

# Spermatogenesis and Fertilization

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# Disclosure

- No conflicts of interest to disclose
- No off-label use of medications discussed

# Learning Objectives

- Describe Spermatogenesis and Sertoli Cell Function
- List the Spermatogenesis Phases
- Describe the function and structure of epididymus

# Topics to be covered

- Spermatogenesis
- Sertoli Cell function
- Sperm motility and structure
- Function and structure of epididymus
- Semen and seminal plasma
- Fertilization
  - Capacitation, zona pellucida interaction, syngamy
- ICSI

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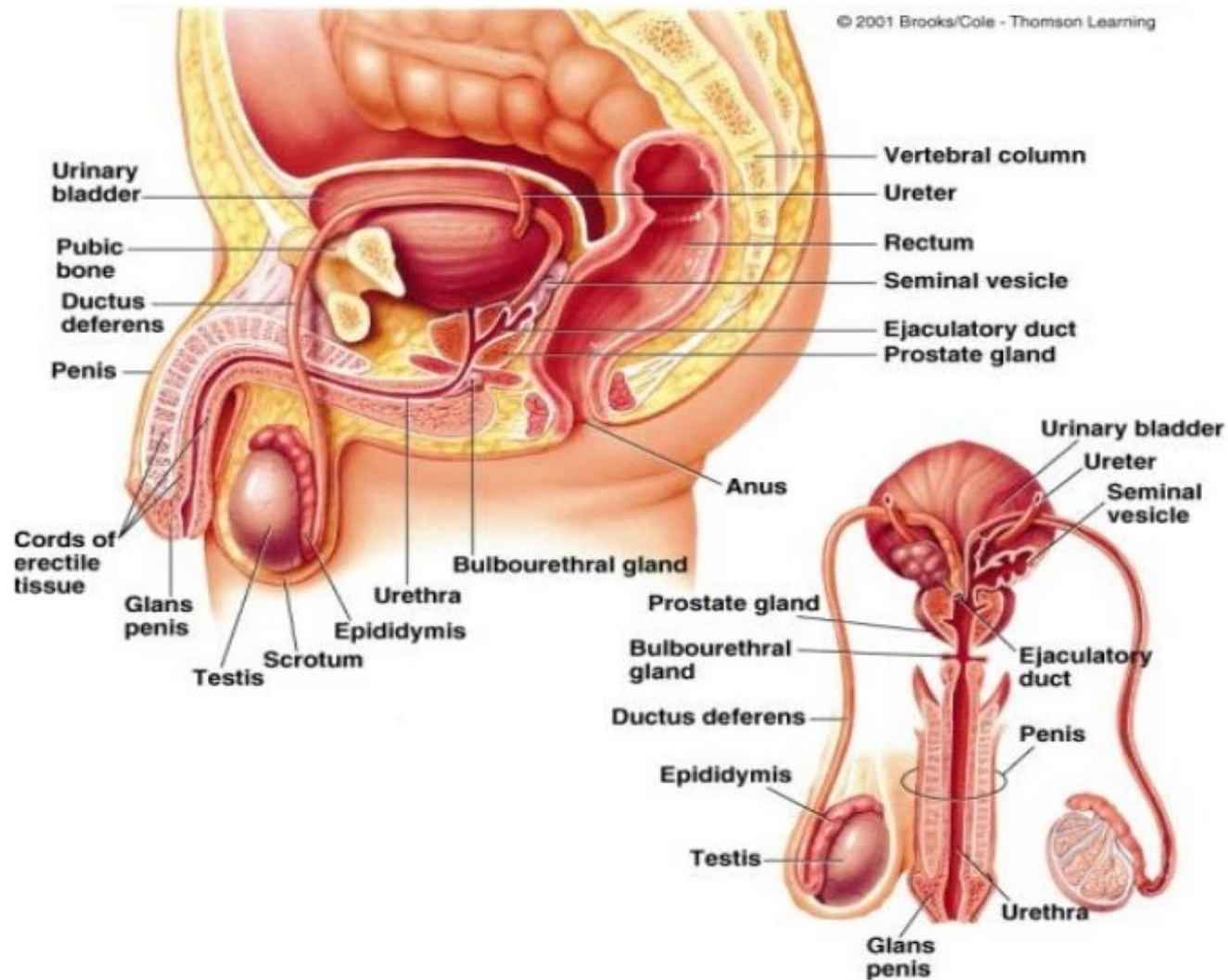
# Motivation

“You were born a winner, a warrior, one who defied the odds by surviving the most gruesome battle of them all - ***the race to the egg***. And now that you are a giant, why do you even doubt victory against smaller numbers and wider margins?”

