ANA 301H1
EMBRYOLOGY LECTURE #1

GAMETOGENESIS

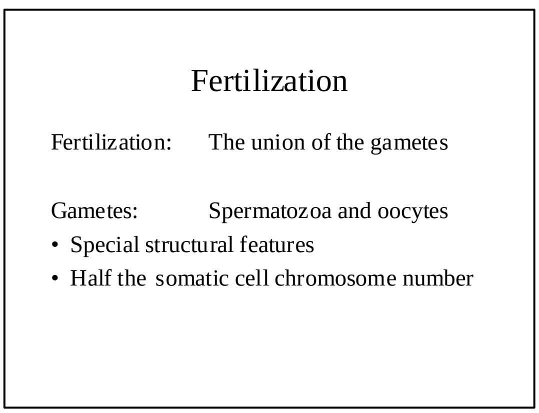
Objectives:
- Understand the significance of gametes.
- Review the process of meiosis and understand the significance of meiotic non-disjunction.
- Describe the process of gametogenesis in males and females and the steps in ovulation.
- Understand the transit of sperm cells from the seminiferous tubules to the site of fertilization; describe the glands producing seminal fluid and understand the process of capacitation.

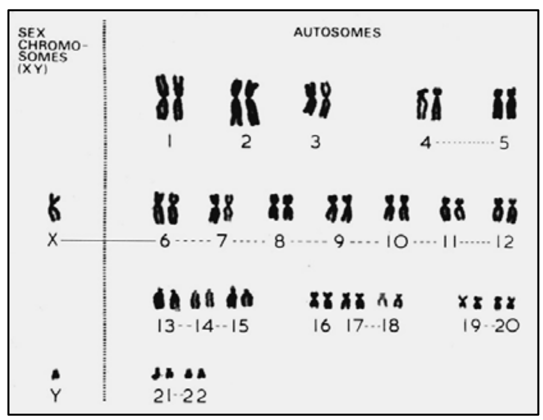
GAMETOGENESIS

Refers to the production of gametes, a population of cells specialized for the purpose of fertilization. Gametogenesis involves:

- the acquisition of the structural and functional characteristics necessary to ensure successful union.

- meiosis, a type of cell division restricted to the gamete lineage. Meiosis occurs in two stages and halves the number of chromosomes in the gamete nucleus by separating the 23 pairs of chromosomes into 23 single chromosomes.

