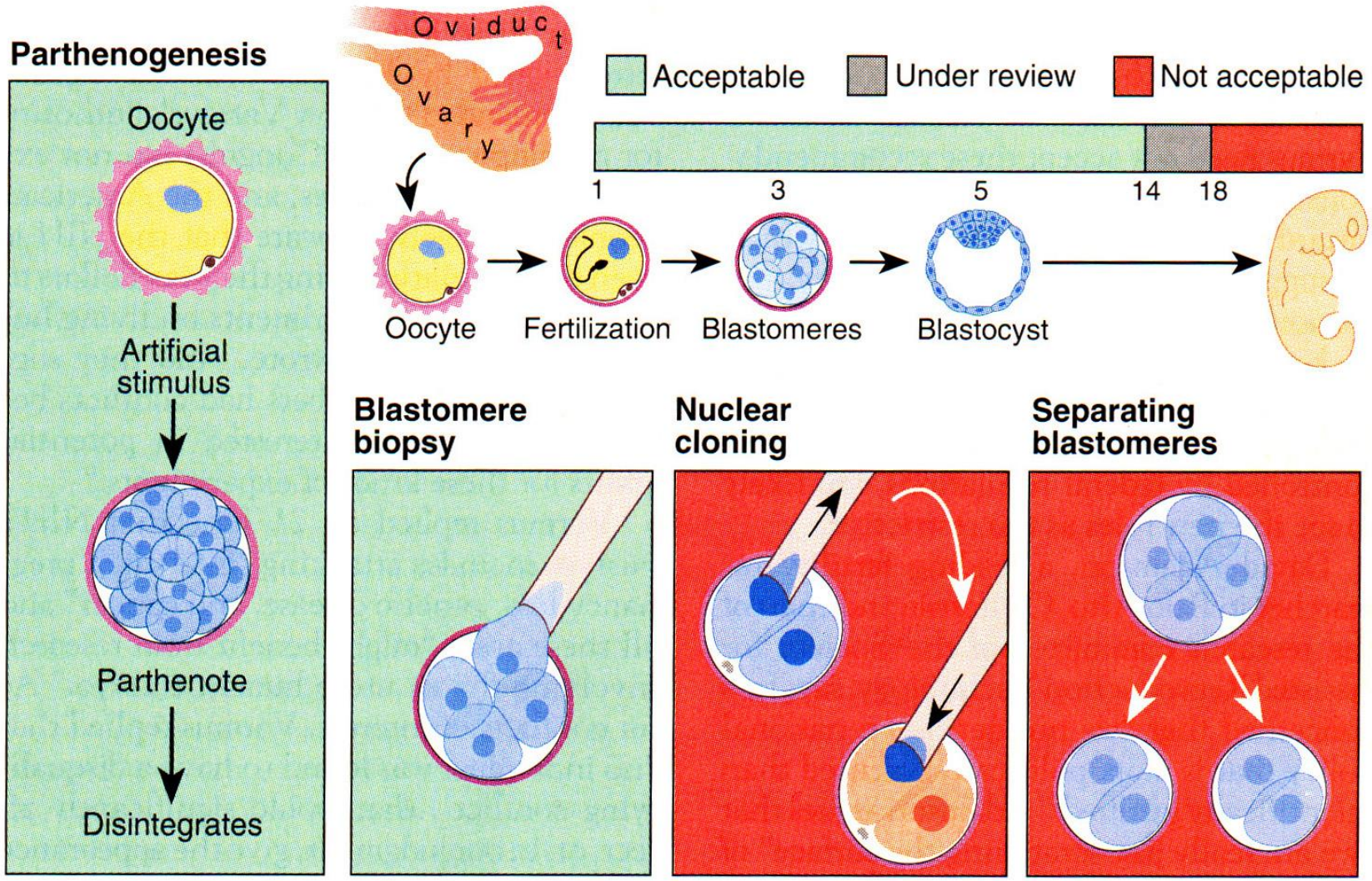




人類基因與胚胎轉殖的可行性與相關道德倫理及法律問題



美國國家衛生研究院(NIH)對人類胚胎研究規範



Fertile grounds for debate. The NIH panel's decisions were based on timing with respect to fertilization *(above)* as well as on general ethical considerations *(below)*; as a result of the latter, "twinning" by separating blastomeres was ruled out along with some other procedures.

MULLER PANEL'S GUIDELINES—IN GESTATION

FUND NOW, WITH NIH CASE-BY-CASE APPROVAL

- Research on existing, unused in vitro embryos, up to 14th day
- Limited creation of in vitro embryos for baseline data, but only for "compelling" research
- Cell extraction (blastomere biopsy) from embryos before implantation
- Derivation of cell lines from existing unused embryos
- Maturing unfertilized eggs (parthenotes) for research

NEEDING FURTHER CONSIDERATION

- Use of fetal oocytes to create embryos for research only
- Research on existing embryos beyond 14th day to neural tube closure
- Cloning by blastomere or blastocyst separation, research only
- Use of existing embryos for research when one progenitor was an anonymous gamete donor who received monetary compensation, or cannot be located to give explicit consent

NIH Muller Panel's Guidelines – in Gestation

NOT ACCEPTABLE

- Transfer of human embryos to animals for gestation
- Transfer of research embryos or parthenotes to humans
- Research on embryos beyond neural tube closure (18th day)
- Twinning (separation of blastomeres) for gestation
- Cloning of embryos by nuclear transplantation
- Creation of human-human or human-animal chimeras
- Creation of embryos strictly for research material, e.g., stem cells
- Cross species fertilization with human gametes, except clinical testing of sperm penetration (with hamster eggs)
- Transfer of embryos to cavity other than uterus
- Sex selection of embryos, except to prevent x-linked diseases
- Use of sperm, eggs, or embryos from donors who did not give explicit consent to research
- Use of sperm, eggs, or embryos for which donors received more than reasonable compensation