Volgograd state medical university

Department of histology, embryology, cytology

Progenesis
Germ Cells. Fertilization. Cleavage.

for the 1st course
English medium students

Objectives

1. To evaluate the differences in the structure of the male and female gametes.
2. To characterize meiosis and to reveal its discriminative features with mitosis.
3. To understand the process of fertilization and to assess significance of its stages.
4. To describe cleavage of the human zygote as the initial stage of the pre-embryonic period.

Embryology is a study of the embryonic development.

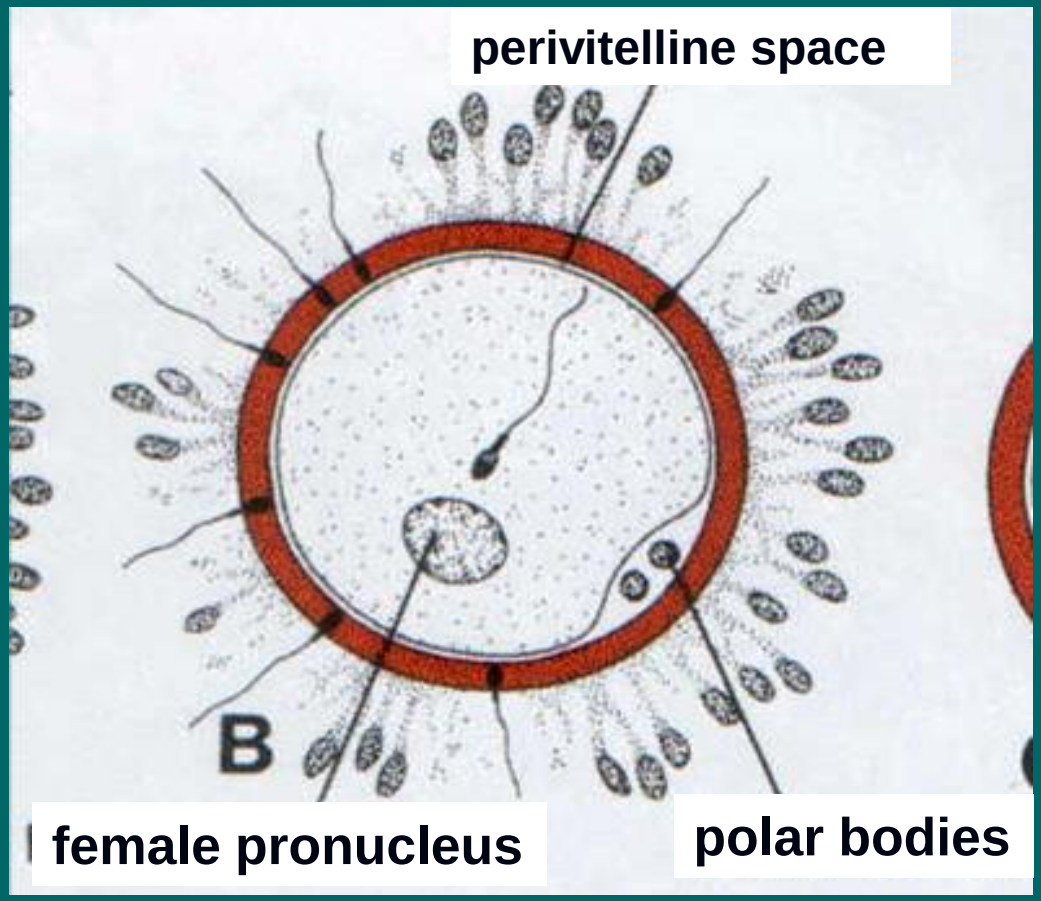
- Embryon – conceptus, logos – study (Greek)

- Embryogenesis is a stage of the ontogenesis.

- Embryogenesis is closely related to the progenesis (or gametogenesis = development and maturation of the germ cells) and early postnatal ontogenesis.

- Ontogenesis is a brief repetition of phylogenesis (the biogenetic principle by Heckel- Mueller, the middle of the 19th century).

The development of a human being begins with fertilization, a process by which the spermatozoon from the male and the oocyte from the female unite to give rise to a new organism, the zygote.



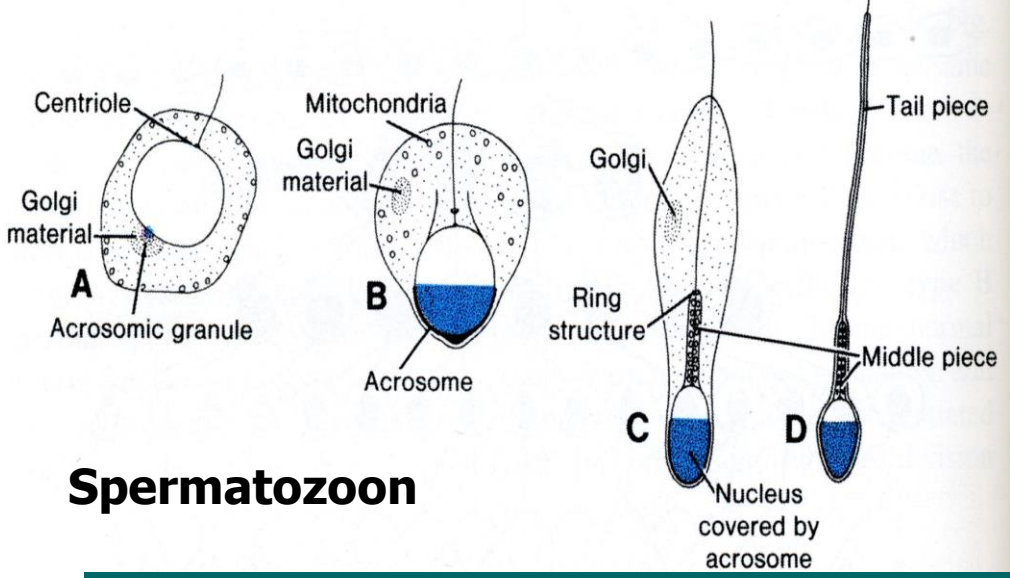
FERTILIZATION

MEIOSIS

Meiosis is essential for sexual reproduction and procreation. In preparation for fertilization, both male and female germ cells undergo meiosis and cytodifferentiation. The purpose of these processes is twofold:

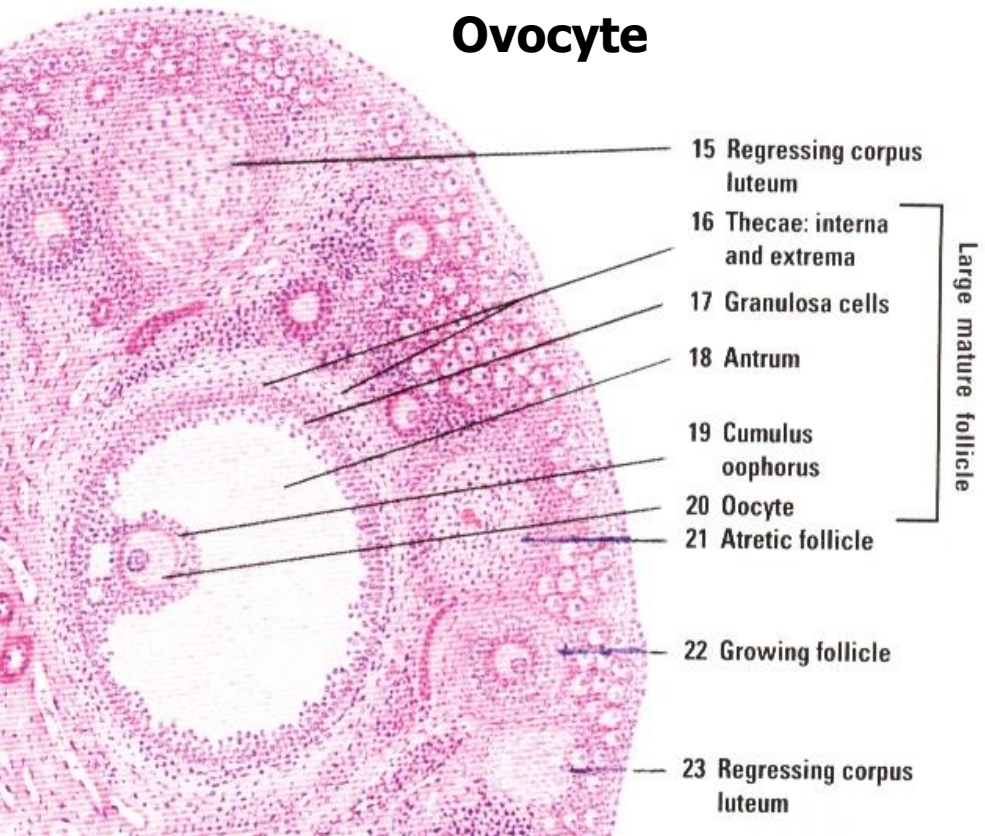
1.To reduce the number of chromosomes from the diploid number of 46 observed in somatic cells, to the haploid number of 23 observed in the gametes (ploidy refers to the number of copies of each chromosome). This is accomplished by meiotic or maturation divisions and is necessary, since fusion of a male and female germ cell would otherwise result in an individual with twice the number of chromosomes of the parent cells.

MEIOSIS



Spermatozoon

Ovocyte

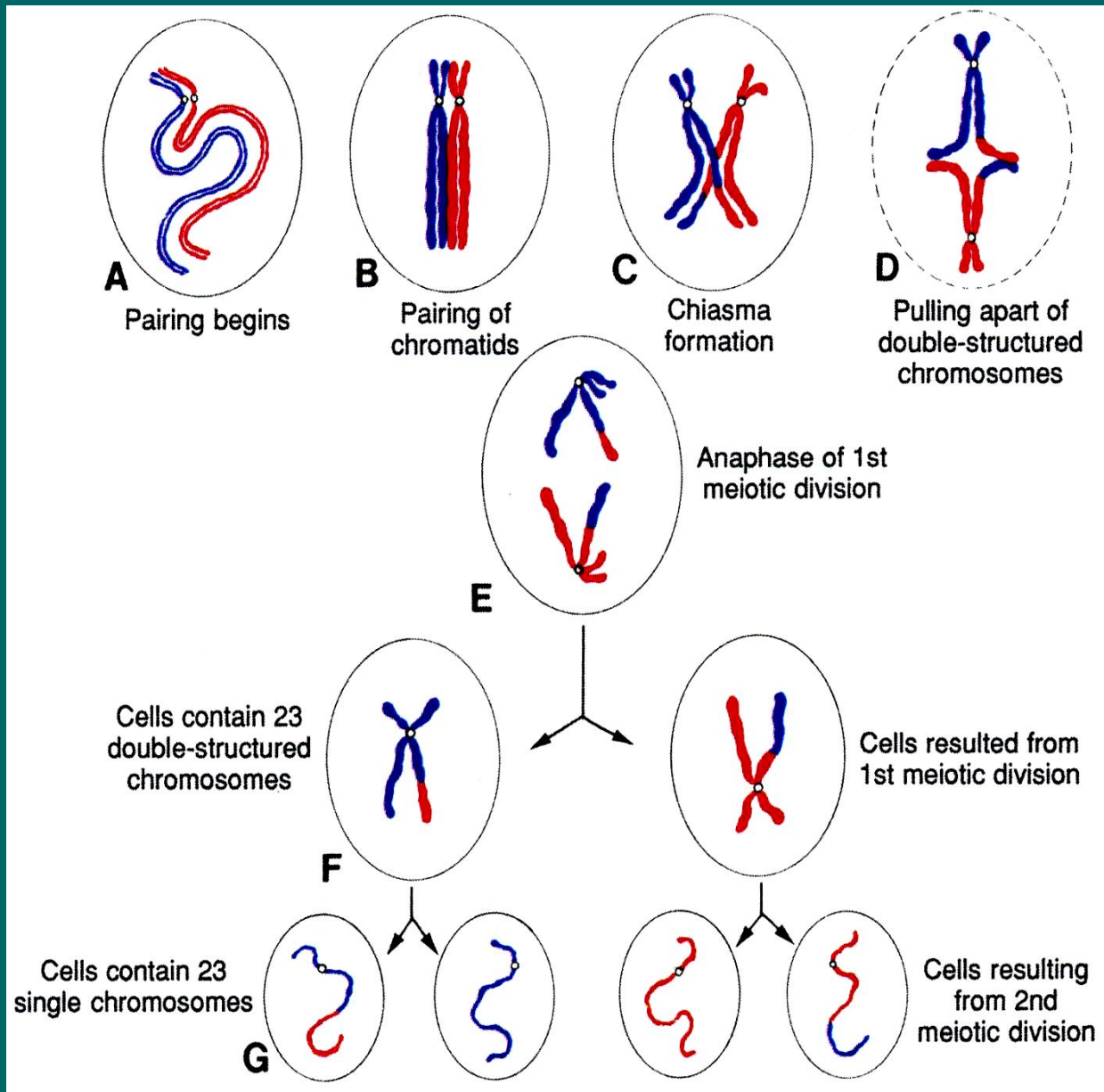


2.To alter the shape of the germ cells in preparation for fertilization. The male germ cell, initially large and round, loses practically all of its cytoplasm and develops a head, neck and tail. The female germ cell, on the other hand, gradually becomes larger as the result of an increase in the amount of cytoplasm. At maturity, the oocyte has a diameter of about 120 mçm.

Meiosis takes diploid cells with homologous pairs of chromosomes (one maternal and one paternal) and allows for recombination between genetic information provided by mother and father for each chromosome.

MEIOSIS

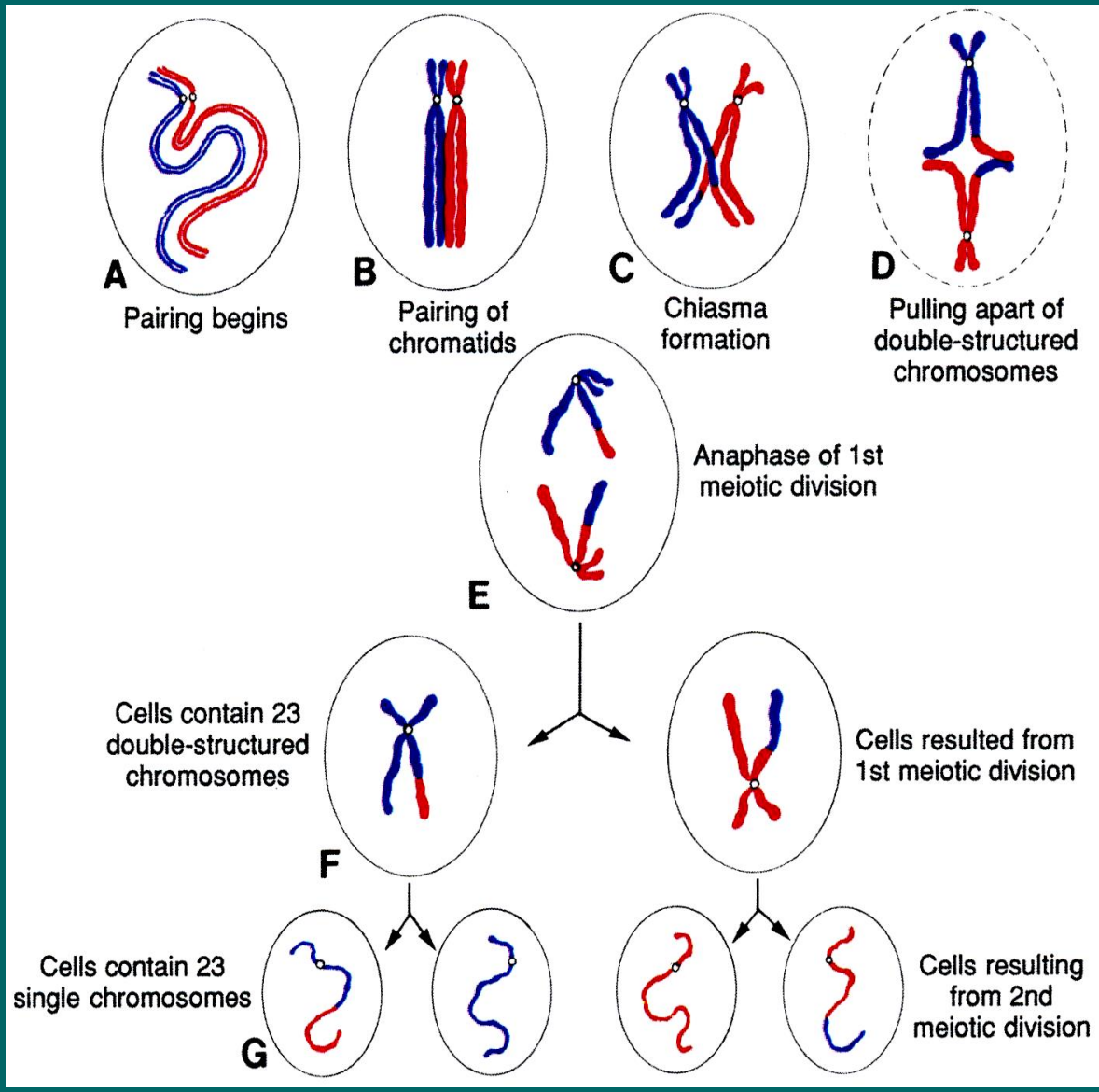
Meiosis is divided into two parts:
During the 1st meiotic division crossingover and exchange of paternal and maternal information occurs between the sister chromatids. Meiosis I requires accurate recognition and pairing of maternal and paternal chromosomes to form a complex structure (synaptonemal complex) for the exchange of genetic information.