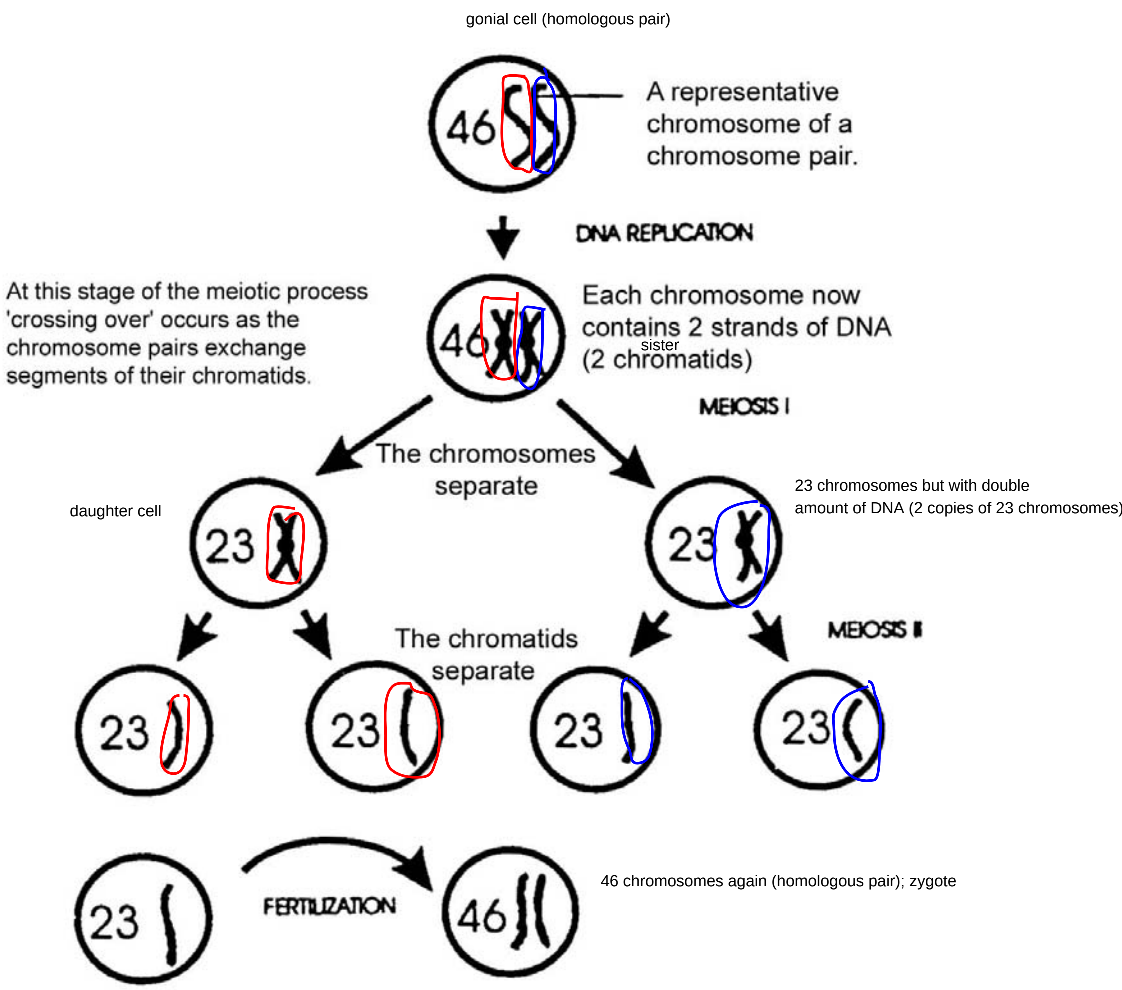




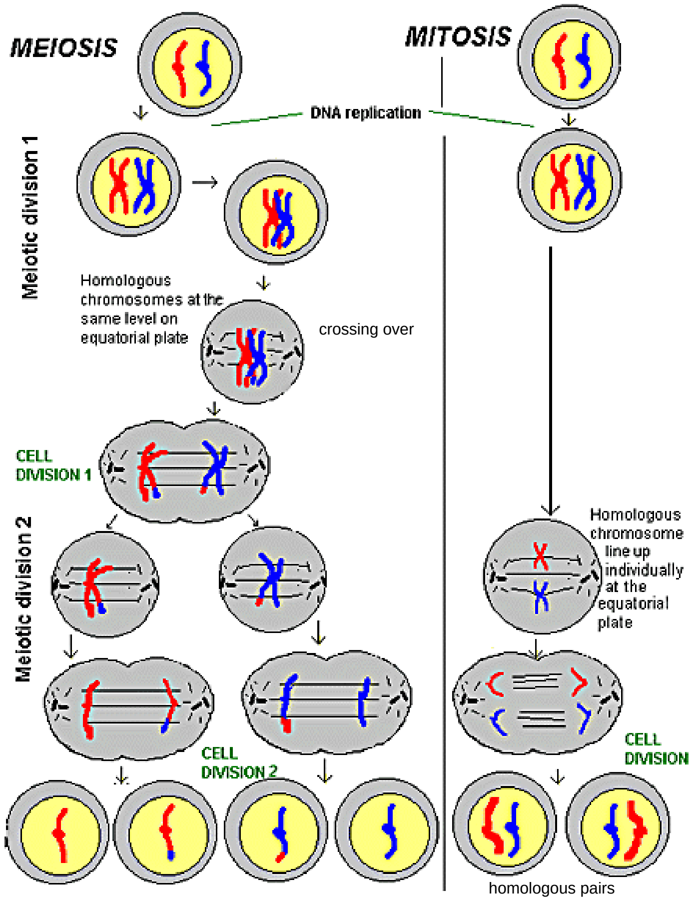
The figure above is a metaphase spread of chromosomes prepared from a cell that was about to undergo a mitotic division. The DNA in each of the 46 chromosomes has replicated so that each chromosome consists of 2 DNA strands (chromatids).

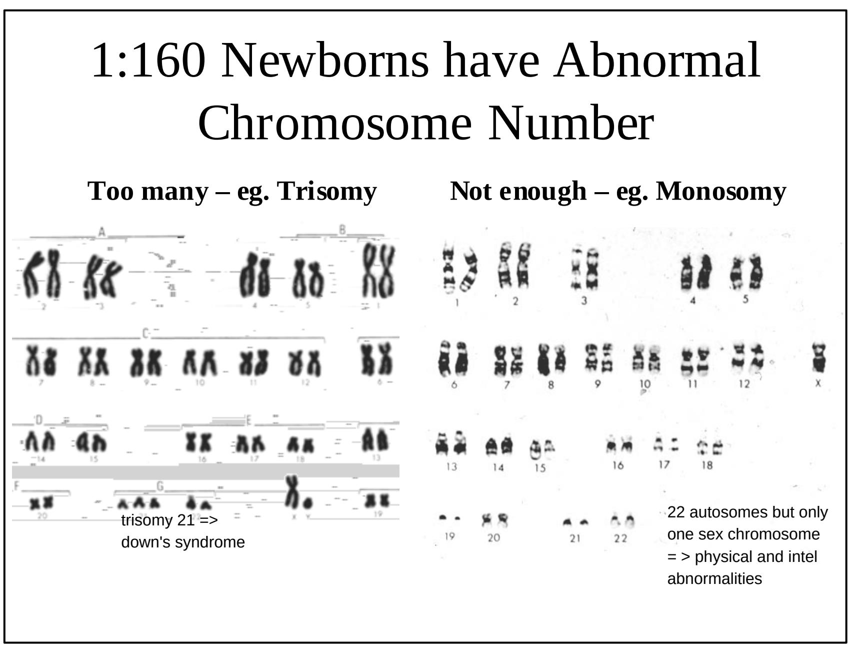
Somatic cells and Gametes

Somatic Cell Nuclei	Gamete Nuclei
- 46 chromosomes (diploid #)	- 23 chromosomes (haploid #)
- 23 pairs of chromosomes	- 23 single chromosomes
- 22 pairs of autosomes	- 22 autosomes
- XX or XY sex chromosomes	- X or Y sex chromosome



Meiosis and Mitosis





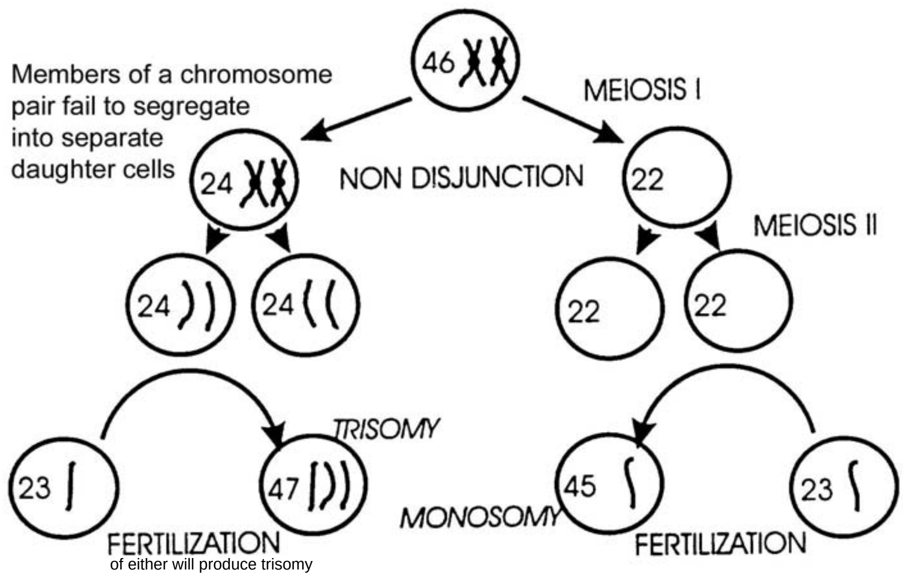
NON-DISJUNCTION

Failure of chromosomes to distribute normally during meiosis leads to the formation of gametes with abnormal numbers of chromosomes. This affects 2 or 3% of sperm and 20 to 60% of oocytes (in later years). If such a gamete participates in fertilization, the resulting embryo will have an abnormal number of chromosomes in its cells (aneuploidy). Approximately 1:160 newborns have abnormal chromosome number. The majority of aneuploid embryos never come to term.

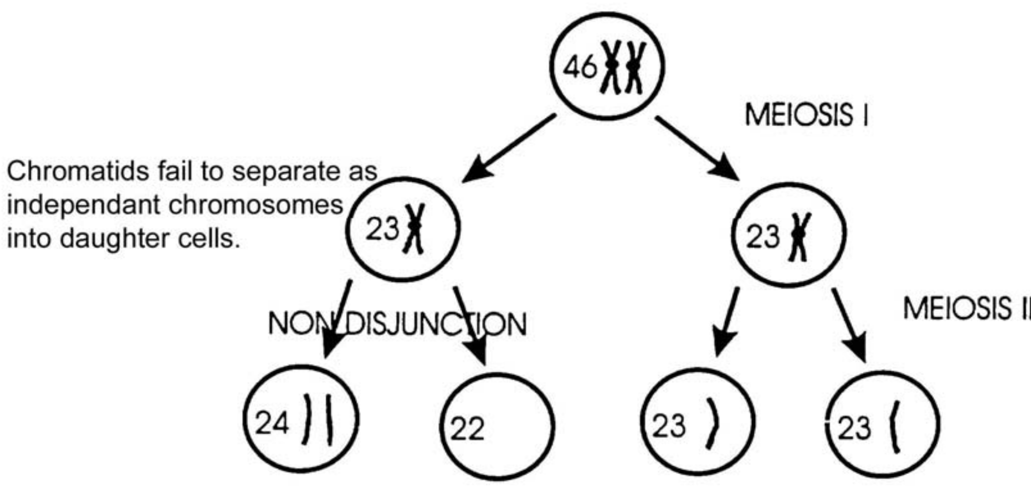
Failure of paired chromosomes to separate during cell division is called non-disjunction.