— Suzy Kassem, *Rise Up and Salute the Sun*

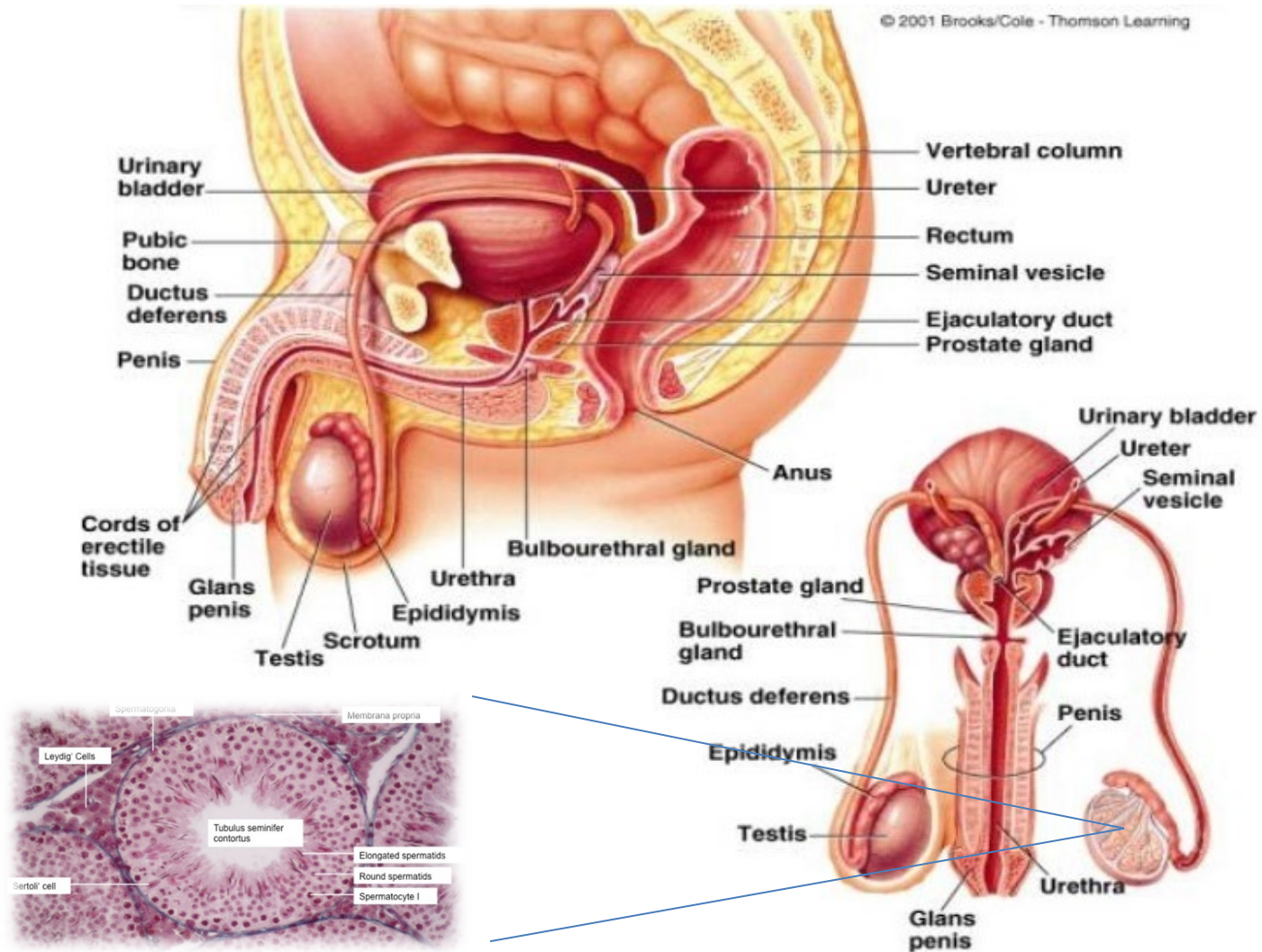
# **SPERMATOGENESIS**

# Spermatogenesis - Anatomy

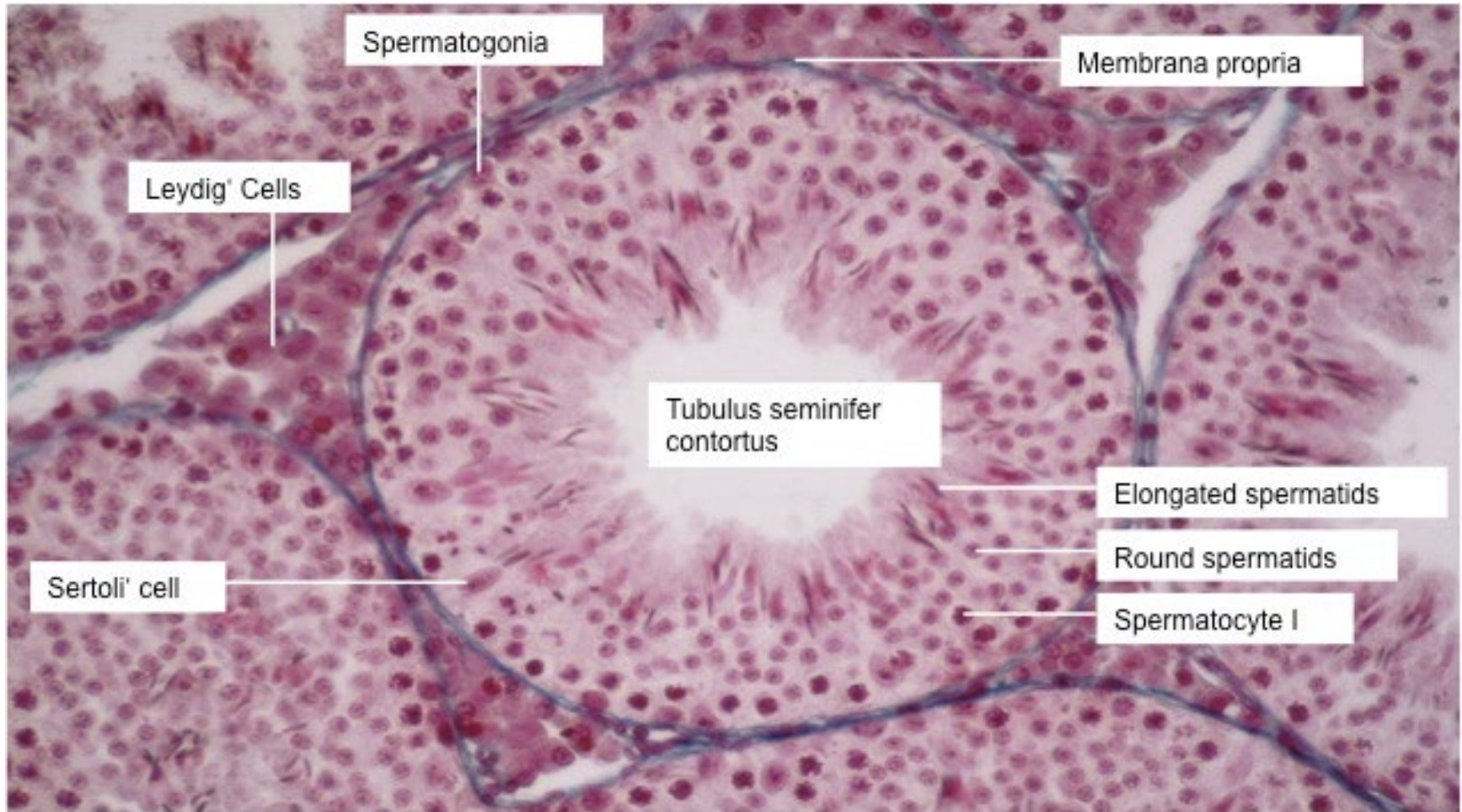




# Spermatogenesis - Anatomy



# Spermatogenesis - Anatomy



# Testes in Health & Reproduction

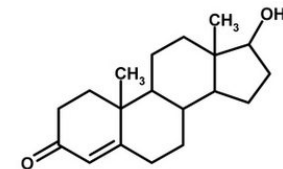
- Exocrine

- Spermatogenesis
- Involves the Sertoli cells and seminiferous epithelium

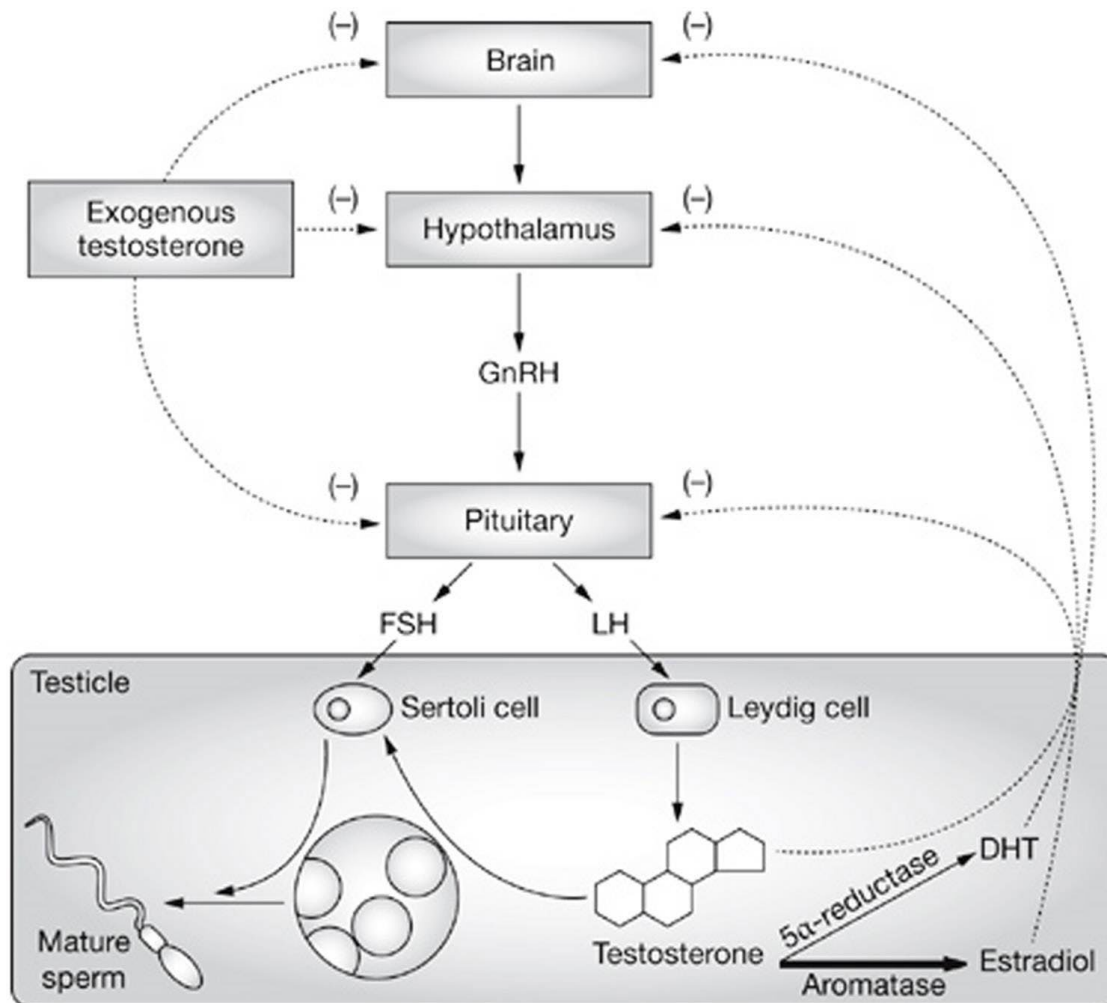


- Endocrine

- Steroidogenesis
- Involves Leydig cells and interstitial compartment



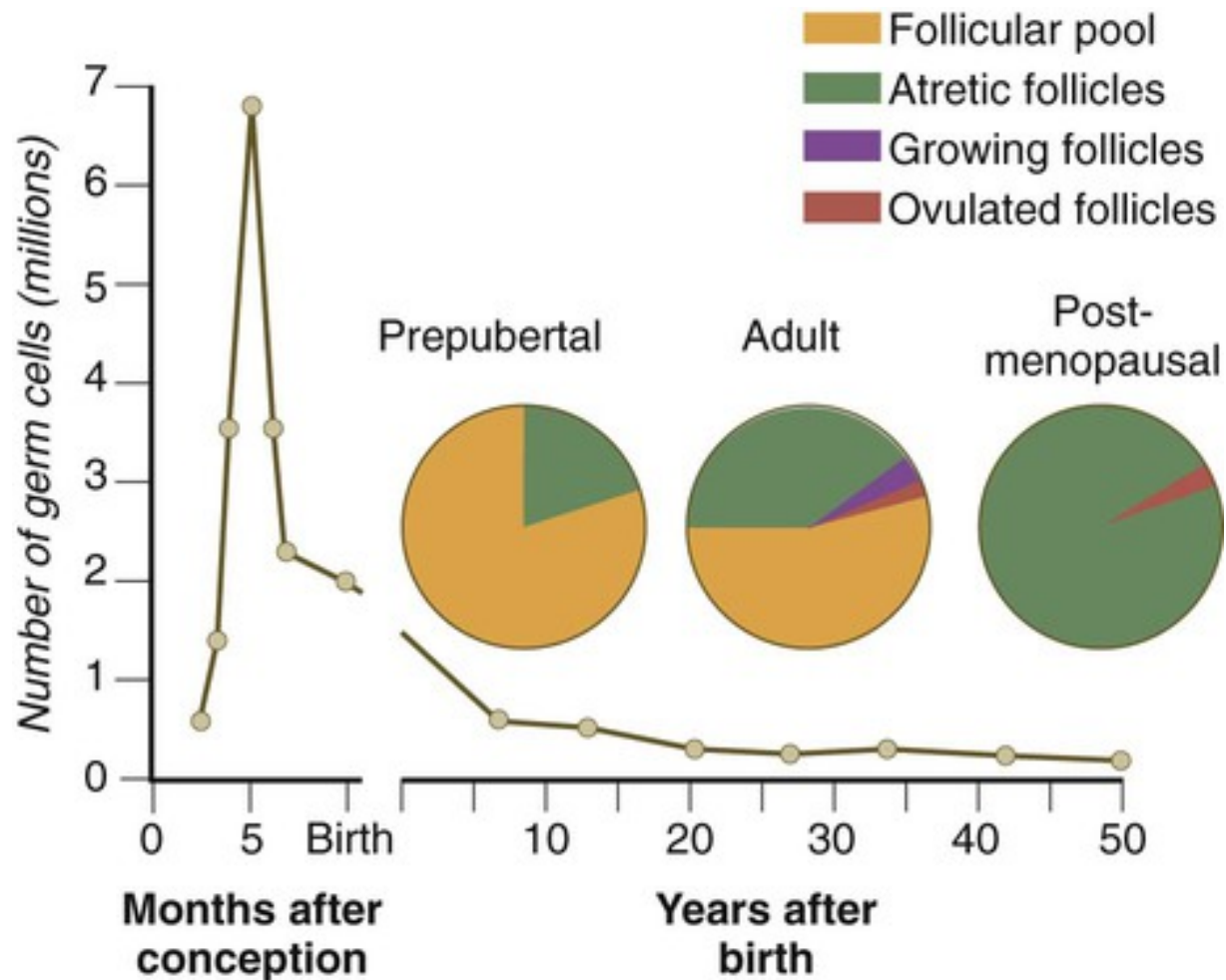
# Hormonal Production and Feedback



Testosterone  
Inhibin B



# Gametogenesis: The oocyte

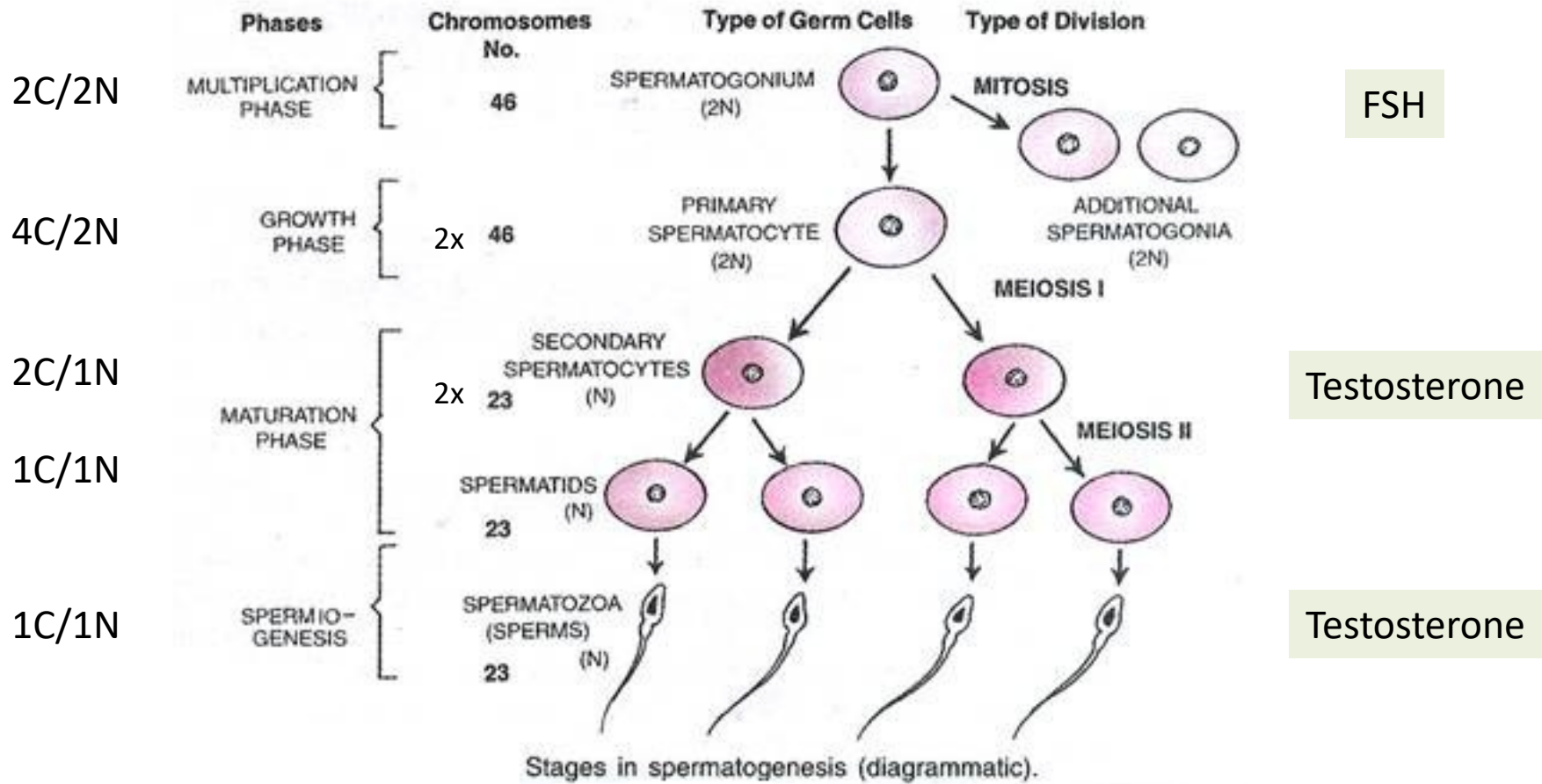


# Spermatogenesis

- In honor of Dr. Brooks Keel...
- The male
  - Produces 2 trillion sperm
  - Ejaculates 700 billion sperm
  - Ejaculates 7 liters of semen



# Spermatogenesis



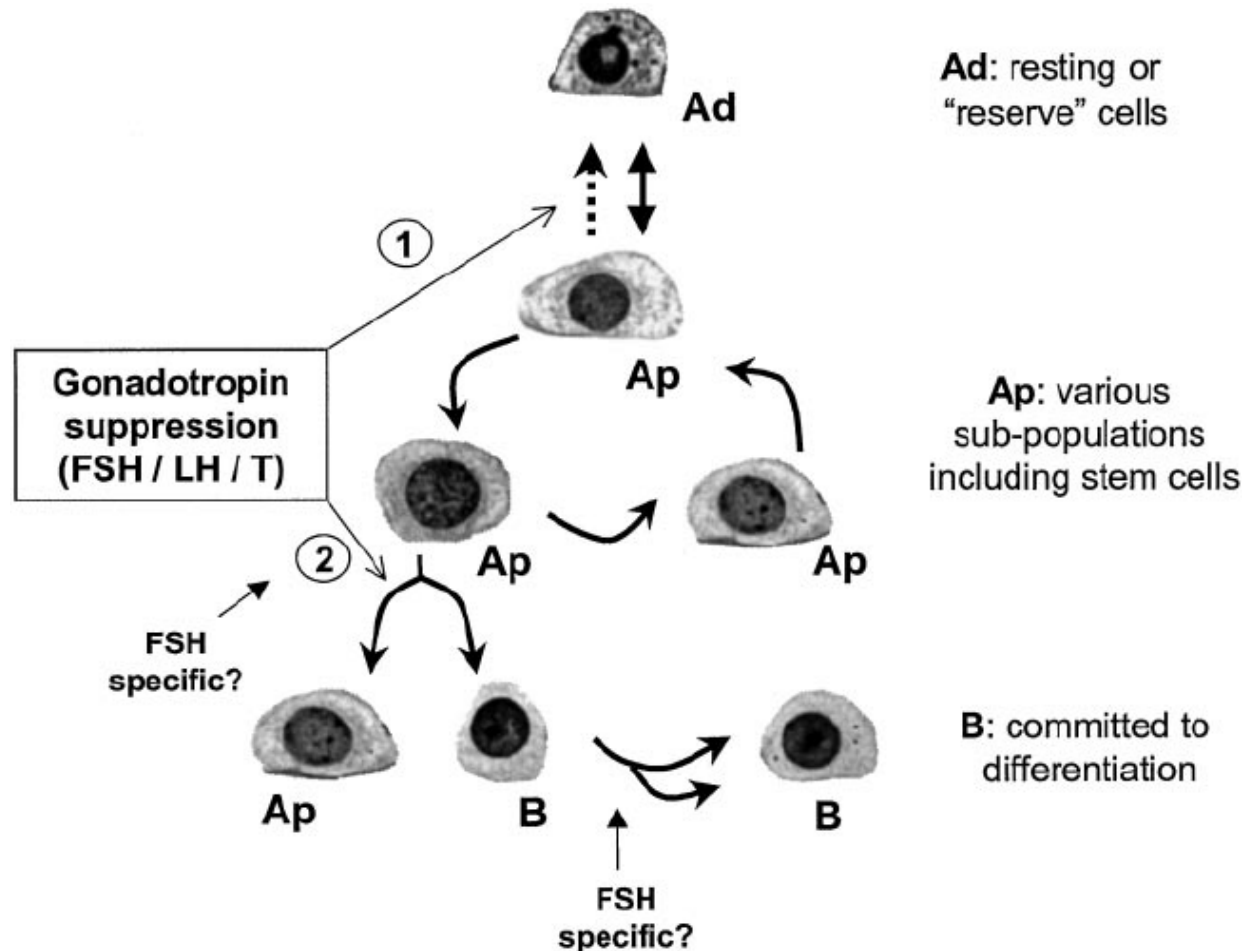
Goal: Know/understand the cells and phases

# Spermatogenesis Phases

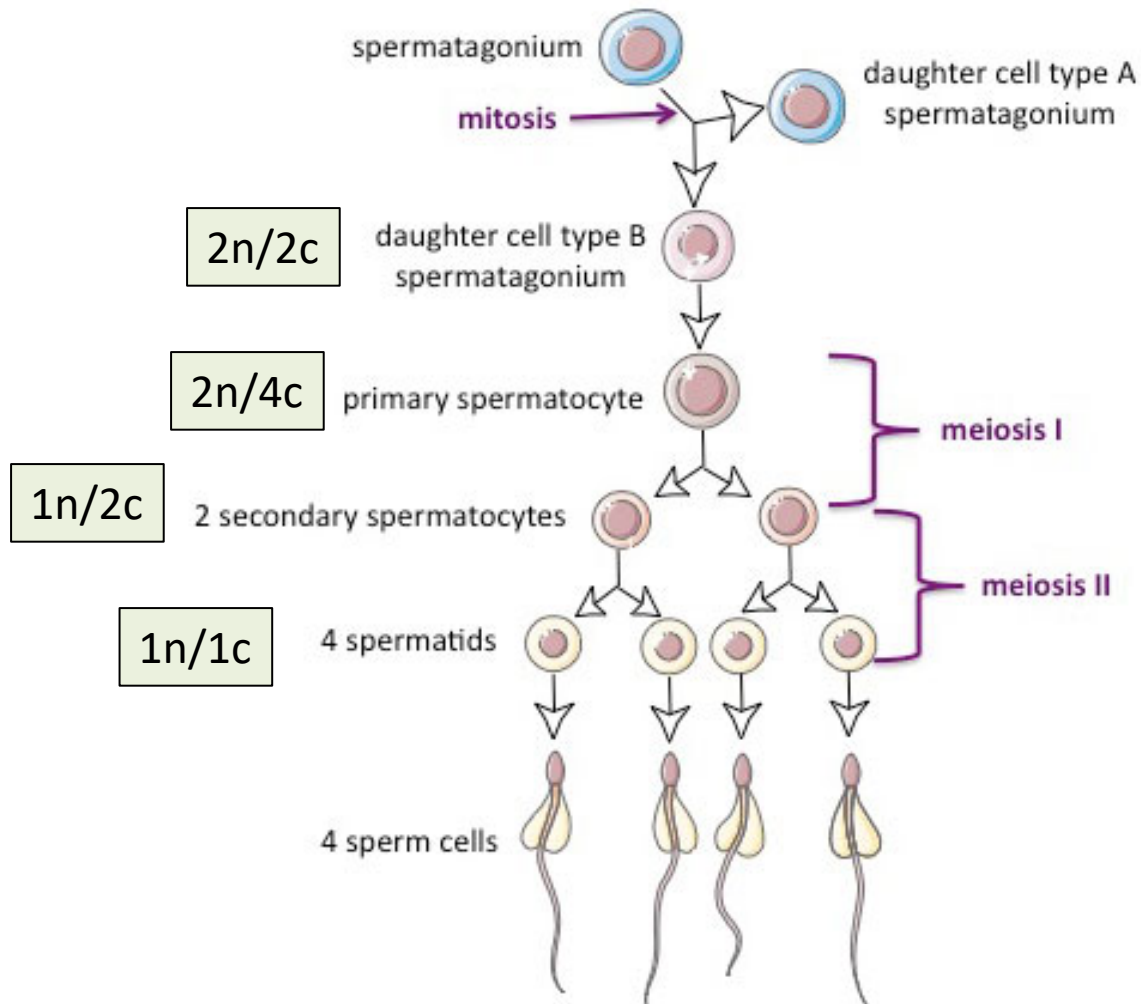
- Proliferation
  - Spermatogonia give rise to spermatocytes
  - Spermatogonia regenerate
  - FSH dependent process
- Meiotic Phase
  - Spermatocytes undergo maturation division
  - Reduce chromosome number by  $\frac{1}{2}$
  - Testosterone dependent process
- Spermiogenic Phase
  - Spermatids mature into spermatozoa
  - Testosterone dependent process