MEIOSIS

Synapsis is the process of pairing which finally results in alignment of the two chromosomes of each homologous pair along their entire length.

The synaptonemal complex provides a means of splicing together the crossed over strands from paternal and maternal chromatids. The stages of meiosis all center around the formation and breakdown of the synaptonemal complex which contains recombination nodules (large multiprotein complexes) which facilitate chromatid exchange.



MEIOSIS I

Meiosis I is divided into stages which are similar to mitosis: interphase, prophase, metaphase, anaphase and telophase.

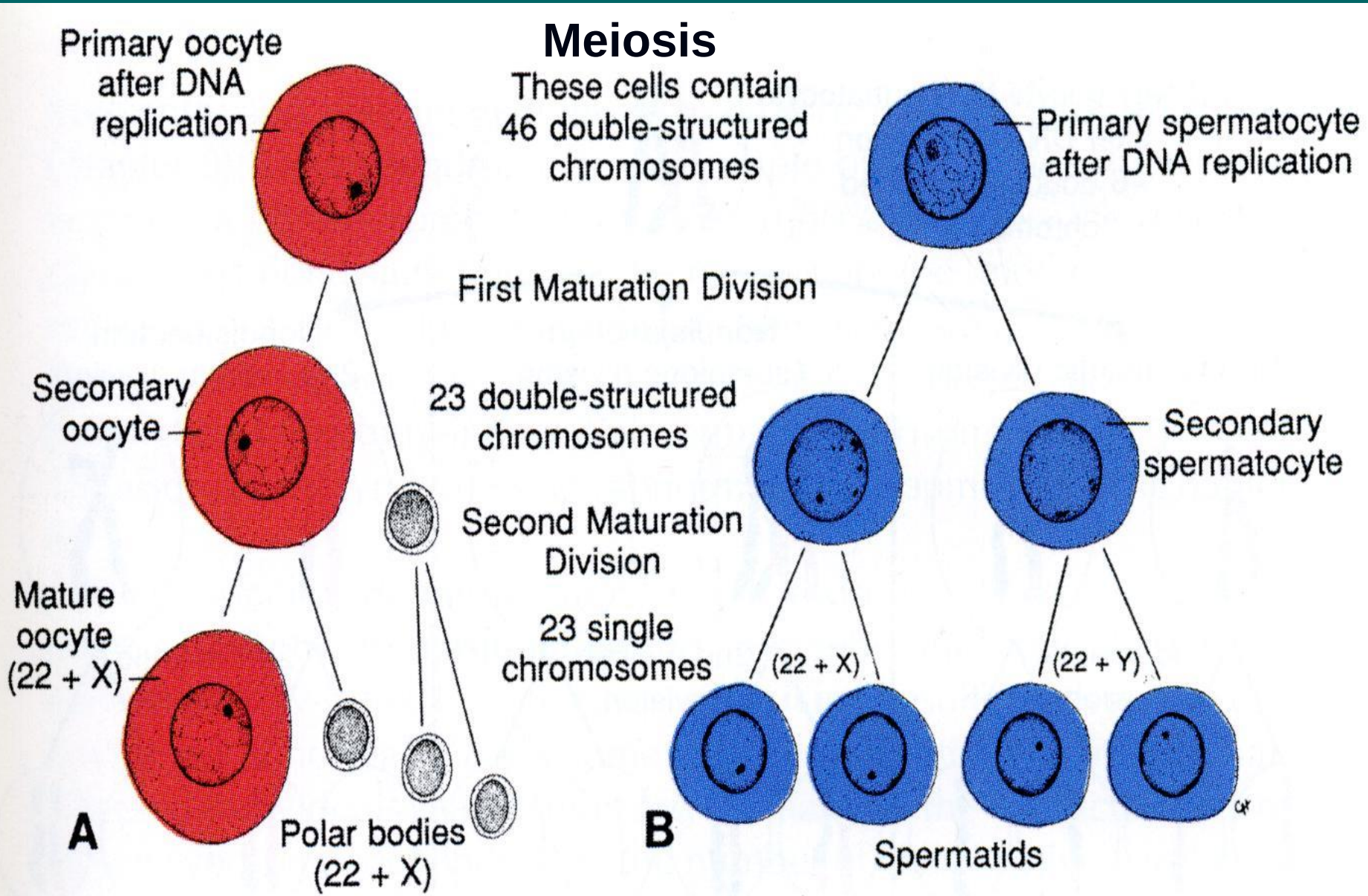
1.Interphase consists of G1, S, and G2 as in mitotic interphase. During S phase duplication of DNA occurs in the parental (diploid) cells so that each chromosome is represented by two sister chromatids.

2.Prophase I consists of 5 substages: leptotene, zygotene, pachytene, diplotene and diakinesis. During prophase I the chromosomes pair and recombination of genetic material occurs with a transition from separate maternal and paternal sister chromatids to the assembly of the synaptonemal complex, which facilitates the exchange of genetic information between sister chromatids in paternal and maternal chromosomes.

SUBSTAGES OF PROPHASE I

substage of prophase I	event occurring in the stage
leptotene	initial condensation of the chromosomes, initiation of assembly of the central component of synaptonemal complex
zygotene	continued formation of the synaptonemal complex; chromosomes pair
pachytene	synapis complete, recombination occurs. Pachytene is the stage which assures that each person is not a clone.
diplotene	beginning of disassembly of the synaptonemal complex (initiation of desynapsis); transcription of RNA
diakinesis	completion of disassembly (desynapsis), condensation is complete.

During 2nd meiotic division the haploid gametes form. Meiosis II establishes a metaphase similar to mitosis in which the homologous pairs separate into individual haploid gametes.



The distinguishing features of the meiosis (compared to mitosis):

1. **Before the onset of the 1st meiotic division the number of DNA is 4n and the number of chromosomes in 2n (as in mitosis)**

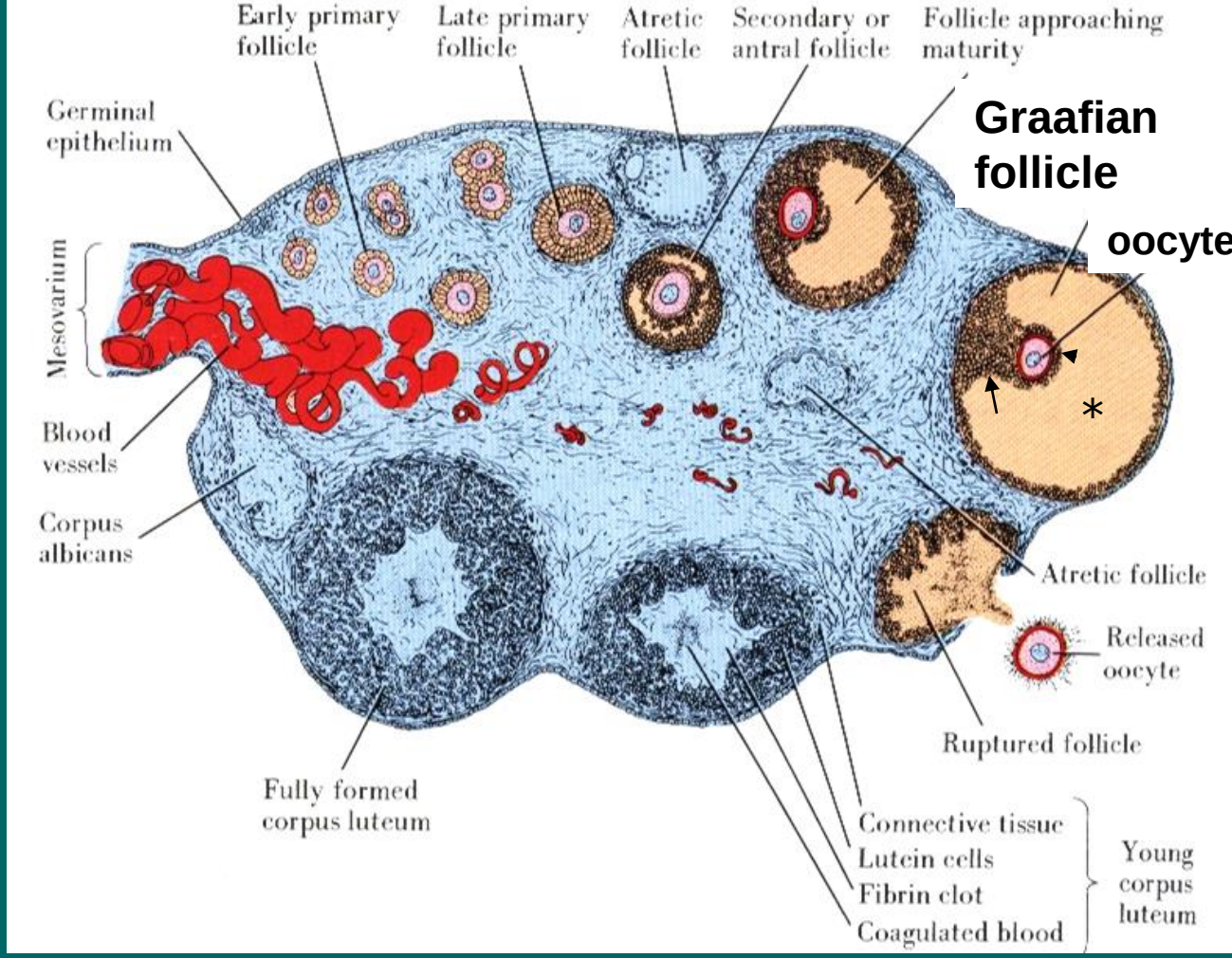
2. **The first characteristic feature of the 1st meiotic division is pairing of homologous chromosomes which are referred to as bivalents (except for the X-Y combination, while in mitotic division homologous chromosomes never pair.**

3. **The 2nd characteristic feature of the 1st meiotic division is called cross(ing)over and consists of the interchange of chromatid segments between two paired homologous chromosomes.**

4. **After the 1st meiotic division the two daughter cells contain one member from each pair of homologous chromosomes (in mitosis each daughter cells contains one of the sister chromatids from 46 double structured chromosomes).**
5. **No DNA synthesis occurs in advance of the 2nd meiotic division (on the contrary to mitosis). Before the 2nd meiotic division the number of chromosomes is 1n while the number of DNA is 2n.**
6. **After the 2nd meiotic division the amount of chromosomes is 1n and of the DNA – 1n.**

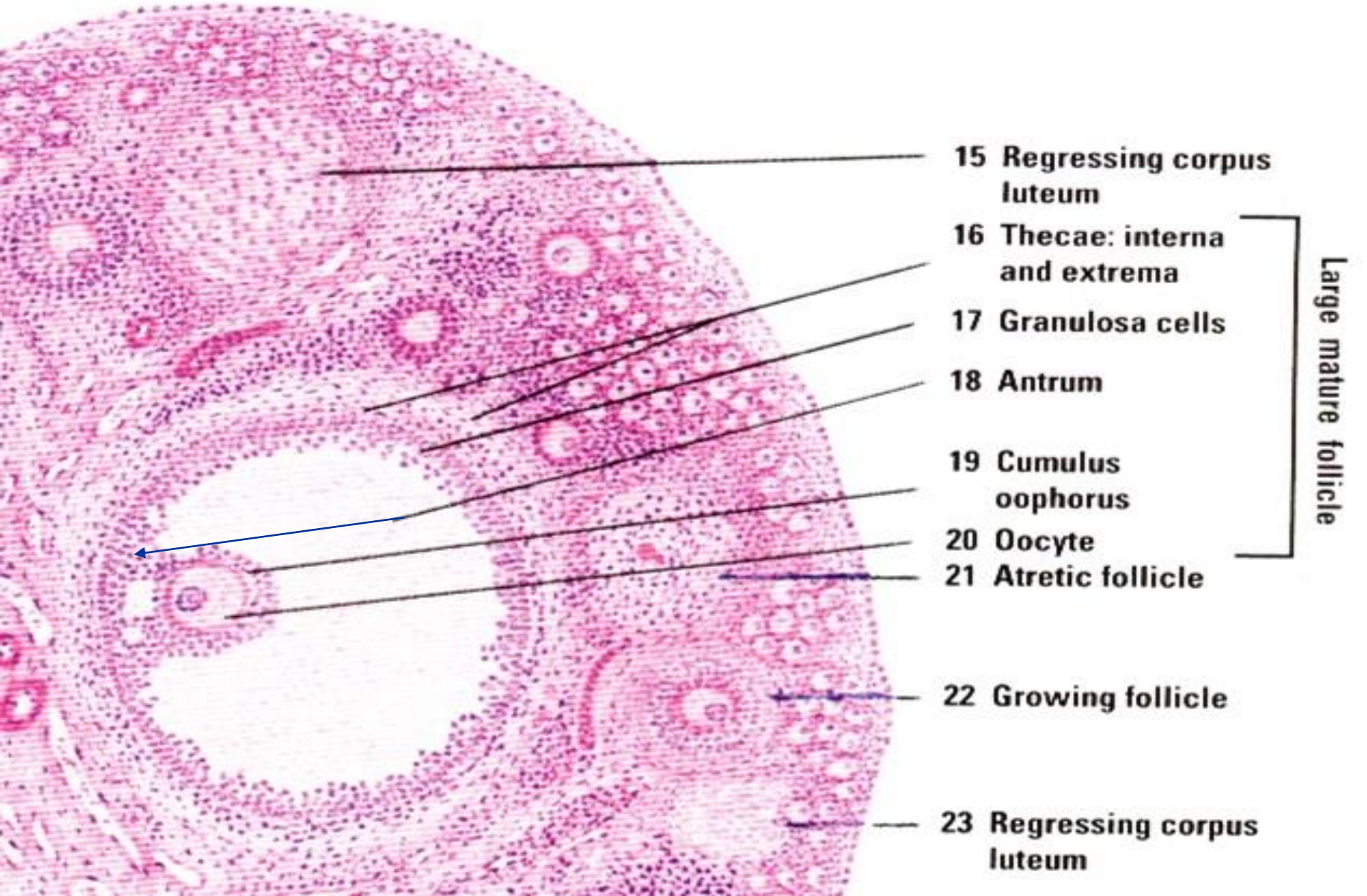
Diagram of the Ovary

Mature ovarian follicle (tertiary or graafian) is a vesicular formation measuring 10-20 mm bulging from ovarian surface. Most of it is occupied by the antrum folliculi (asterisk) filled with liquor.



Primary oocyte is situated in a local eccentric thickening of the stratum granulosum – cumulus oophorus (arrow). One or more layers of columnar granulosa cells – corona radiata (arrowhead) – are attached to the oocytes and accompany it after ovulation. Zona pellucida is up to 10 mcm thick. Both theca externa and interna reach maximal development. Pre-ovulatory follicle occupies full thickness of ovarian cortex.

FEMALE OVARY WITH A TERTIARY FOLLICLE, H & E.



