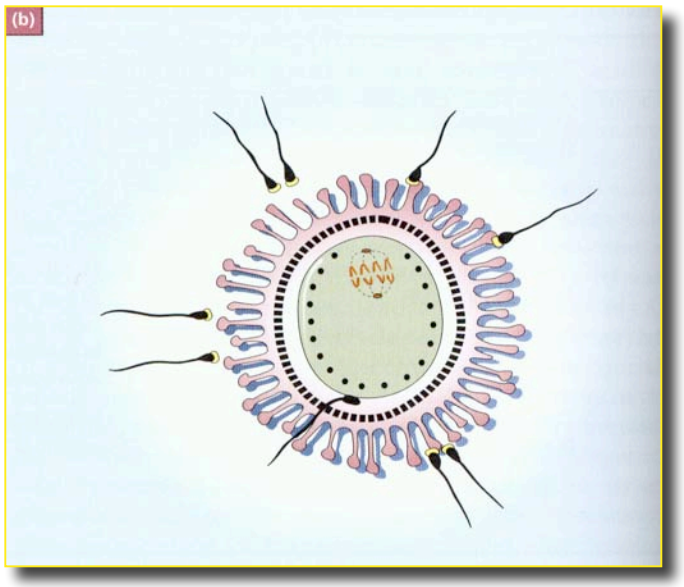
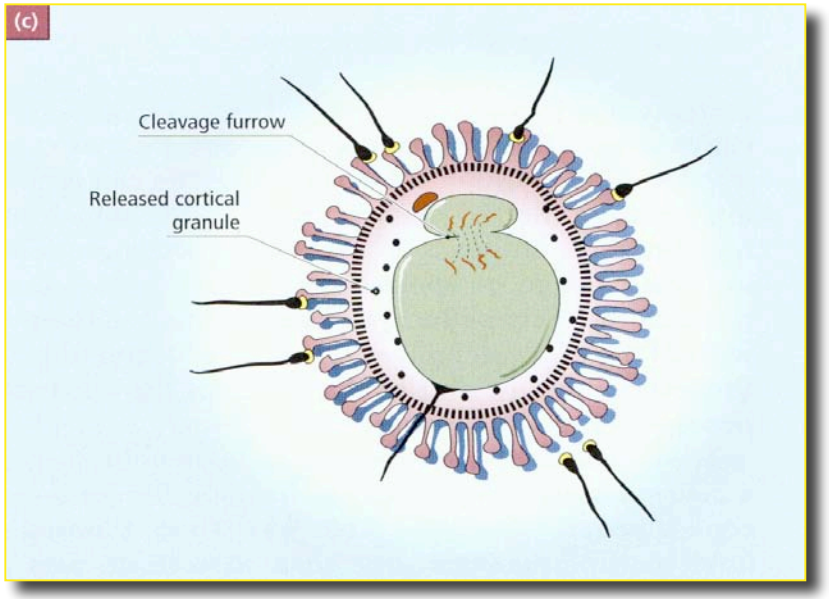
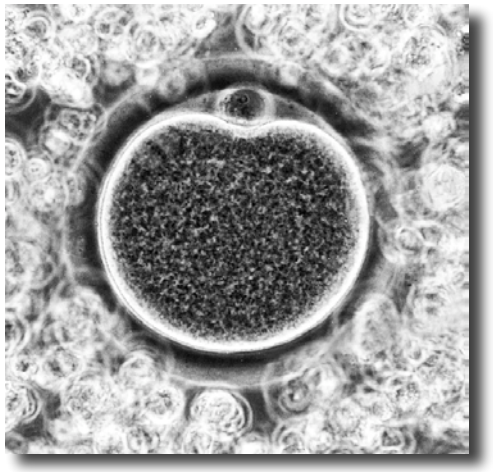
# Fertilization



# Egg Maturation



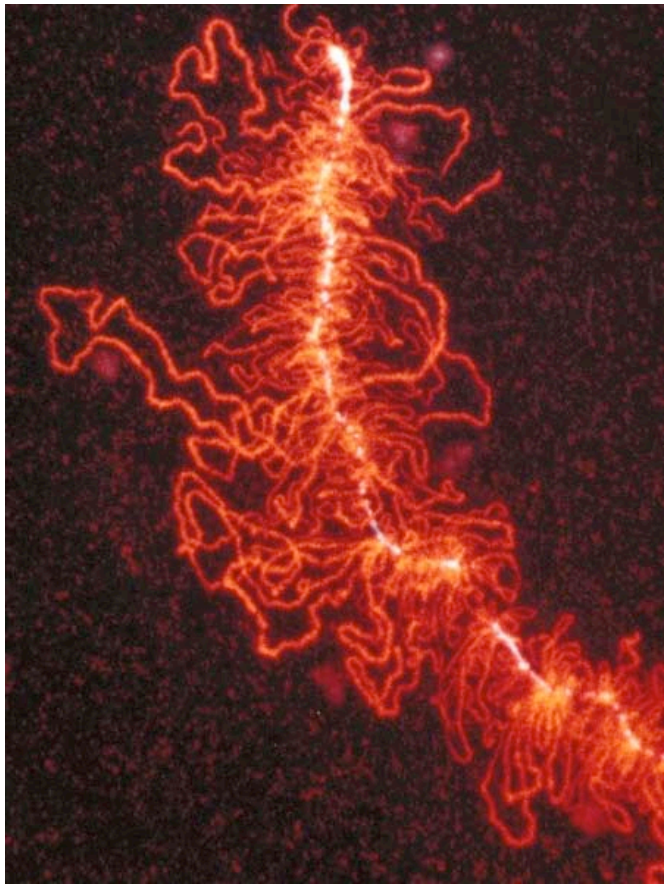
- All material necessary for beginning of new life
- Contains
  - Proteins
  - Ribosomes and tRNA
  - mRNA
    - Enough for 25 - 50,000 different proteins!
  - Morphogenic factors
  - Protective chemicals

