

Lecture - Fertilization

From Embryology

Embryology - 18 Aug 2015    Translate [Expand]

Introduction

This lecture and the associated laboratory will cover male and female gametogenesis and fertilisation.

Development is 1 embryonic cell producing about 10^{13} (100,000,000,000,000) cells in the adult at any one time (over time with cell death and ongoing replacement this is substantially more).

This is where the first embryonic cell begins! Fertilization is the fusion of haploid gametes, egg (oocyte) and sperm (spermatozoa), to form the diploid zygote. Note though there can be subtle differences in the fertilization process which occurs naturally within the body or through reproductive technologies outside the body, the overall product in both cases is a diploid zygote.

Some Recent Research - Meiosis Podcast Biosights 18 March 2013 - Breaking egg symmetry (<http://jcb.rupress.org/content/200/5/567/suppl/DC2>) | JCB 16 June 2014 How sperm get into the zona (<http://jcb.rupress.org/content/205/6/754.2.full>)

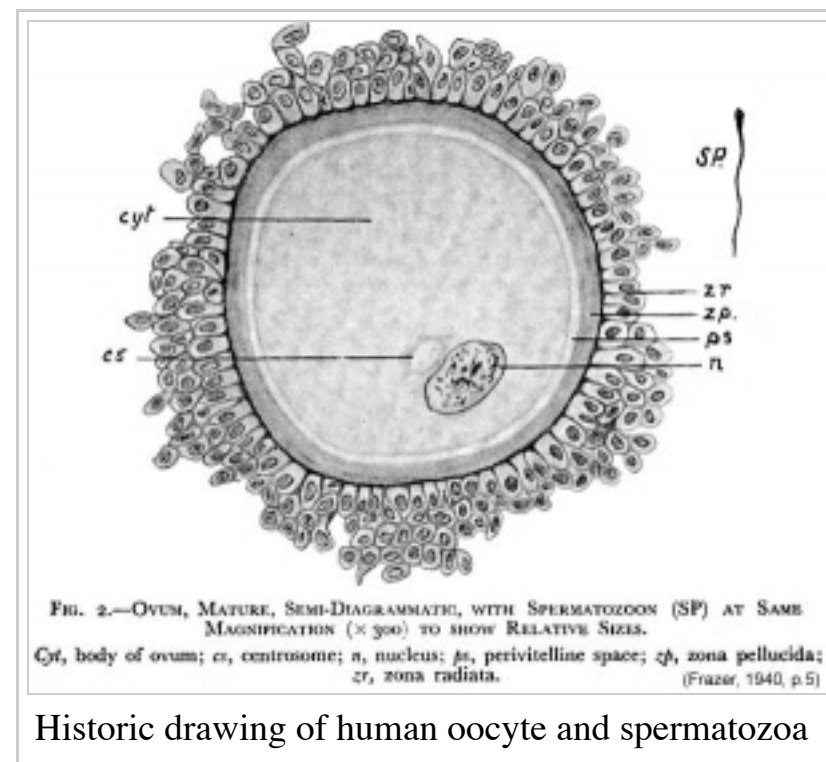
Lecture - Print PDF

Lecture Archive: 2014 Lecture PDF | 2013 | 2012 (http://embryology.med.unsw.edu.au/embryology/index.php?title=Lecture_-_Fertilization&oldid=96435) | 2011 (http://embryology.med.unsw.edu.au/embryology/index.php?title=Lecture_-_Fertilization&oldid=60496) | 2010 | 2009

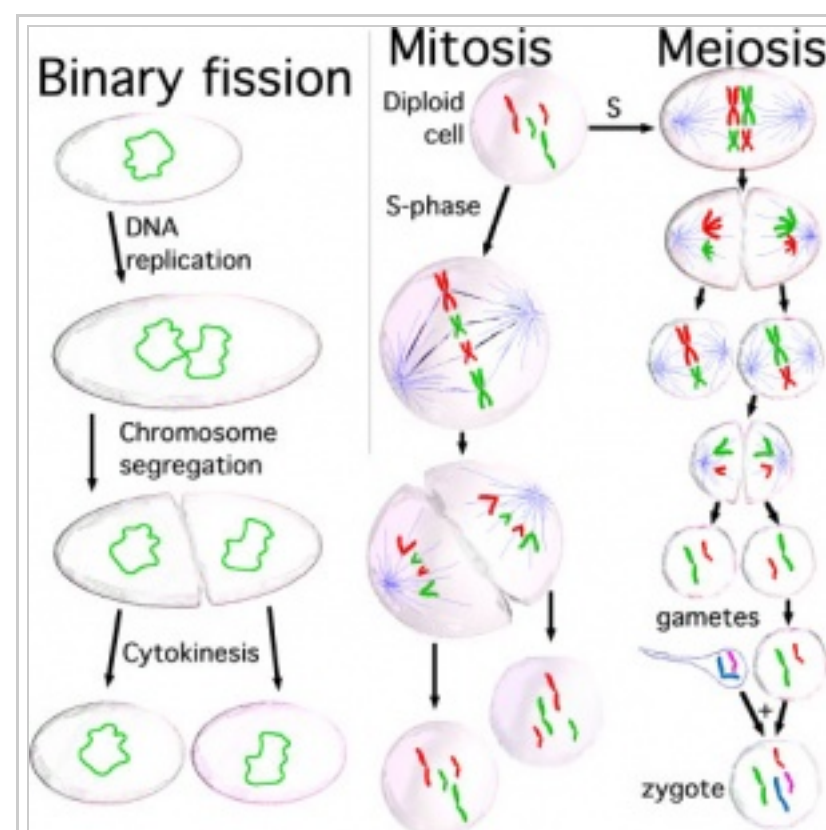
Lecture Objectives

1. Broad understanding of reproductive cycles.
2. Understand the key features of gametogenesis.
3. Understand the differences in male and female gametogenesis.
4. Brief understanding of the differences between mitosis and meiosis.
5. Understanding of the events in fertilization.

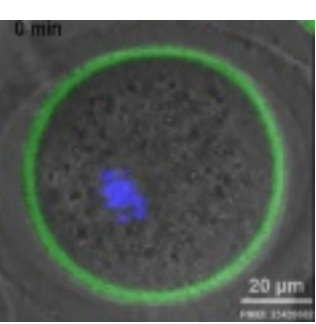

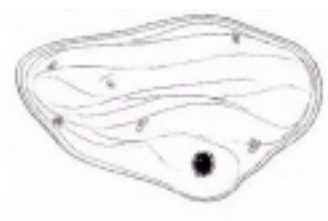

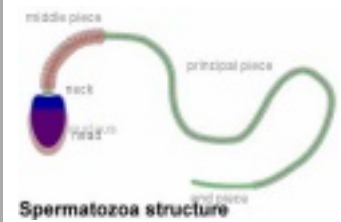
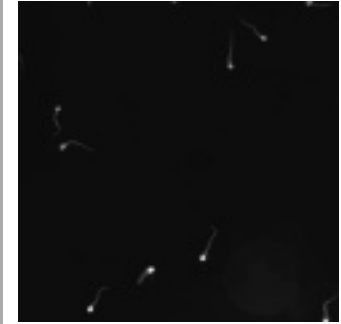

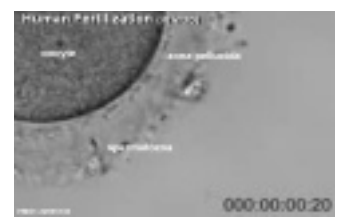


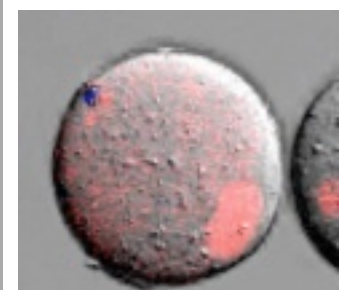
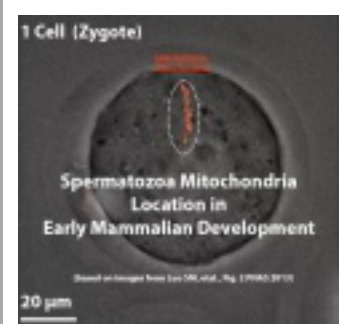
Lecture Resources






Historic drawing of human oocyte and spermatozoa



Cell division - 3 types

Movies [Collapse]					
 Oocyte Meiosis Page Play	 Ovulation Page Play Audio	 Ovulation Page Play	 Oocyte Transport Page Play	 Spermatozoa Page Play	 Spermatozoa Motility Page Play
 Human Fertilization Page Play	 Human Fertilization Page Play	 Fertilization Page Play	 Pronuclear Fusion Page Play	 Ectopic Polar Body Page Play	 Male Mitochondria Page Play