Ovulation:

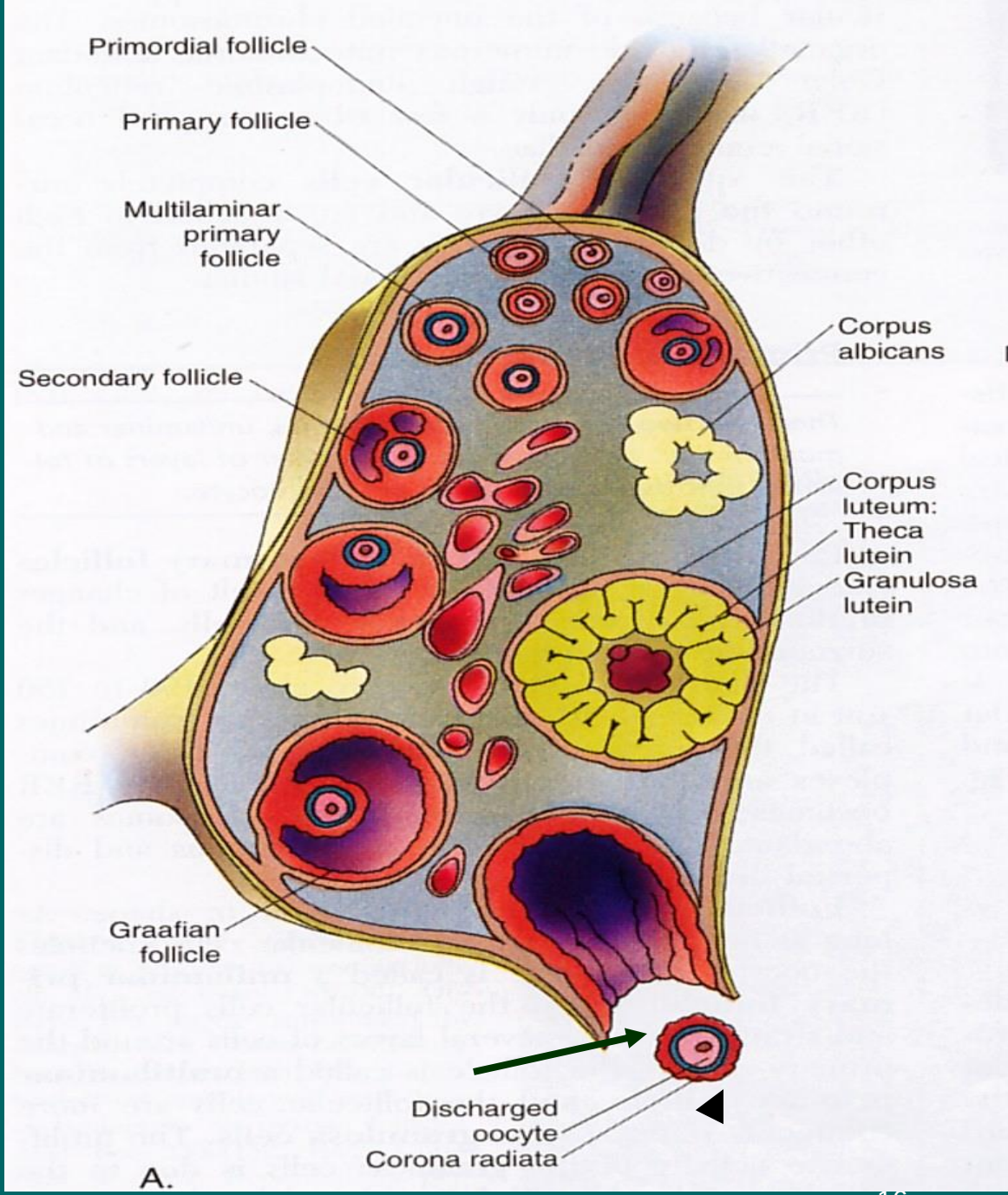
- is the process of rupture of the mature follicle and releasing the secondary oocyte from the graaffian follicle,

  - it occurs in the middle of the menstrual cycle (the 14th day) when the blood level of estrogens produced by the developing graaffian follicle and secondary follicles is elevated enough to induce sudden surge of the LH released by the basophile cells of the pituitary;

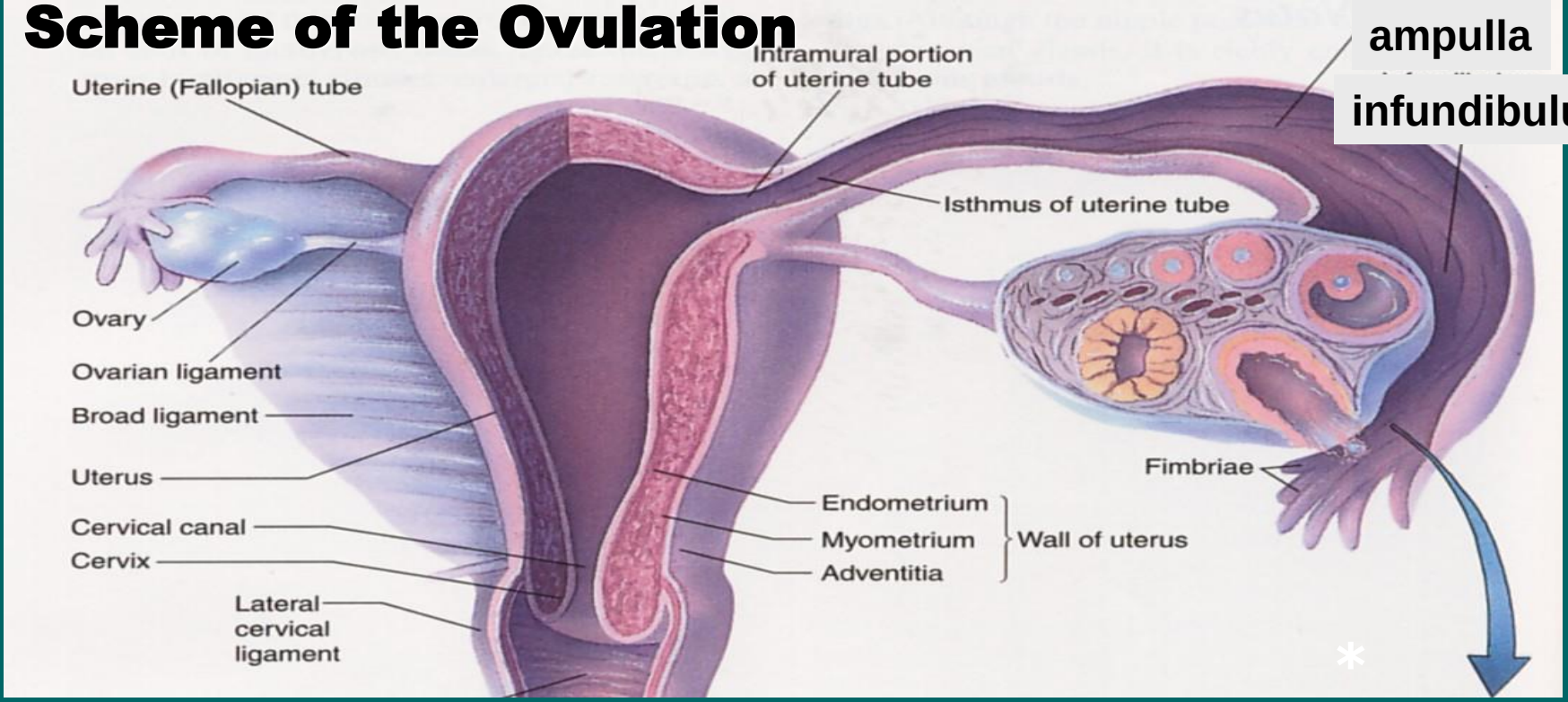
  - the oocyte resumes and completes the 1st meiotic division resulting in the formation of the 1st polar body and secondary oocyte arrested in the metaphase of the 2nd meiotic division.

During ovulation secondary oocyte (arrowhead) with corona radiata are released from the ovary. By that time cumulus oophoron is already detached by liquor folliculi from its base and oocyte with coats floats freely within it. This happens exactly 14 days before the next menstruation.

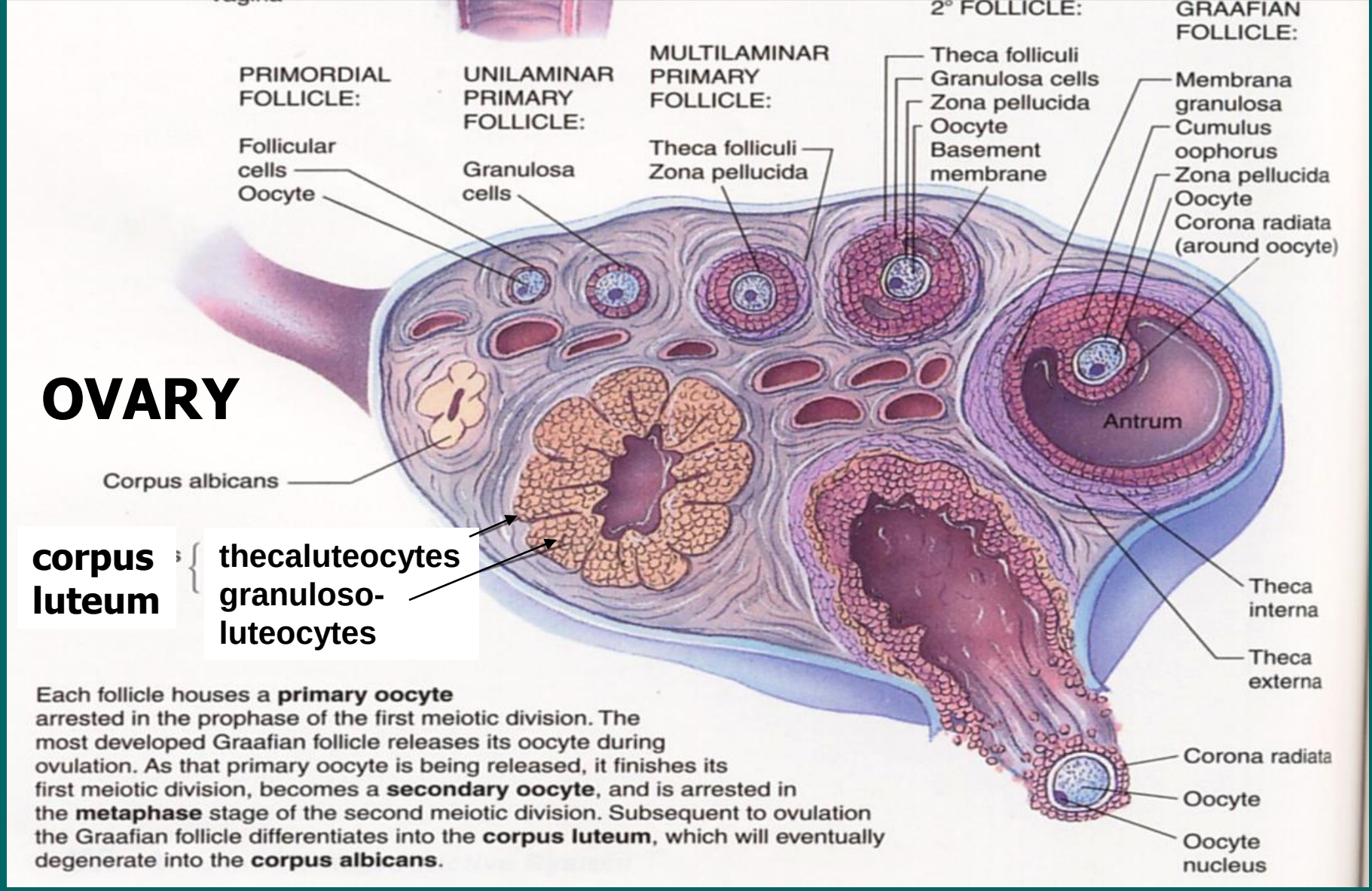
OVULATION



Scheme of the Ovulation



Shortly before ovulation fimbrae of the oviduct begin to cover the surface of the ovary and the tube itself starts to contract rhythmically. The distal end of the oviduct (asterisk) whisks the secondary oocyte with follicular cells into the infundibulum of the oviduct by sweeping movements of the fimbrae and by motion of cilia on the epithelial lining. The oocyte continues the journey to the ampulla for about 25 minutes where it may be fertilized. In humans fertilized oocyte reaches the uterine lumen in approximately 3-4 days. If not fertilized within 24 hours the secondary oocyte degenerates and is phagocytosed.



The corpus luteum is formed from the remnants of the ovulated graafian follicle. It is a temporary endocrine gland manufacturing and releasing hormones that support the uterine endometrium and retain the embryo once implantation occurred.

CLINICAL CORRELATES

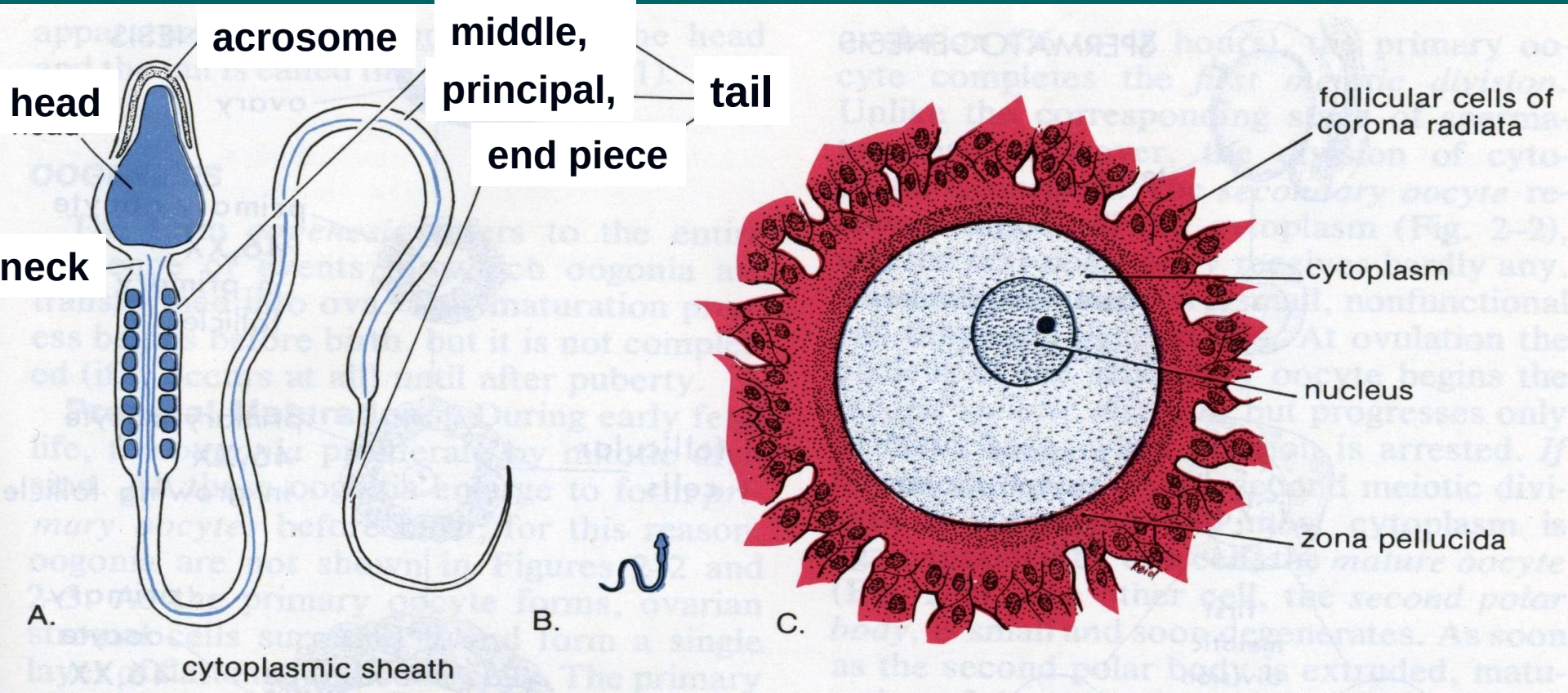
1.During ovulation some women have slight pain which is known as middle pain because it normally occurs near the middle of the menstrual cycle.

2.Ovulation is often accompanied by a rise in basal temperature which may be monitored to aid in determining when release of the oocyte occurs.