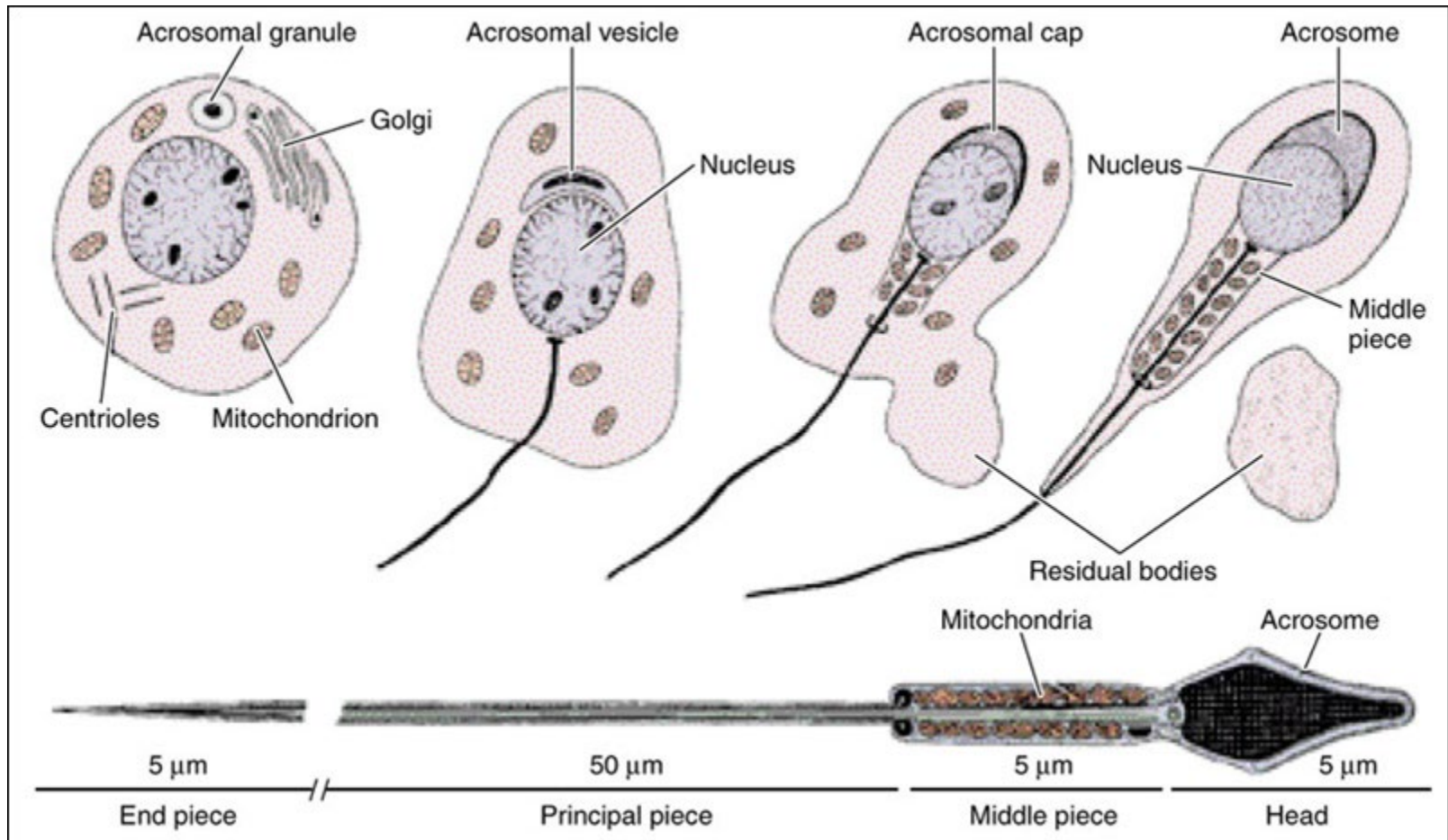
# Spermatogonial Proliferation Phase



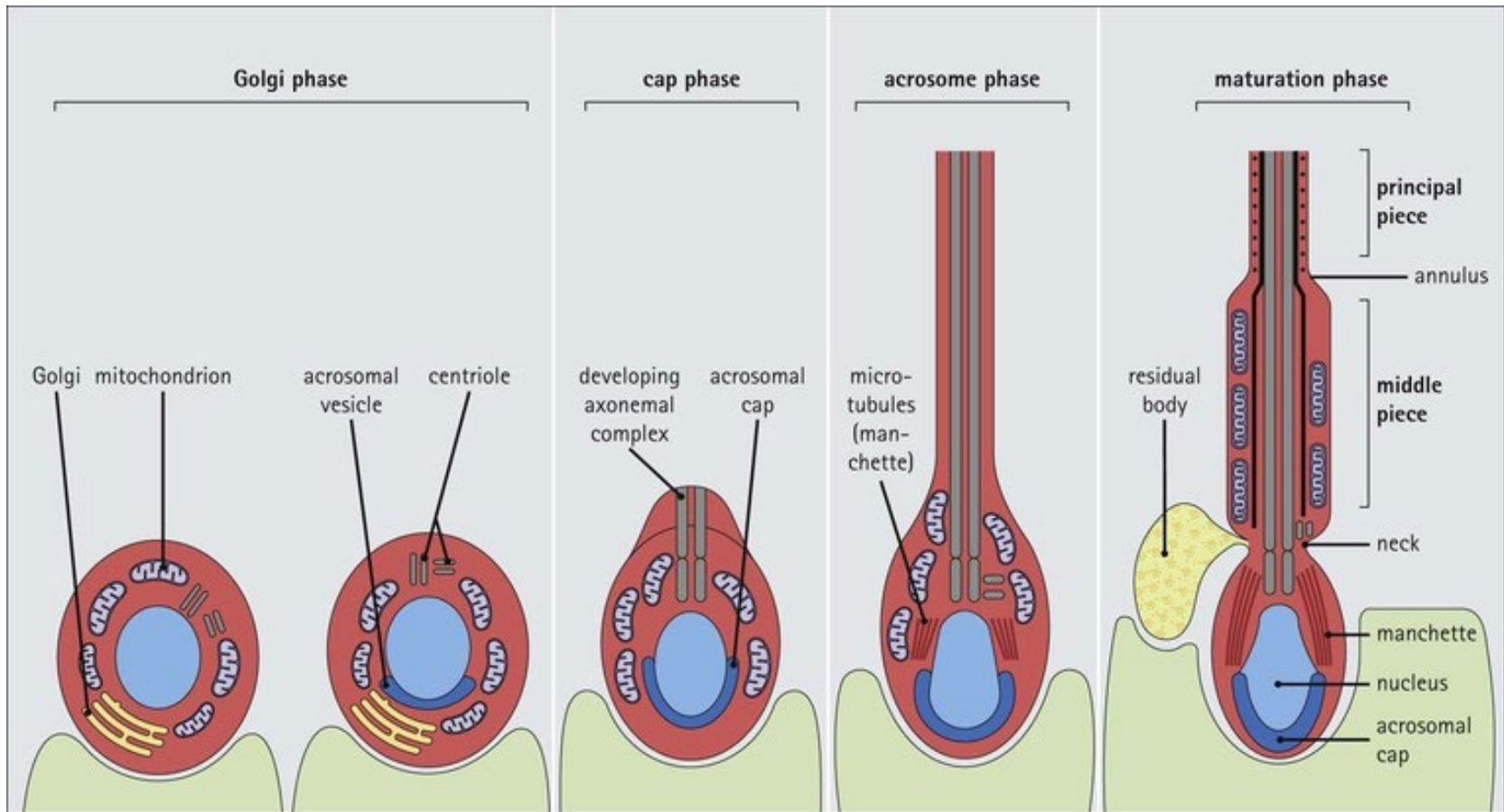
# Meiotic Phase



# Spermiogenesis Phase



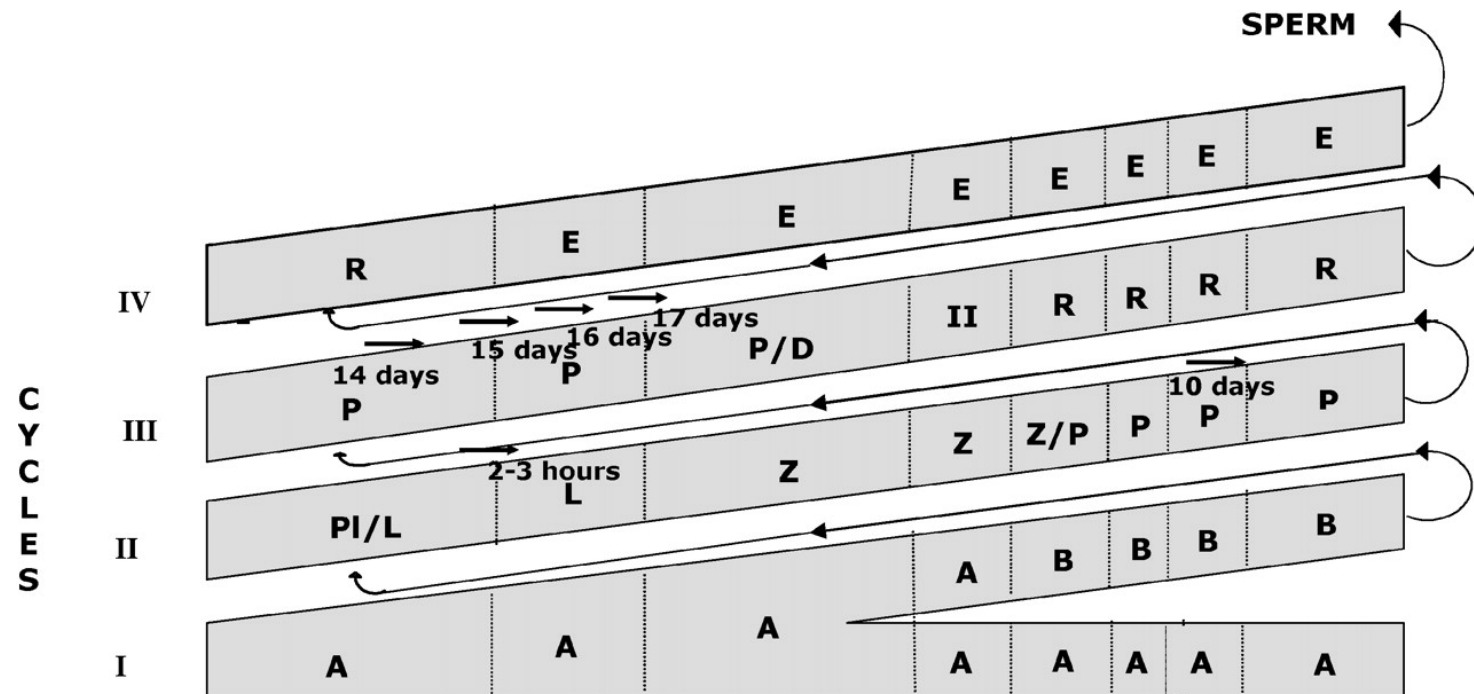
# Spermiogenesis Phase



- Golgi and acrosomal vesicle fusion
- Centriole migration

- Histone → protamine
- Nuclear condensation

# Summary: Seminiferous Epithelium



STAGES	1	2	3	4	5	6	7	8
FREQUENCY (%)	24.0	12.48	22.8	8.08	9.57	4.9	5.51	12.66
DURATION (dias)	3.68	1.92	3.52	1.23	1.47	.76	.84	1.94

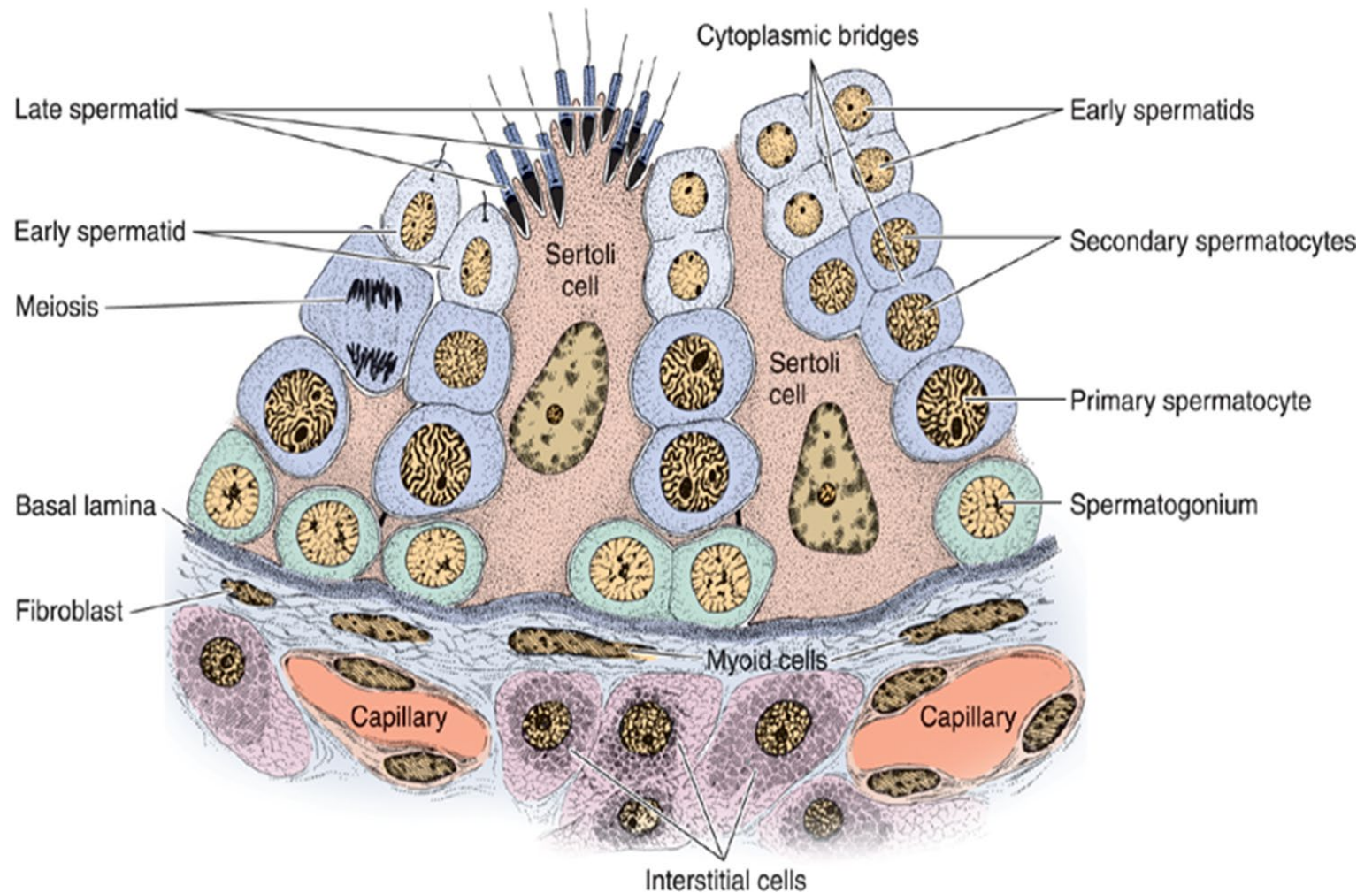
**Spermatogonia initiate a new cycle every ~16 days with 4 cycles (4 X 16 = 64 days)**

**Another 10 days is required for movement to epididymis (total of 74 days)**

# **SERTOLI CELLS**

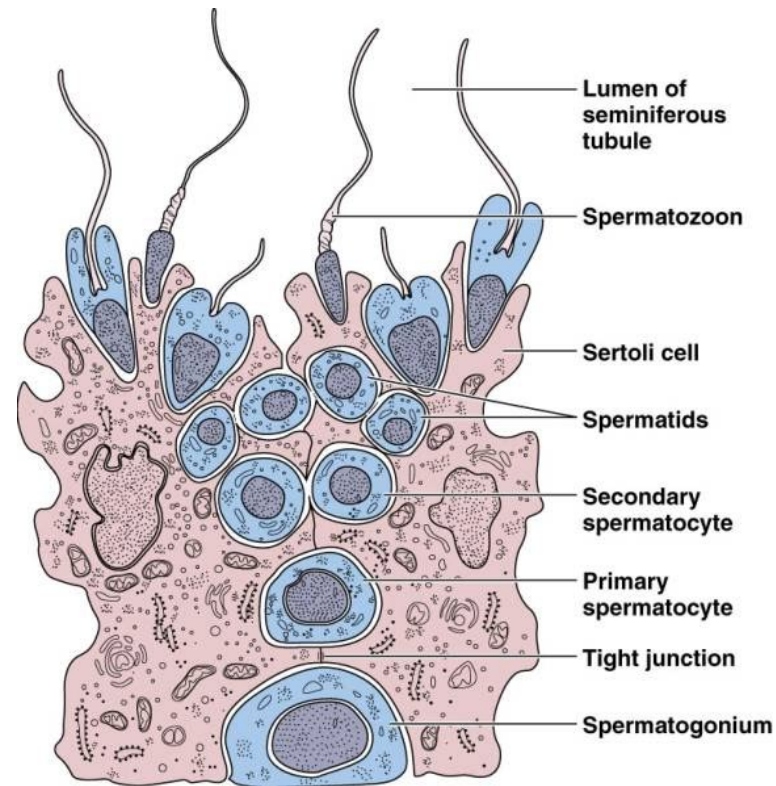


# Sertoli Cell Function



# Sertoli Cell Roles: Support

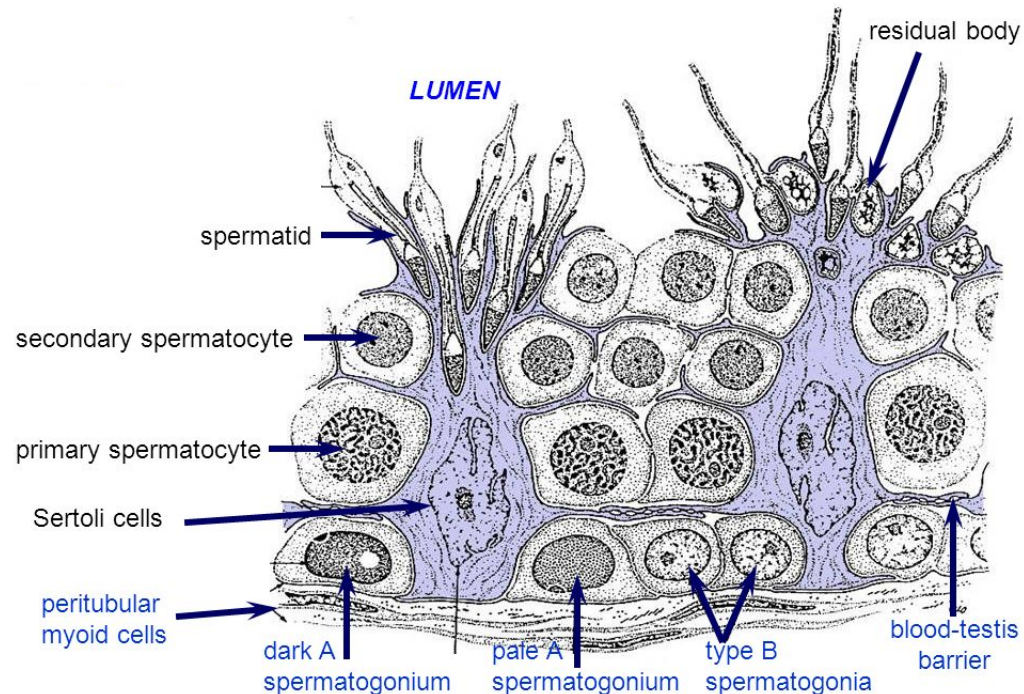
- Support and nutrition for developing germ cells
- Gateway for substances from capillaries to germ cells