References	[Collapse]
 Hill, M.A. (2015). <i>UNSW Embryology</i> (15th ed.) Retrieved August 18, 2015, from https://embryology.med.unsw.edu.au	<ul style="list-style-type: none"> Cell Division Links: Meiosis Mitosis Lecture - Cell Division and Fertilization Spermatozoa Development Oocyte Development Fertilization Zygote Menstrual Cycle Oocyte Zona pellucida Spermatozoa Meiosis Fertilization Mitosis Week 1
 Moore, K.L., Persaud, T.V.N. & Torchia, M.G. (2011). <i>The developing human: clinically oriented embryology</i> (9th ed.). Philadelphia: Saunders.	The following chapter links only work with a UNSW connection. <ul style="list-style-type: none"> First Week of Human Development (http://www.unsw.ebib.com.wwwproxy0.library.unsw.edu.au/patron/Read.aspx?p=1430154&pg=35) Second Week of Human Development (http://www.unsw.ebib.com.wwwproxy0.library.unsw.edu.au/patron/Read.aspx?p=1430154&pg=63)
 Schoenwolf, G.C., Bleyl, S.B., Brauer, P.R., Francis-West, P.H. & Philippa H. (2015). <i>Larsen's human embryology</i> (5th ed.). New York; Edinburgh: Churchill Livingstone.	The following chapter links only work with a UNSW connection. <ul style="list-style-type: none"> Chapter 1 - Gametogenesis, Fertilization, and First Week (https://login.wwwproxy0.library.unsw.edu.au/login?url=http://www.unsw.ebib.com/patron/FullRecord.aspx?p=2074524)

ECHO360 Recording[Expand]

Human Reproductive Cycle

Sexual reproduction in most species is regulated by regular endocrine changes, or cycles, in the female. These cycles begin postnatally, function for variable times and can then decrease or cease entirely.

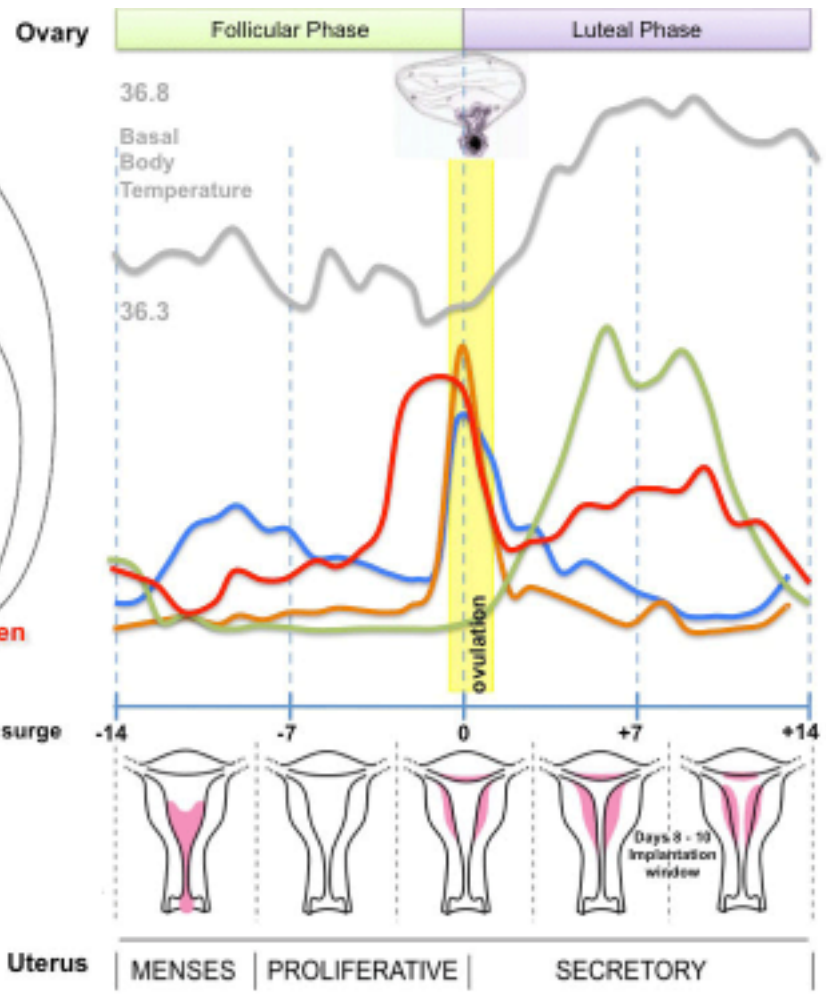
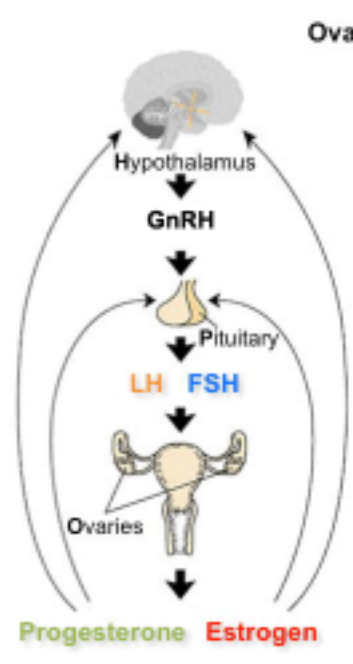
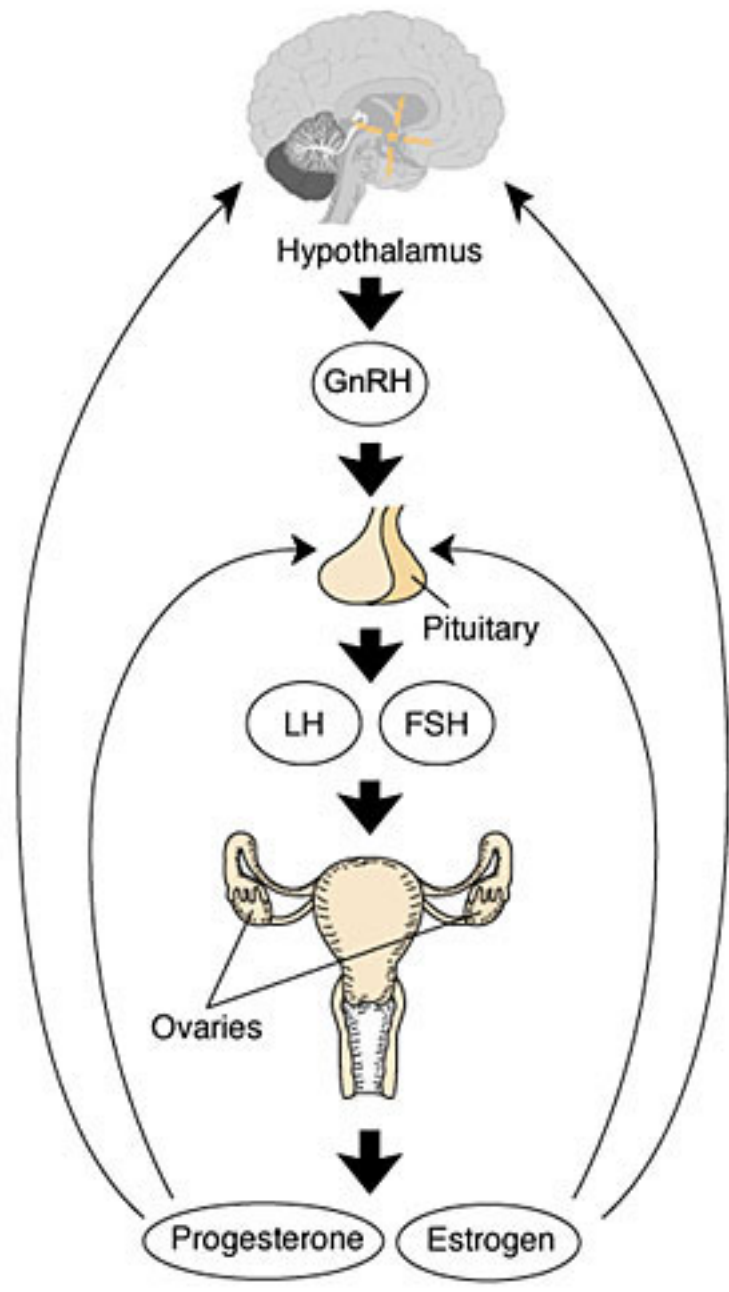
- Human reproduction is regulated in females by the **menstrual cycle**, a regular cyclic hormonal change which coordinate changes in the ovary and internal reproductive tract. This cycle commences at puberty and ends at menopause.
- Non-primates (rats, mice, horses, pig) reproduction is regulated in females by the **estrous cycle** (British spelling, oestrous).