can be meiosis
or mitosis

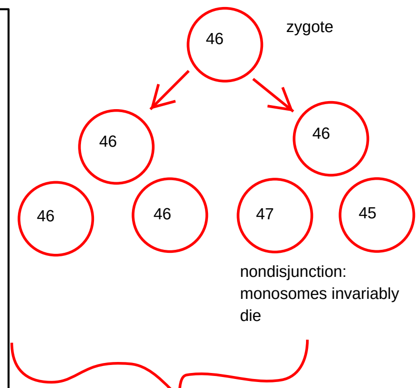
Non-disjunction in meiosis I



Non-disjunction in meiosis II



the earlier nondisjunction occurs, the more devestating the effects



mosaic individual made of cells w/ mix of 46 and 47 chromosomes

1:160 Newborns have Abnormal Chromosome Number

- **<u>Non-disjunction</u>** . . . Failure of paired chromosomes to separate during cell division
- 2 or 3% of sperm
- 20 % of oocytes (up to 60% in later years)
- The majority of aneuploid embryos never come to term

if up to 60% of oocytes have abnormal chromosome number (due to non-disjxn), why only 1/161 newborns have abnormal chromosome number?
- b/c natural processes eliminate most abnormal gametes and abnormal embyros

1. SPERMATOGENESIS

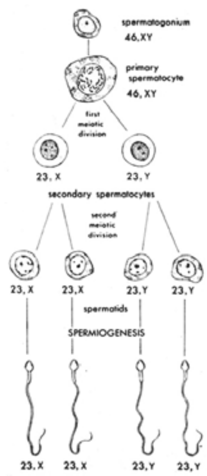
- Beginning at puberty and continuing for the lifetime. (falls off a bit w/ age)
- In the seminiferous tubules of the testes.
- a 2 phase process

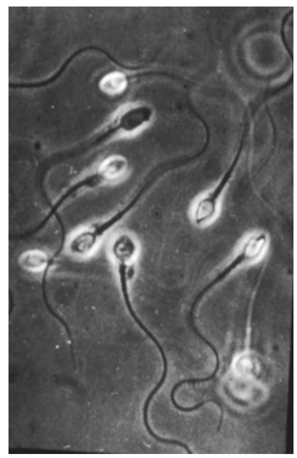
First phase	spermatogonia	Meiosis I → Meiosis II	spermatids (23 chromosomes)
Second phase	spermatids	Spermiogenesis →	spermatozoa

- From spermatogonium to spermatozoon takes about 10 weeks.

Spermatogenesis

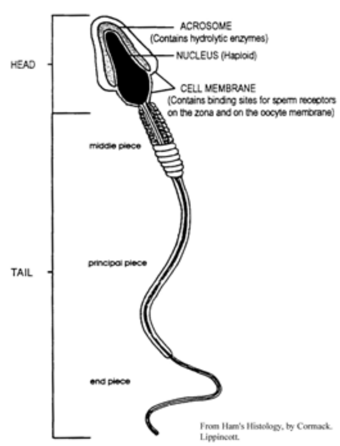
- Starts at puberty and is a continuous process
- Produces 50 to 150 x 10^6 sperm cells/day

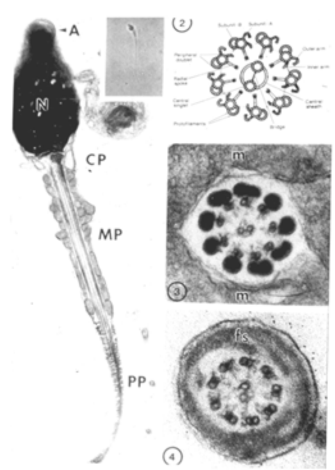


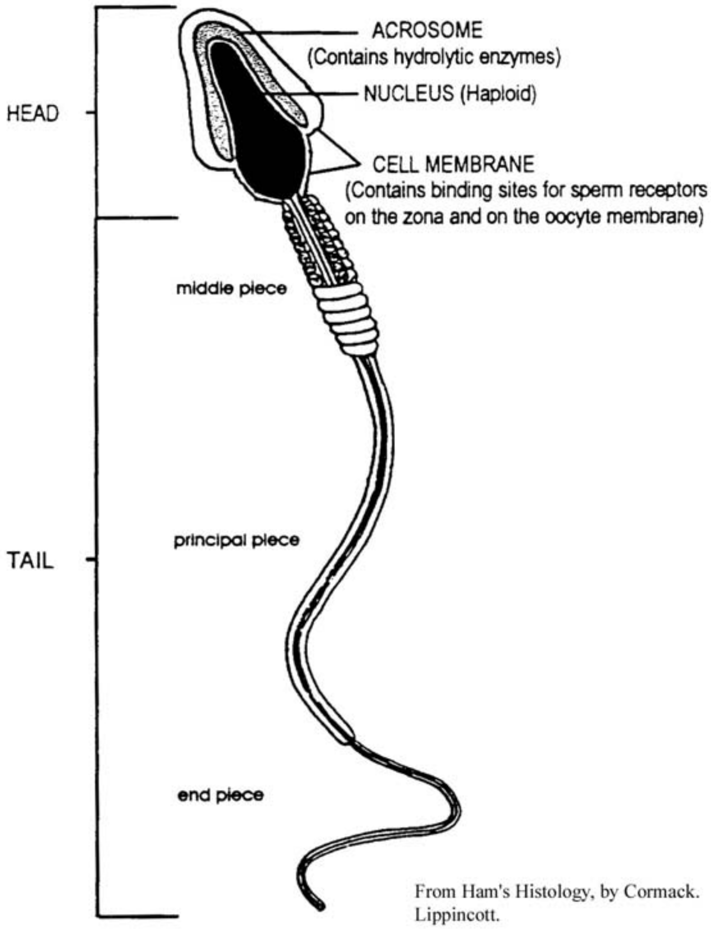


Spermatozoon

FIGURE 1
The human infant preformed in the sperm as depicted by Nicolas Hartsoeker (1694).



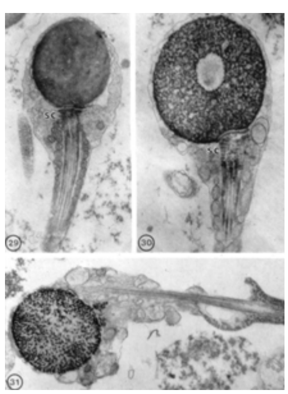


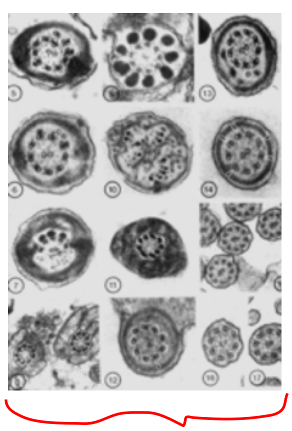


From Ham's Histology, by Cormack. Lippincott.