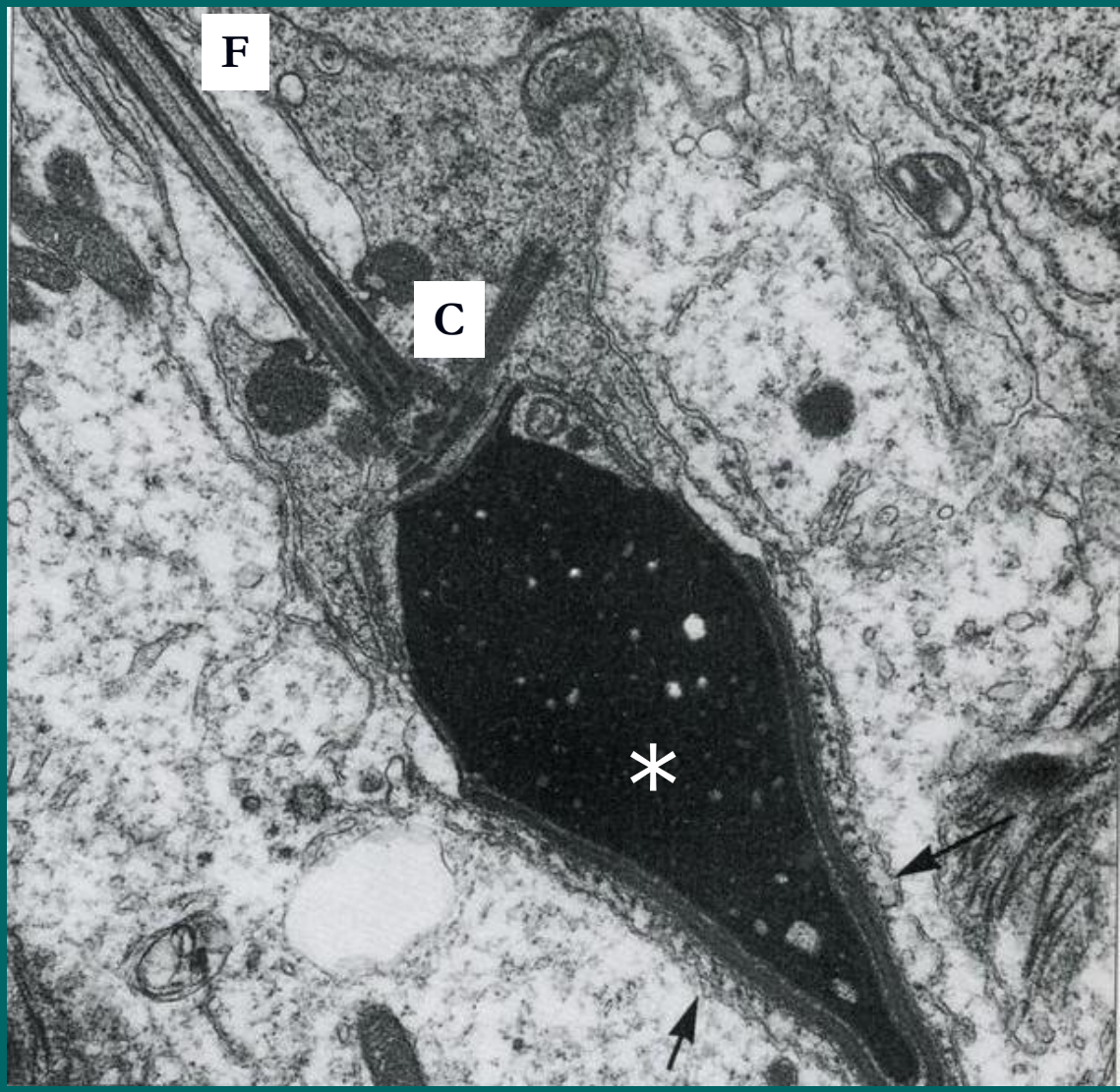
3.Some women fail to ovulate because of a low concentration of gonadotropins. In such cases medications which stimulate the release of the gonadotropins may be prescribed. Although such drugs are effective they often produce multiple ovulation, so that the risk of multiple pregnancies is 10 times as high in these women as in the general population.

The mature sperm is a free-swimming actively motile cell consisting of a head, a tail, and a neck which is a junction between the head and the tail. It is much smaller than the oocyte (picture B and C are in the same scale).

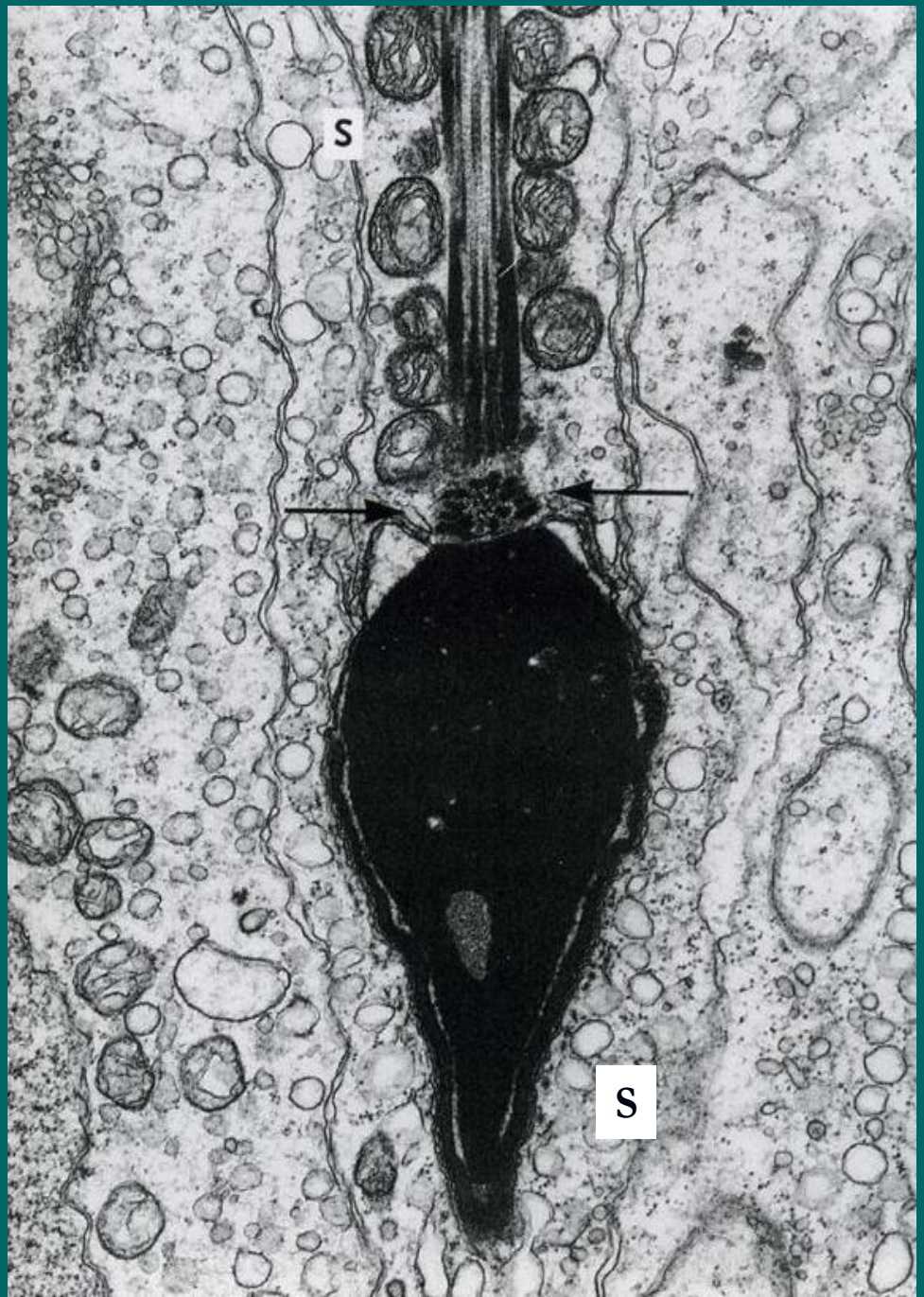
GERM CELLS



The head is composed mostly of the nucleus (asterisk) the anterior two thirds of which are covered by the acrosome (acrosomal cap, arrows) – an organelle containing lytic enzymes playing an important role in the fertilization. Chromatin in the nucleus is greatly condensed. The neck contains the centriol compex. The tail (F) includes middle piece, principal piece and end piece. The mitochondria in the middle piece generate energy for sperm motility.

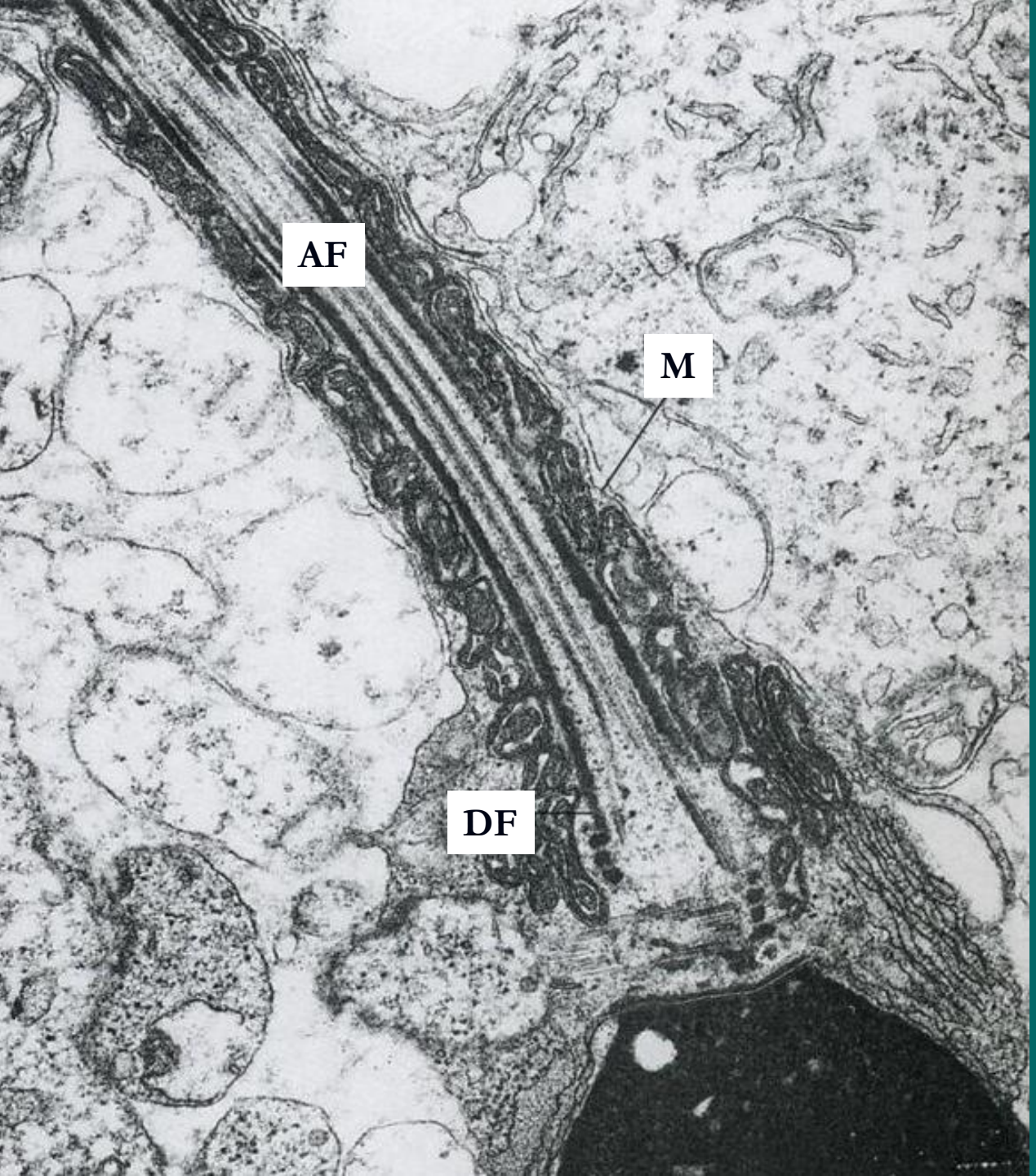


Spermatid, early maturation phase



LATE SPERMATID, TEM

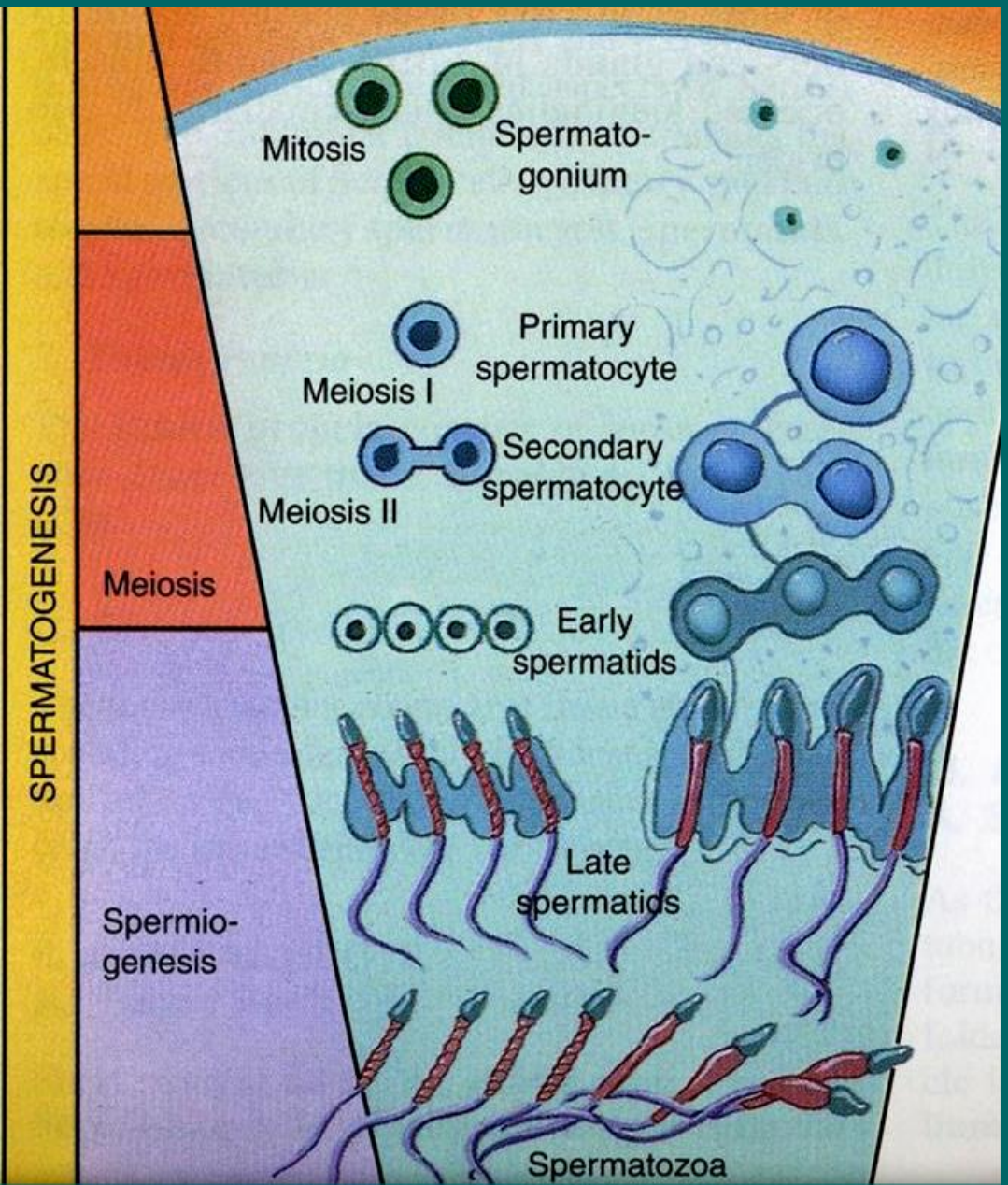
The tail, or flagellum, is associated with mitochondria (in the middle piece) and attached to the head at the neck region via connecting piece (arrows) of segmented dense columns. The spermatid is embedded in a column of Sertoli cells cytoplasm (S).



SPERMATOZOON

The middle piece of the tail shows a central axial filament (AF, 9+2 microtubules as in cilia), outer dense fibers (DF, attached to the neck) and a sheath of mitochondria (M) for tail motility. Dense fibers and axial filaments extend caudally with tapering, for about 40 mcm, forming the principle and end piece of the tail.