Female

- Menstrual Cycle a regular cycle of reproduction (28 days)
- begins at puberty, release of 1 egg (oocyte) every cycle
- Endocrine controlled (HPG axis) **H**ypothalamus - **P**ituitary - **G**onad

Male

- continuous production of sperm (spermatozoa)
- begins at puberty, release millions of spermatozoa
- Endocrine controlled (HPG axis) **H**ypothalamus - **P**ituitary - **G**onad



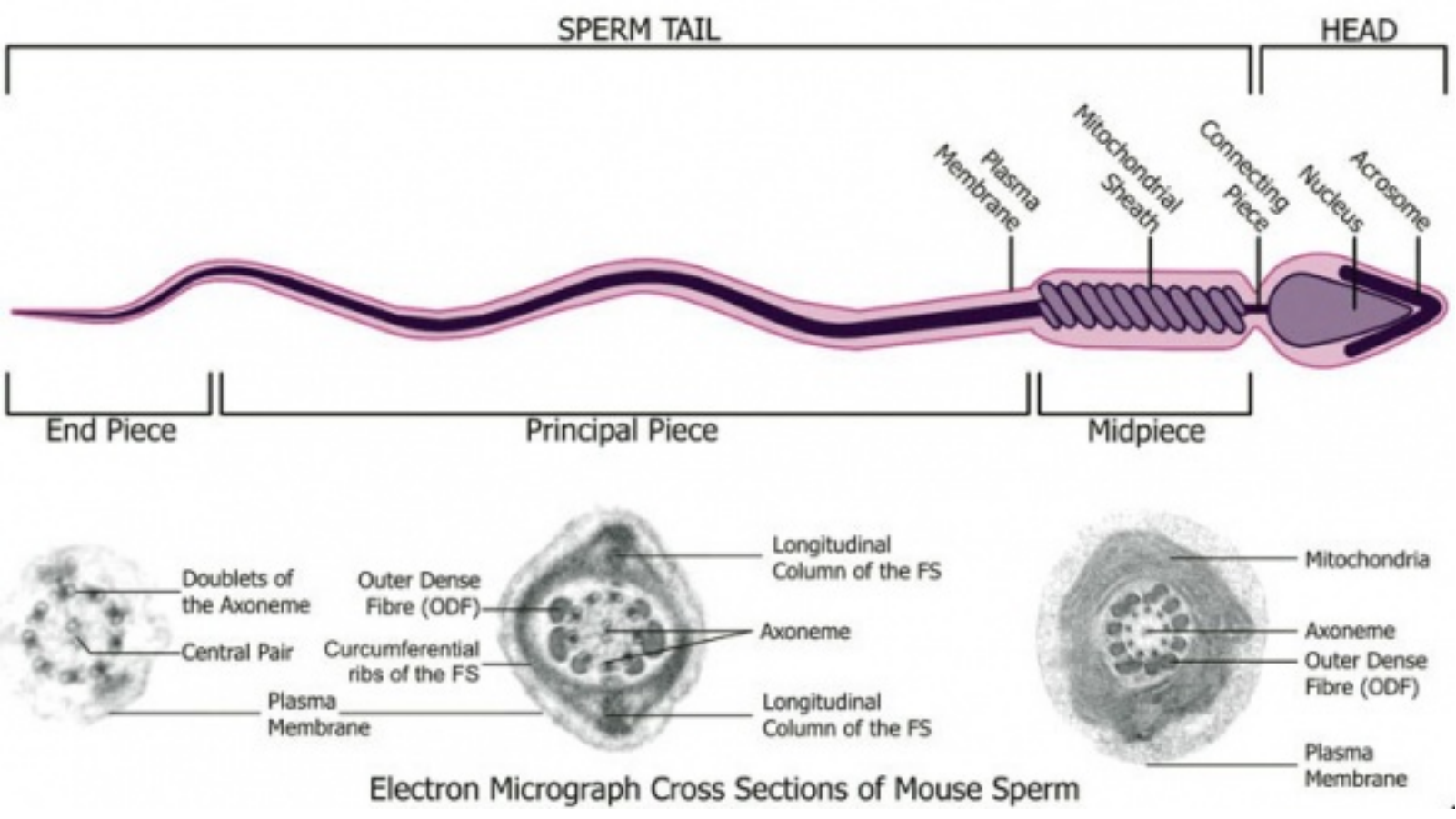
Gametogenesis

Meiosis in the gonad (ovary or testis) produces the haploid gametes, oocyte and spermatozoa (egg and sperm). Meiosis time course and final gamete number differs between female and male.

Male - Spermatogenesis



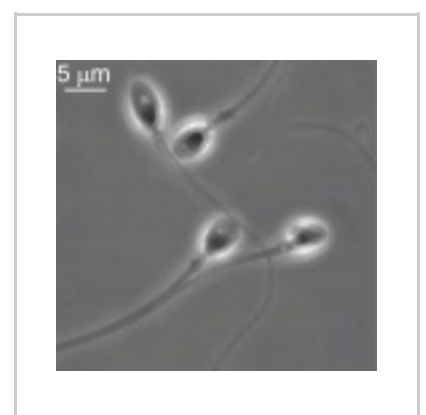
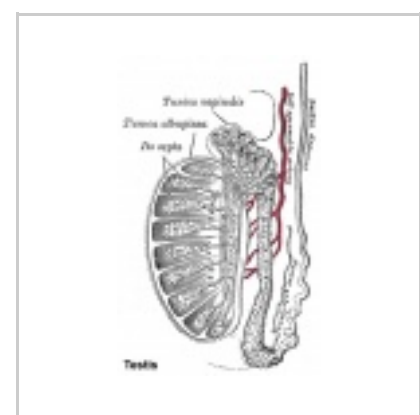
Human spermatozoa (electron microscope)



Mouse spermatozoa (electron microscope)

The testes have two functions.

1. produce the male gametes or **spermatozoa**
2. produce male sexual hormone, **testosterone** (internal and external genitalia, sex characteristics)



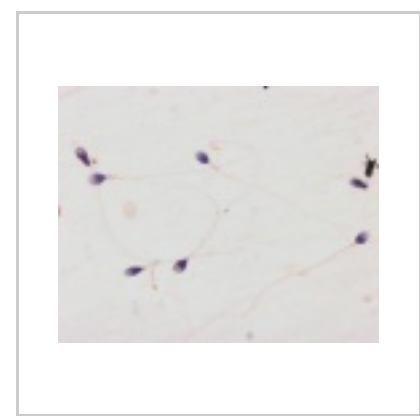
Historic testis drawing

Child Seminiferous tubule

Adult Seminiferous tubule

Seminiferous tubule cross-section and supporting cells

Human spermatozoa



Human Spermatozoa

Human spermatozoa take about **48 days** from entering meiosis until morphologically mature spermatozoa.

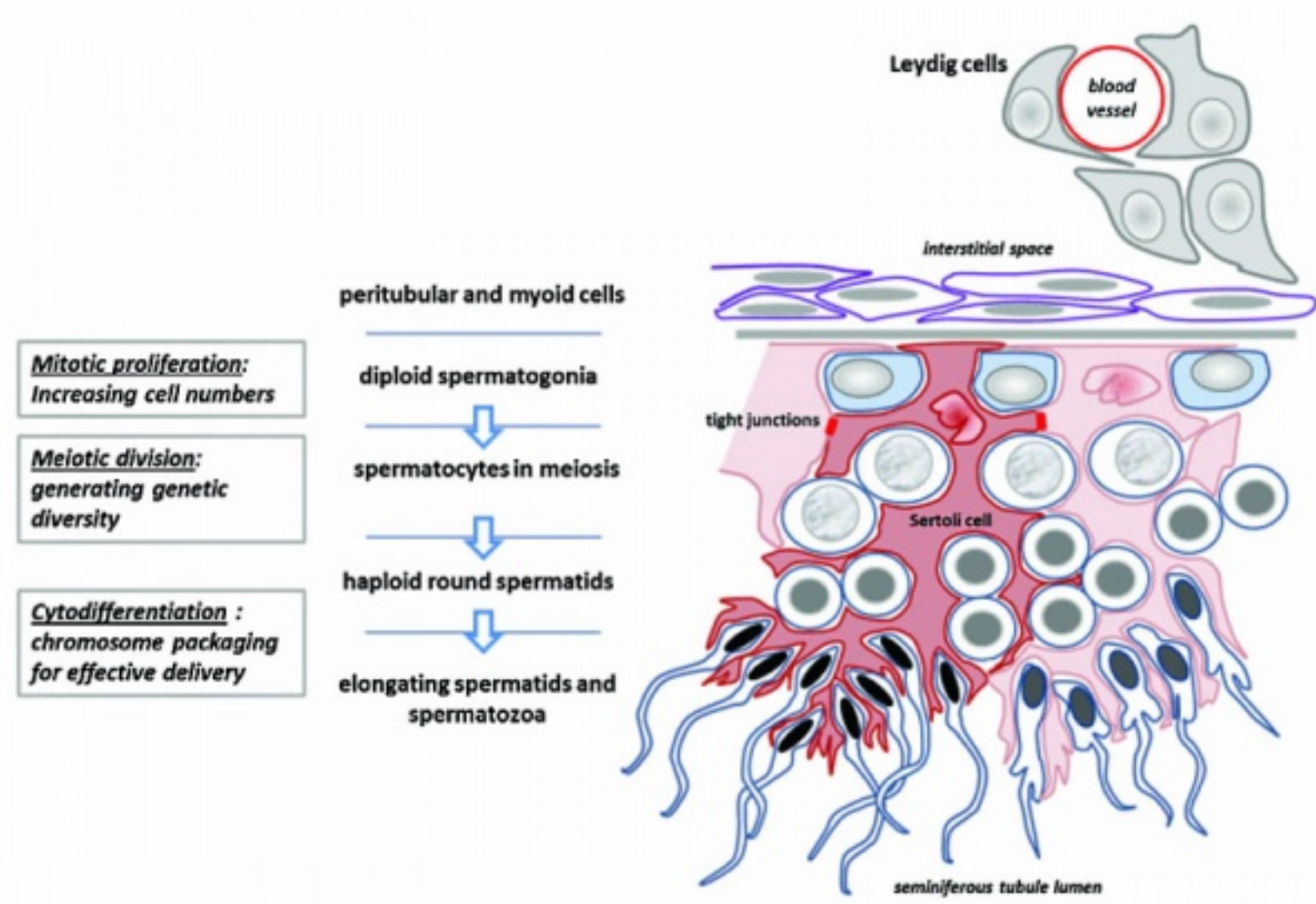
- **Spermatogonia** - are the diploid first cells of spermatogenesis
- **Primary spermatocytes** - large, enter the prophase of the first meiotic division
- **Secondary spermatocytes** - small, complete the second meiotic division
- **Spermatid** - immature spermatozoa
- **Spermatozoa** - differentiated gamete