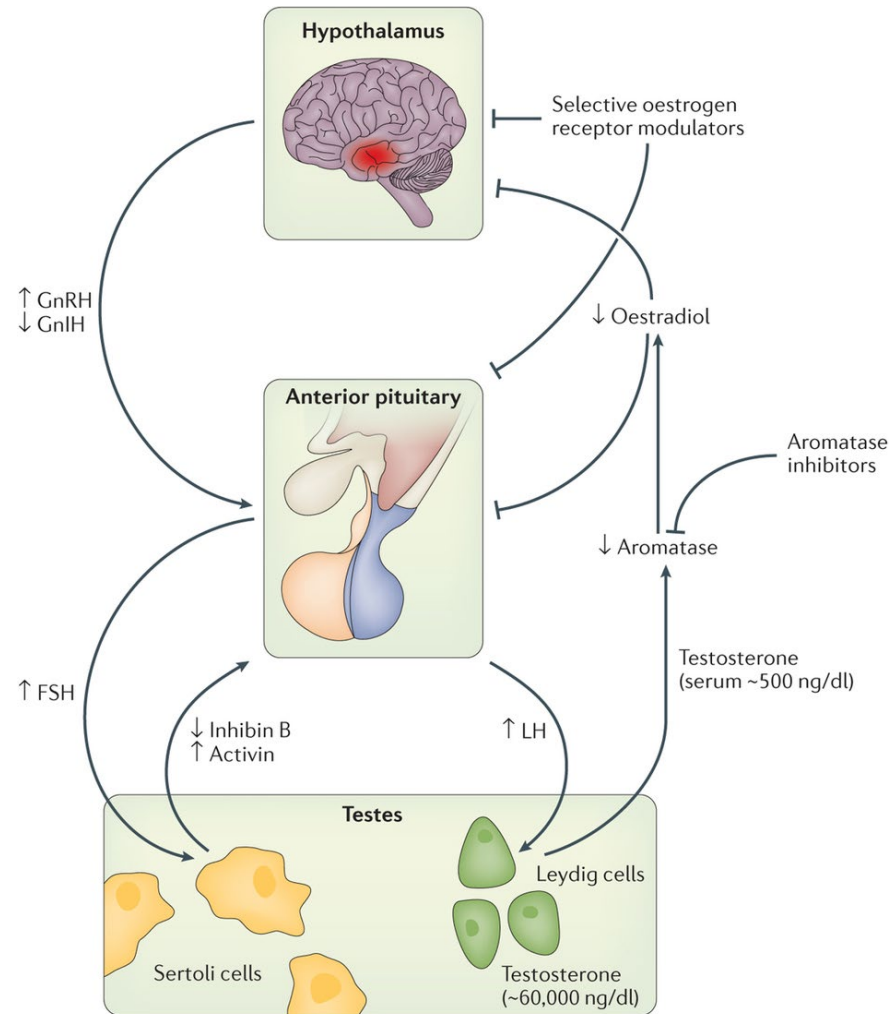


# Sertoli Cell Roles: Spermiation

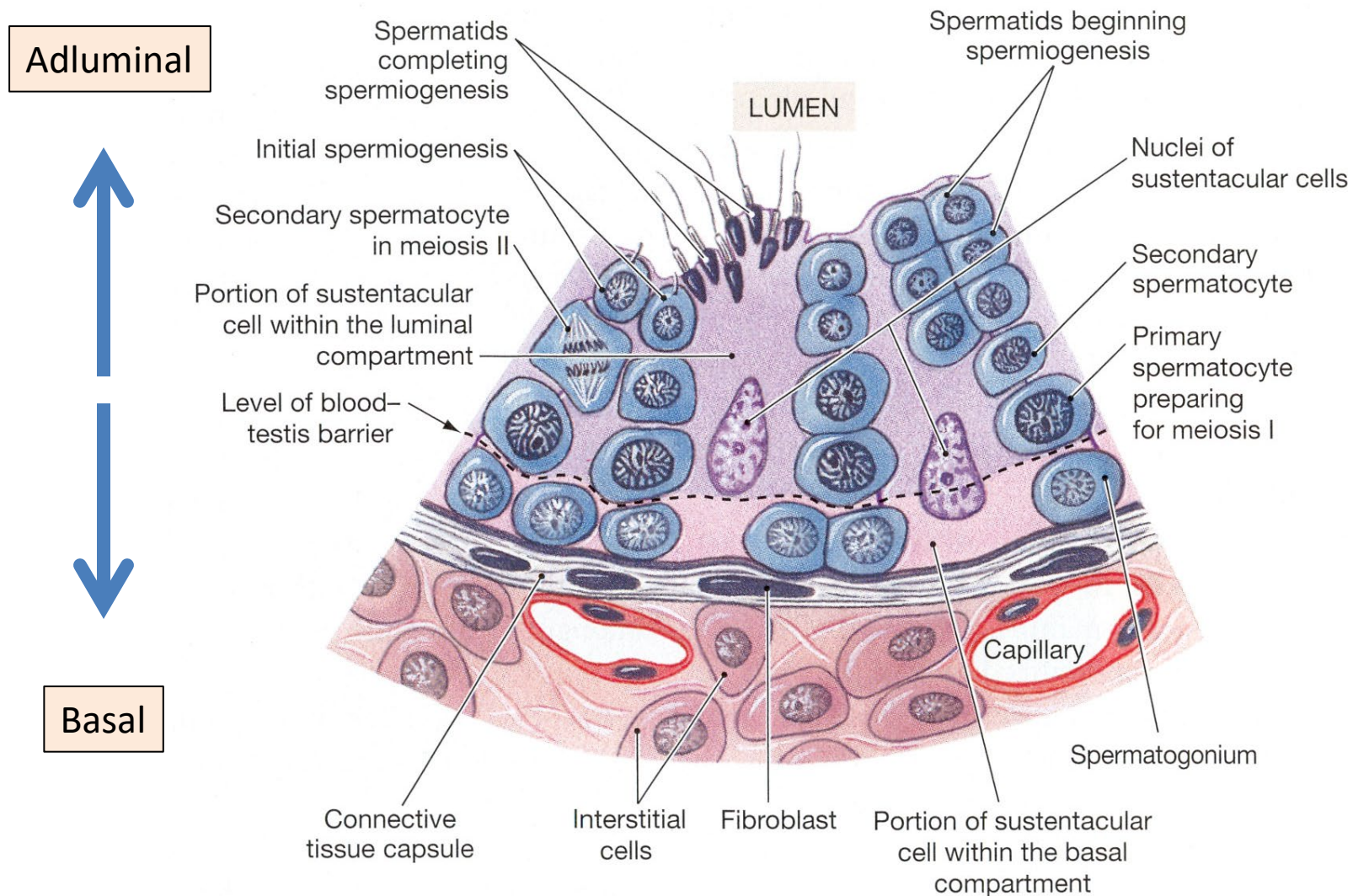
- Spermiation
  - Phagocytosis of degenerating germ cells and residual body
  - Dependent on FSH and Testosterone



# Sertoli Cell Roles: Endocrine



# Sertoli Cell Roles: Blood Testis Barrier

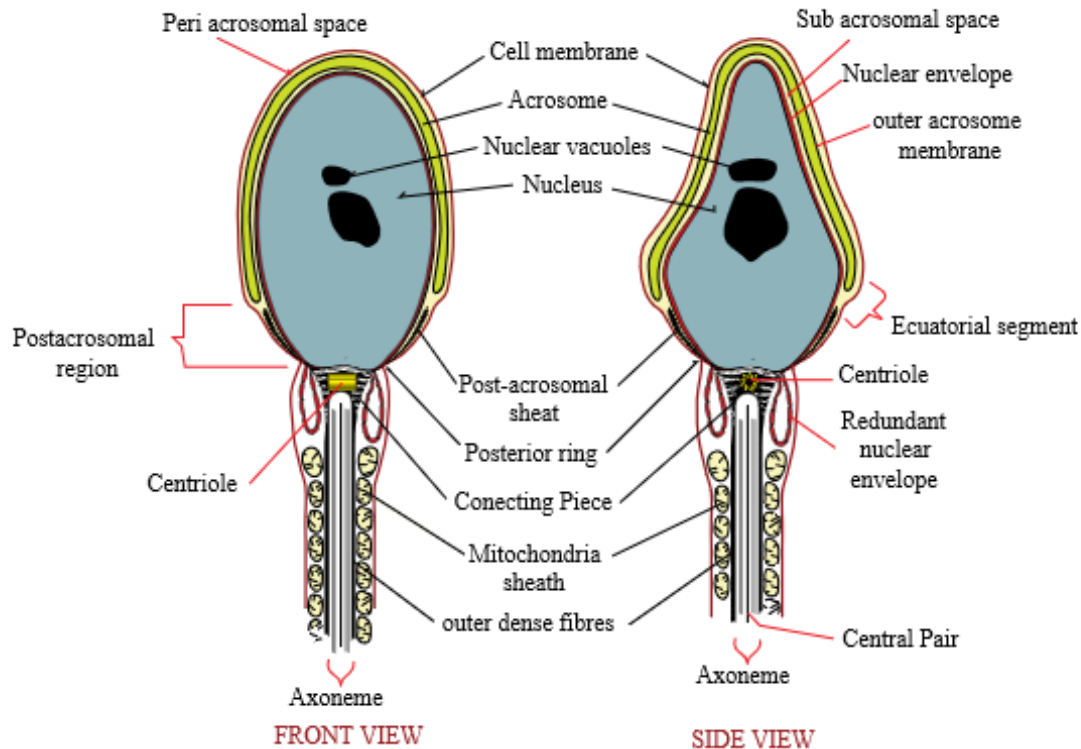
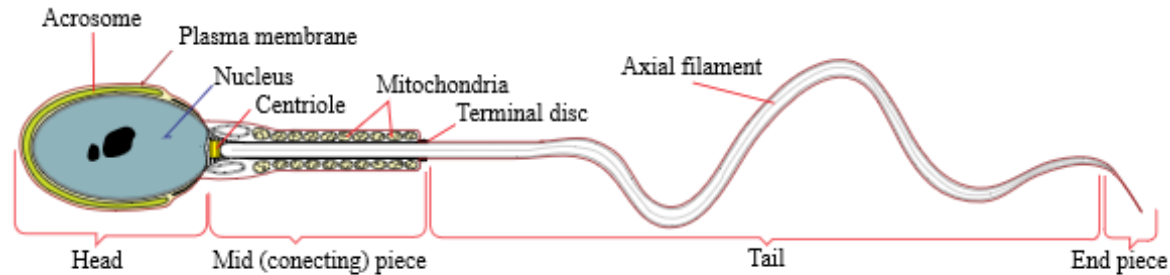