- tail = flagellum/cilium
- head: nucleus = very dark; 23 chromosomes; very condensed; heterochromatic
- acrosome = modified lysosome ; proteolytic enzymes to digest barriers
- cell membrane of sperm cell = contains molecules that help interact w/ and recognize the egg

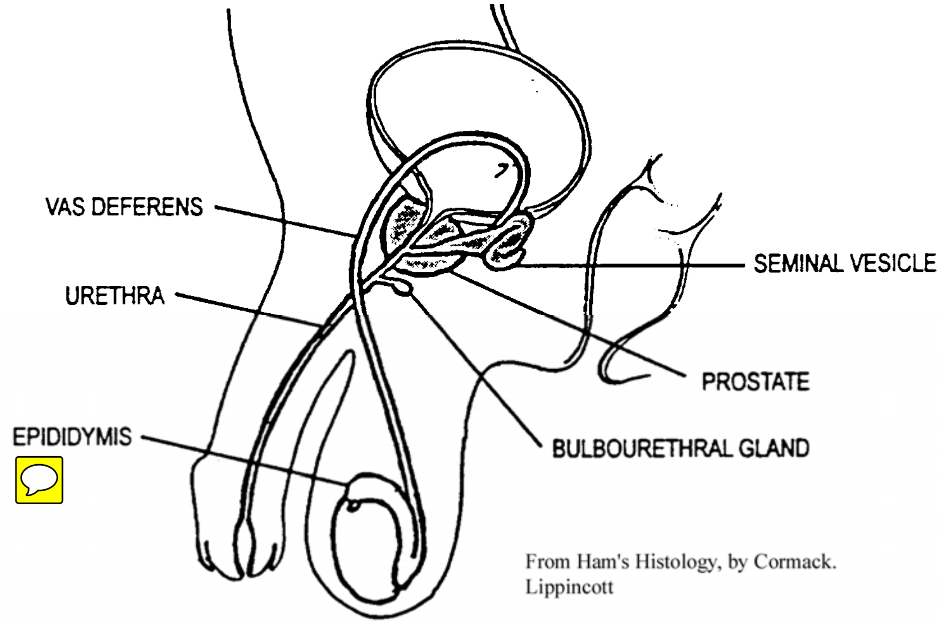
Abnormal Forms are Very Common





deficient microtubules or flagella

Seminal Fluid



From Ham's Histology, by Cormack. Lippincott

The seminal fluid is produced principally by the paired seminal vesicles (60%), the prostate gland (30%) and the paired bulbourethral glands (10%).

- At the time of ejaculation, the seminal fluid is added to the spermatozoa to constitute the semen.

(monosaccharides)

- The seminal fluid functions to provide a nutrient source for the spermatozoa, to neutralize vaginal acidity and promote sperm motility.
(organic acids of vag neutralized by alkaline seminal fluid; sperm flagella operate optimally at alkaline pH)

- A concentration of 40 - 200 X 10^6 spermatozoa per ml of semen is considered normal.

Azoospermia – A lack of spermatozoa in the semen (Zero)

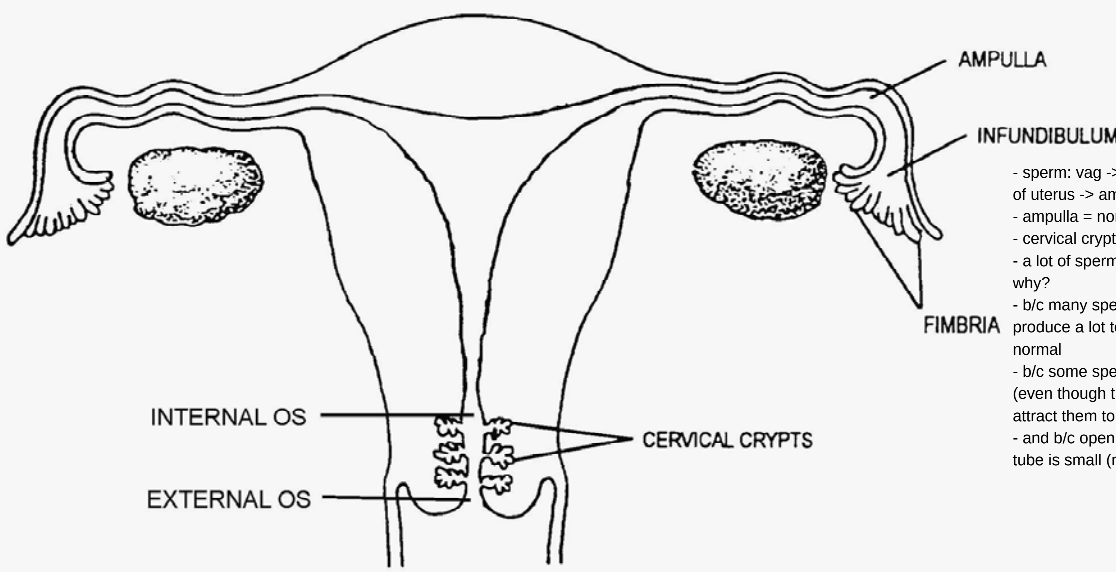
Oligospermia – A deficiency of spermatozoa in the semen (Some, but not a normal number)

Sperm Transit

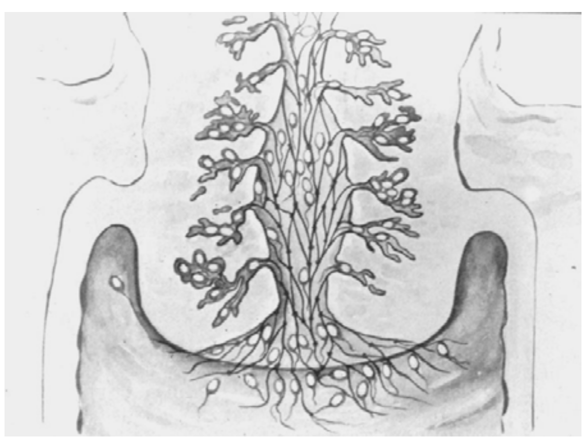
- The normal site of fertilization is in the ampulla of the uterine tube.

- Within minutes of ejaculation, some spermatozoa are carried from the vagina to the ampulla [redacted] by contractions of the uterine and tubal musculature. The remaining spermatozoa (the majority) colonize the crypts of the cervix.

- Over the following several days, sperm cells leave the cervix and swim to the ampullae by means of the sperm tail beat. Most of the cells can survive for at least 2 days. Some might be able to survive as long as 5 or 6 days.