Spermatozoa development:

primordial germ cell - spermatogonia - primary spermatocyte - secondary spermatocytes - spermatid - spermatozoa

Sertoli cells (support cells)

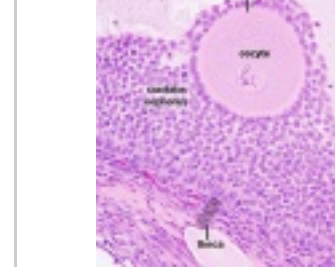
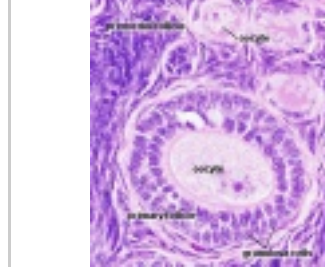
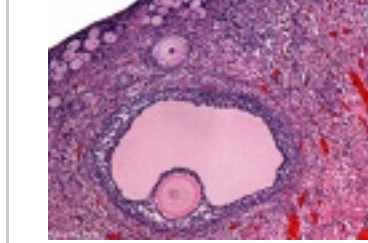
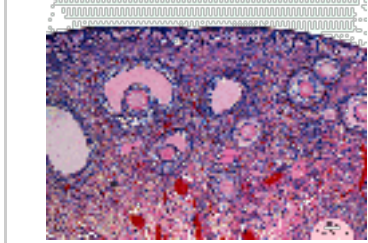
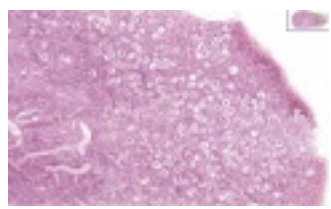
Interstitial cells or **Leydig cells** (produce hormone)



Female - Oogenesis

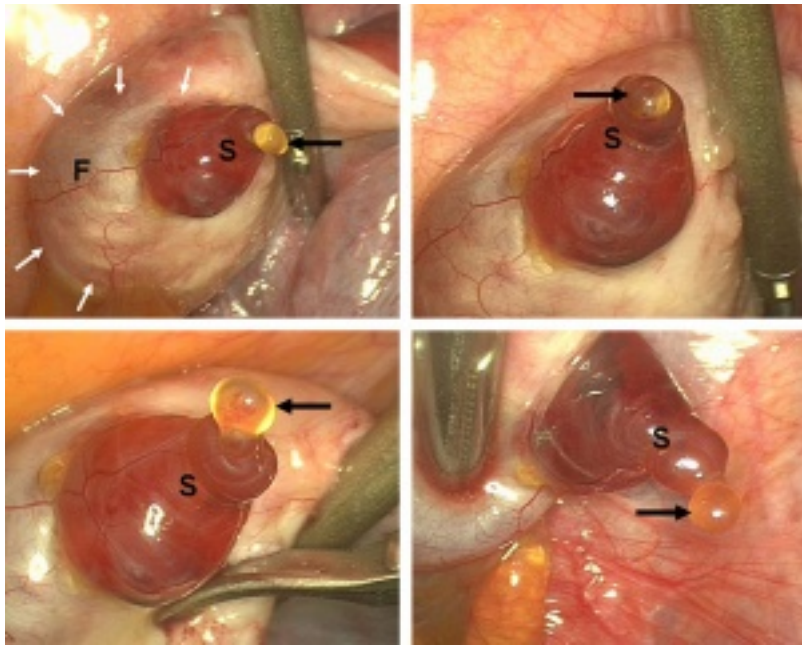
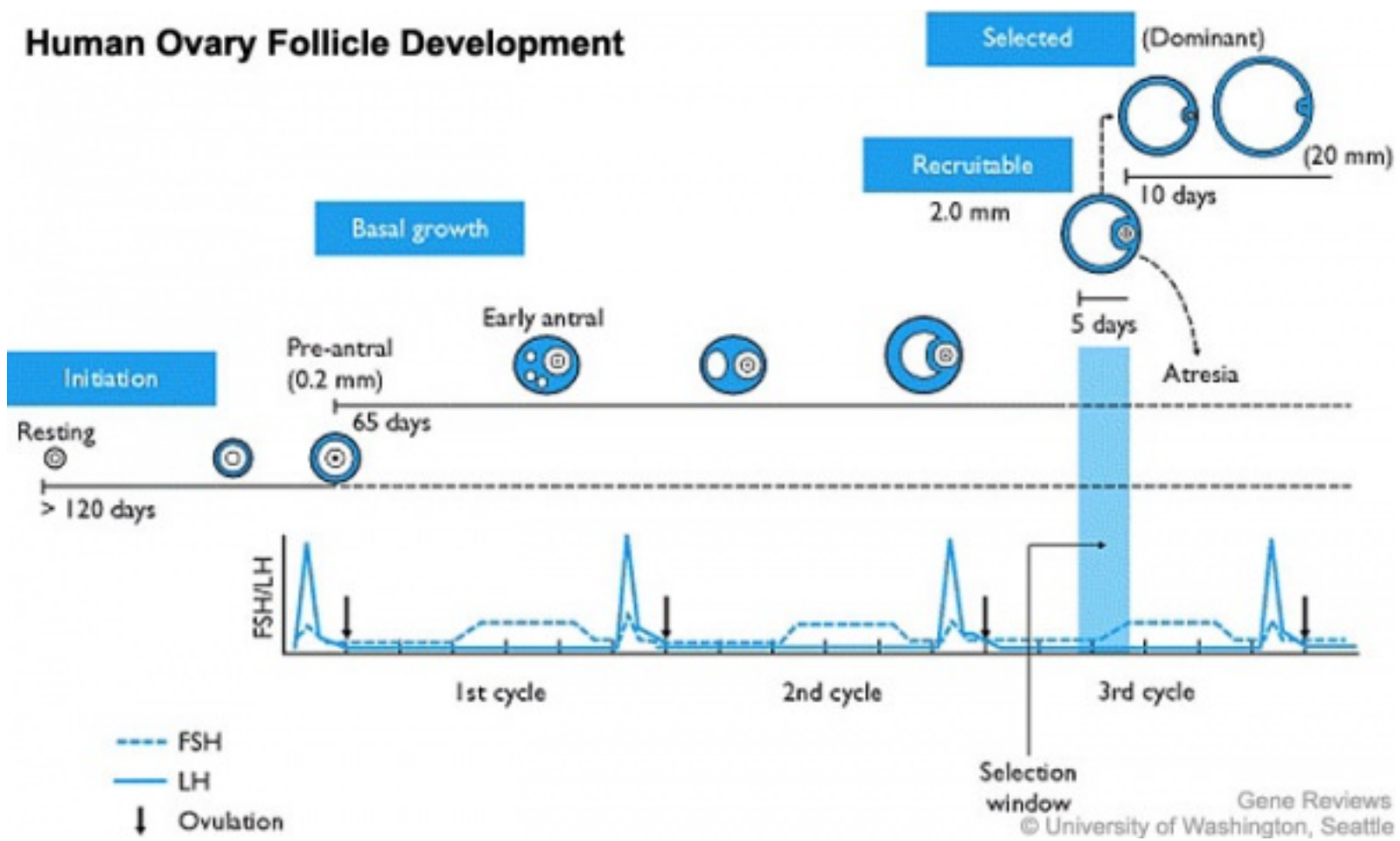
The ovaries have two functions.