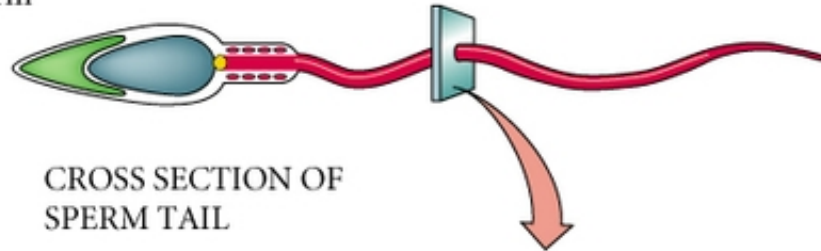
# **STRUCTURE AND MOTILITY**

# Structure and Motility



# Axoneme

(A) Sperm



(B) Plasma membrane

AXONEME

Radial spoke

Spoke head

Nexin

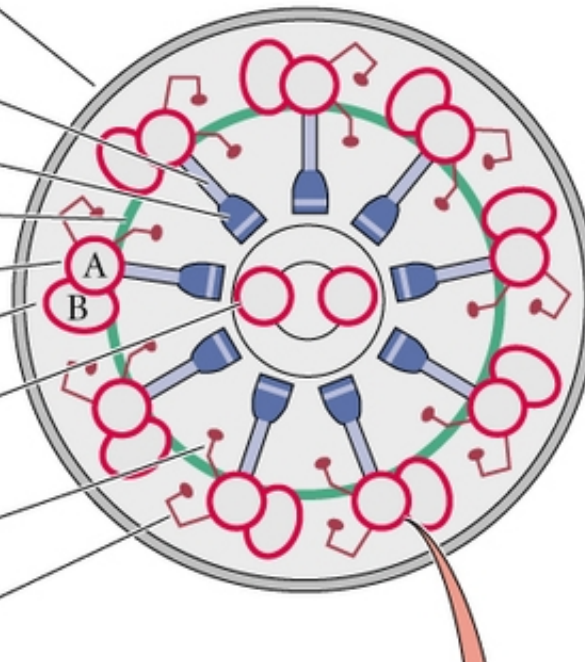
Subfiber A

Subfiber B

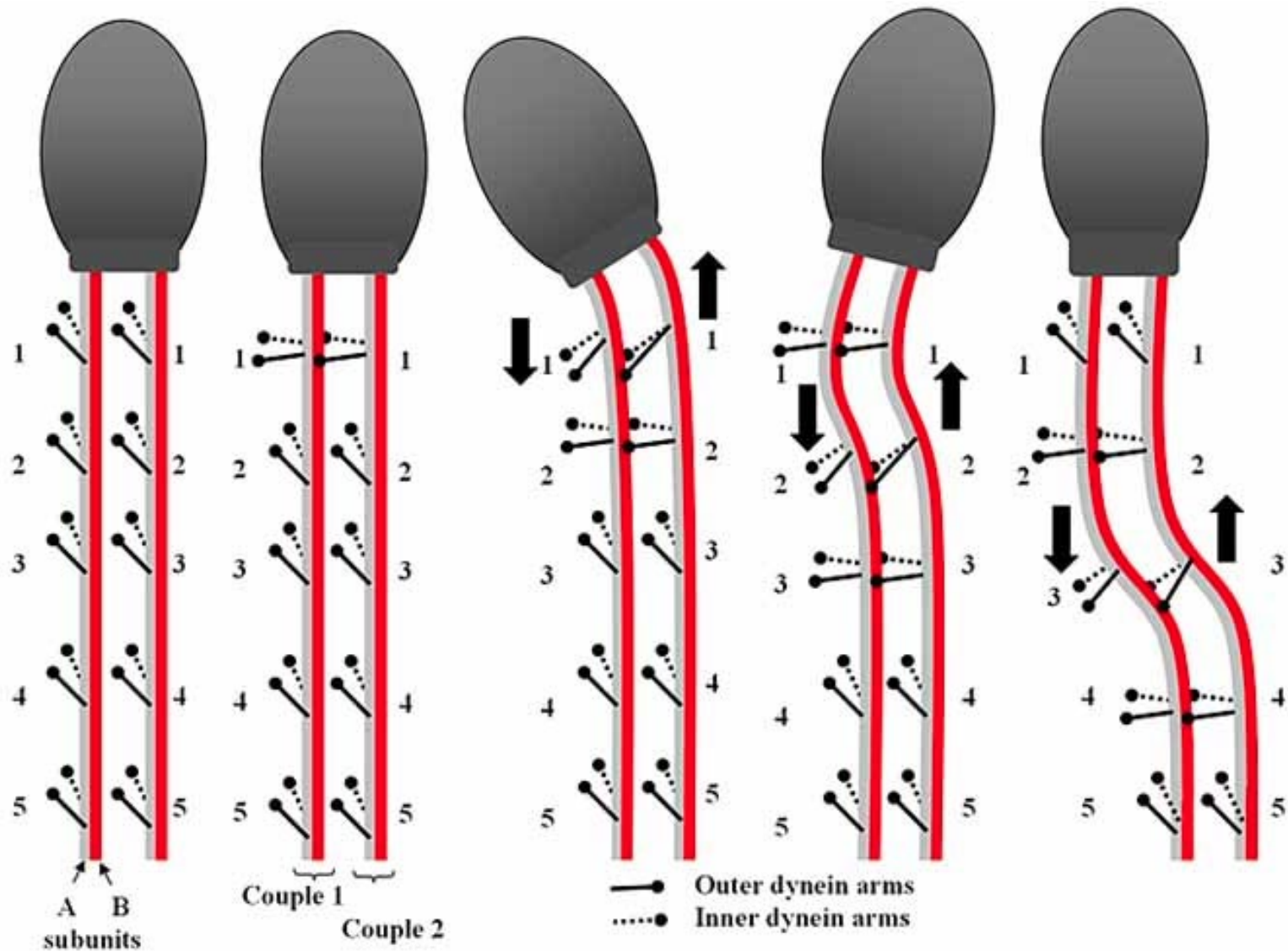
Central singlet microtubule

Inner dynein arm

Outer dynein arm



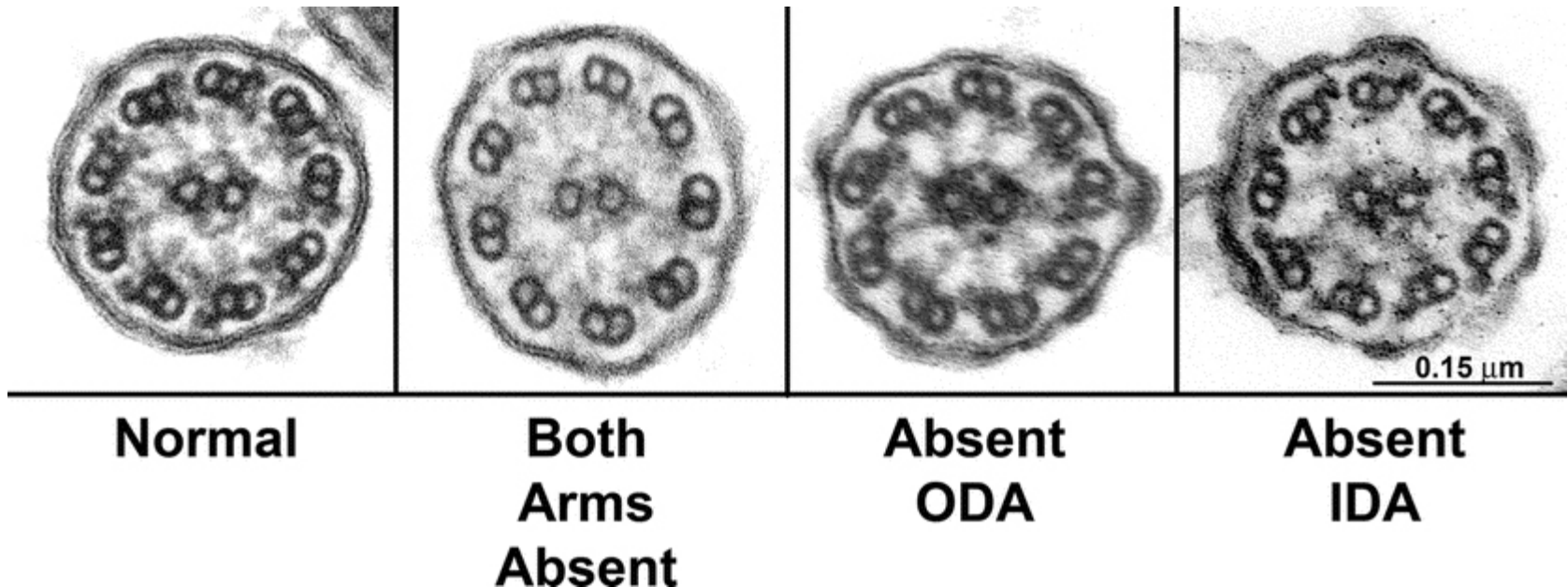
# Microtubule Motility





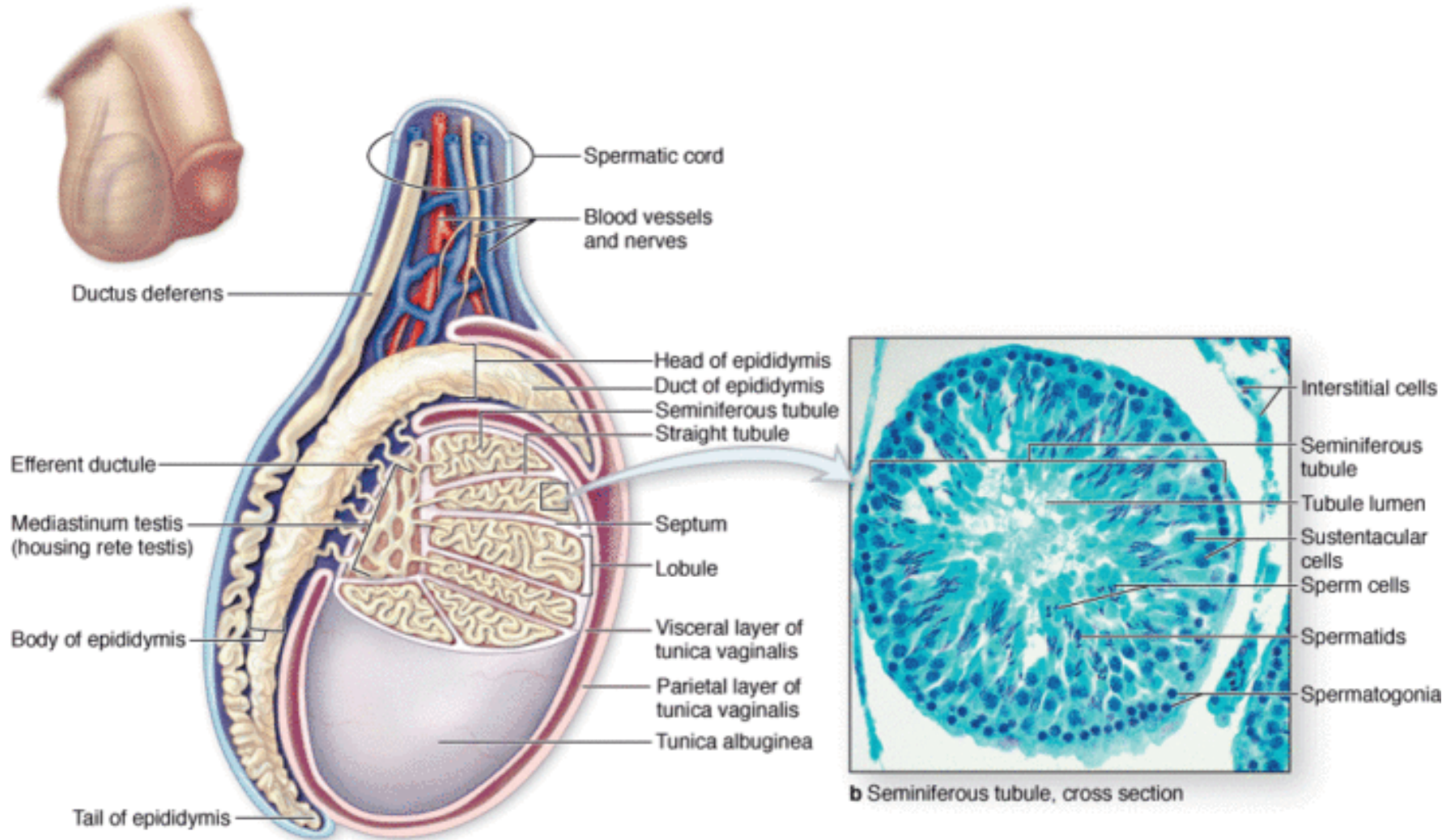
# Primary Ciliary Dyskinesia

- Kartagener syndrome (situs inversus, chronic sinusitis, and bronchiectasis)
- Autosomal recessive



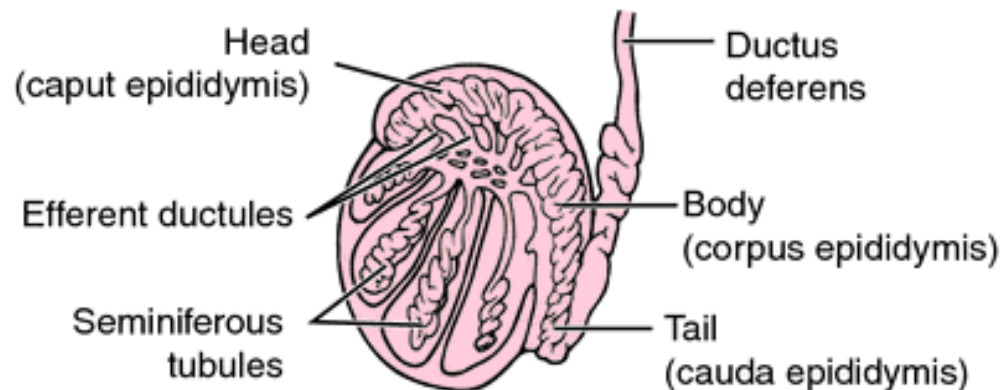
**EPIDIDYMISS**

# Epididymis Anatomy



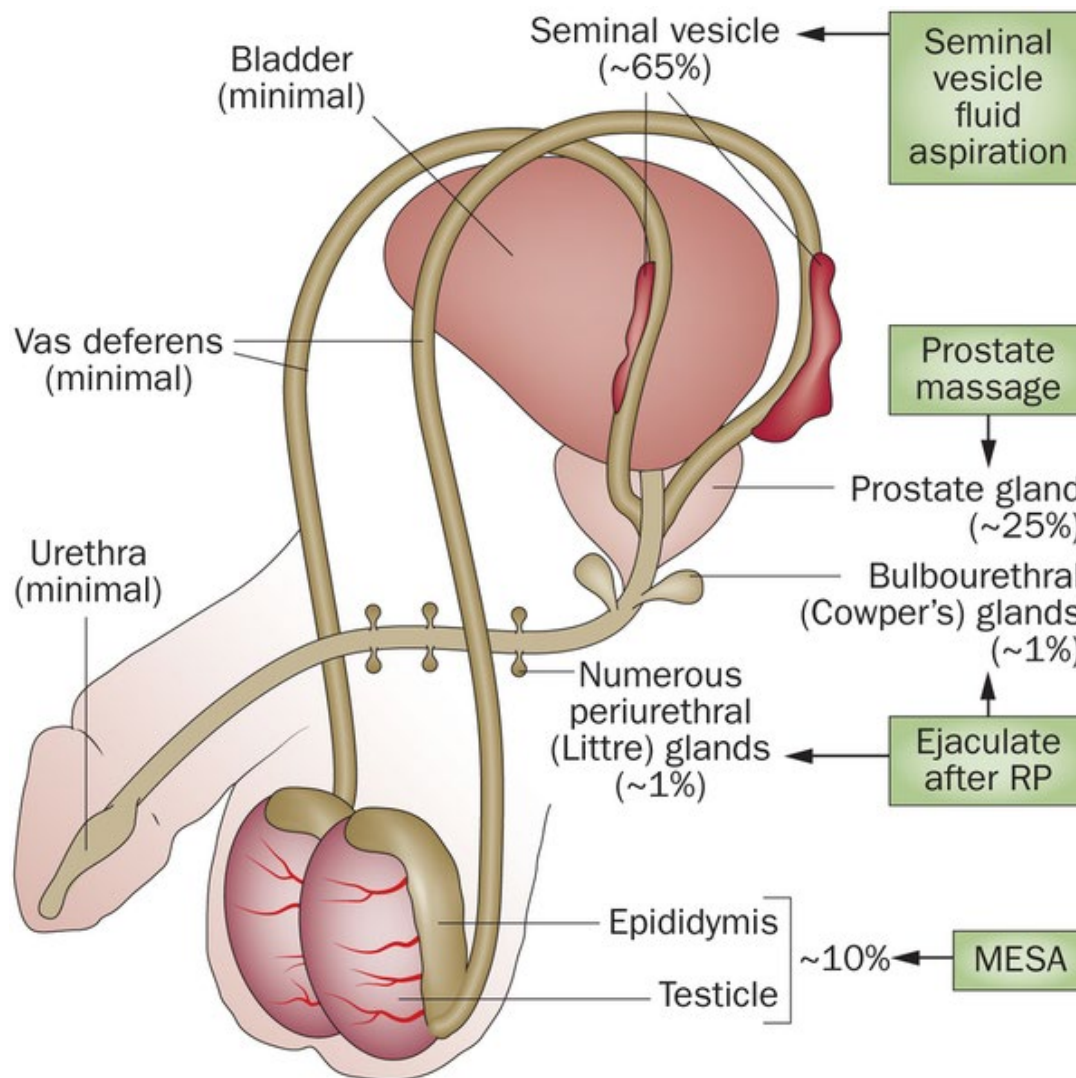
# Epididymis Function

- Sperm migration (10-16 days)
- Maturation of sperm and acquisition of motility
- Sperm storage (cauda) and release
- Phagocytosis of aged sperm