# Egg Maturation - *Xenopus*

| Component | Excess over normal cell |
|---|---|
| Mitochondria | 100,000 |
| RNA polymerase | 60-100,000 |
| DNA polymerase | 100,000 |
| Ribosomes | 200,000 |
| tRNA | 10,000 |
| histones | 15000 |

Laskey, 1979

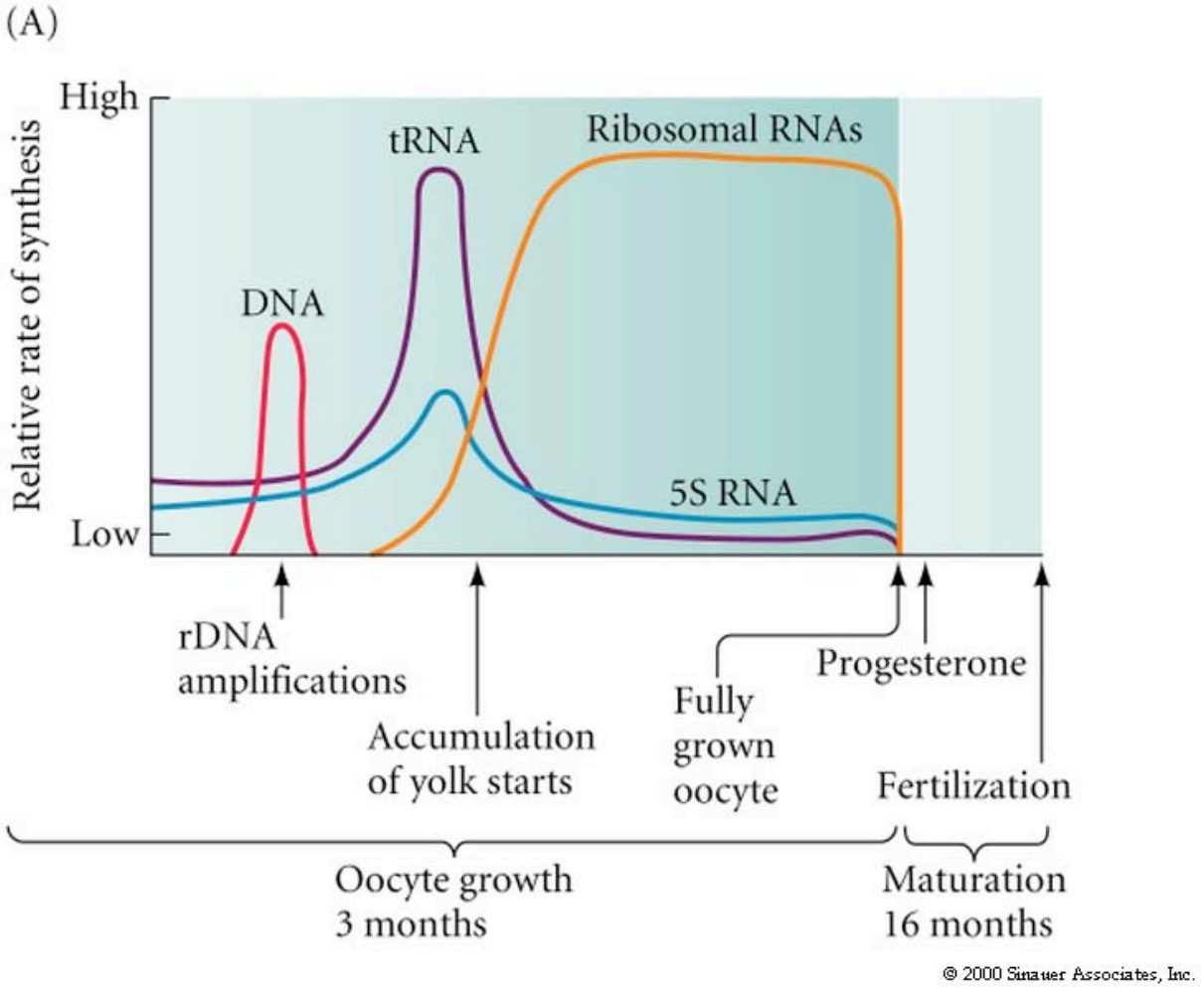
# Oocyte RNA synthesis



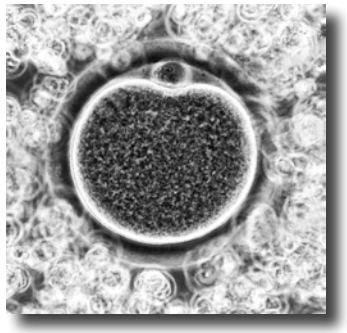
Lampbrush Chromosome

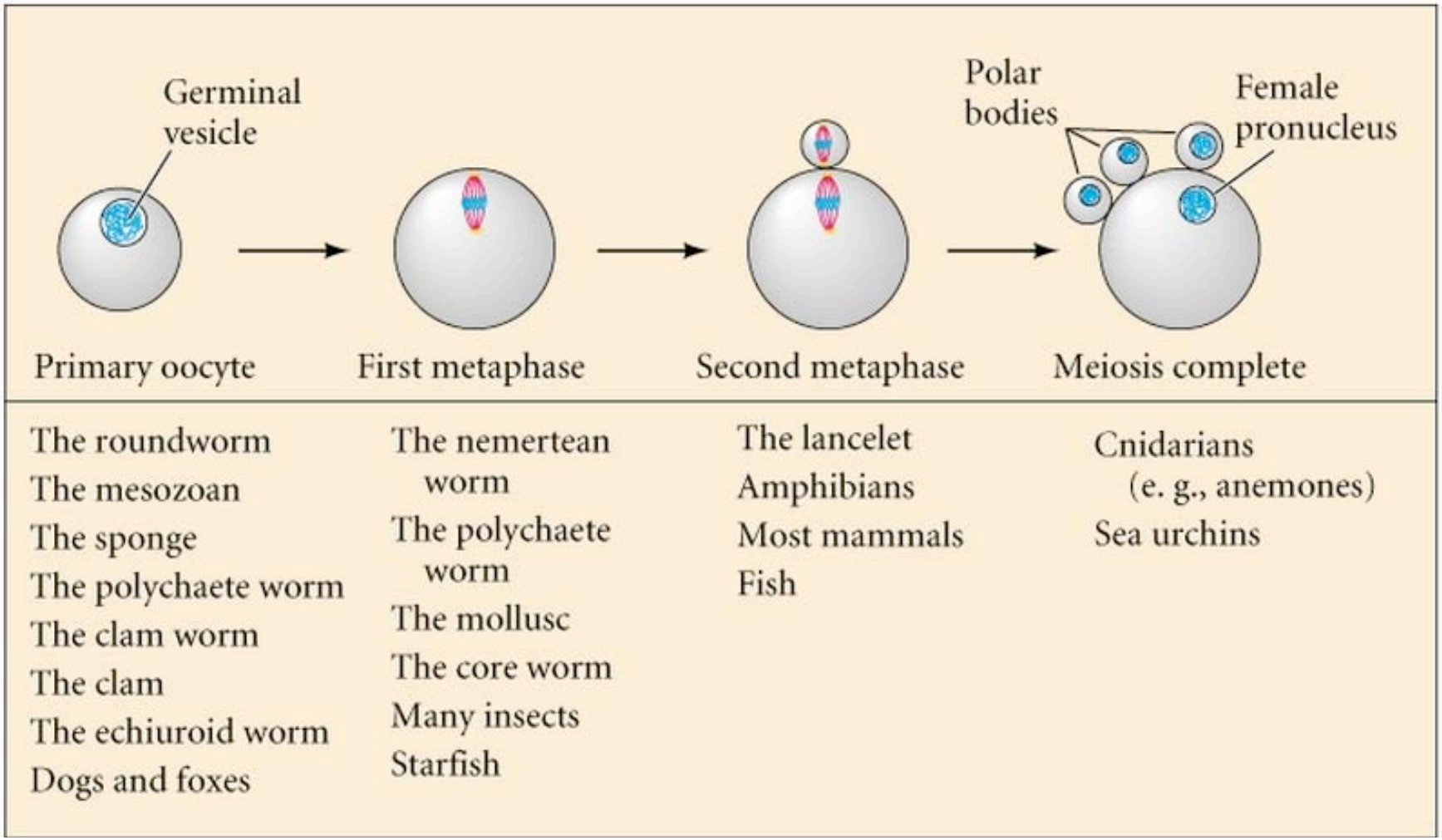
- During final stages of oocyte development
  - Massive RNA synthesis
    - For cytoplasmic RNA
    - For protein synthesis late in development (ZP proteins)
  - Lampbrush chromosomes