1. produce the female gametes or **oocytes**
2. produce female hormones, estrogen and progesterone (secondary sex characteristics, menstrual cycle)



infant ovary overview of ovary three stages of follicle development primary follicle tertiary follicle

In an adult human female the development of a primordial follicle containing an oocyte to a preovulatory follicle takes in excess of **120 days**.



Human Ovulation

Human Follicle Development

Human ovary follicle development

Ovarian Follicle Stages: primordial follicle - primary follicle - secondary follicle - tertiary follicle - preovulatory follicle

Follicle cells (support cells) **Theca cells** (produce hormone)

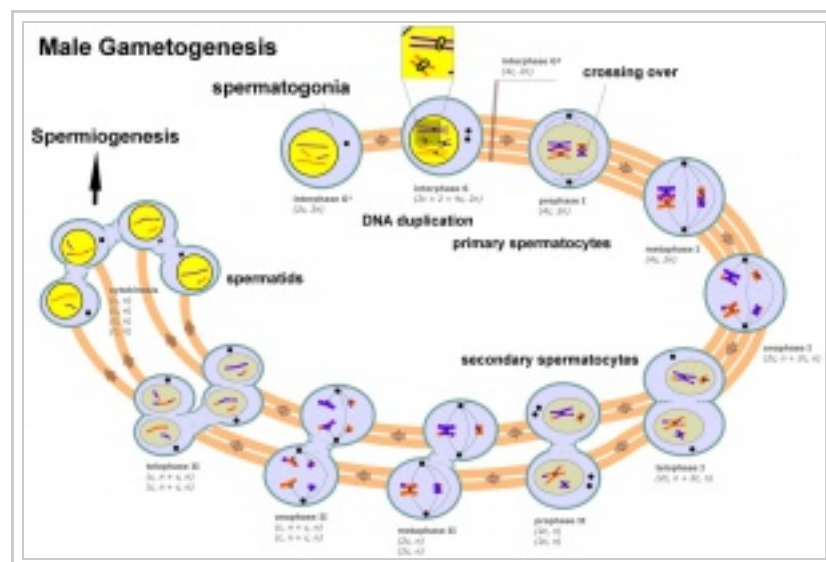
[Ovulation Movie\[Expand\]](#)

Meiosis Differences

Male Meiosis

- Meiosis initiated continuously in a mitotically dividing stem cell population
- 4 gametes produced / meiosis
- Meiosis completed in days or weeks
- Meiosis and differentiation proceed continuously without cell cycle arrest
- Differentiation of gamete occurs while haploid after meiosis ends
- Sex chromosomes excluded from recombination and transcription during first meiotic prophase

MBoC - Figure 20-27. The stages of spermatogenesis
<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mboc4.figgrp.3734>



Male gametogenesis

Female Meiosis

- Meiosis initiated once in a finite population of cells
- 1 gamete produced / meiosis
- Completion of meiosis delayed for months or years

- Meiosis arrested at 1st meiotic prophase and reinitiated in a smaller population of cells
- Differentiation of gamete occurs while diploid in first meiotic prophase
- All chromosomes exhibit equivalent transcription and recombination during meiotic prophase

The Cell - Figure 14.37. Meiosis of vertebrate oocytes
(<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=cooper.figgrp.2492>)

Polar Bodies

- In female gametogenesis only a single (1) haploid egg is produced from meiosis. In male gametogenesis four (4) haploid sperm are produced from meiosis. So what happens to all the extra DNA in producing this single egg?
 - In Meiosis 1 the "extra" DNA is excluded to the periphery as a 1st polar body, which encloses the extra DNA.
 - In Meiosis 2 the "extra" DNA is once again excluded as a 2nd polar body. The first polar body may also undergo meiosis 2 producing a 3rd polar body.
- These polar bodies are not gametes.
- Polar bodies appear to have no other function other than to dispose of the extra DNA in oogenesis.
 - Recent research in mice suggest that the position of oocyte polar body may influence fertilization site.

[Meiosis Polar Body Movie\[Expand\]](#)

Fertilization

Gamete formation, menstrual cycle and fertilisation will also be covered in detail in this week's Laboratory. Fertilization is the complete process resulting in the fusion of haploid gametes, egg and sperm, to form the diploid zygote. The recent development of aided fertilization is described as in vitro fertilization (in vitro = "in glass", outside the body, IVF). Clinically, all these aided fertilization techniques are grouped as Assisted Reproductive Technologies or ART.

- Oogenesis - 1 gamete produced/meiosis + 3 polar bodies, meiosis is slow, 1 egg produced and released at ovulation
- Spermatogenesis - 4 gametes produced/meiosis, meiosis is fast, 200-600 million sperm released at ejaculation

[Fertilization Movies\[Expand\]](#)

Fertilization Site

- Fertilization resulting in embryo development usually occurs in first 1/3 of uterine tube (oviduct, Fallopian tube)
- The majority of fertilized oocytes do not go on to form an embryo
- Fertilization can also occur outside uterine tube associated with Assisted Reproductive Technologies (IVF, GIFT, ZIFT...) and ectopic pregnancy
- Oocyte ovulation - release from the ovary with associated cells, into peritoneal cavity, uterine tube fimbria then into uterine tube (oviduct, uterine horn, fallopian tube) and epithelial cilia mediated movement.
- Spermatozoa ejaculation - deposited in vagina, movement of tail to "swim" in uterine secretions through cervix, uterine body and into uterine tube, have approximately 24-48h to fertilize oocyte.

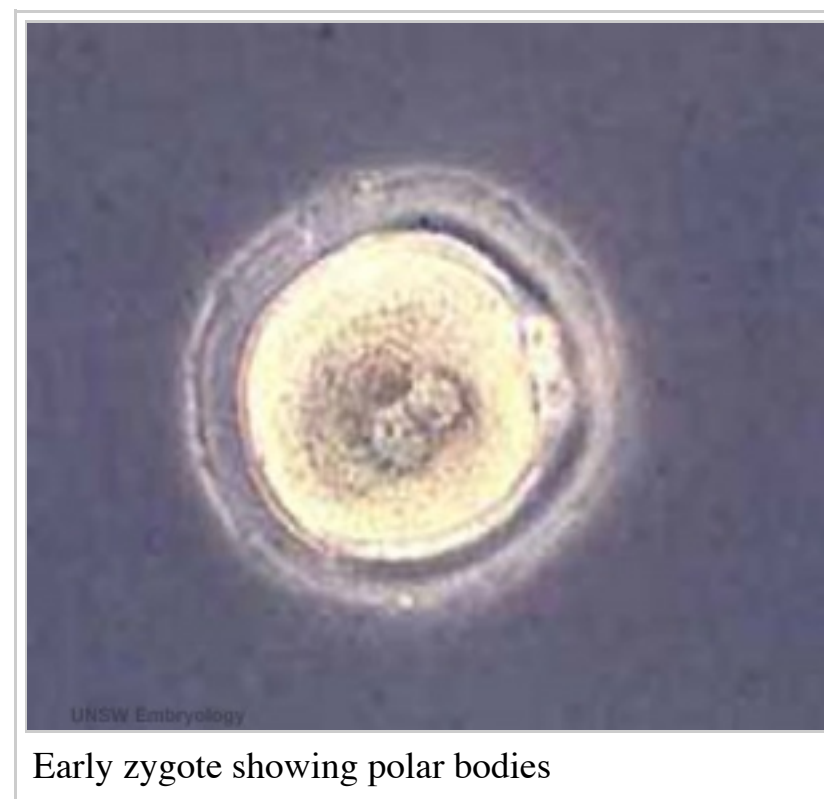
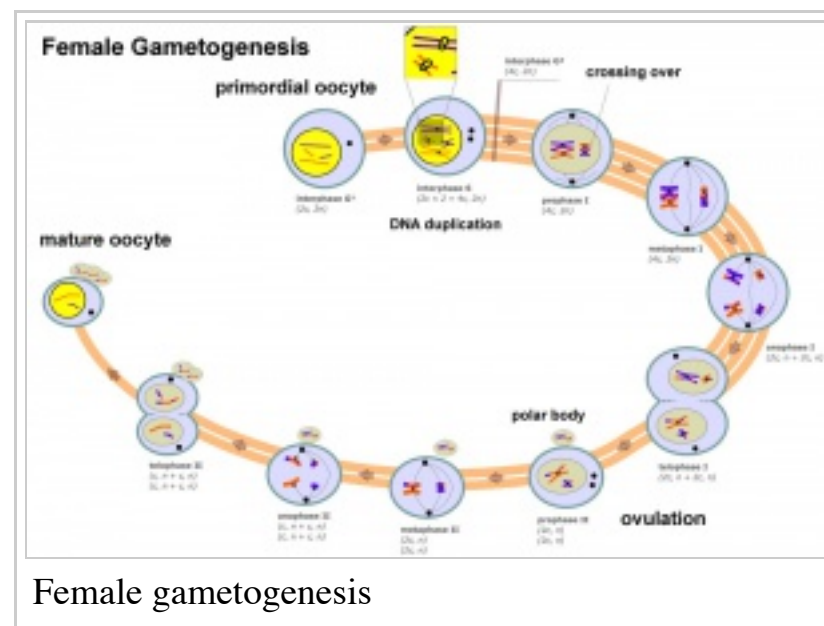
Prior to the fertilization process commencing both the gametes complete a number of biological processes.