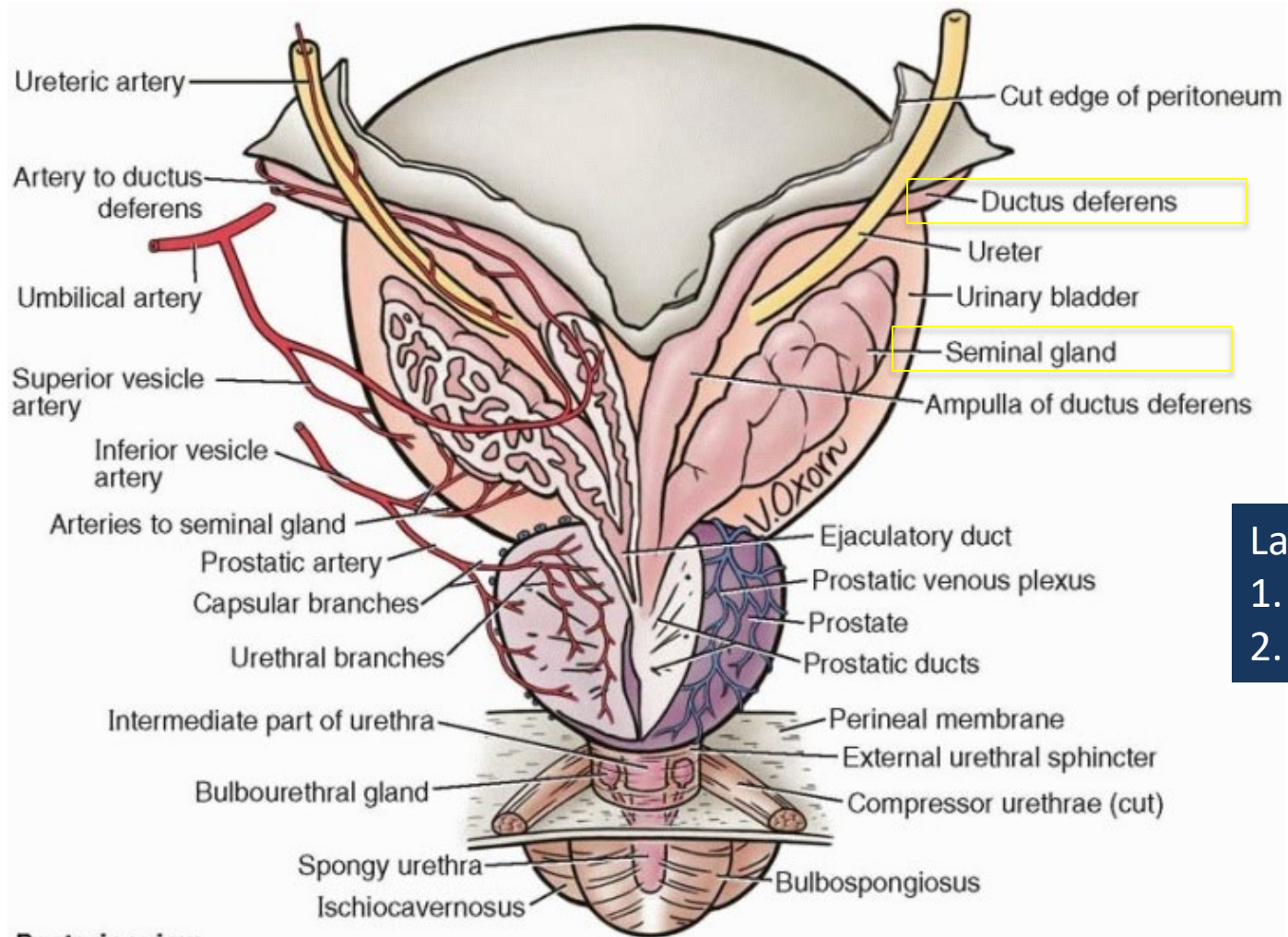
# **SEMINAL PLASMA AND SEMEN**

# Seminal Plasma and Semen





# Seminal Vesicles and CBAVD



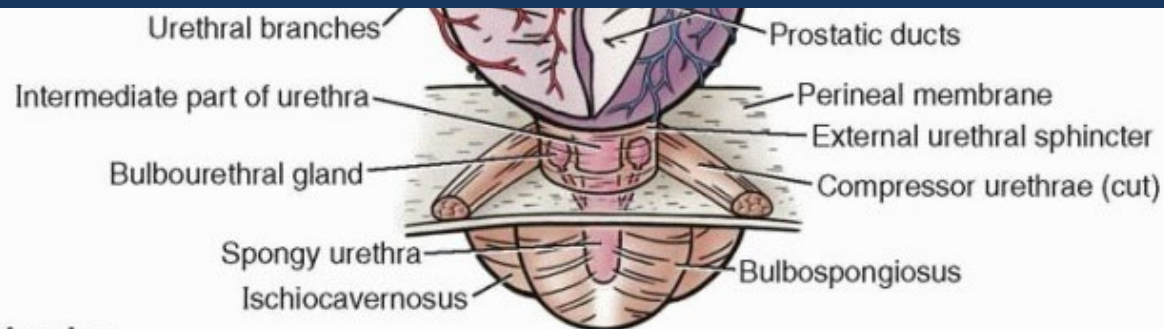
Posterior view

Lack:  
 1. Sperm  
 2. Fructose

# Seminal Vesicles and CBAVD



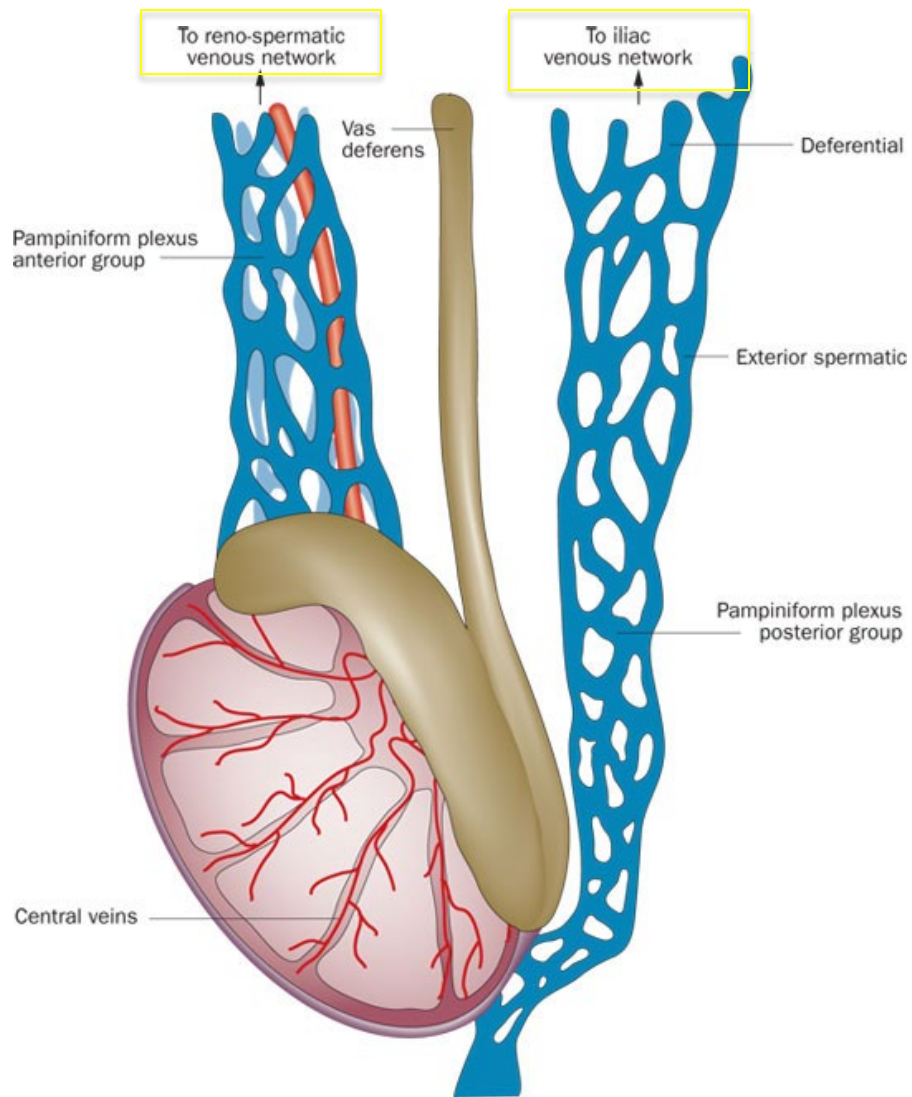
- 1-2% of infertile men have congenital bilateral absence of the vas deferens
- Most have mutation in the cystic fibrosis transmembrane conductance regulator (CFTR)
- May or may not have other findings (respiratory or pancreatic)



Posterior view

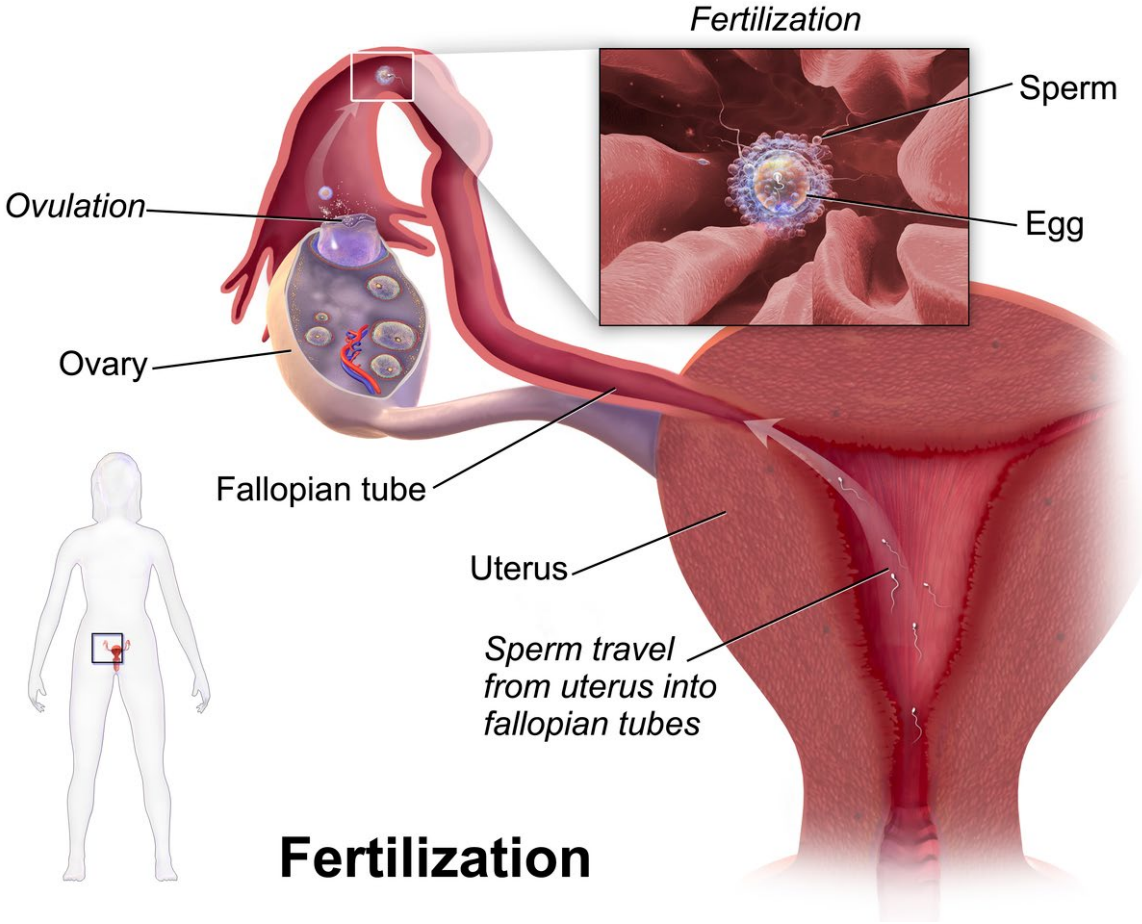


# Pampiniform Plexus



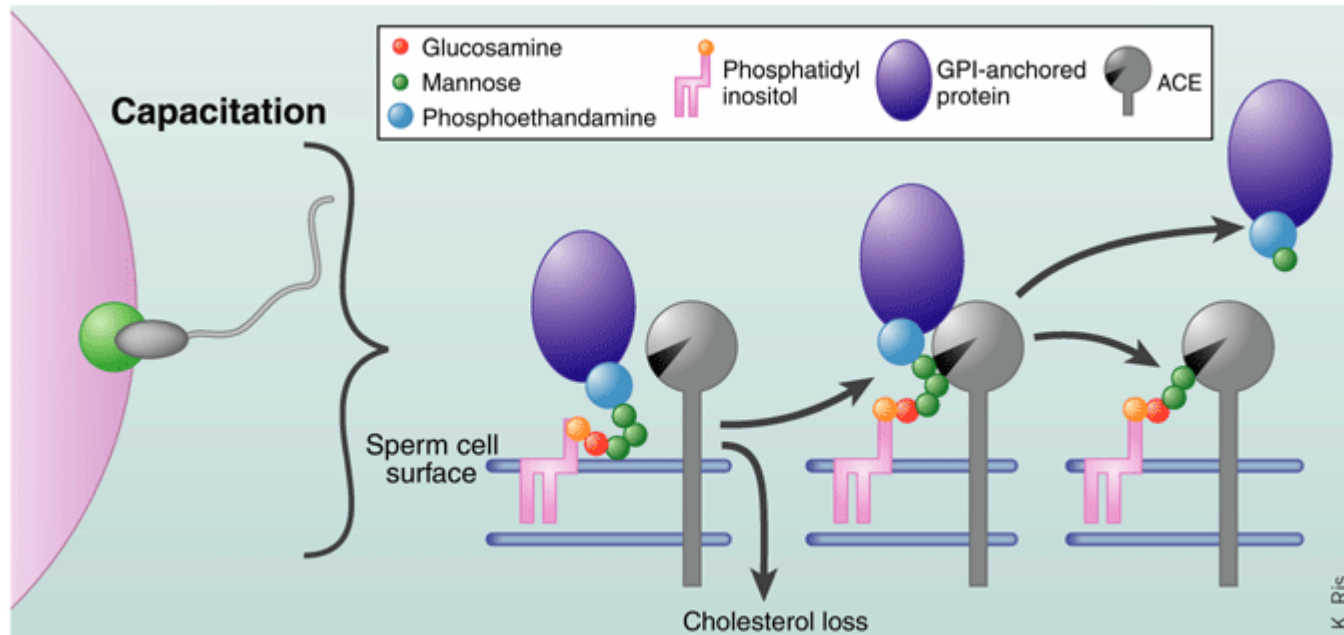
# FERTILIZATION

# Fertilization



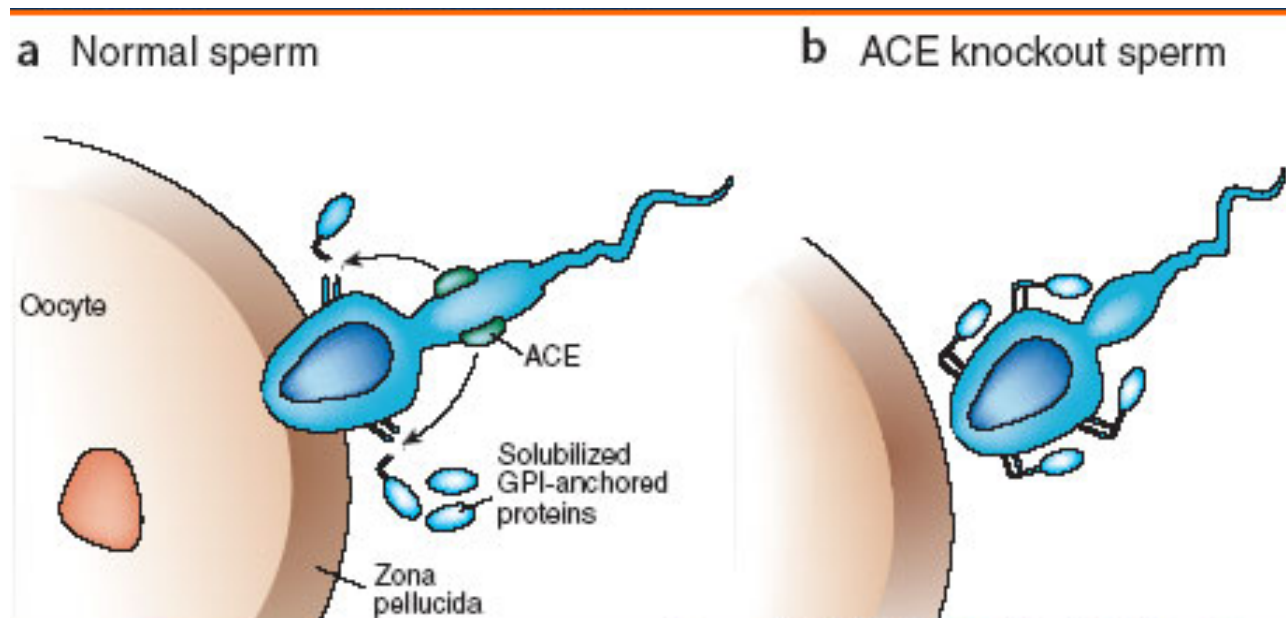
# Capacitation

- Removal of sterols and non-covalently bound epididymal/seminal glycoproteins
- Increased membrane fluidity and  $\text{Ca}^{2+}$  permeability



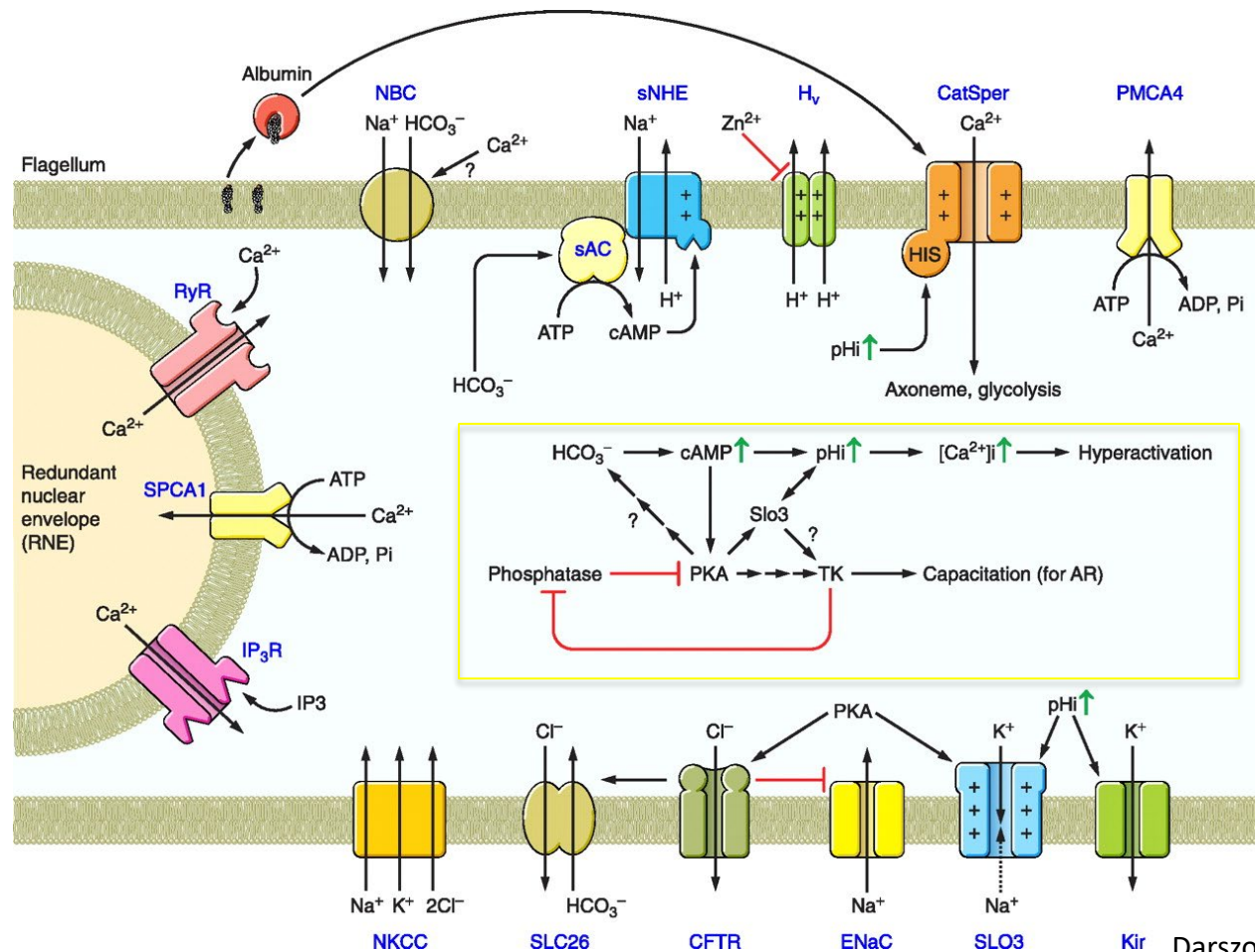
# Capacitation: Testicular ACE

- Angiotensin I converting enzyme (ACE) cleaves glycosylphosphatidylinositol (GPI)-anchored proteins