    - Stretched out loops of DNA
    - Allows gene transcription

# Xenopus oocytes



# Egg Stage at Fertilization





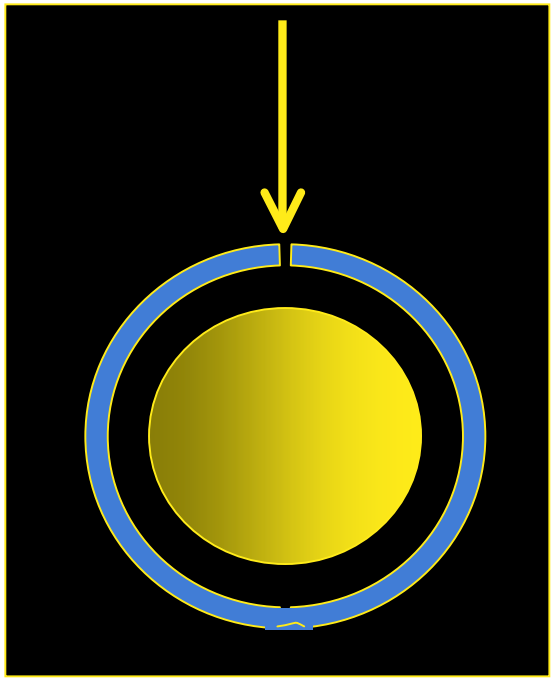

| Primary oocyte | First metaphase | Second metaphase | Meiosis complete |
|---|---|---|---|
| The roundworm | The nemertean worm | The lancelet | Cnidarians (e. g., anemones) |
| The mesozoan | The polychaete worm | Amphibians | Sea urchins |
| The sponge | The mollusc | Most mammals | |
| The polychaete worm | The core worm | Fish | |
| The clam worm | Many insects | | |
| The clam | Starfish | | |
| The echiuroid worm | | | |
| Dogs and foxes | | | |