- Oocyte Meiosis** - completes Meiosis 1 and commences Meiosis 2 (arrests at Metaphase II).
- Spermatozoa Capacitation** - following release (ejaculation) and mixing with other glandular secretions, activates motility and acrosome preparation.
- Migration** - both oocyte and spermatozoa.

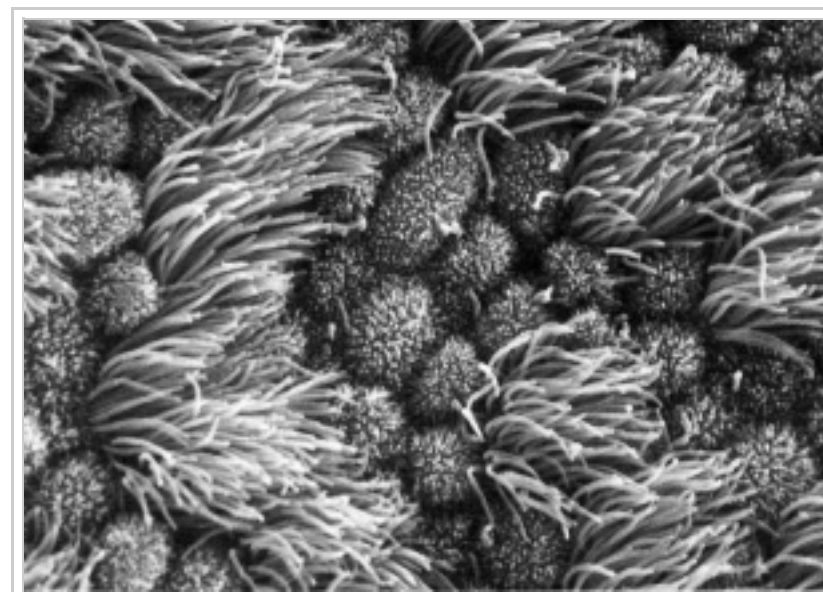
Endocrinology - Diagram of the comparative anatomy of the male and female reproductive tracts
(<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=endocrin&part=A972&rendertype=box&id=A1230>)

[Gamete Movement Movies\[Expand\]](#)

Fertilization - Male



Early zygote showing polar bodies



Human uterine tube ciliated epithelium

Spermatozoa: Ejaculation - Capacitation - Spermatozoa motility - Chemotaxis - Binding to zona pellucida - Acrosome reaction - Membrane fusion

Ejaculation

- about 3.5 ml, containing 200 - 600 million spermatozoa
- by volume less than 10 % spermatozoa
- accessory glands contribute majority of volume (60 % seminal vesicle, 10 % bulbourethral, 30 % prostate)

Male Infertility

- Oligospermia (Low Sperm Count) - less than 20 million sperm after 72 hour abstinence from sex
- Azoospermia (Absent Sperm) - blockage of duct network
- Immotile Cilia Syndrome - lack of sperm motility

Capacitation

- spermatozoa activation process - removal of glycoprotein coat and seminal proteins and alteration of sperm mitochondria

Spermatozoa motility

- tail of spermatozoa provide movement by microtubules
- energy for this movement is provided by mitochondria in tail initial segment

Chemotaxis

- oocyte cumulus cells release **progesterone** (may also be other oocyte and follicular fluid factors)

Spermatozoa Binding

- Zona pellucida protein ZP2 acts as receptor for spermatozoa binding (species specific)

Acrosome Reaction

- exocytosis of acrosome contents (calcium mediated) MBoC - Figure 20-31. The acrosome reaction that occurs when a mammalian sperm fertilizes an egg (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.3741>)
- enzymes to digest the zona pellucida
- exposes sperm surface proteins to bind ZP2

Membrane fusion

- between spermatozoa and oocyte cell membranes, allows sperm nuclei passage into egg cytoplasm
- membrane fusion also initiates oocyte processes to block polyspermy

Fertilization - Oocyte

Oocyte: Membrane depolarization - Cortical reaction - Meiosis 2 completion

Membrane Depolarization

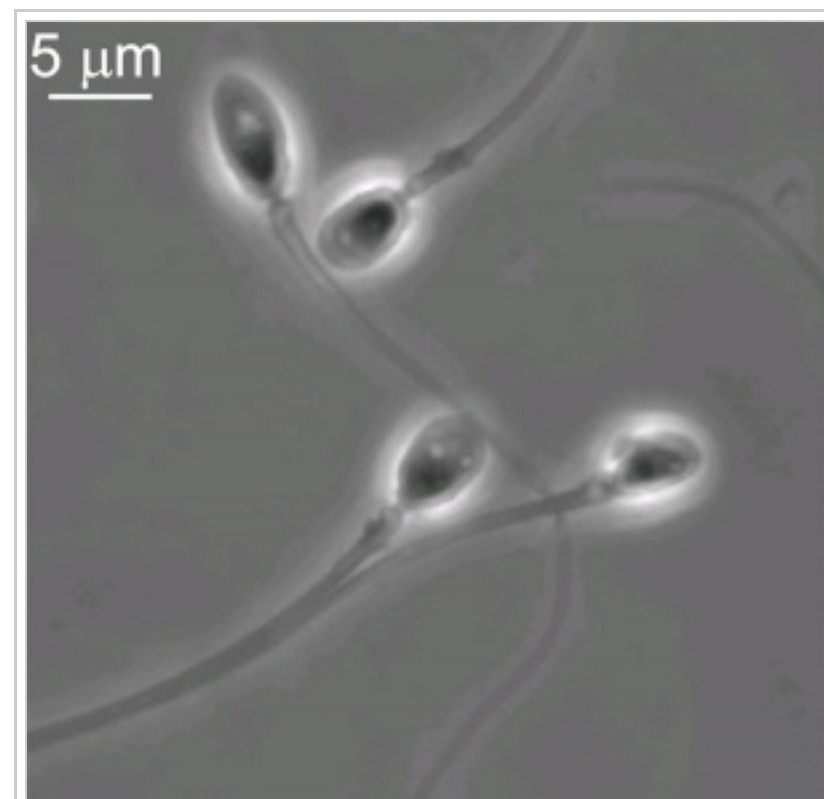
- caused by spermatozoa membrane fusion, acts as primary block to polyspermy (fertilisation by more than one spermatozoa)

Cortical Reaction

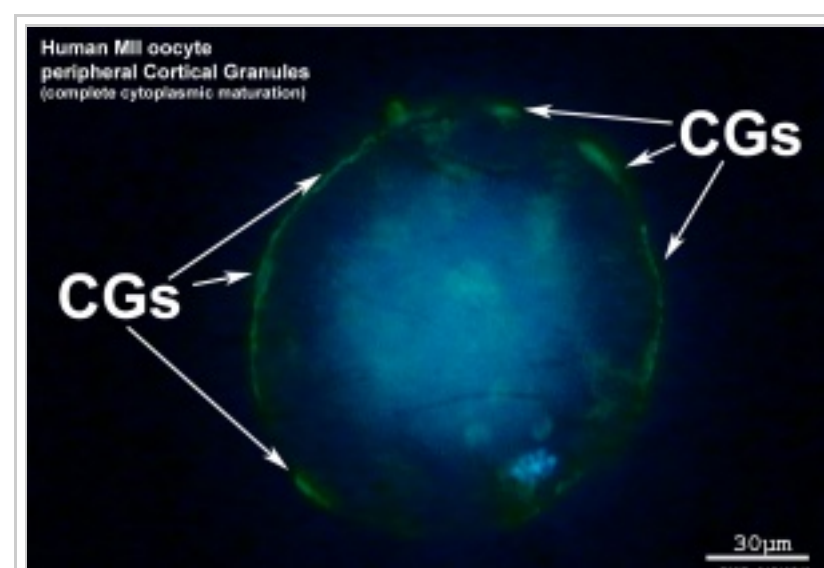
- Inositol triphosphate (IP3) pathway elevates intracellular calcium, exocytosis of cortical granules
- enzyme alters ZP2 so it will no longer bind sperm plasma membrane
- MBoC - Figure 20-32. How the cortical reaction in a mouse egg is thought to prevent additional sperm from entering the egg (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.3743>)

Meiosis 2

- completion of 2nd meiotic division
- forms second polar body (third polar body may be formed by meiotic division of the first polar body)