SPERMATOGENESIS IN THE TESTIS

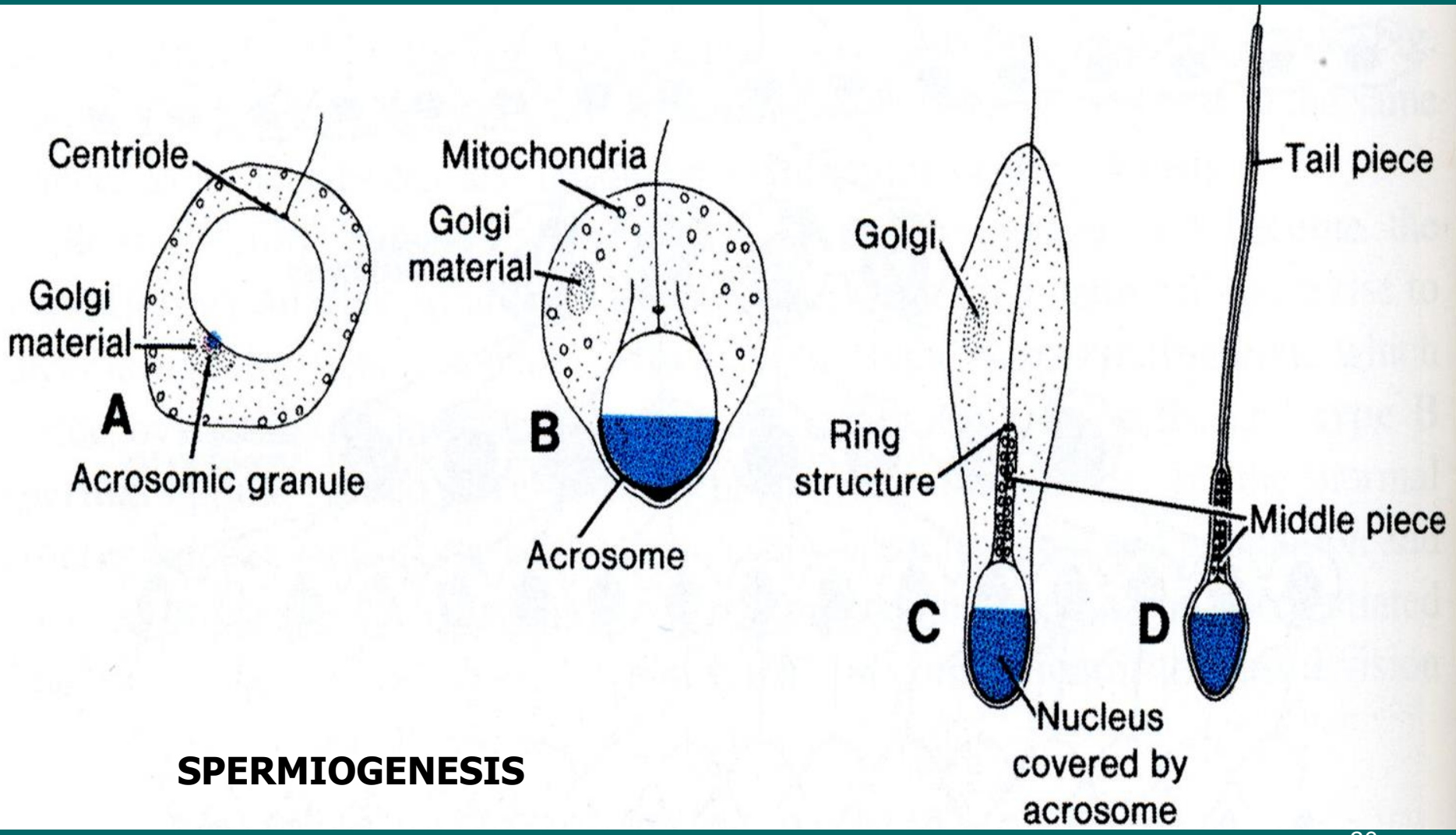


Male sex cells develop in the testis. The germinal epithelium of the seminiferous tubule of testis contains spermatogenic cells which undergo mitosis, meiosis and spermiogenesis. Thus spermatogonia differentiate into primary spermatocytes, secondary spermatocytes, spermatids (early and late) and spermatozoa.

SPERMIOGENESIS

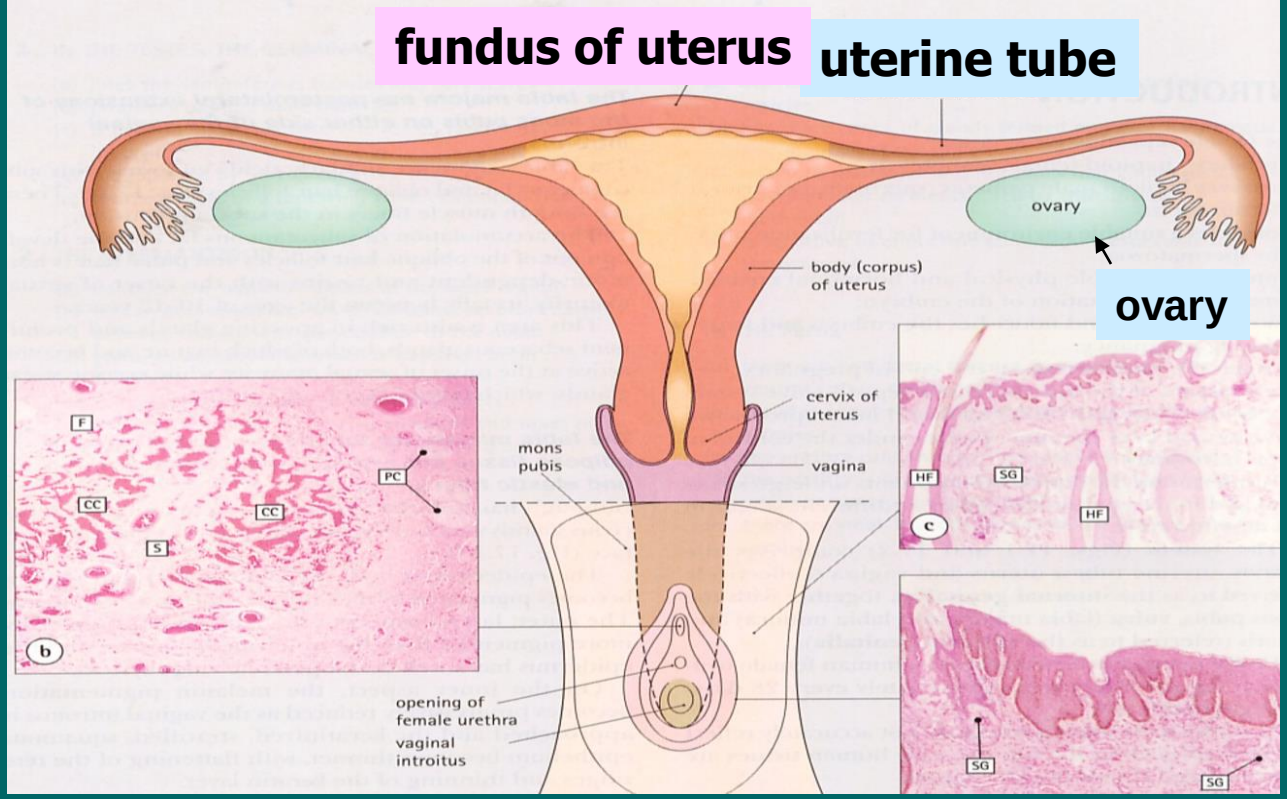
Spermiogenesis is a process of cytodifferentiation of the spermatids into spermatozoa and includes no cell division. Instead the spermatid loses much of its cytoplasm, forming an acrosomal granule, a long cilium and associated outer dense fibers and a coarse fibrous sheath.

The spermatozoon that is formed and released into the lumen of the seminiferous tubule is nonmotile and incapable of fertilizing an ovum. The spermatozoa remain immotile until they leave the epididymis. They become capable of fertilizing once they have been capacitated in the female reproductive system.



SPERMIOGENESIS

The Diagram of the Female Reproductive System



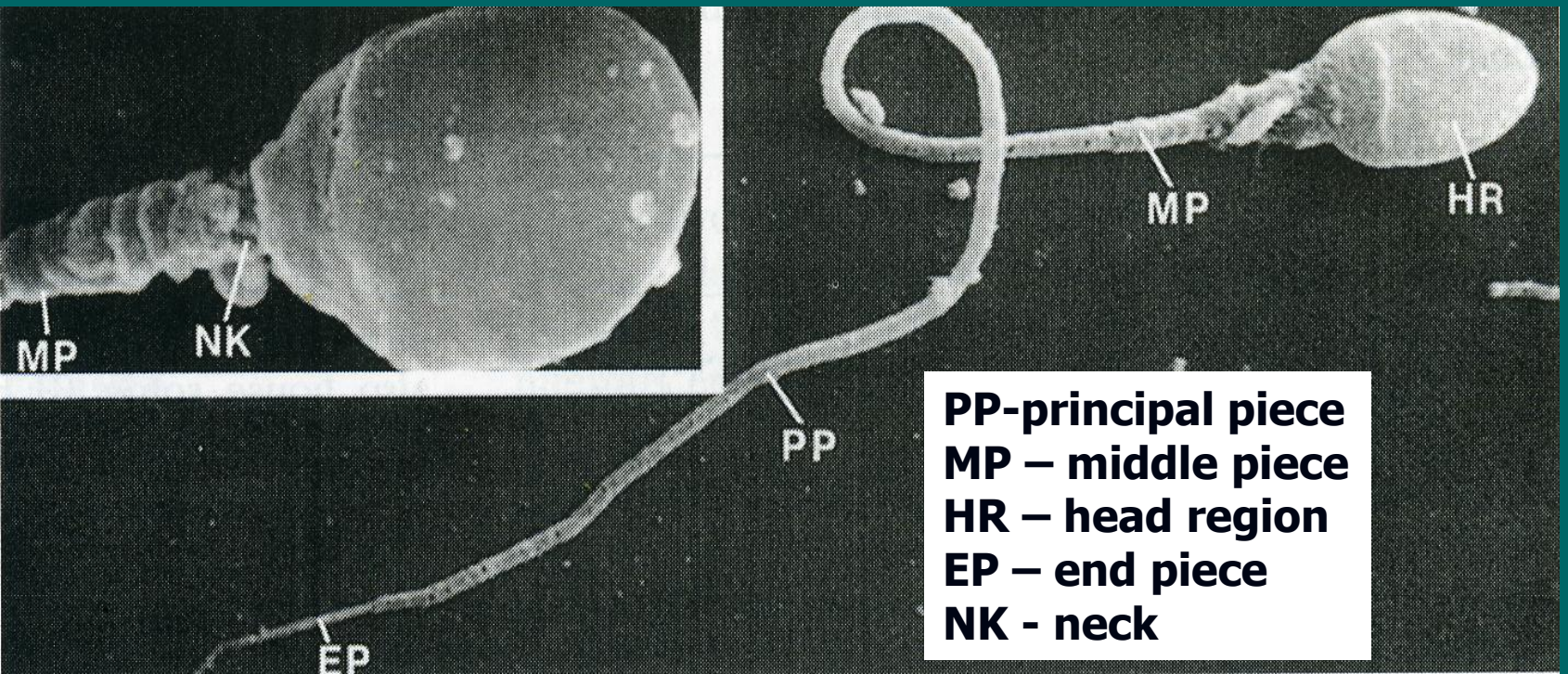
Usually 200-500 million sperms are deposited on the cervix and in the posterior fornix of the vagina at intercourse. The sperm passes by movements of their tails through the cervical canal, while passage through the uterus and uterine tube is assisted by muscular contractions of these organs.

Stimulated by prostaglandins of the semen spermatozoa reach rapidly the isthmus of each of the uterine tube. Once in the uterine tube, the isthmus serves as a sperm reservoir, and movement from this region to the ampulla is a synchronized process. The first spermatozoa reach fertilization site within 5 minutes, but some sperms take up to 45 minutes to reach the ampulla. Not more than 300-500 sperms reach fertilization site. They may remain viable there for several days (up to 3 days).

FERTILIZATION

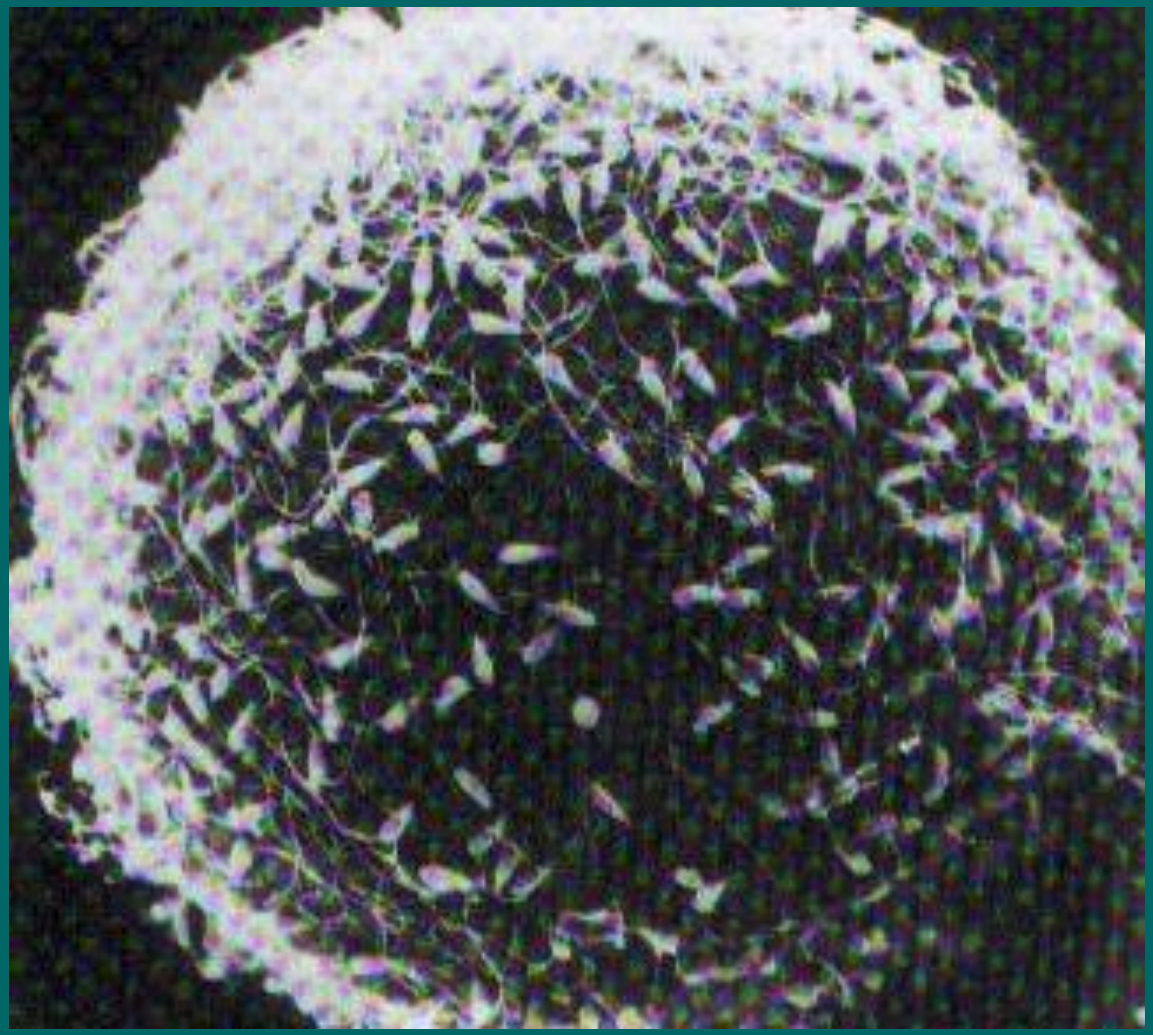
Spermatozoa are not able to fertilize the oocyte immediately upon arrival in the female genital tract but must undergo capacitation and the acrosome reaction to acquire this capability.

Capacitation is a period of conditioning in the female reproductive tract that in the human lasts approximately 7 hours. During this time a glycoprotein coat and seminal plasma proteins are removed from the plasma membrane that overlies the acrosomal region of the spermatozoa. Only capacitated sperm can pass through the corona cells and undergo the acrosomal reaction.



Sperm, SEM

Scanning electron microphotograph of sperm binding to the zona pellucida.