# Recognition of Sperm and Eggs

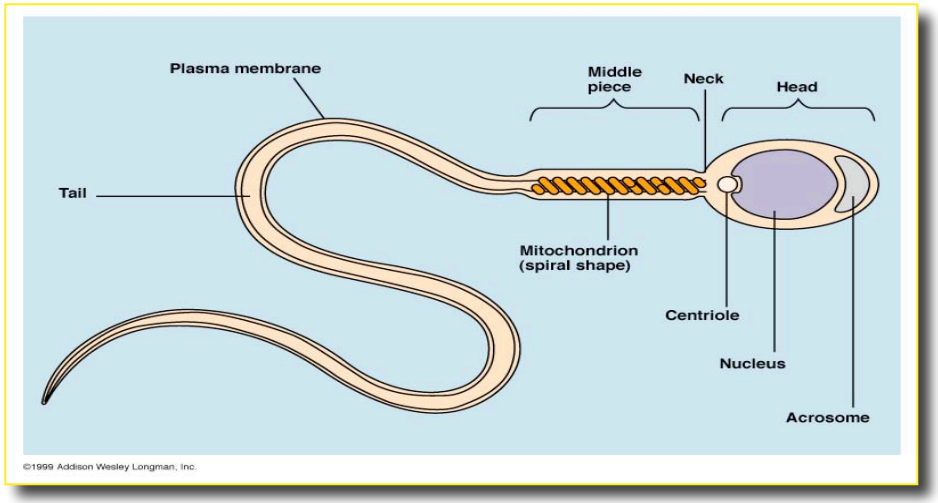
- Sperm Attraction - External
  - 1. Egg attracts sperm by Chemotaxis
  - 2. Not common phenomenon in nature
    - common in marine organisms
    - chemotaxis is species specific

# Chemotaxis

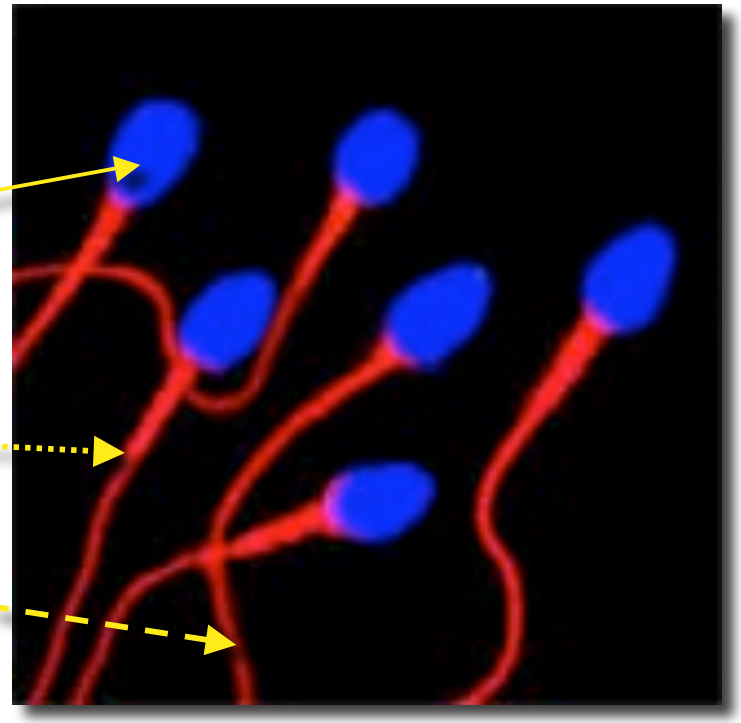
- Found in invertebrates and some fishes
- Herring
  - egg has chorion that surrounds oocyte
  - chorion has single opening - the micropyle
  - the sperm must pass thru to fertilize egg
  - c. chorion releases chemical in area of micropyle
    - herring - steroid
    - sturgeon - glycoprotein