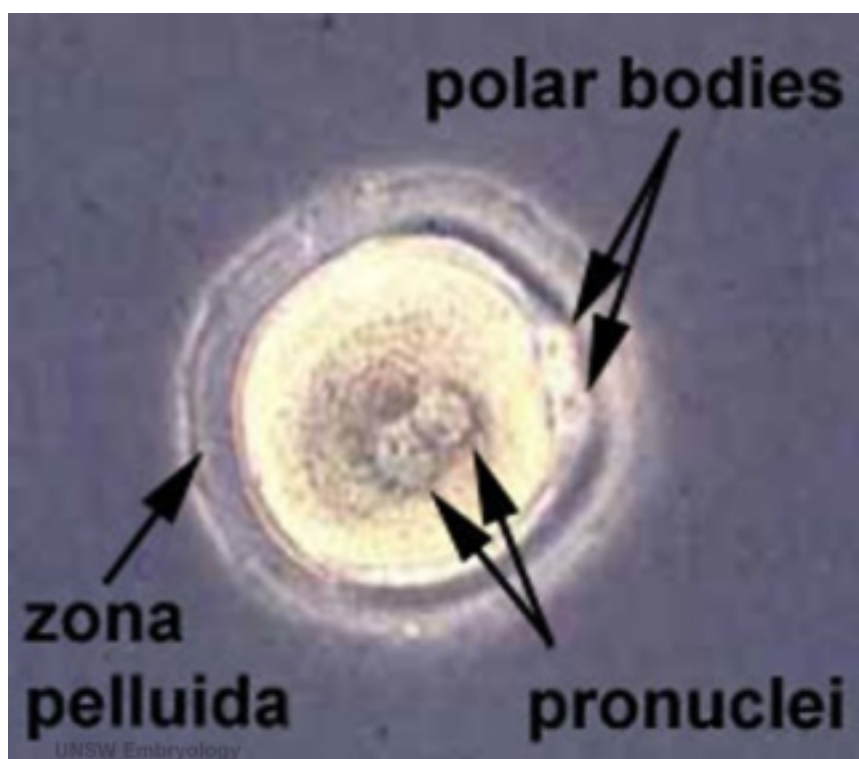
Human spermatozoa (light microscope)



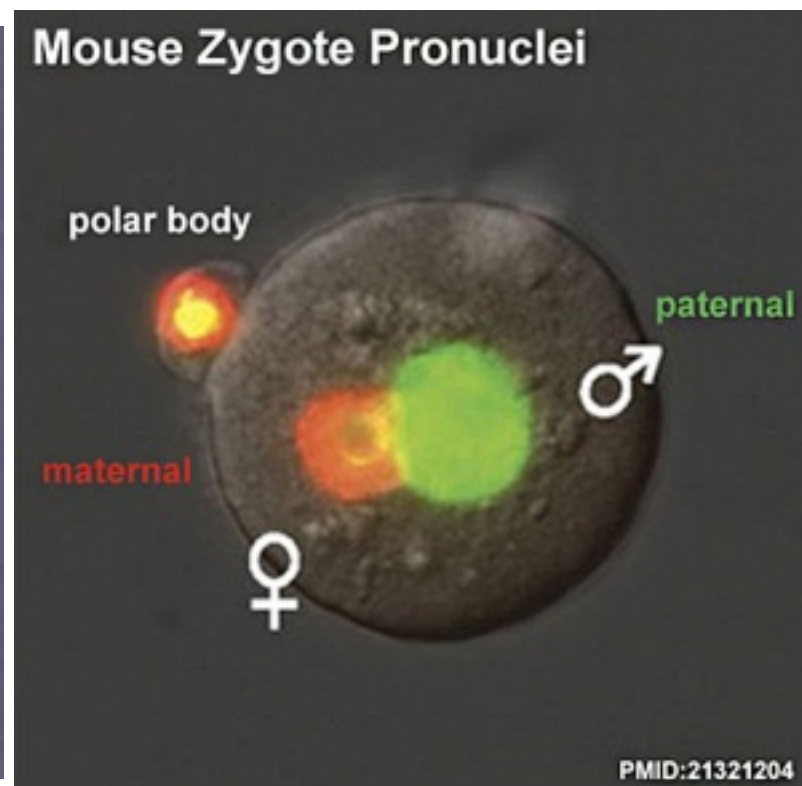
Human MII oocyte cortical granules

Formation of the Zygote

Early Zygotes



Human Zygote



Mouse Zygote

- Pronuclei - Male and Female haploid nuclei approach each other and nuclear membranes break down
- chromosomal pairing, DNA replicates, first mitotic division
- Sperm contributes - centriole which organizes mitotic spindle
- Oocyte contributes - mitochondria (maternally inherited)

Sex Determination

- based upon whether an X or Y carrying sperm has fertilized the egg, should be 1.0 sex ratio.
- actually 1.05, 105 males for every 100 females, some studies show more males 2+ days after ovulation.
- cell totipotent (equivalent to a stem cell, can form any tissue of the body)

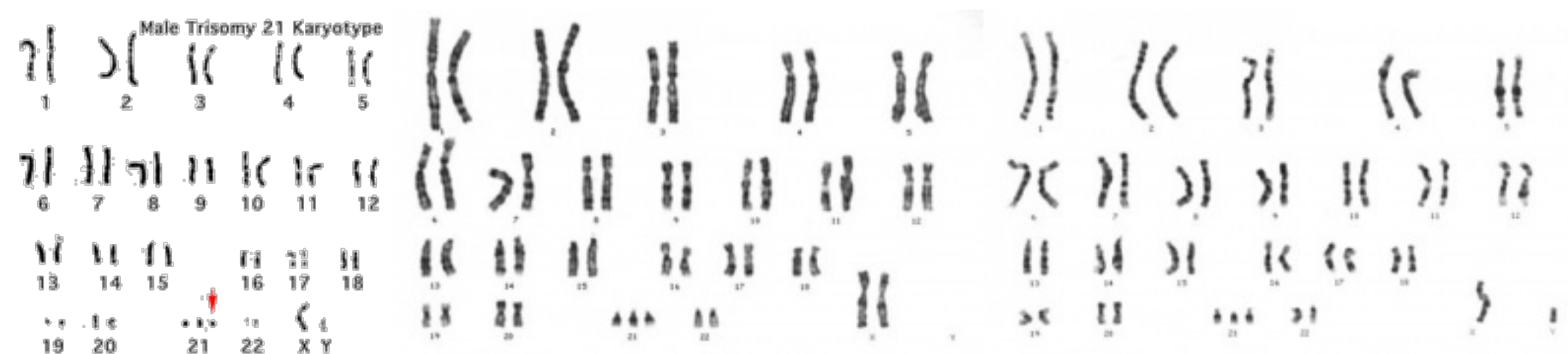
Men - Y Chromosome

- Y Chromosome carries Sry gene, protein product activates pathway for male gonad (covered in genital development)

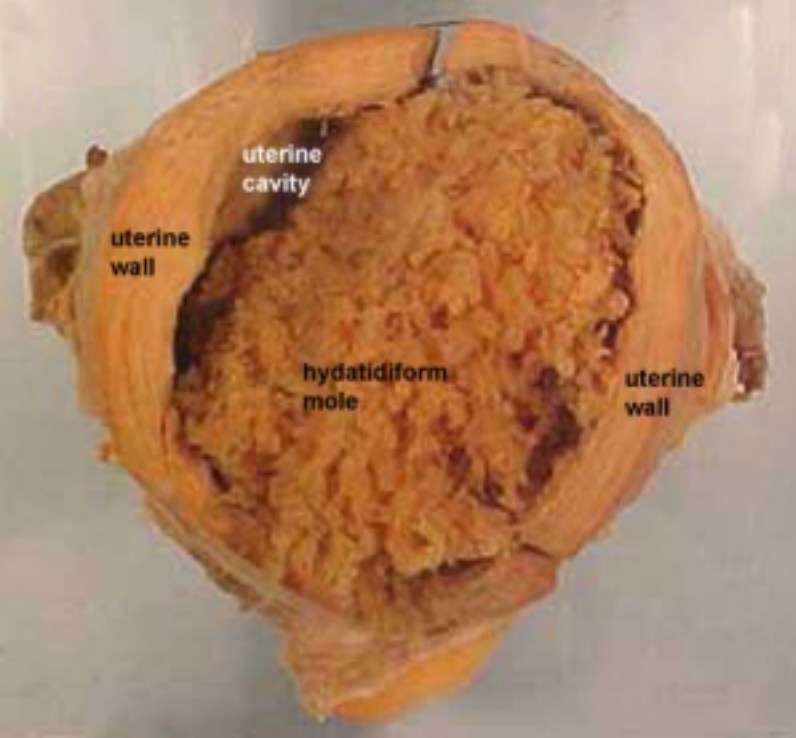
Women - X Chromosome

- Gene dosage, one X chromosome in each female embryo cell has to be inactivated
- process is apparently random and therefore 50% of cells have father's X, 50% have mother's X
- Note that because men only have 1 X chromosome, if abnormal, this leads to X-linked diseases more common in male than female where both X's need to be abnormal.