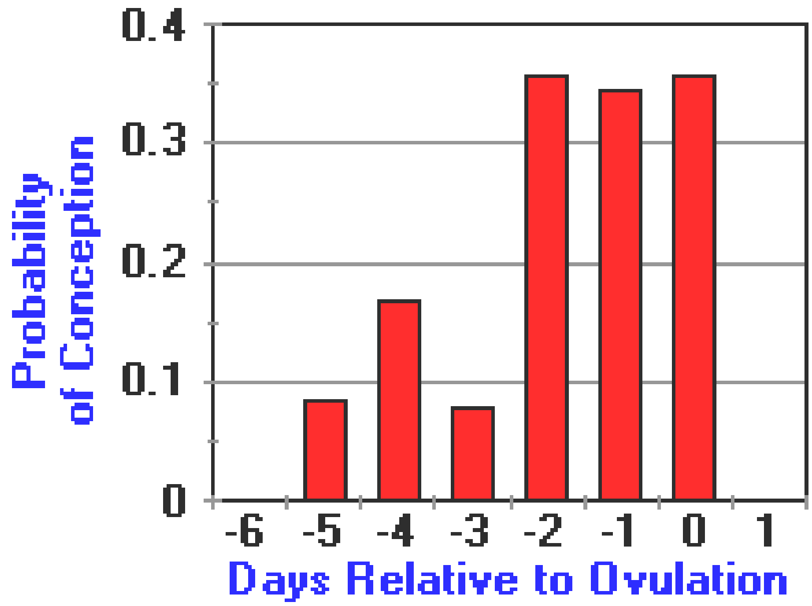


- sperm: vag -> external os -> internal os -> body of uterus -> ampulla
- ampulla = normal site of fertilization
- cervical crypts = glands produce mucous
- a lot of sperm is required to achieve fertilization; why?
- b/c many sperm cells are abnormal => need to produce a lot to ensure there are enough that are normal
- b/c some sperm cells end up in the wrong tube (even though there are chemotactic signals that attract them to the egg)
- and b/c opening b/w body of uterus to uterine tube is small (need more sperm to find)



sperm cells colonize cervix after ejac

THE PROBABILITY OF CONCEPTION ON SPECIFIC DAYS NEAR THE DAY OF OVULATION



- at ovulation (highest probability of conception), there's spike in body temp
- a day later, probability = 0 b/c oocyte dead
- one or two days before ovulation: probability just as high as at ovulation
- good sperm can live up to 5 days (the sperm that die at -5, -4 and -3 are relatively weak)

The bars represent the probabilities calculated from data on 129 menstrual cycles in which sexual intercourse was recorded to have occurred on only a single day during the six day interval ending on the day of ovulation (day 0). The solid line shows daily probabilities based on all 625 cycles, as estimated by the statistical model.

Capacitation sperm cells must go through this process before fertilization can occur

- Normally occurs from 4 to 6 hours after the sperm cells enter the female reproductive tract.
- Capacitation is characterized by an increase in sperm motility and metabolism (hyperactivation). mechanism not entirely clear: something is washed off sperm cell membrane by female buffer fluids -> Ca2+ influxes into sperm cell -> overdrive
- Normally necessary for sperm - egg binding and the acrosome reaction.
- Capacitation is not a species-specific phenomenon and can be readily induced in the laboratory for the purpose of *in vitro* fertilization.

2. OOGENESIS

- In the ovaries
- Beginning during fetal life and lasting until menopause.

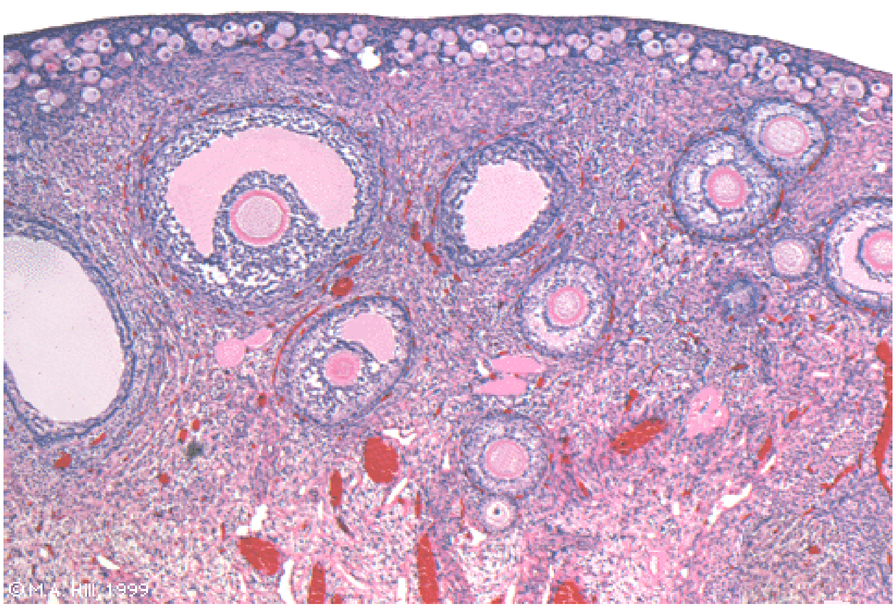
Primary oocyte —— Meiosis I begins in the fetal ovary ——> Secondary oocyte

but stops and does not complete until just before ovulation so all females retain 1' oocytes from birth until about menopause; later oocytes have been affected by many environ factors; children of older women more likely to develop abnormalities

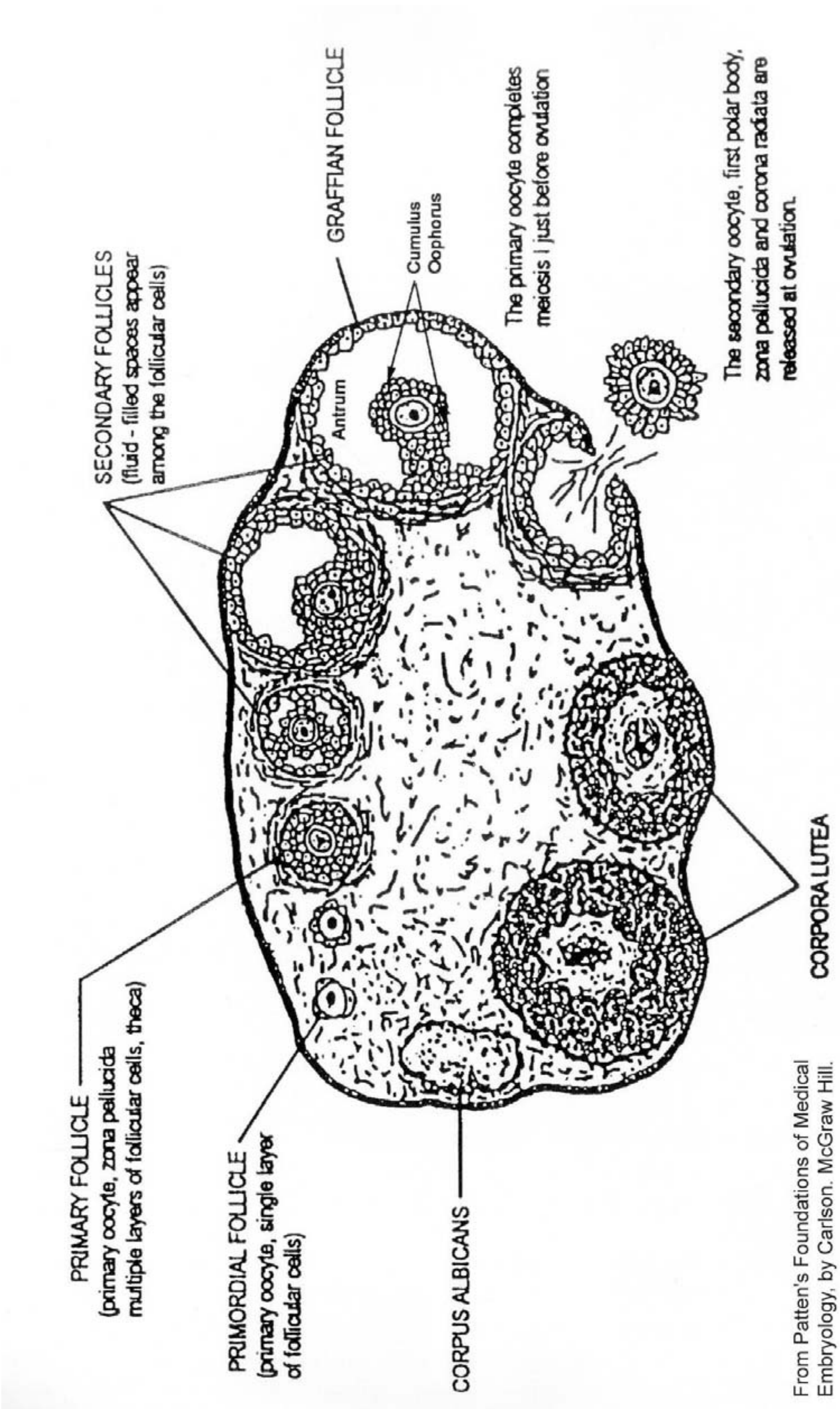
Secondary oocyte —— Meiosis II begins just before ovulation ——> at which point it becomes Ovum