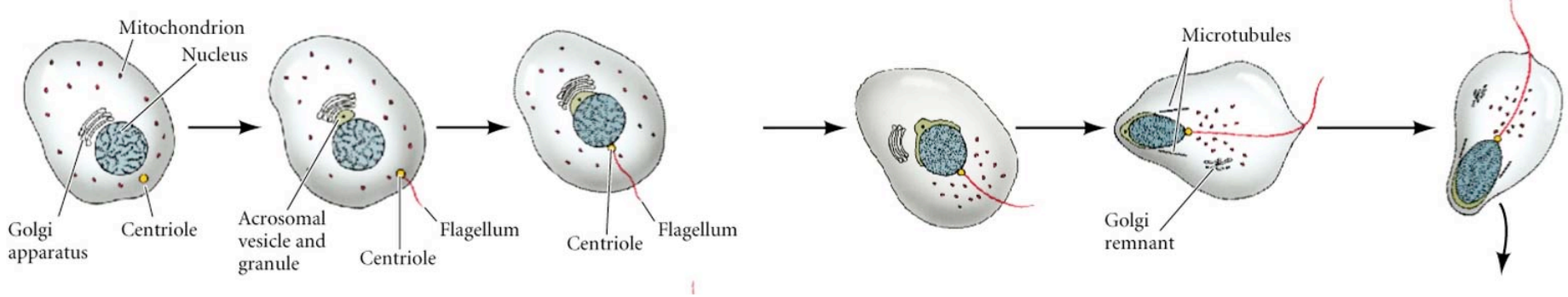
# Sperm Anatomy



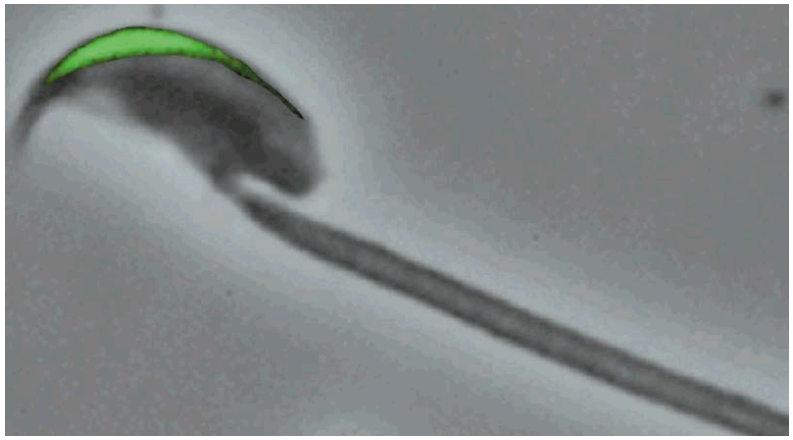

- 4 sperm/ meiotic event
- Sperm has
  - Head
  - mid piece
  - tail

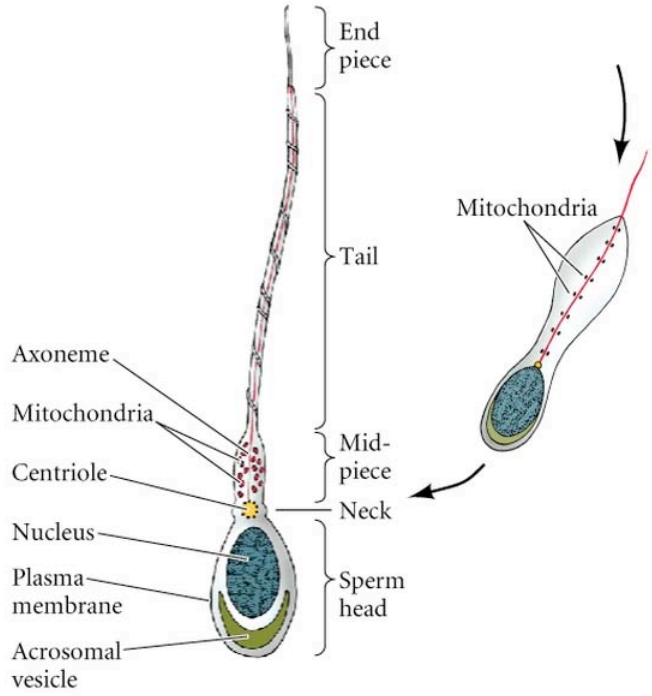


(A)