# Capacitation and Hyperactivation

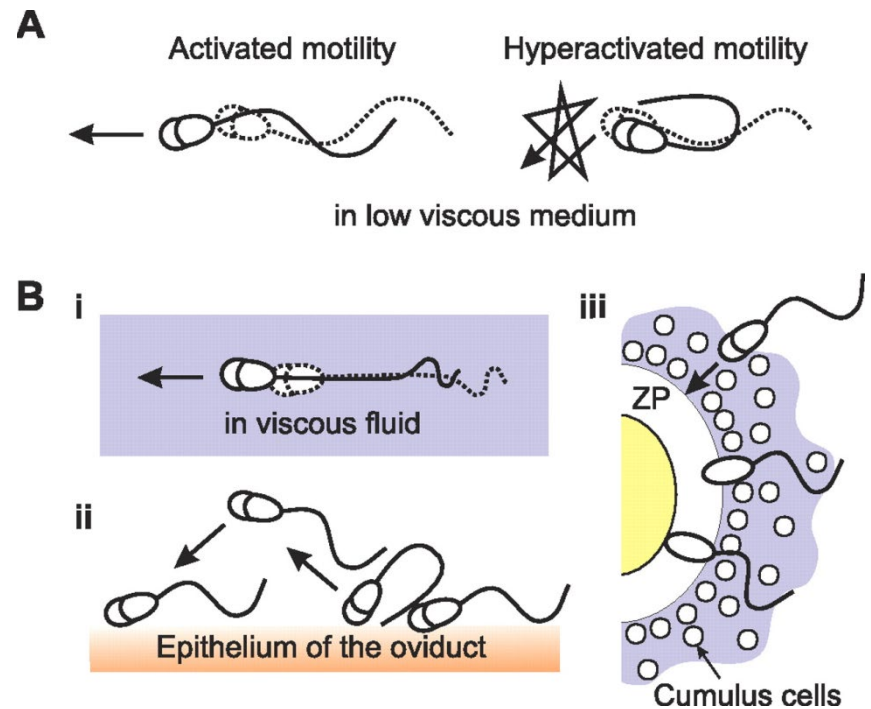
- Increased  $\text{Ca}^{2+}$ ,  $\text{HCO}_3^-$ , and cAMP; increased pH





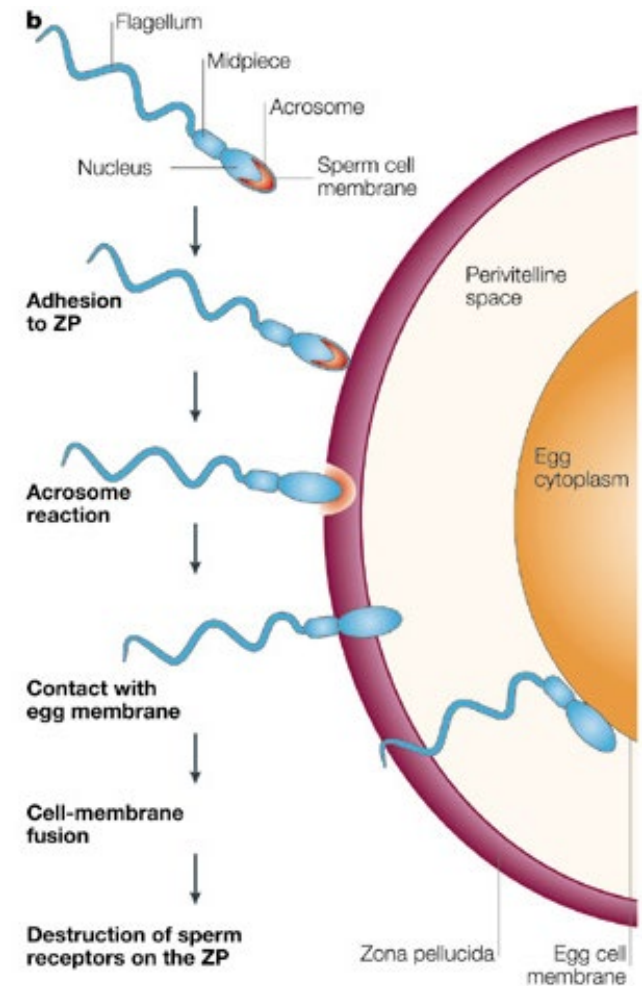
# Hyperactivated Motility Purpose

- Effectiveness of motility depends on fluid viscosity
- Allows for
  - Movement in viscoelastic fluids in female genital tract,
  - Detachment from isthmic reservoir
  - Penetration of cumulus matrix

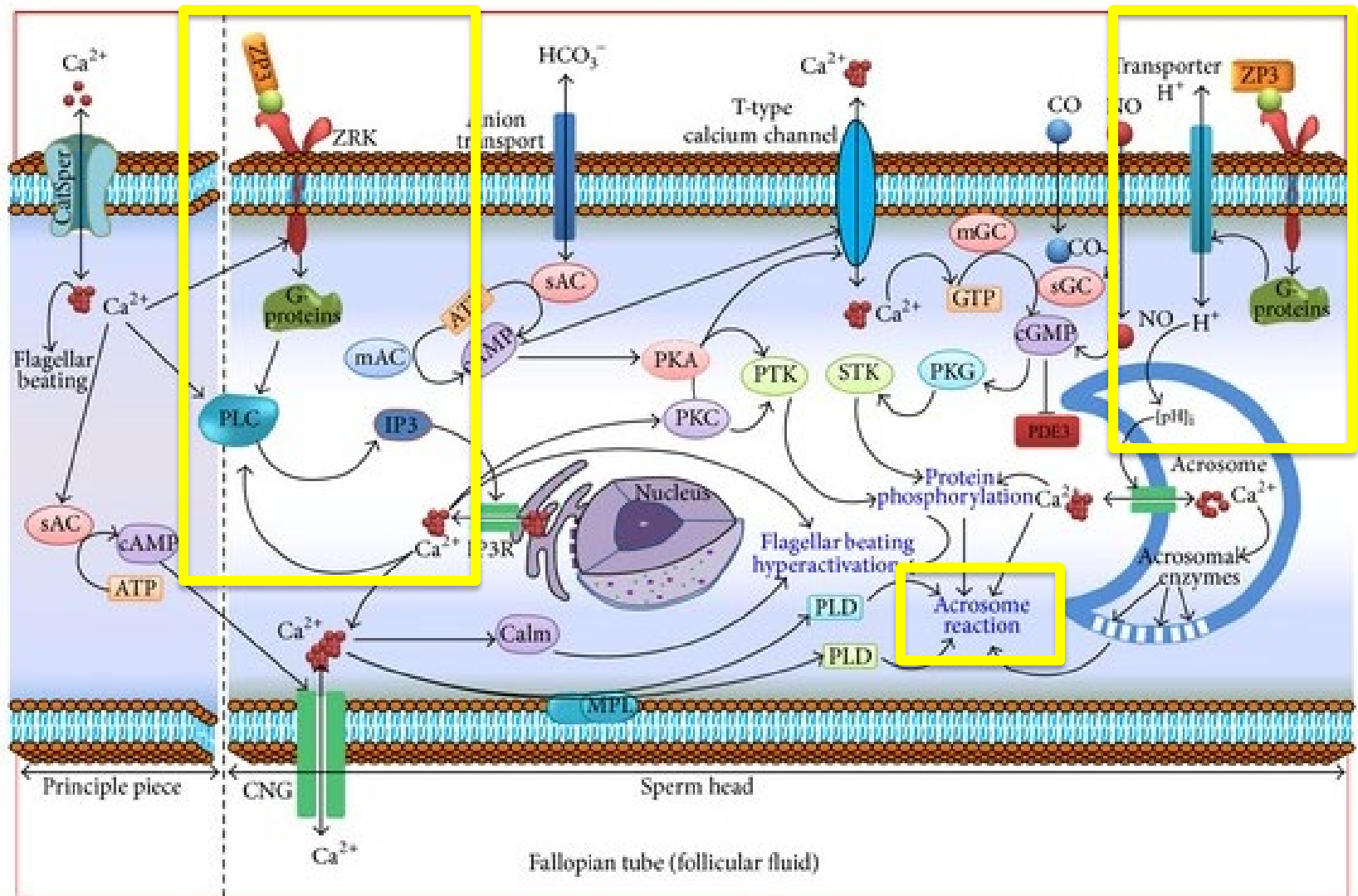


# Acrosome Reaction: Overview

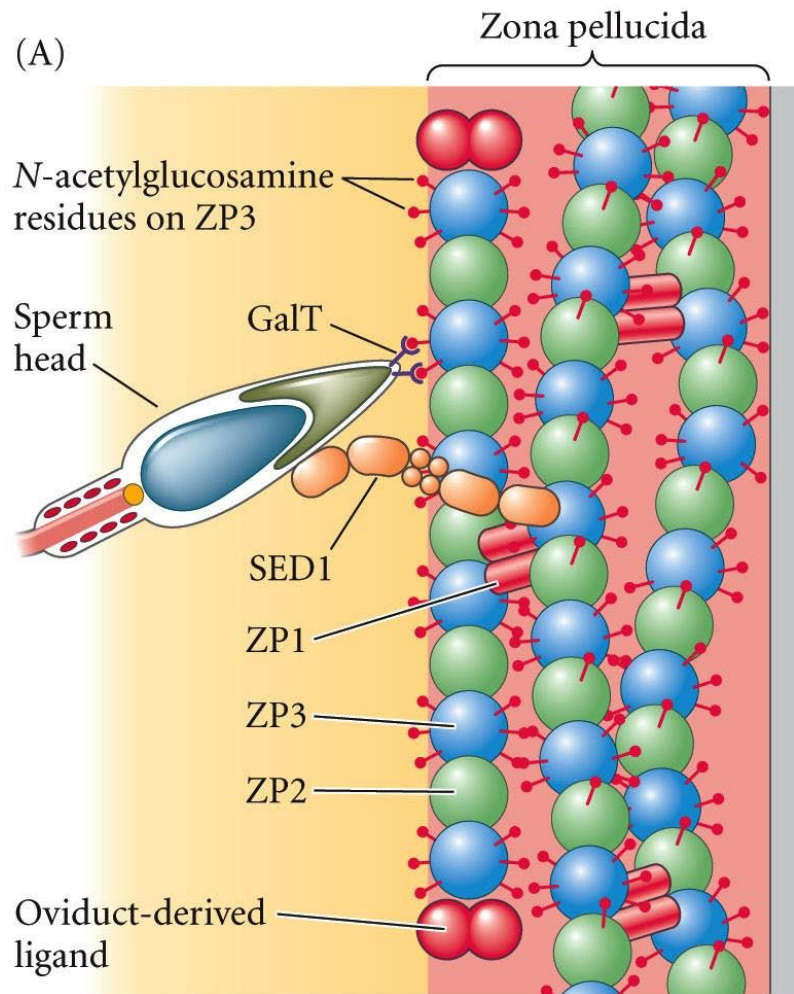
- Requires capacitated sperm
- Initiated with sperm membrane binding to **ZP3**
- Acrosome reaction allows tight binding to **ZP2**
- Enzymes from the acrosomal vesicle digest through the zona pellucida
  - Hyaluronidase, neurominidase-like factor, cumulus-dispersing factor, and acrosin (protease)
- Allows for the equatorial segment fusion with oolema



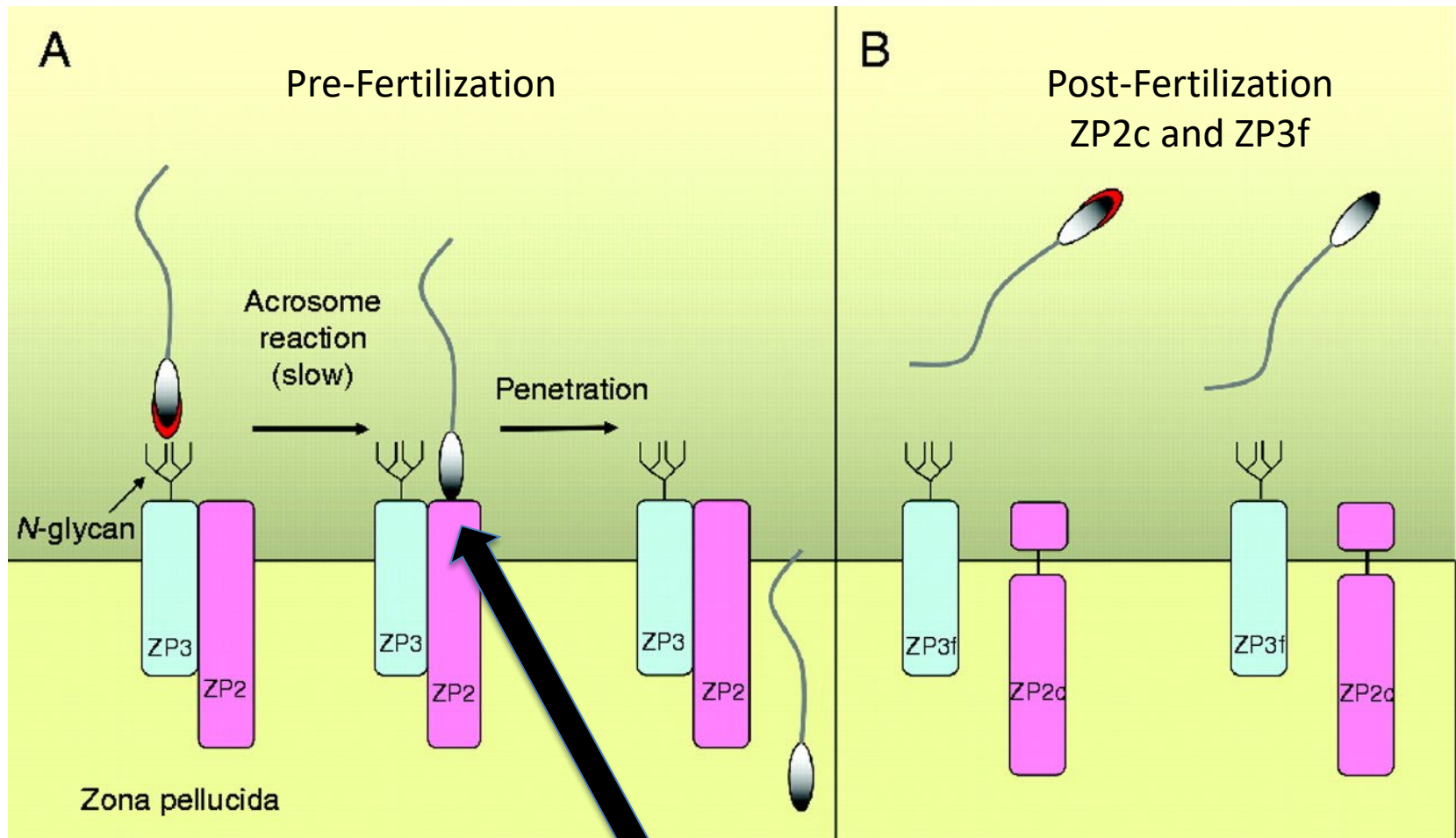
# Acrosome Reaction: $\text{Ca}^{2+}$ and ZP3