But stops and does not complete unless the egg is fertilized

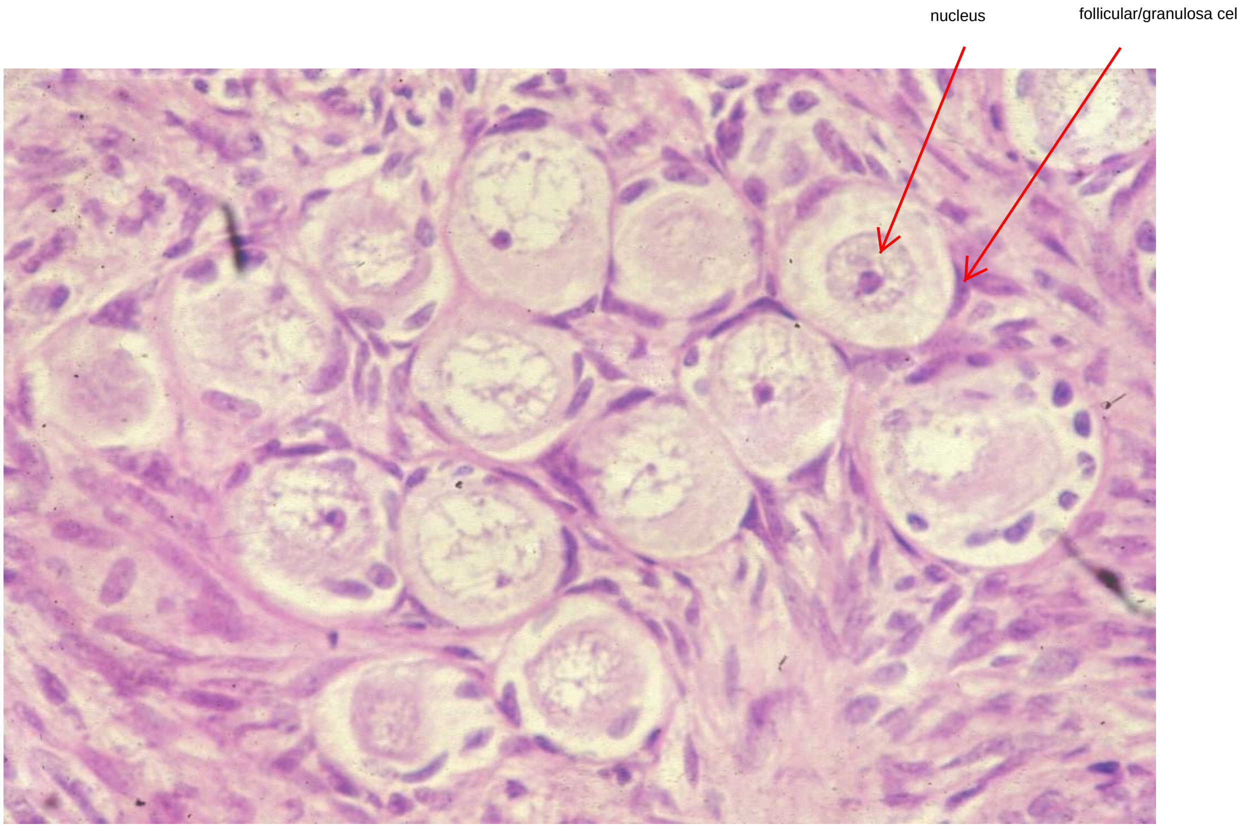
Follicles – The cellular units in the ovary that contain the developing egg (oocyte).



ovarian follicles


PRIMARY FOLLICLE
(primary oocyte, zona pellucida
multiple layers of follicular cells, theca)
PRIMORDIAL FOLLICLE
(primary oocyte, single layer
of follicular cells)
CORPUS ALBICANS
SECONDARY FOLLICLES
(fluid - filled spaces appear
among the follicular cells)
GRAFFIAN FOLLICLE
Antrum
Cumulus
Oophorus
The primary oocyte completes
meiosis I just before ovulation
The secondary oocyte, first polar body,
zona pellucida and corona radiata are
released at ovulation.
CORPORA LUTEA
From Patten's Foundations of Medical
Embryology, by Carlson. McGraw Hill.

Primordial follicles – consist of a primary oocyte, arrested in prophase of the first meiotic division. ~~It is a large cell with a large pale nucleus that is sometimes referred to as the germinal vesicle.~~ The primary oocyte is surrounded by a single layer of flattened cells called follicular cells. Each month, beginning at puberty, a number of these follicles will begin to develop in complexity and grow in size in preparation for ovulation. Typically, however, only one will reach full development and ovulate. The rest will degenerate along the way. At birth the ovaries contain some 2 million primordial follicles. Only about 400 of these of these will ever ovulate during the reproductive years of the individual.