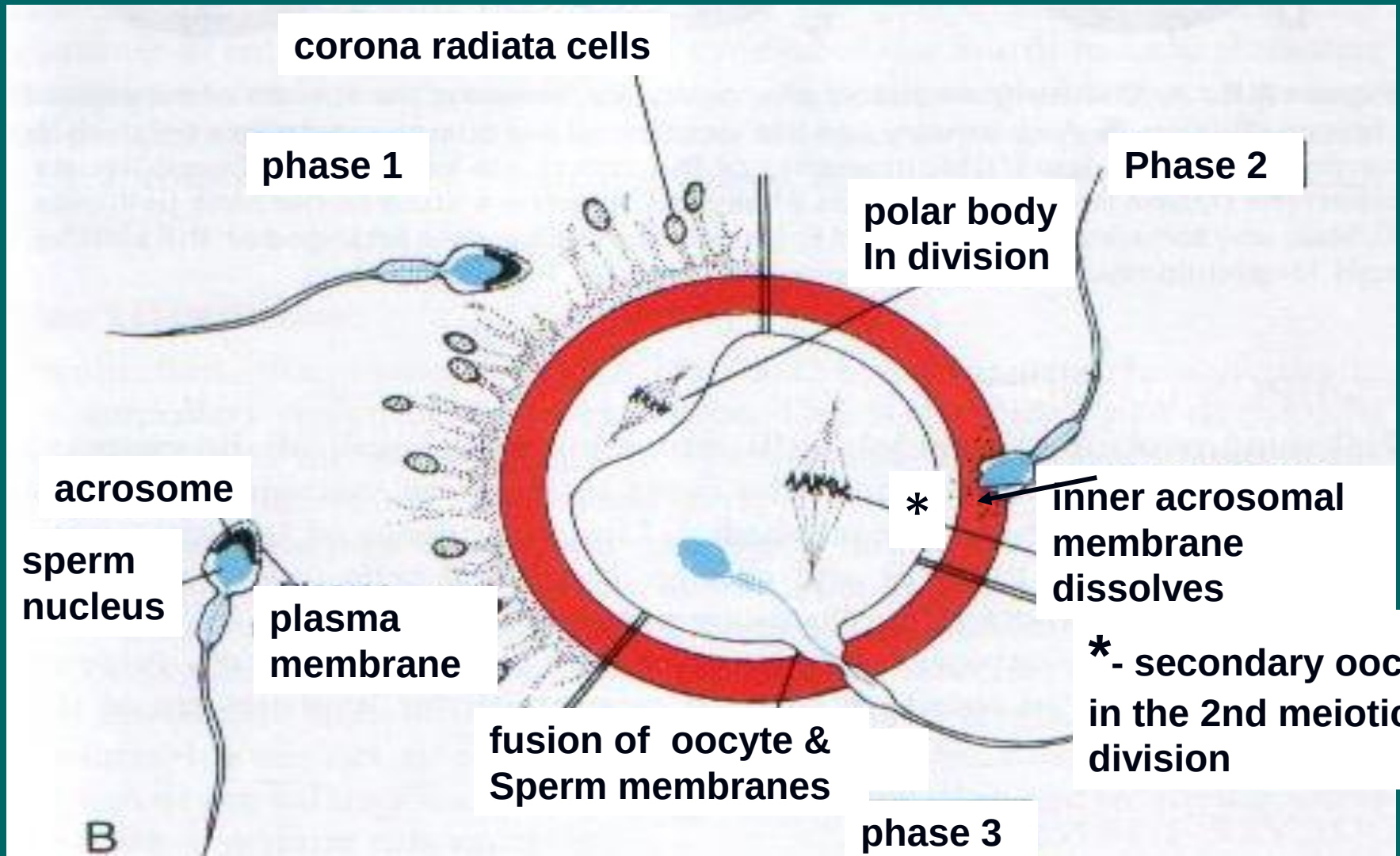


The acrosomal reaction which occurs after binding to the zona pellucida, is induced by zona proteins. This reaction culminates in the release of enzymes needed to penetrate the zona pellucida, including acrosin and trypsin-like substances.

PHASES OF FERTILIZATION

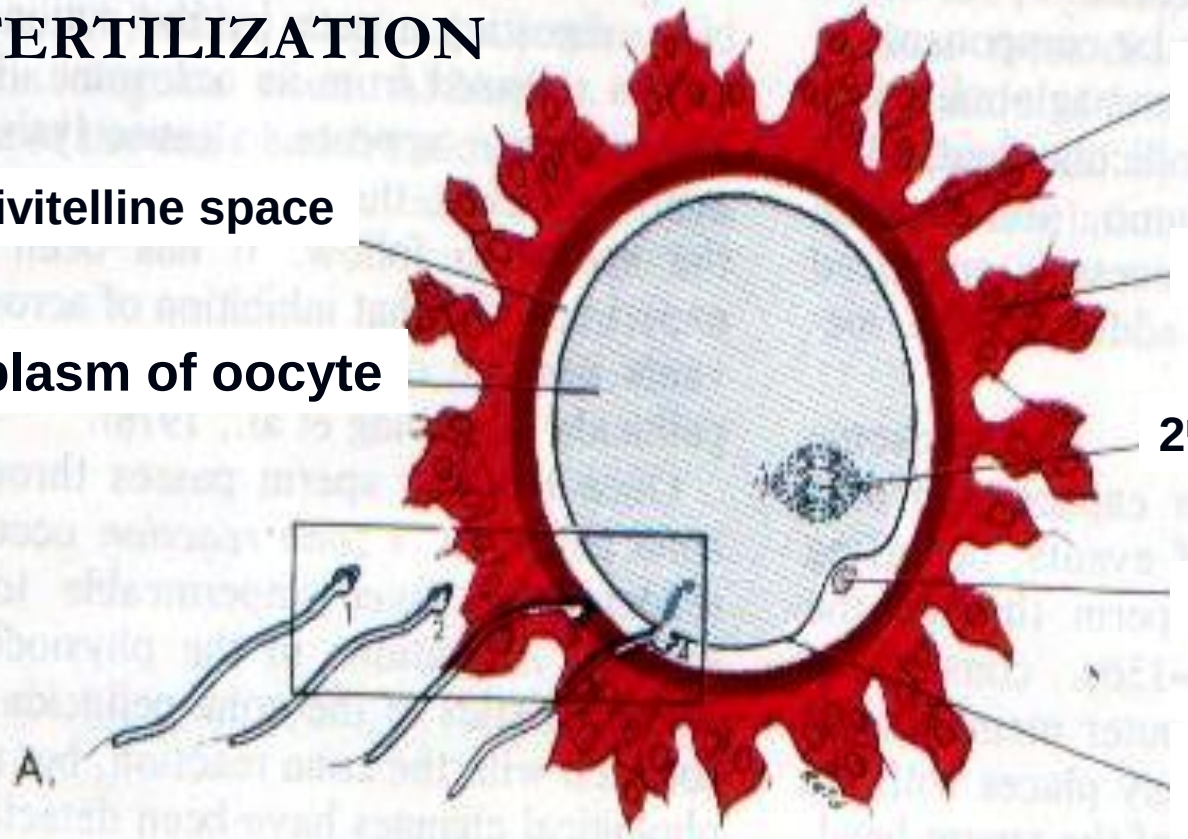
The phases of fertilization include:

1. **Penetration of the corona radiata**
2. **Penetration of the zona pellucida**
3. **Fusion of the oocyte and sperm cell membranes.**



Of the 300-500 sperms reaching the fertilization site only one fertilizes the egg. Capacitated sperm passes easily through corona cells. The zona is a glycoprotein shell surrounding the egg that facilitates and maintains sperm binding and induces the acrosomal reaction. Binding is mediated by the ligand ZP3, a zona protein, and receptors on the sperm plasma membrane. Release of acrosomal enzymes (acrosin) allows sperm to penetrate the zona thereby coming in contact with the oocyte surface.

FERTILIZATION



zona pellucida

perivitelline space

corona radiata

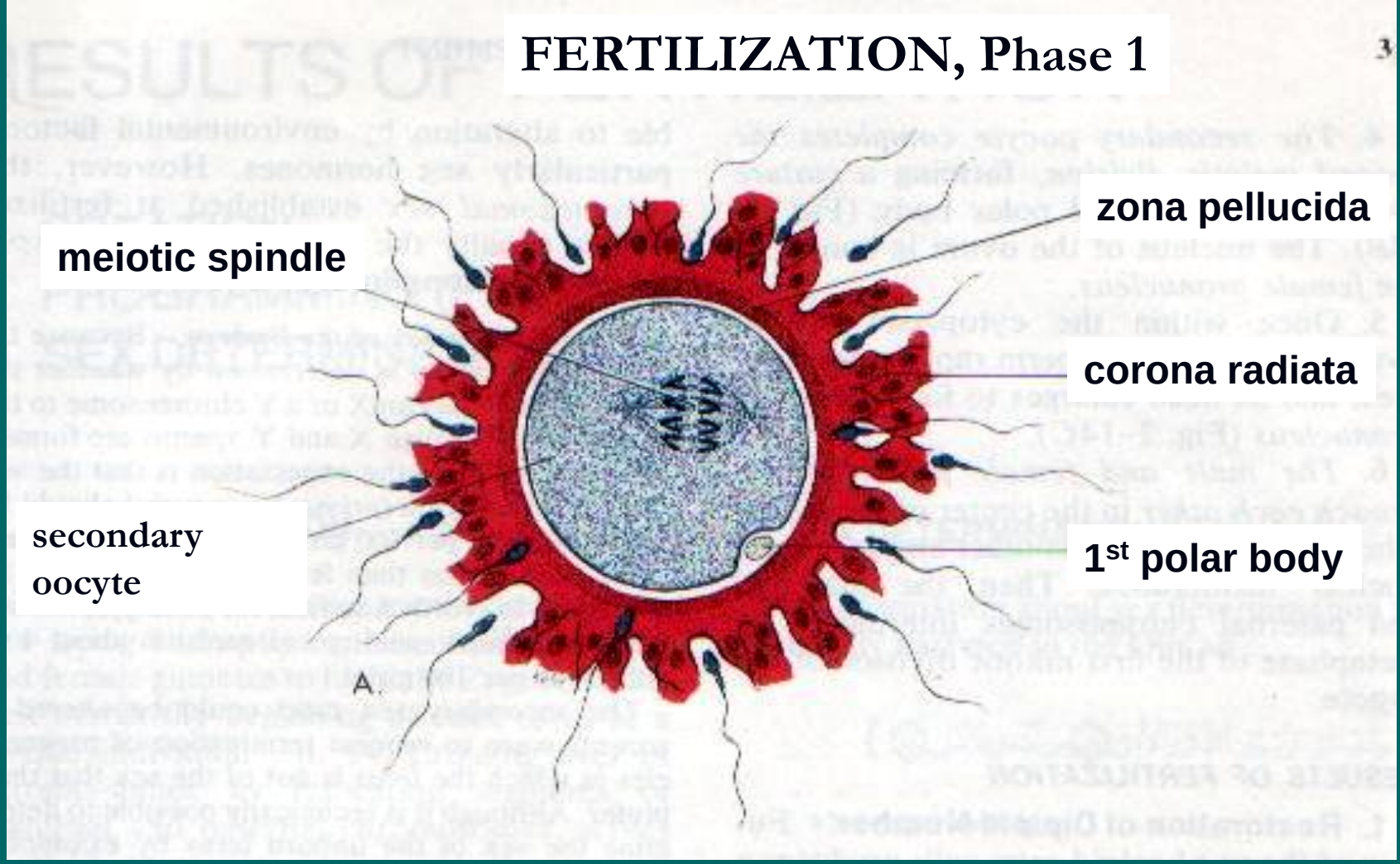
cytoplasm of oocyte

2nd meiotic metaphase

1st polar body

plasma membrane of oocyte

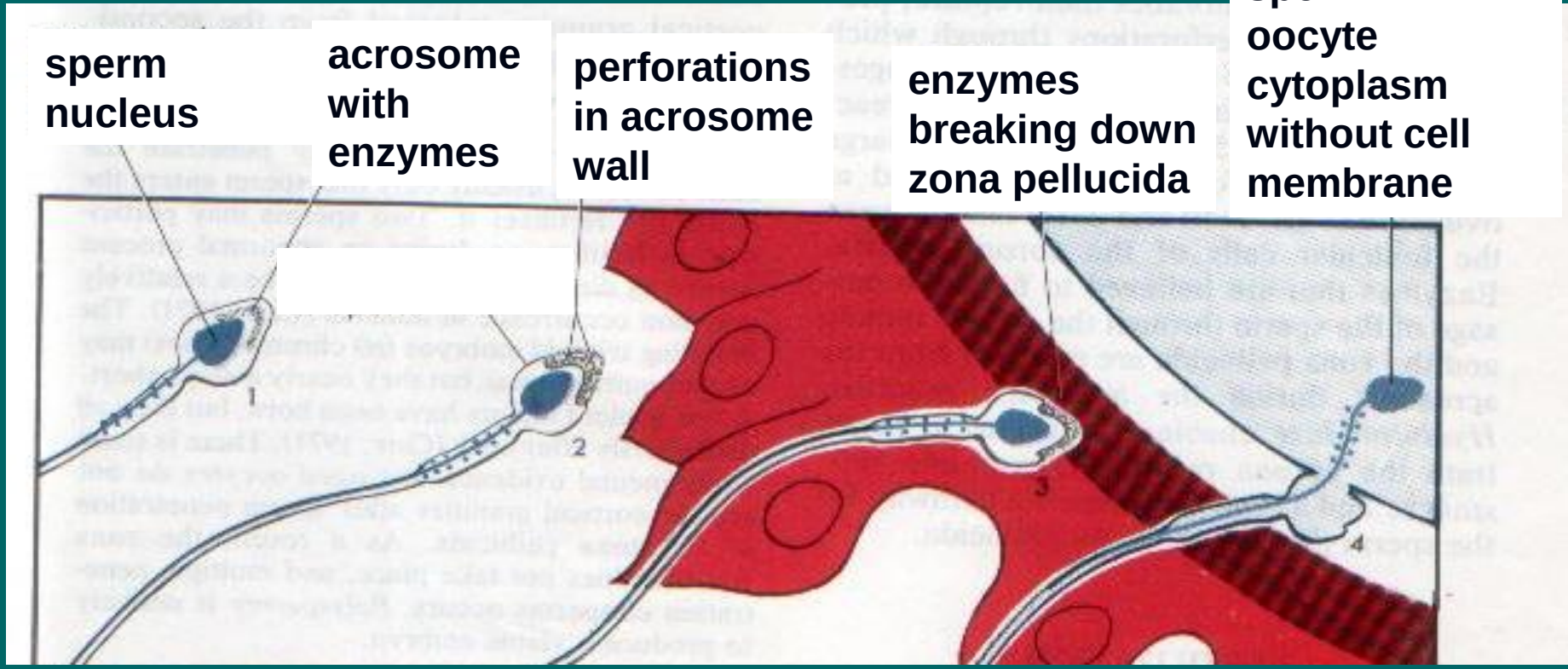
FERTILIZATION, Phase 1



The initial adhesion of sperm to the oocyte is mediated in part by the interaction of integrins on the oocyte and their ligands, disintegrins, on sperm.

Following adhesion the plasma membranes of the sperm and oocyte fuse. Since the plasma membrane covering the acrosomal head cap disappears during the acrosome reaction, actual fusion is accomplished between the oocyte membrane and the membrane that covers the posterior region of the sperm head. In the human both the head and the tail of the spermatozoon enter the cytoplasm of the oocyte, but the plasma membrane is left behind on the oocyte surface. As soon as the spermatozoon has entered the oocyte, the egg responds in three ways:

FERTILIZATION



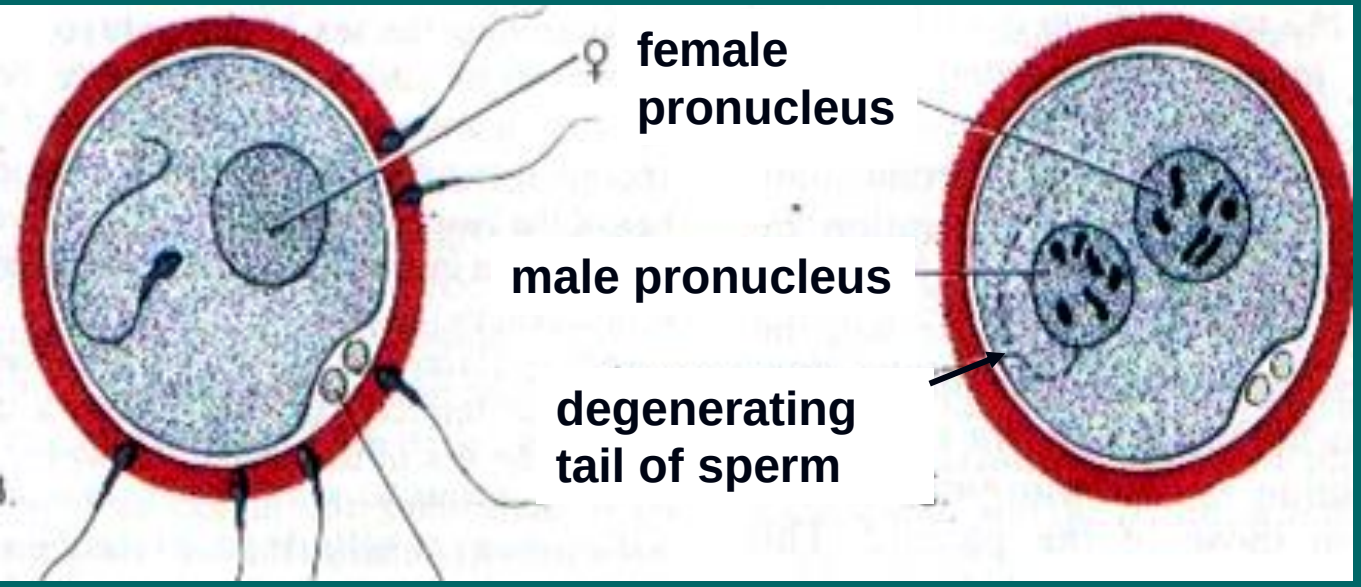
FERTILIZATION: OOCYTE RESPONCE

1. Cortical and zona reaction. After the binding of the sperm to the plasmalemma of the oocyte, as a results of the release of cortical oocyte granules lining the oocyte plasmalemma, the oocyte membrane becomes impenetratable to other spermatozoa.