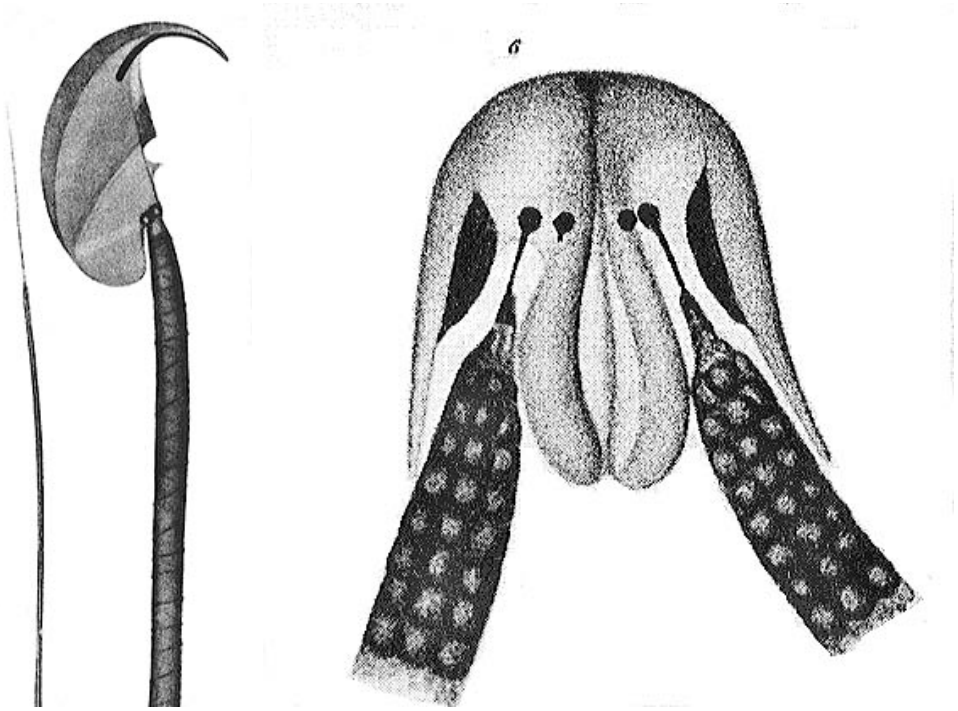






# Sperm Heads



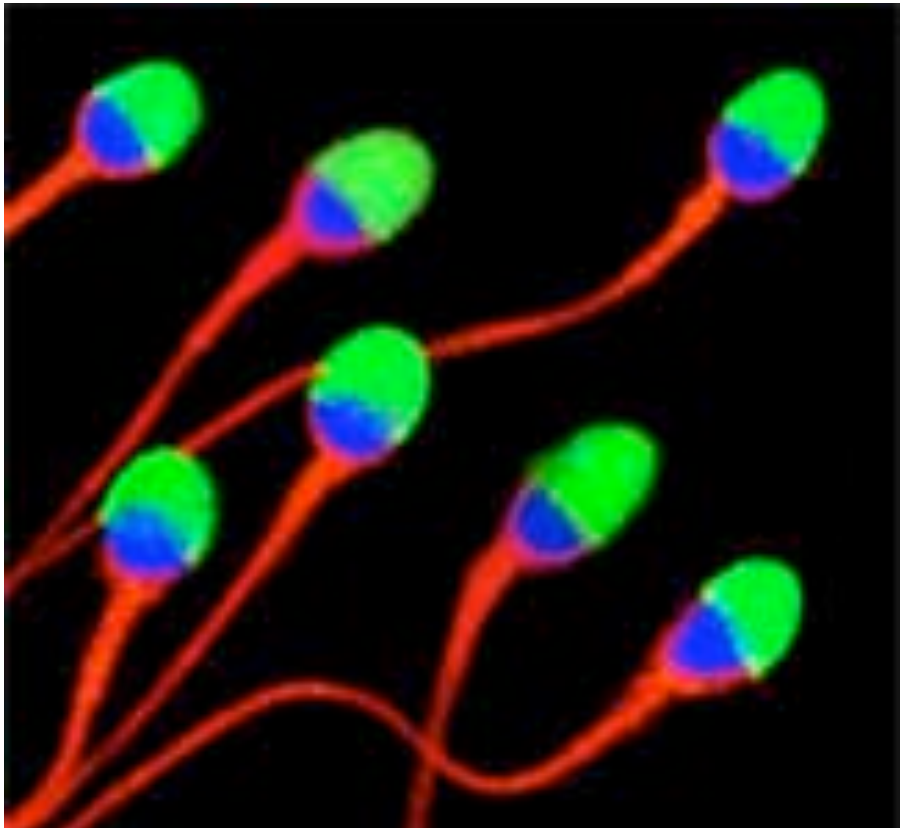
Mouse Opossum

- Vary in shape
- Contain
  - Condensed DNA
  - Acrosome
    - Highly modified lysosome
  - Proteins

# Acrosome

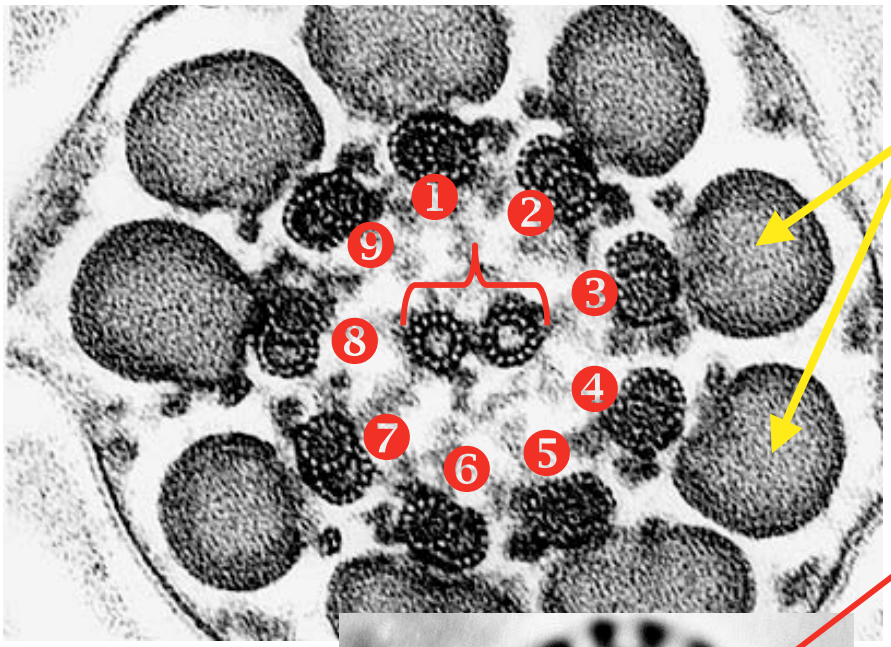
Silver nitrate stain
and Giemsa

- Modified lysosome
  - Contains proteolytic enzymes

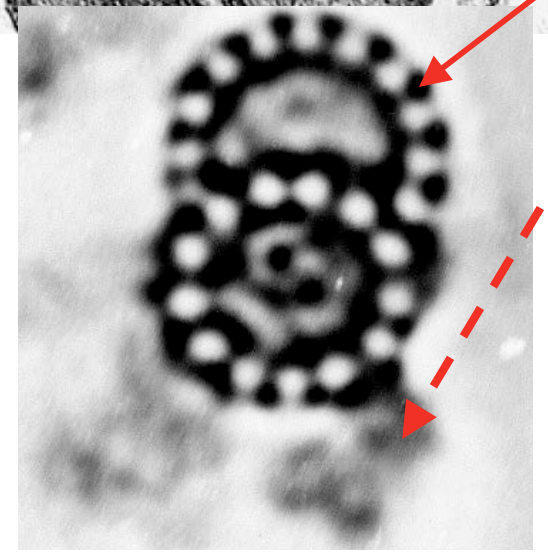


FITC lectin = green = acrosome
TOTO-3 iodide = blue = DNA
Nile red = membrane lipid = tail