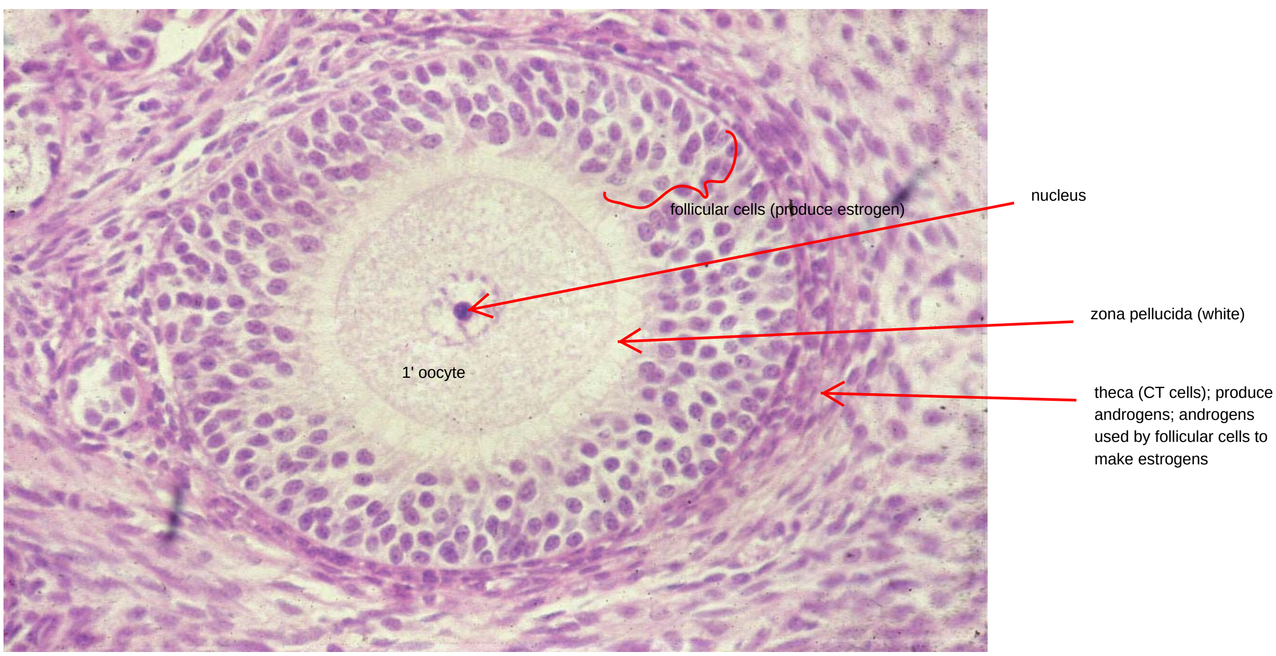
Primary follicles – As primordial follicles evolve into primary follicles:

1. The primary oocyte grows in size, but it remains arrested in meiosis I.

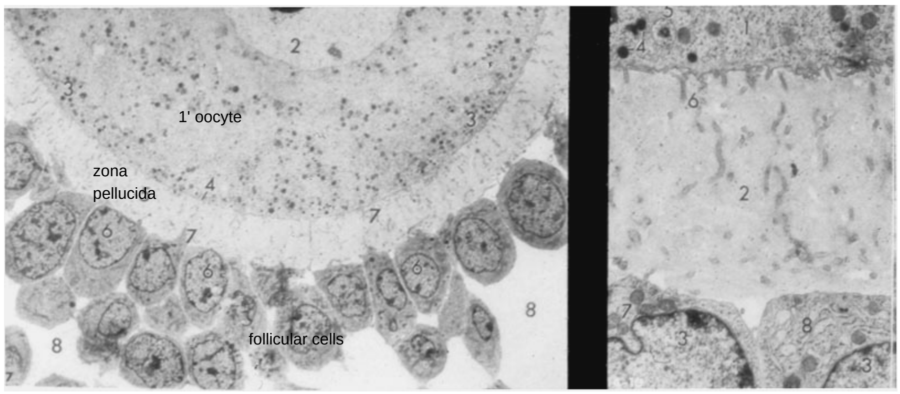
2. The follicular cells become cuboidal in shape and multiply and secrete estrogen. They are sometimes referred to as granulosa cells. The granulosa cells are connected to one another by gap junctions which will allow the direct transfer of small molecules from one cell to another. The primary oocyte is also connected by gap junctions to the follicular cells in the innermost layer. These connections are thought to provide a mechanism whereby the follicular cells can assist in the metabolic support of the primary oocyte and in keeping it suspended in the first meiotic division.

3. The connective tissue immediately surrounding the granulosa cells becomes thickened and well-vascularized and forms a layer called the theca. The cells of the theca synthesize androgens which the granulosa cells use as a substrate in the production of estrogen.

4. The zona pellucida is laid down. The zona pellucida is an extracellular matrix that lies between the primary oocyte and the innermost layer of the follicular cells.



The follicular cells and the primary oocyte are connected



oocyte

zona pellucida (contains gap jxns = cytoplasmic continuity b/w oocyte and follicular cells)

follicular cells

Gap junctions connect the follicular cells to each other and the oocyte to the follicular cells

exchange of nutrients, gases, and waste

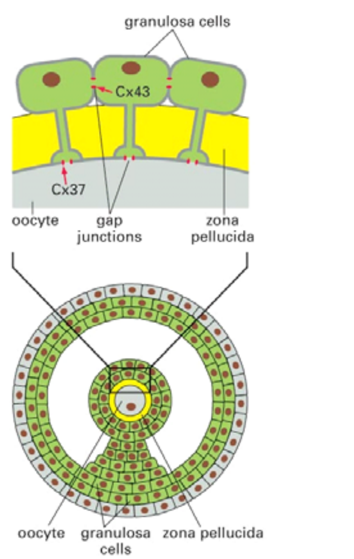


Figure 19–17. Molecular Biology of the Cell, 4th Edition.

- oocytes don't have much cell membrane, so they take advantage of follicular cells' greater extent of membrane for more efficient exchange
- follicular cells secrete cAMP to keep oocyte suspended in meiosis I
- evidence: if you take away follicular cells, oocyte completes meiotic division I
- evidence: follicular cells have LH receptors (oocytes don't); with LH spike, the follicular cells disassemble their gap jxns => cAMP cut off from oocyte => oocyte will complete meiotic division I

Secondary follicles – Secondary follicles are similar to primary follicles except for an increase in size and the appearance of fluid-filled spaces between the follicular cells. As these spaces enlarge, they coalesce together into one large space called the antrum.

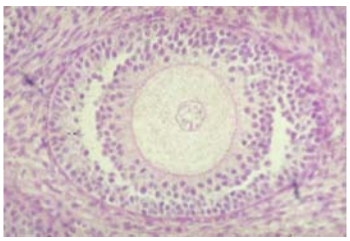
- The follicular cells that form the walls of the antrum are called the mural follicular cells. They will remain behind in the ovary after ovulation and form the granulosa lutein cells of the corpus luteum.

- The oocyte and the follicular cells immediately surrounding it form a complex called the cumulus oophorus that projects into the antrum.

Graffian follicles – the secondary follicles continue to grow in size until they reach a diameter of about 2.5 cm. At this point they are so large that they will cause the surface of the ovary to bulge. On the surface of this elevation a pale avascular spot called a "stigma" can be identified. These large secondary follicles are often called Graffian follicles after the 17th century Dutch physician who first described them, Renier de Graaf.

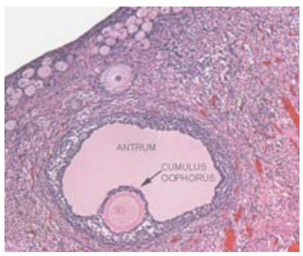
The time it takes for a primordial follicle in a human ovary to develop into a primary follicle is not known, but it is thought to take approximately 3 months for a primary follicle to develop into a mature, pre-ovulatory secondary follicle.