Abnormalities



- The most common chromosome abnormality is **aneuploidy**, the gain or loss of whole chromosomes.
- Caused by **meiotic nondisjunction**, the failure of chromosomes to correctly separate homologues during meiosis I or sister chromatids during meiosis II.
- Down Syndrome - caused by an extra copy of chromosome 21. Abnormal Development - Trisomy 21 (Down Syndrome) Maternal Age
- **Chromosomal translocations** occur when there is an inappropriate exchange of chromosomal material. Philadelphia chromosome (<http://visualsonline.cancer.gov/retrieve.cfm?imageid=7153&dpi=72&fileformat=jpg>)
- Philadelphia chromosome - piece of Chr9 exchanged with Chr22 Generates truncated abl, overstimulates cell production, leads to chronic myelogenous leukemia



Hydatidiform Mole

- **Complete Mole** - Only paternal chromosomes.
- **Partial Mole** - 3 sets of chromosomes ((triploidy) instead of the usual 2.

Next

- Lab 1 - Gametogenesis and Fertilisation

Homework

Beginning your online work - Working Online in this course

1. Make your own page.
 1. **Log-in** to the embryology website using your student ID and Zpass.
 2. Click your **student number** (shown in red at the top right of the screen following log-in)
 3. **Create** page using the tab at the top of the page, and save.
2. How would you identify your Type in a group and add to your page.
3. What was the most interesting thing you learnt in today's lecture?

If you have done the above correctly your ZID should be blue and not red on this page ANAT2341 2015 Students.

UNSW Embryology Links

- Spermatozoa Development Oocyte Development Fertilization Trisomy 21 (Down Syndrome)
- 2012 Lecture (http://embryology.med.unsw.edu.au/embryology/index.php?title=Lecture_-_Fertilization&oldid=96435)

Cell Division Links: Meiosis | Mitosis | **Lecture - Cell Division and Fertilization** | Spermatozoa Development | Oocyte Development | Fertilization | Zygote

References

Online Textbooks

- **Developmental Biology** by Gilbert, Scott F. Sunderland (MA): Sinauer Associates, Inc.; c2000 Figure 2.9. Summary of meiosis (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?highlight=meiosis&rid=dbio.figgrp.200>) | fusion of egg and sperm plasma membranes (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=dbio.figgrp.1360>)
- **Molecular Biology of the Cell** 4th ed. Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Raff, Martin; Roberts, Keith; Walter, Peter New York and London: Garland Science; c2002 - IV. Internal Organization of the Cell Chapter 17. The Cell Cycle and Programmed Cell Death Programmed Cell Death (<http://www.ncbi.nlm.nih.gov:80/books/bv.fcgi?db=Books&rid=mboc4.chapter.3167>) | An Overview of the Cell Cycle (<http://www.ncbi.nlm.nih.gov:80/books/bv.fcgi?db=Books&rid=mboc4.section.3169>) | Figure 17-1. The cell cycle (<http://www.ncbi.nlm.nih.gov:80/books/bv.fcgi?db=Books&rid=mboc4.figgrp.3168>) | Fertilization (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mboc4.section.3738>)
- **Molecular Cell Biology** by Lodish, Harvey; Berk, Arnold; Zipursky, S. Lawrence; Matsudaira, Paul; Baltimore, David; Darnell, James E. New York: W. H. Freeman & Co.; c1999 Chapter 13. Regulation of the Eukaryotic Cell Cycle Regulation of the Eukaryotic Cell

Cycle (<http://www.ncbi.nlm.nih.gov:80/books/bv.fcgi?db=Books&rid=mcb.chapter.3432>) | Overview of the Cell Cycle and Its Control (<http://www.ncbi.nlm.nih.gov:80/books/bv.fcgi?db=Books&rid=mcb.section.3463>) | Figure 13-2. Current model for regulation of the eukaryotic cell cycle (<http://www.ncbi.nlm.nih.gov:80/books/bv.fcgi?db=Books&rid=mcb.figgrp.3467>) | **Movies** Proposed alternative mechanisms for chromosome congression. (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mcb.figgrp.5522>) | Centromeric attachment of microtubules. (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mcb.figgrp.5509>) | The stages of mitosis and cytokinesis in an animal cell. (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mcb.figgrp.5500>)

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Reviews

Dennis W Stacey, Masahiro Hitomi **Cell cycle studies based upon quantitative image analysis**. *Cytometry A*: 2008, 73(4);270-8 [PubMed:18163464]

Christoph Schorl, John M Sedivy **Analysis of cell cycle phases and progression in cultured mammalian cells**. *Methods*: 2007, 41(2);143-50 [PubMed:17189856]

Terms

- **asthenozoospermia** - (asthenospermia) Term for reduced sperm motility and can be the cause of male infertility.
- **blood-testis barrier** - (BTB) Formed by tight junctions, basal ectoplasmic specializations, desmosome-like junctions and gap junctions between adjacent Sertoli cells near the basement membrane of the seminiferous epithelium.
- **diploid** - (Greek, *di* = double + *ploion* = vessel) Having two sets of chromosomes, the normal state for all cells other than the gametes.
- **haploid** - (Greek, *haploos* = single) Having a single set of chromosomes as in mature germ/sex cells (oocyte, spermatozoa) following reductive cell division by meiosis. Normally cells are diploid, containing 2 sets of chromosomes.
- **Leydig cell** - (interstitial cell) Male gonad (testis) cell which secrete the androgen testosterone, beginning in the fetus. These cells are named after Franz von Leydig (1821 - 1908) a German scientist who histologically described these cells.
- **meiosis** - The cell division that occurs only in production of germ cells where there is a reduction in the number of chromosomes (diploid to haploid) which is the basis of sexual reproduction. All other non-germ cells in the body divide by mitosis.
- **mitosis** - The normal division of all cells, except germ cells, where chromosome number is maintained (diploid). In germ cell division (oocyte, spermatozoa) meiosis is a modified form of this division resulting in reduction in genetic content (haploid). Mitosis, division of the nucleus, is followed by cytokinesis the division of the cell cytoplasm and the cytoplasmic contents. cytokinesis overlaps with telophase.
- **sperm annulus** - (Jensen's ring; Latin, *annulus* = ring) A region of the mammalian sperm flagellum connecting the midpiece and the principal piece. The annulus is a septin-based structure formed from SEPT1, 4, 6, 7 and 12. Septins are polymerizing GTPases that can act as a scaffold forming hetero-oligomeric filaments required for cytokinesis and other cell cycle roles.
- **spermatogenesis** - (Greek, *genesis* = origin, creation, generation) The term used to describe the process of diploid spermatogonia division and differentiation to form haploid spermatazoa within the testis (male gonad). The process includes the following cellular changes: meiosis, reorganization of DNA, reduction in DNA content, reorganization of cellular organelles, morphological changes (cell shape). The final process of change in cell shape is also called spermiogenesis.
- **spermatogenesis** - (Greek, *genesis* = origin, creation, generation) The maturation process of the already haploid spermatazoa into the mature sperm shape and organization. This process involves reorganization of cellular organelles (endoplasmic reticulum, golgi apparatus, mitochondria), cytoskeletal changes (microtubule organization) and morphological changes (cell shape, acrosome and tail formation).

- **spermatogonia** - The cells located in the seminiferous tubule adjacent to the basal membrane that either divide and separate to renew the stem cell population, or they divide and stay together as a pair (Apr spermatogonia) connected by an intercellular cytoplasmic bridge to differentiate and eventually form spermatazoa.
- **spermatozoa head** - Following spermiogenesis, the first region of the spermatozoa containing the haploid nucleus and acrosome. In humans, it is a flattened structure (5 μm long by 3 μm wide) with the posterior part of nuclear membrane forming the basal plate region. The human spermatozoa is about 60 μm long, actively motile and divided into 3 main regions (head, neck and tail).
- **spermatozoa neck** - Following spermiogenesis, the second region of the spermatozoa attached to basal plate, transverse oriented centriole, contains nine segmented columns of fibrous material, continue as outer dense fibres in tail. In humans, it forms a short structure (1 μm). The human spermatozoa is about 60 μm long, actively motile and divided into 3 main regions (head, neck and tail).
- **spermatozoa tail** - Following spermiogenesis, the third region of the spermatozoa that has a (head, neck and tail). The tail is also divided into 3 structural regions a middle piece, a principal piece and an end piece. In humans: the middle piece (5 μm long) is formed by axonema and dense fibres surrounded by mitochondria; the principal piece (45 μm long) fibrous sheath interconnected by regularly spaced circumferential hoops; the final end piece (5 μm long) has an axonema surrounded by small amount of cytoplasm and plasma membrane.
- **spermatogonial stem cells** - (SSCs) The spermatogonia cells located beside the seminiferous tubule basal membrane that either divide and separate to renew the stem cell population, or they divide and stay together as a pair (Apr spermatogonia) connected by an intercellular cytoplasmic bridge to differentiate and eventually form spermatazoa.
- **sperm protein 56** - A component of the spermatozoa acrosomal matrix released to the sperm surface during capacitation.

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