# Mid Piece




Axoneme



- 'motor' of the sperm is the AXONEME
- High # mitochondria
- Microtubules in 9 + 2 arrangement
  - Sliding filament model
  - Tubules = tubulin
  - Protein arms = dynein
    - Hydrolyzes ATP
    - Kartagener triad
      - These people lack dynein
      - Males infertile
  - Histone H1
    - Stabilizes microtubules

(A) Sperm

CROSS SECTION OF
SPERM TAIL

(B) Plasma membrane

AXONEME

Radial spoke

Spoke head

Nexin

Subfiber A

Subfiber B

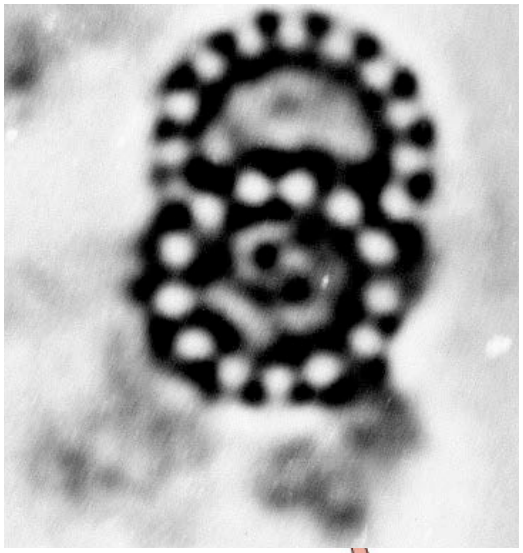
Central singlet
microtubule

Inner dynein arm

Outer dynein arm

A

B



MICROTUBULE
DOUBLET

(C)