In turn the lysosomal enzymes released from the cortical granules alter the structure and the composition of the zona pellucida (zona reaction) to prevent sperm binding and penetration through inactivation of the species-specific receptor sites for spermatozoa on the zona surface.

Other spermatozoa have been found embedded in the zona pellucida but only one seems to be able to penetrate the oocyte. This reaction prevents polyspermy (penetration of more than 1 sperm into the oocyte).

FERTILIZATION

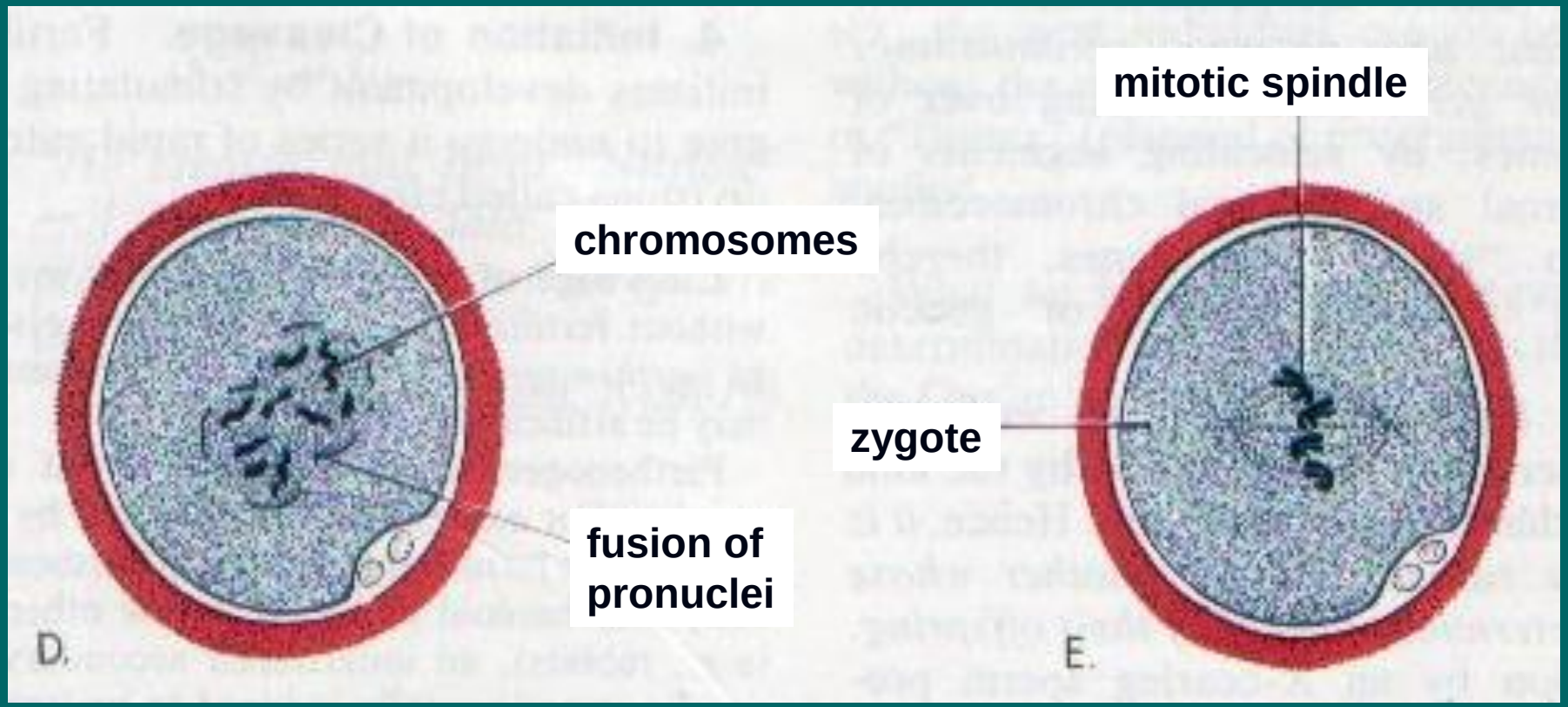


2.Resumption of the 2nd meiotic division. The oocyte finishes its 2nd meiotic division immediately after entry of the spermatozoon. One of the daughter cells, which receives hardly any cytoplasm, is known as the 2nd polar body, the other daughter cell is definite oocyte. Its chromosomes (22+x) arrange themselves in a vesicular nucleus known as female pronucleus.

3. Metabolic activation of the egg. The activating factor is probably carried by the spermatozoon. Postfusion activation may be considered to encompass the initial cellular and molecular events associated with early embryogenesis.

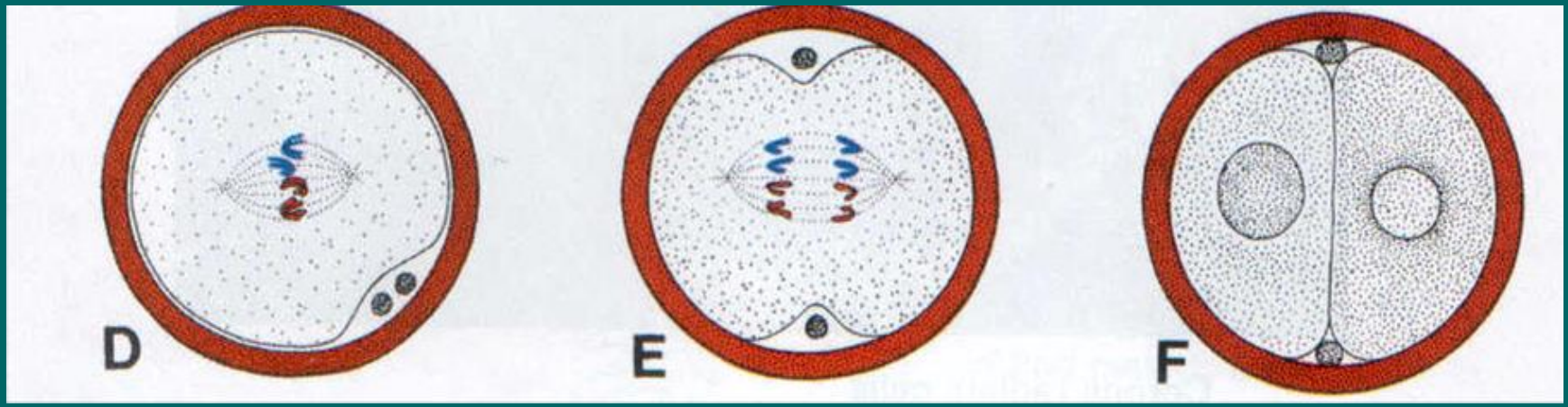
The spermatozoon meanwhile moves forward until it lies close to the female pronucleus. The tail detaches and degenerates. The male nucleus becomes swollen and forms the male pronucleus. Morphologically the male and female pronuclei are indistinguishable, and eventually they come into close contact and...

FERTILIZATION



lose their nuclear envelopes (D). During growth of male and female pronuclei (both haploid), each pronucleus must replicate its DNA. Immediately after DNA synthesis, chromosomes organize on the spindle in preparation for normal mitotic division (E).

FERTILIZATION



The 23 maternal and 23 paternal (double) chromosomes split longitudinally at the centromere (D), and sister chromatids move to the opposite poles (E), providing each cell of the zygote with the normal diploid number of chromosomes. As sister chromatids move to the opposite poles, a deep furrow appears on the surface of the cell (F), gradually dividing the cytoplasm into two parts.

The main results of fertilization are as follows:

1. Restoration of the diploid number of chromosomes, half from the father and half from the mother. Hence the zygote contains a new combination of chromosomes different from both patients.
2. Determination of the sex of the new individual. An X-carrying sperm produces a female (XX) embryo, hence the chromosomal sex of the embryo is determined during fertilization.
3. Initiation of cleavage. Without fertilization the oocyte usually degenerates 24 hours after ovulation.

CLINICAL CORRELATES

Contraceptive methods.

1.Barrier technique for contraception include male and female condoms.

2.The contraceptive pills is a combination of estrogen and the progesterone analogue progestin, which together inhibit ovulation but permit menstruation. Both hormones act at the level of FSH and LH, preventing their release from the pituitary. The pills are taken for 21 days and then stopped to allow menstruation after which cycle is repeated.

3. Depo-Provera is a progestin compound that can be implanted subdermally or injected intramuscularly to prevent ovulation for up to 5 years or 23 months respectively.

4. The intrauterine device is placed in the uterine cavity. Its mechanism for preventing pregnancy is not clear but may entail direct effects on sperm and oocytes or inhibition of preimplantation stages of development.

5.The drug RU-486 causes abortion if it is administered within 8 weeks of the previous menses. It initiates menstruation, possibly through its action as an antiprogesterone agent.

6.Vasectomy and tubal ligation are effective means of contraception, and both procedures are reversible, though not in every case.

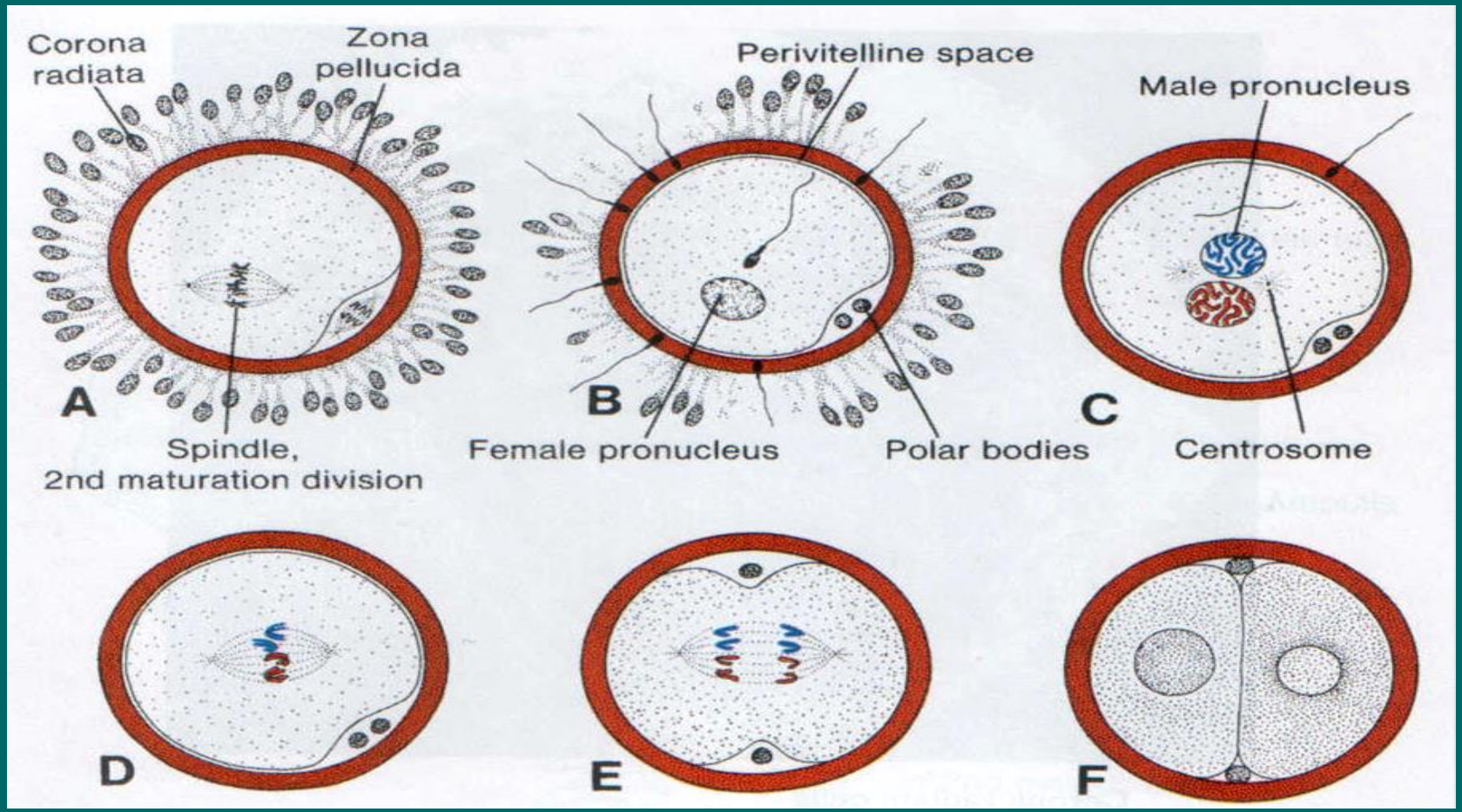
CLINICAL CORRELATES

Infertility.
It is a problem for 15-30% of couples.

1.Male infertility may be due to insufficient number of sperm and/or pure motility (donor sperm for in vitro fertilization or intracytoplasmic sperm injection).
Normally the ejaculate has a volume of 3-4 ml, with approximately 100 million sperm per milliliter. Males with 20 million sperm per ml or 50 million sperm per total ejaculate are usually fertile.

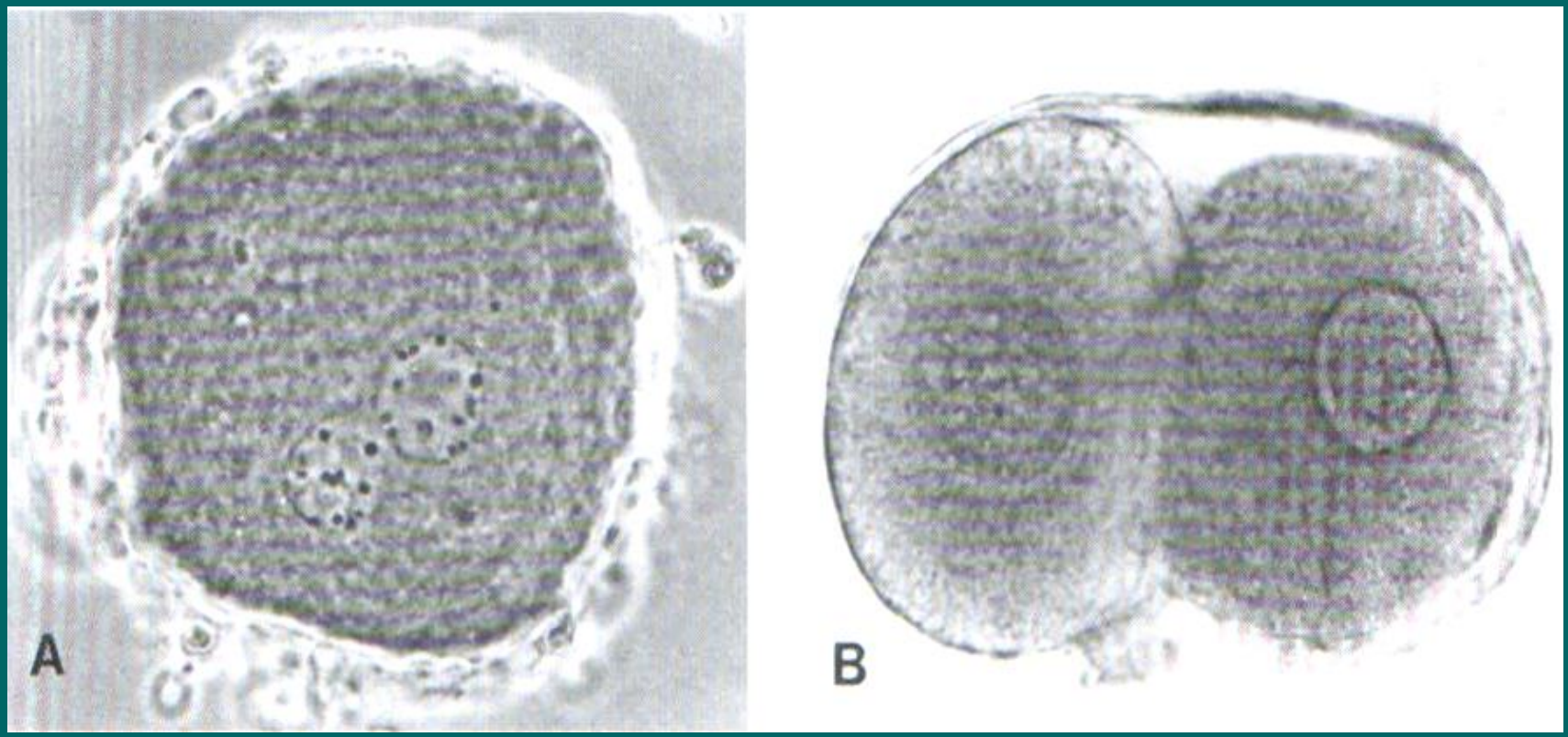
2.Infertility in a woman may be due to a number of causes, including occluded oviduct (most commonly due to pelvic imflammatory disease), hostile cervical mucus, immunity to spermatozoa, absence of ovulation, etc (in vitro fertilization may be applied as preimplantation-stage embryos are resistant to teratogenic insults and the risk of producing malformed offspring is low. The embryo is emplanted in the uterus when it reached the 8-cell stage; gamete intrafallopian transfer into the ampulla or zygote intrafallopian transfer may be also applied).