EARLY SECONDARY FOLLICLE



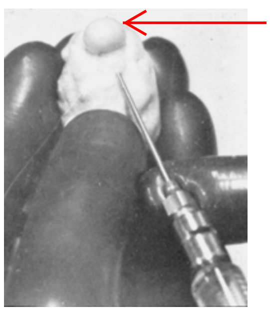
1' oocyte still suspended in meiotic division;
still have ZP and theca

LATE SECONDARY FOLLICLE



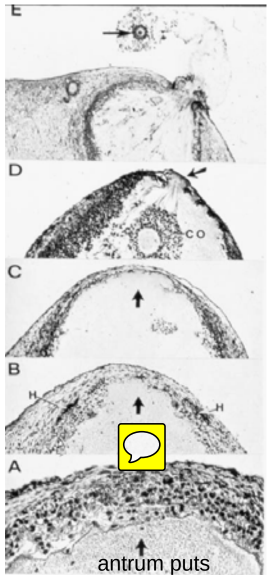
antrum will continue to grow until it deforms surface of ovary

GRAFFIAN FOLLICLE



stigma = area where graffian follicle will burst through ovary at ovulation

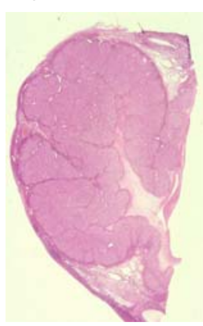
Ovulation



- surface of ovary gets increasingly thin until it opens; oocyte breaks out
- oocyte able to break free b/c follicular cells are disassembling cell/gap jxns

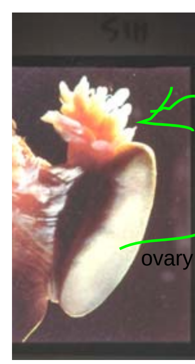
antrum puts pressure on ovary surface cuts off blood supply => ischemia => tissue gets weak and thin

The Corpus Luteum Remains Behind



- oocyte escapes
- remains of follicle becomes corpus luteum
- cells of follicle (theca and mural follicular cells) produce estrogen and progesterone
- signals to uterus to become receptive to implantation
- if no fertilization -> regression/involution -> becomes corpus albicans (scar on ovary)
- if fert -> implant -> embryo will secrete hCG -> maintain corpus luteum -> keep uterus in secretory state

The Egg



enters mouth of uterine tube

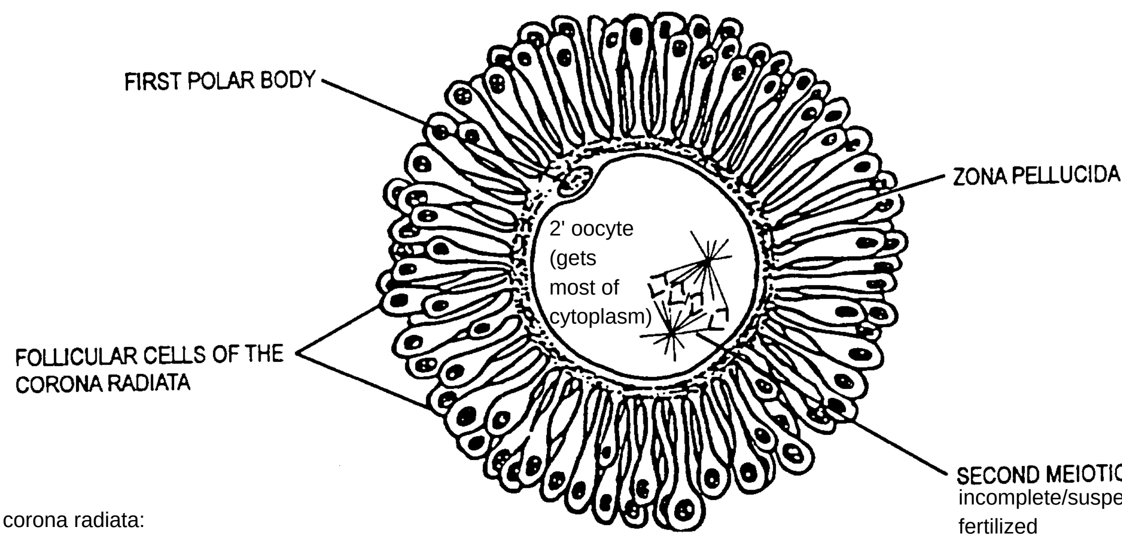
ovary (releases egg)

fuzz around 2' oocyte = corona radiata (follicular cells); many sperm cells trapped

OVULATION

- [redacted]

- The secondary oocyte, the first polar body, the zona pellucida and the follicular cells that immediately surround it are released through the surface of the ovary into the peritoneal cavity and enter the ostium (mouth) of the uterine tube at 'ovum pick - up'. The follicular cells that cover the secondary oocyte at this time are collectively referred to as the corona radiata. They are no longer connected to the oocyte by junctions and so are only loosely associated. They will gradually fall away over the next few days.



*****temp fxns of corona radiata:
- rough surface for cilia to move ovum (like velcro) into uterine tube; facilitates ovum pickup
- also creates bigger target for sperm to find ovulated complex
- fxns as a sperm trap
- guides sperm down to the ZP

- If the oocyte is fertilized and completes meiosis II, cytokinesis of the division is again unequal generating a second polar body. The second polar body will then degenerate.

Some Useful Definitions

Fertilization Age – refers the age on an embryo or fetus counting from the day of fertilization. All ages in this course will refer to fertilization ages.

Gestational Age – Used in clinical practice and refers to the age of an embryo or fetus counting from the first day of last menstrual period. This is 14 days before the day of fertilization and so the gestational age is 2 weeks older than the fertilization age.

Chromosome – a linear thread of DNA and Associated molecules.

Chromatid – Either of 2 parallel filaments, joined at a centromere that make up a chromosome in a dividing cell and which separate in cell division, each going to a different pole of the dividing cell and each becoming a chromosome of one of the two daughter cells.

Ploidy – Refers to the number of copies of each chromosome present in the nucleus.

Haploid (N) – The number of unique chromosomes in a species. In man the haploid number is 23. i.e. there are 23 different chromosomes (22 autosomes and a sex chromosome)

Diploid (2N) – Two sets of chromosomes. In man the diploid number is 46. There are 22 pairs of autosomes and a pair of sex chromosomes.

Polyploid – More than 2 full sets of chromosomes eg) 3N = triploid; 4N = tetraploid.

Aneuploid – A chromosome number that is not an exact multiple of the normal haploid number. e.g. 21 trisomy.

Embryology Textbook References

In alphabetical order:

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Embryology websites:

STF Embryology theme song on YouTube

http://www.youtube.com/watch?v=osWuWjbeO-Y

Online course in embryology for medical students developed by the universities of Fribourg, Lausanne and Bern (Switzerland)

http://www.embryology.ch/indexen.html

University of New South Wales Embryology site

http://embryology.med.unsw.edu.au/

McGill Medical Embryology

http://sprojects.mmi.mcgill.ca/embryology/