# Relationship of ZP Binding Proteins



# Changes in ZP Binding Proteins

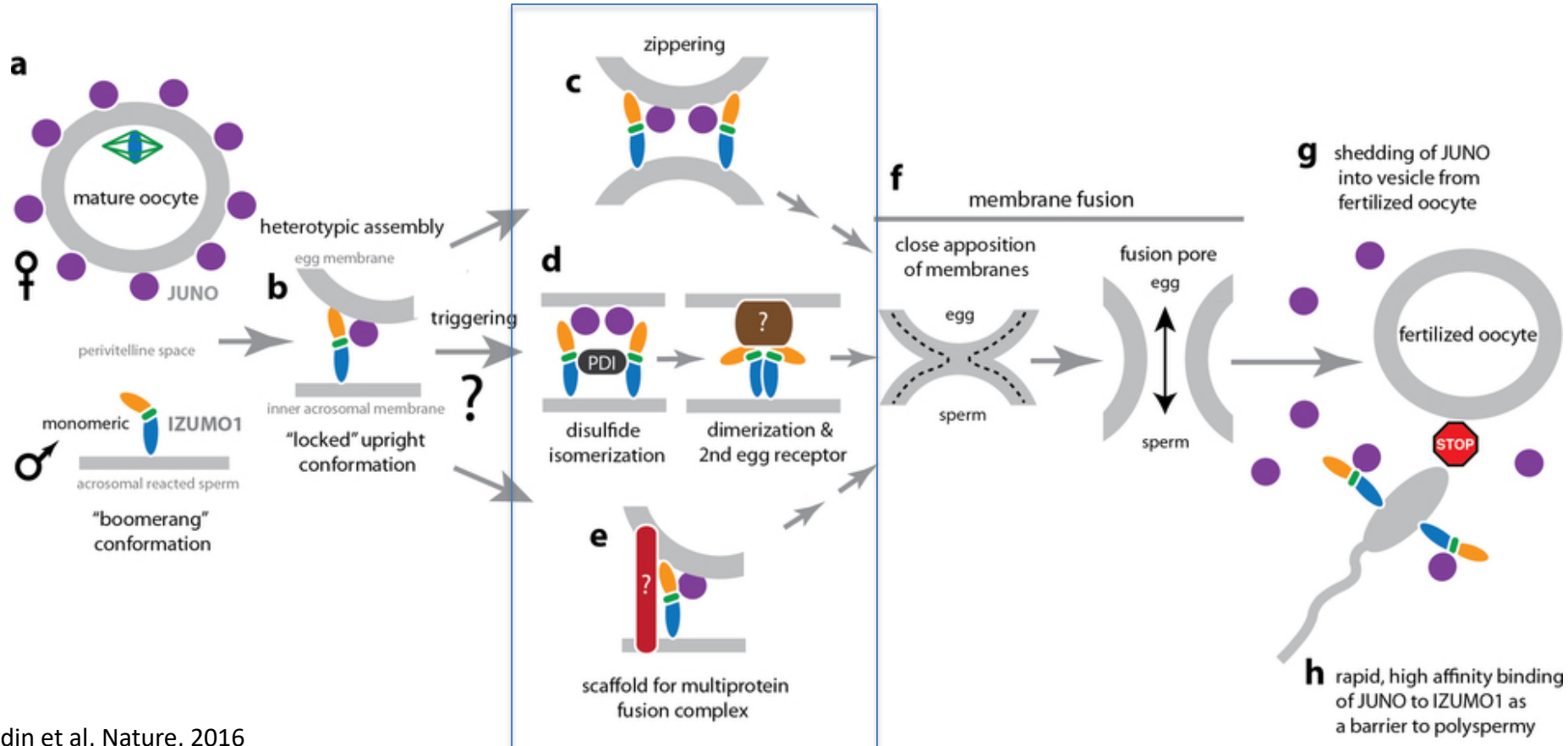


Inner acrosomal membrane binding



# Sperm and Oocyte Fusion

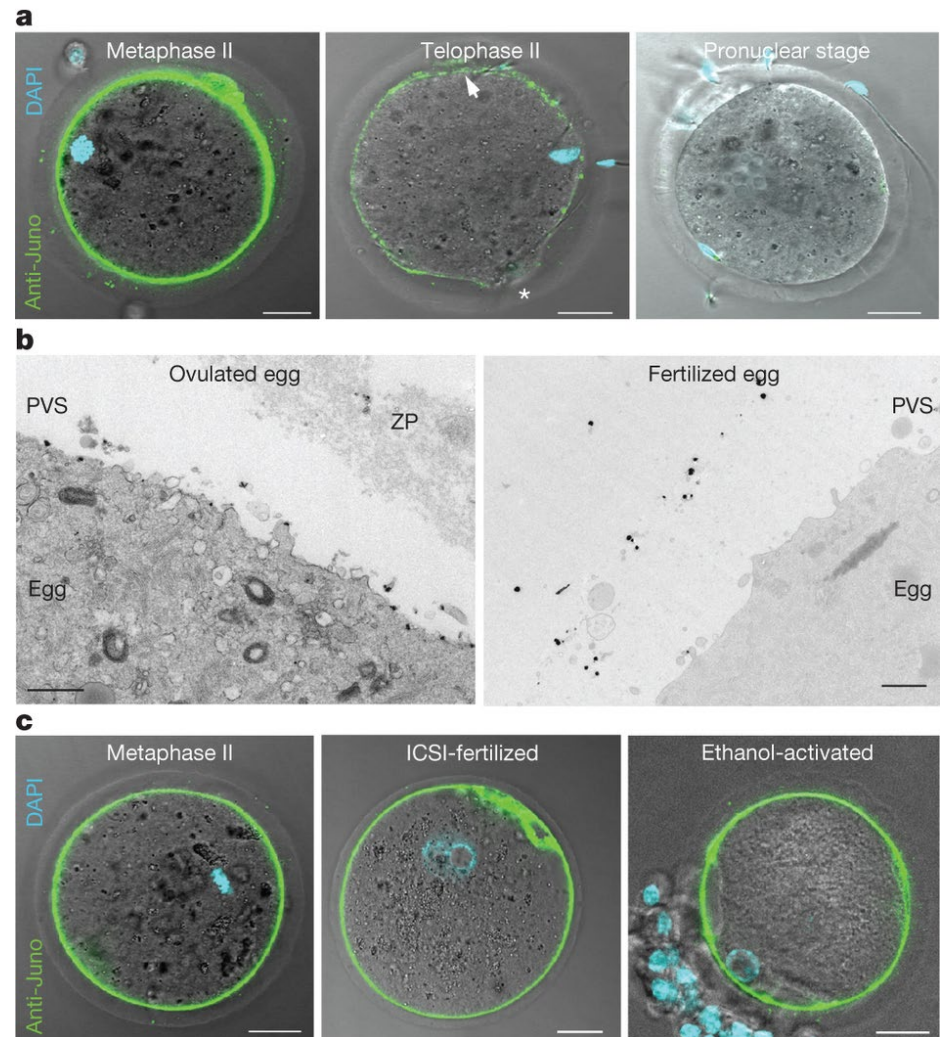
- Interaction of sperm IZUMO1 and oocyte JUNO
- JUNO rapidly shed into perivitelline space after reaction



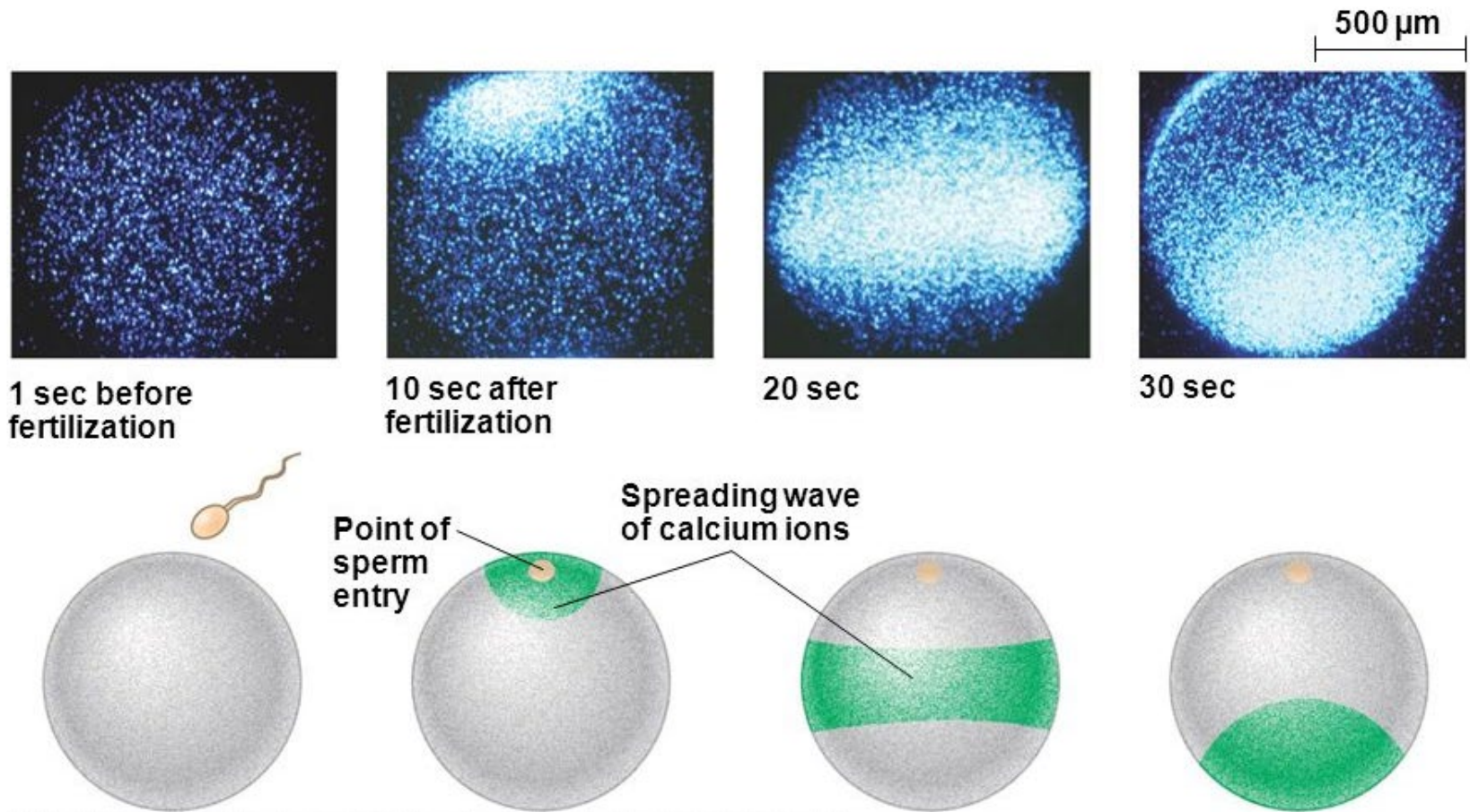


# Juno Role in Blocking Polyspermy

- Juno becomes undetectable after fertilization
- Seen on oolema in unfertilized oocyte and in perivitelline space after fertilization
- Oocyte fertilized via ICSI and parthenodes retain Juno staining

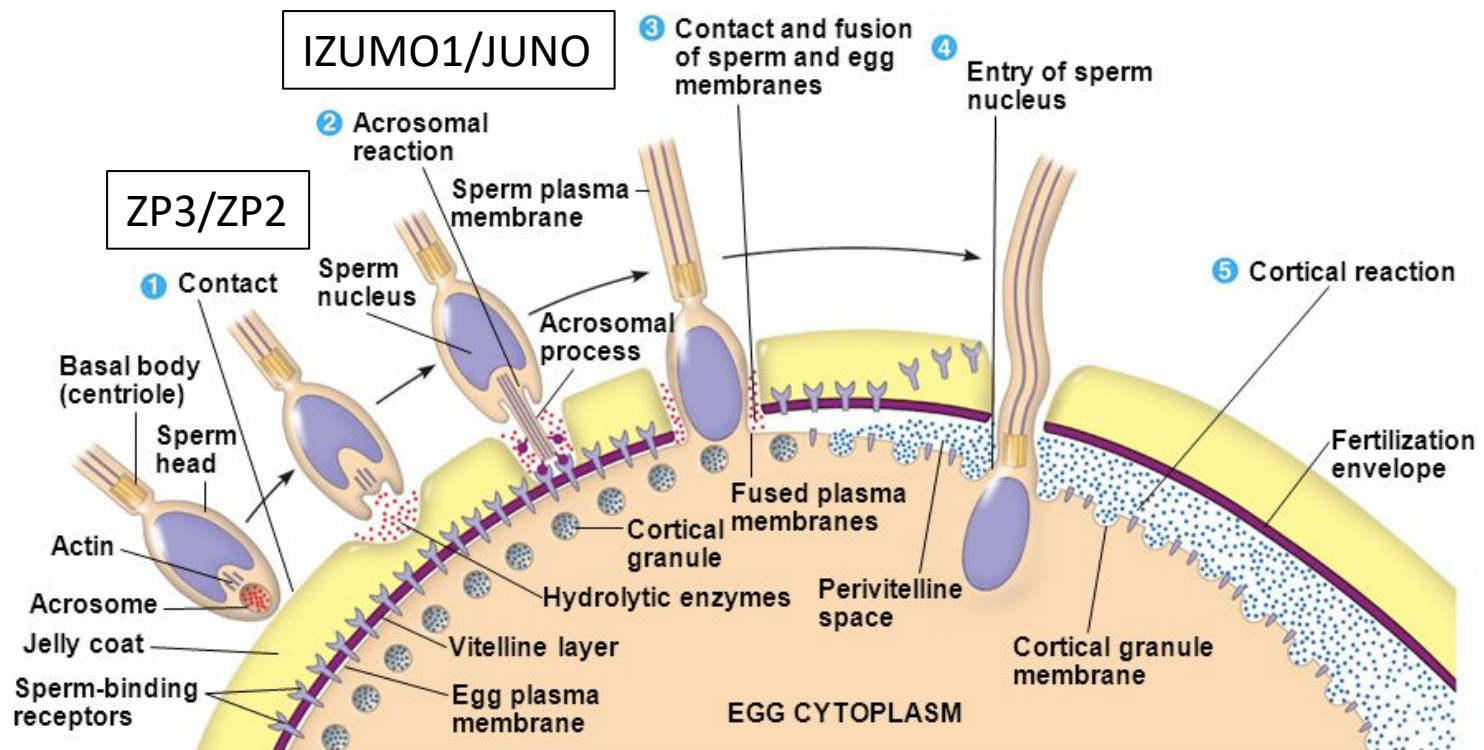


# Block to Polyspermy: Membrane Depolarization



# Cortical Reaction

- Membrane fusion induces rise in  $\text{Ca}^{2+}$  stimulating cortical granules to release contents and fast block to polyspermy



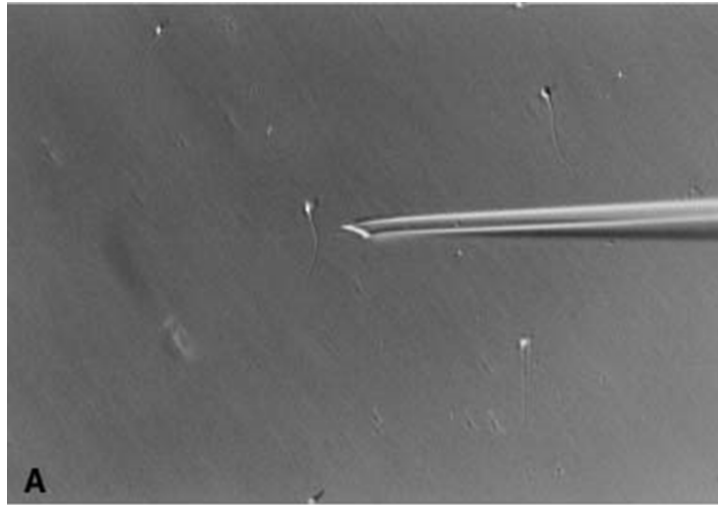


# Syngamy



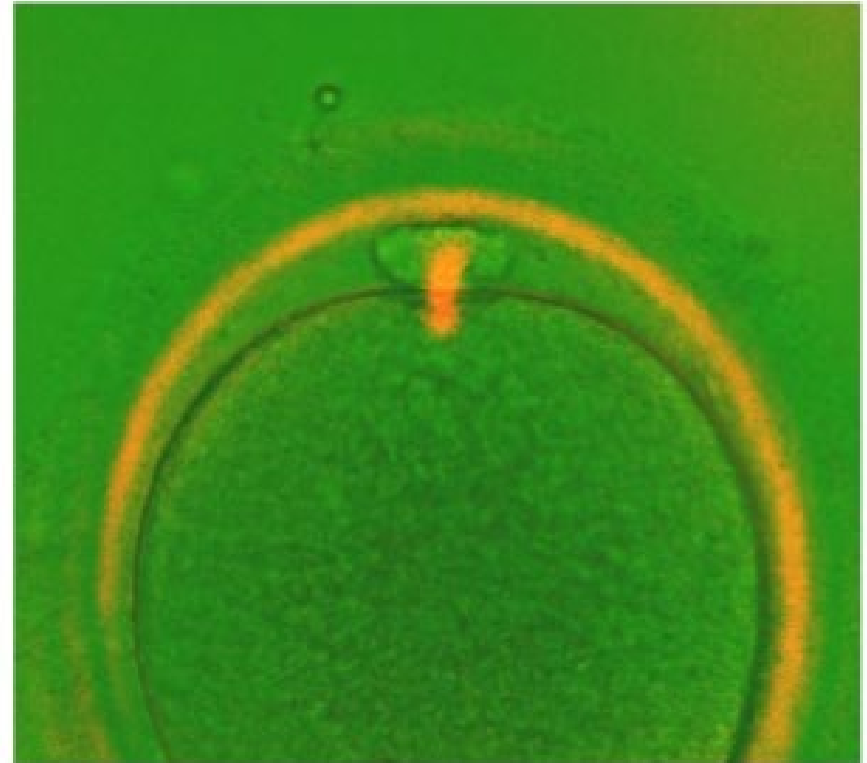


# Intracytoplasmic Sperm Injection (ICSI)



# Injection Point for ICSI

- Position polar body at 12 or 6 o'clock and injection point is at the 3 o'clock position





# Fertilization Results

- Monospermic dygenic (3PN) and single pronucleate (1PN) oocytes may occur more frequently with ICSI
- With conventional fertilization 1PN may be asynchronous PN development
- With ICSI this is due to failure of sperm to decondense fully and form PN

(a) Normally fertilized oocyte – 1 haploid male PN, 1 haploid female PN, 2 PBs



(b) Abnormally fertilized oocyte – 1 haploid female PN, 2 PBs



(c) Abnormally fertilized oocyte – 1 diploid female PN, 1 PB



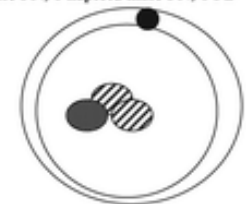
(d) Abnormally fertilized oocyte – 1 haploid or diploid male PN, 2 PBs



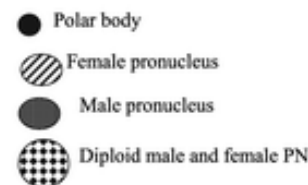
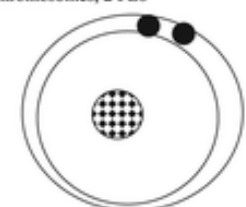
(e) Abnormally fertilized oocyte – 2 haploid male PN, 1 haploid female PN, 2 PBs



(f) Abnormally fertilized oocyte – 2 haploid female PN, 1 haploid male PN, 1 PB



(g) Possible normally fertilized oocyte – 1 diploid 'fused' PN, with male and female chromosomes, 2 PBs



# Take Home

- Understanding the fundamental anatomy and physiology is the basis for being able to answer questions on male factor infertility
  - Sperm maturation
  - Sperm transport
  - Fertilization
- Know commonly tested pathologies (azospermia, CBAVD, normal and abnormal fertilization, male HPG axis, etc.)

Good Luck!