FERTILIZATION

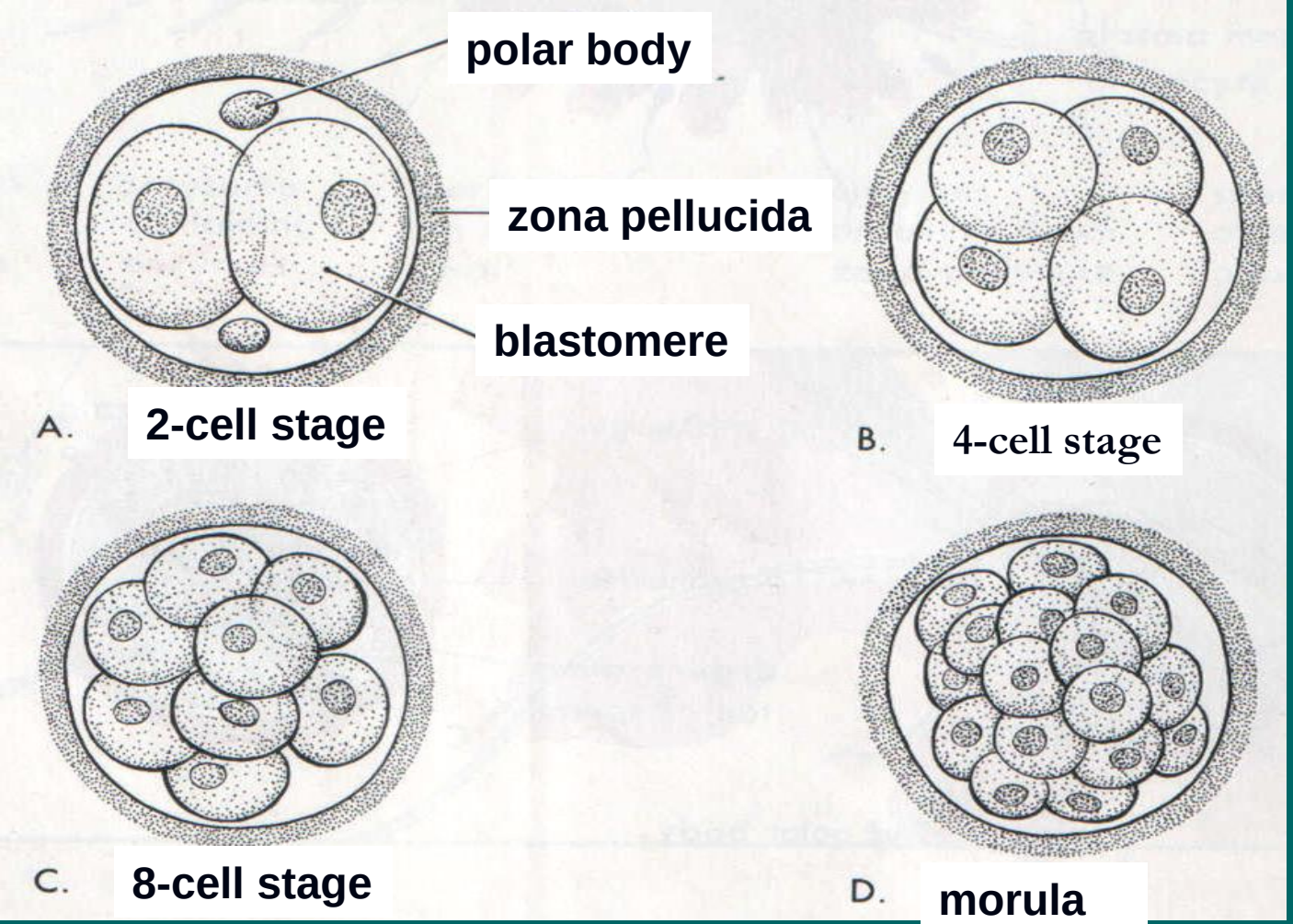


A – oocyte immediately after ovulation, showing the spindle of the 2nd meiotic division; B – a spermatozoon has penetrated the oocyte, which has finished its 2nd meiotic division; chromosomes of the oocyte are arranged in a vesicular nucleus, the female pronucleus. Heads of several sperm are stuck in the zona pellucida. C - male and female pronuclei. D, E – chromosomes become arranged on the spindle, split longitudinally, and move to the opposite poles. F – two-cell stage.

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.



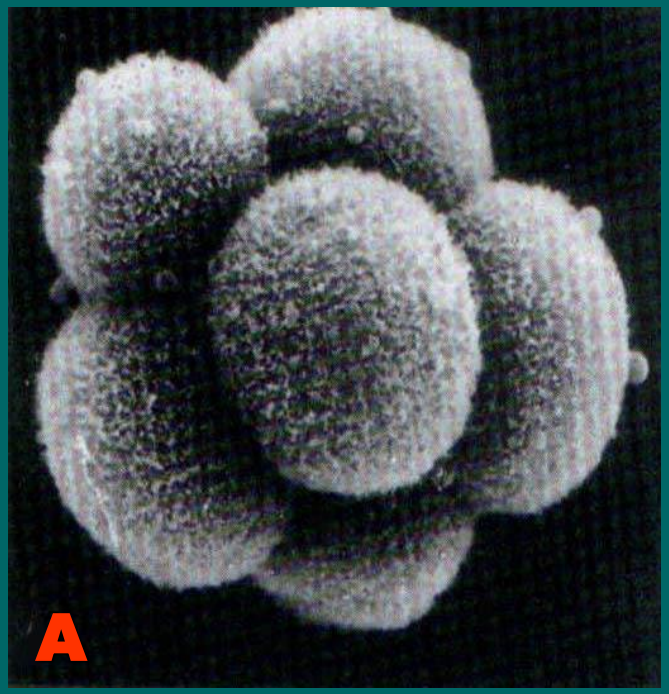
Fertilization of the ovum by a sperm results in the formation of the zygote and initiates early human development by stimulating the zygote to undergo mitotic cell division which begins shortly after fertilization and results in the formation of the two daughter cells called blastomeres.

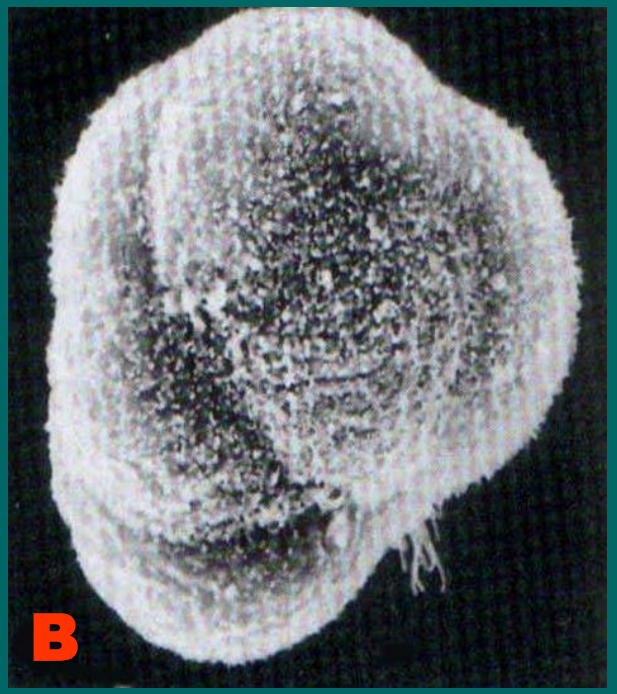


Cleavage of Zygote.

Once the zygote has reached the two-cell stage, it undergoes a series of mitotic divisions, increasing the number of cells. (The polar bodies (A) small, non-functional cells that soon degenerate).
Subsequent divisions follow rapidly upon one another. With each cleavage division blastomeres become smaller, hence the term – cleavage. Until eight-cell stage they form a loosely arranged clump.

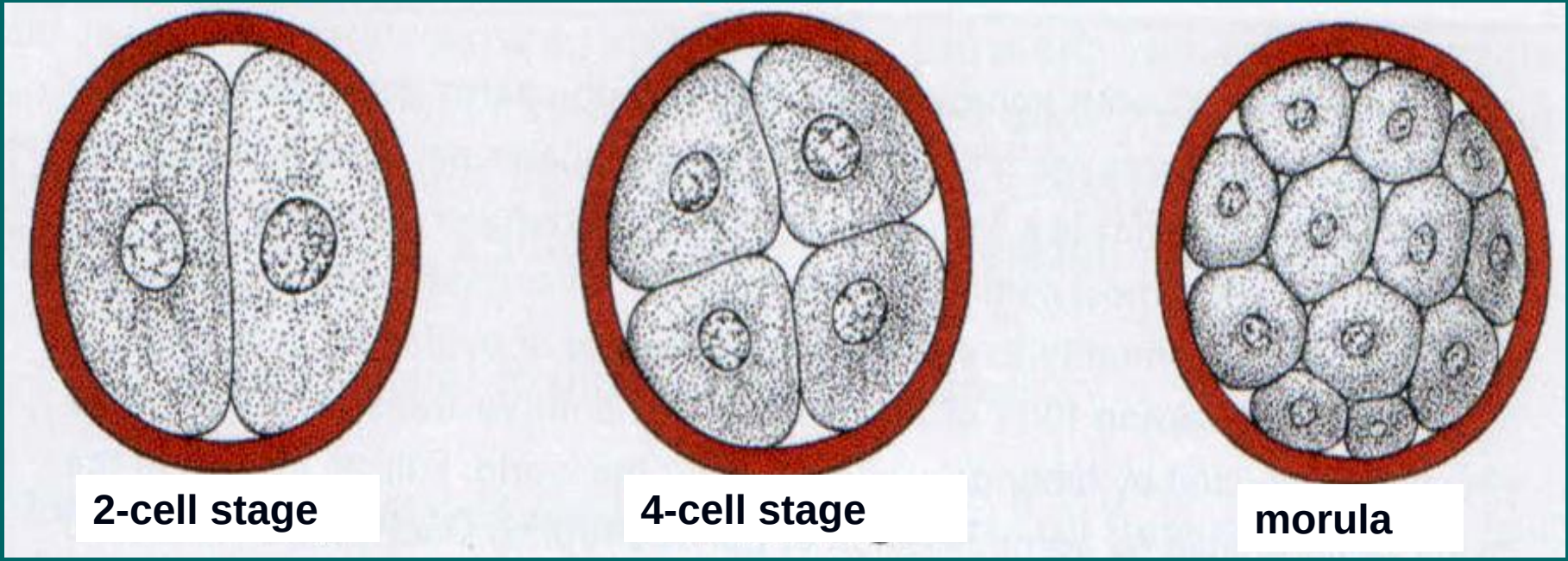
SEM of Uncompacted (A) and Compacted (B) 8-cell Mouse Embryo.





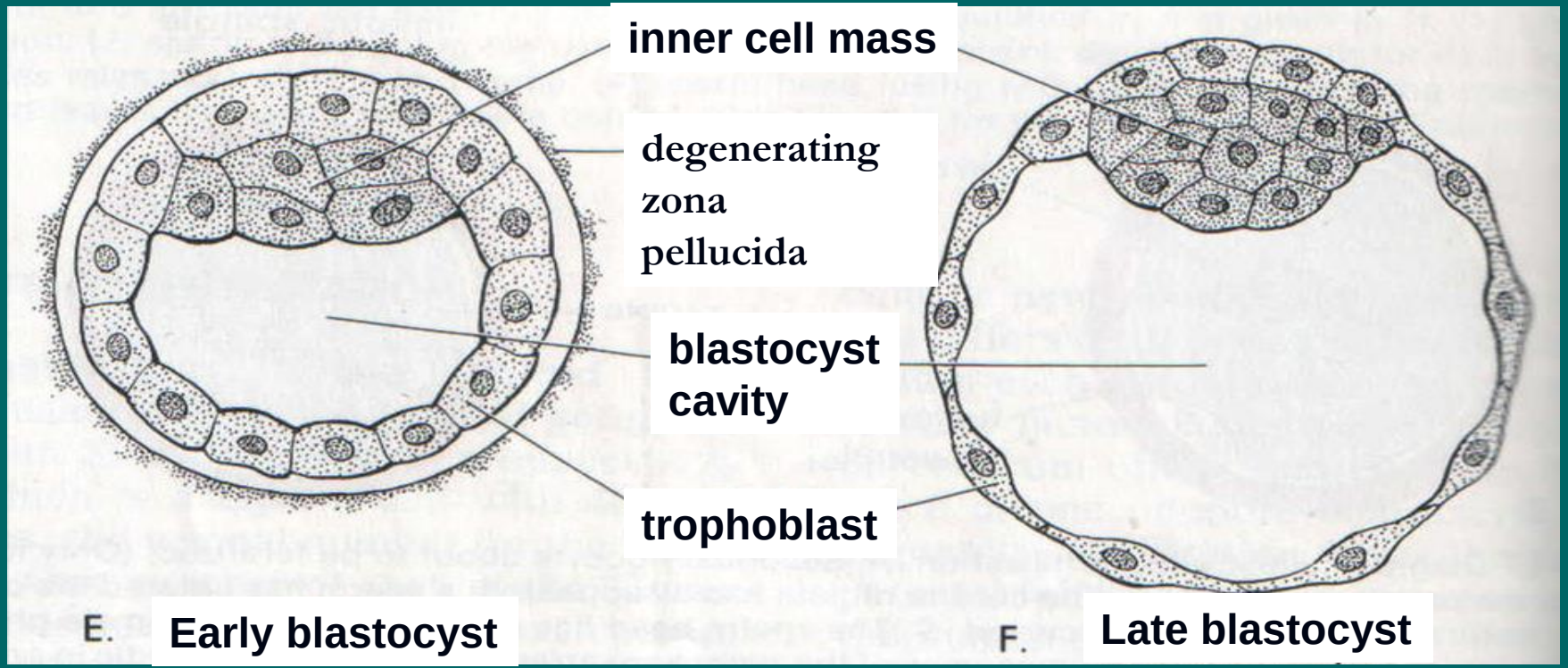
Following the 3rd cleavage, blastomeres maximize their contact with each other, forming a compact ball of cells held together by tight junctions. This process, compaction, segregates inner cells, which communicate extensively by gap junctions, from outer cells. In the uncompacted state outlines of each blastomere are distinct, whereas after compaction cell-cell contacts are maximized and cellular outlines are indistinct.

CLEAVAGE



By the 3rd day a solid ball of 16 or so blastomeres has formed, which is called morula (from the Latin morus, meaning mulberry). The morula is a mulberry-like cellular mass.

Sections of blastocysts. In early blastocyst the zona pellucida is degenerating (E), in late blastocyst (about 5 days) it disappears.



The morula passes down the uterine tube, enters the uterus and fluid passes into the morula from the uterine cavity and collects between the cells. As the fluid increases, it separates these cells into two parts: an outer cell mass called trophoblast and the inner cell mass called embryoblast.

107-cell human blastocyst showing inner cells mass and trophoblast cells

Inner cell mass is surrounded by the outer cell mass. The inner cell mass – embryoblast – gives rise to tissues of embryo proper, and the outer cell mass – to trophoblast (from the Greek trophe meaning "nutrition" and blastos, meaning "germ" or "bud") – later contributes to the placenta.