"A"
MICROTUBULE

1 2 3 4 5 6 7 8 9 10

B

A

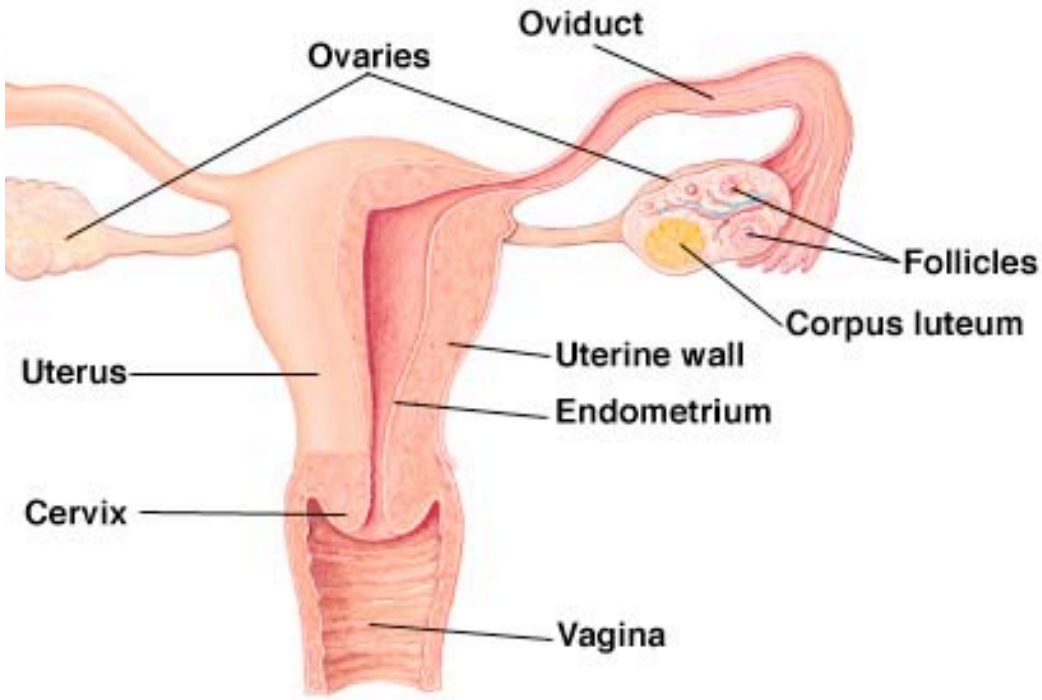
α-Tubulin monomer

β-Tubulin monomer

β α

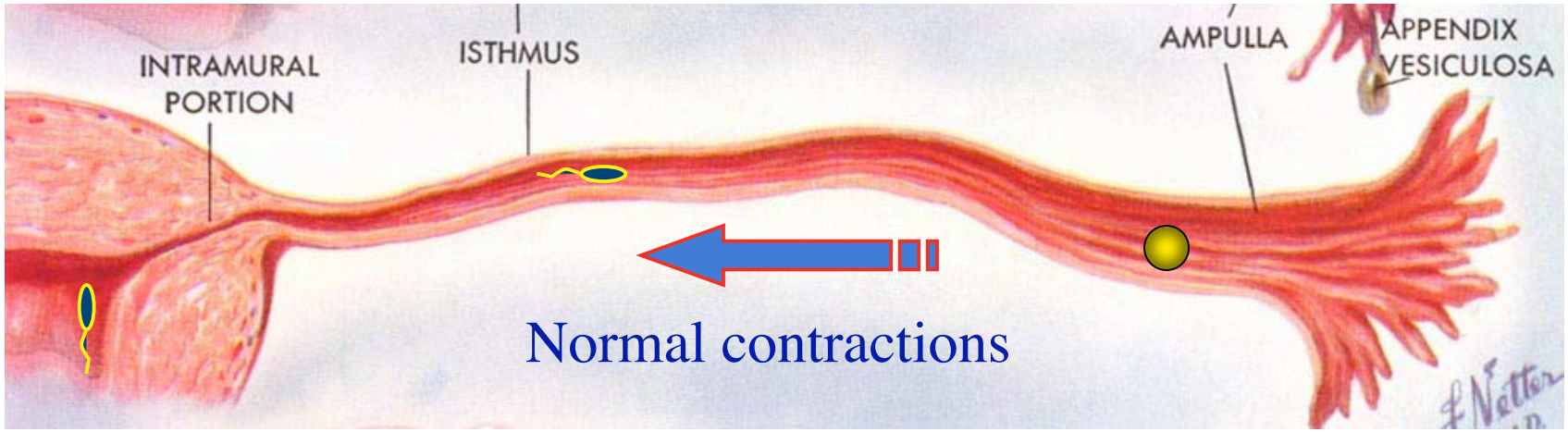
Tubulin
dimer

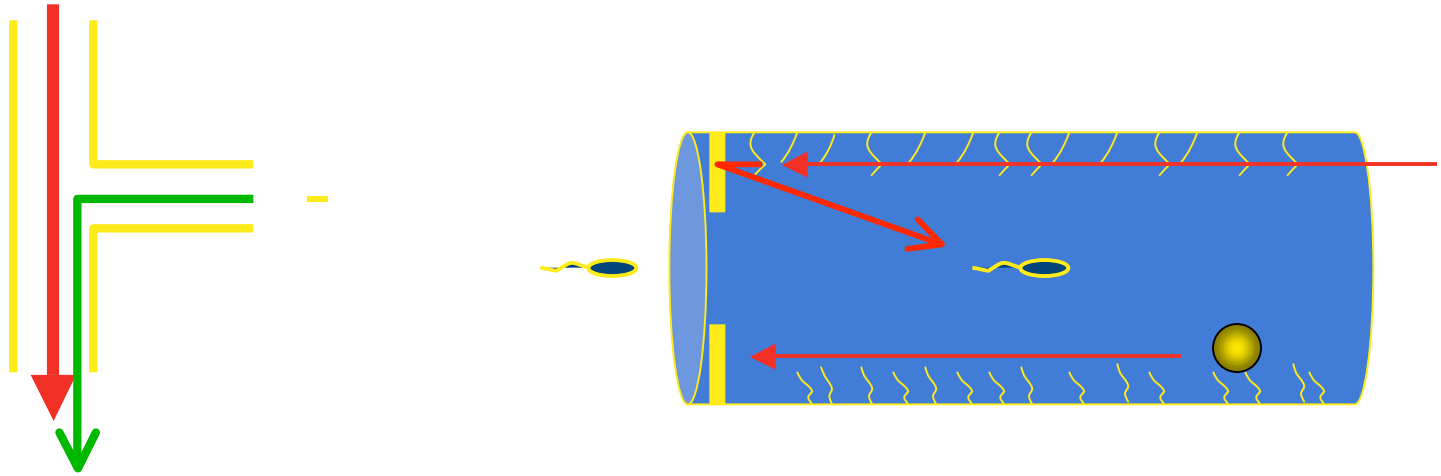
# Sperm transport




- Egg and sperm transported to site of fertilization
- Sperm do NOT get there by swimming
  - Beads of similar size as head arrive in same amount of time
  - Dead sperm do too!
  - So - how are they transported?

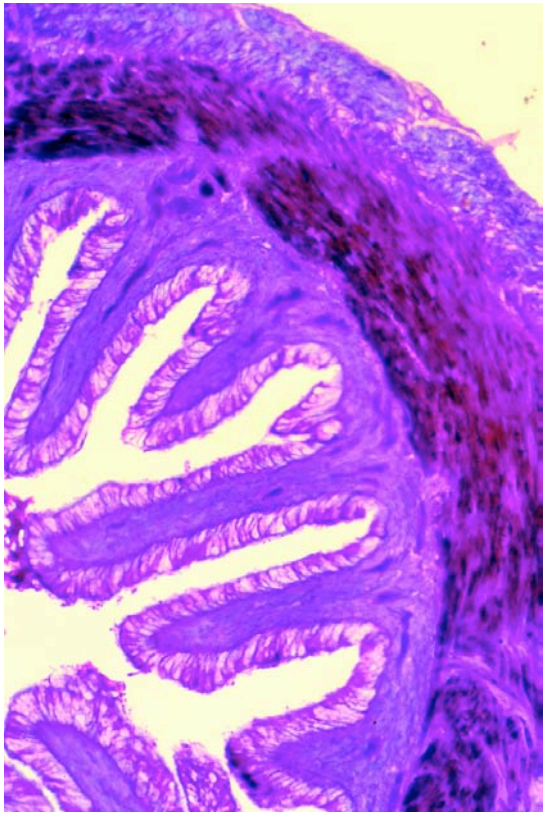
# Venturi Effect



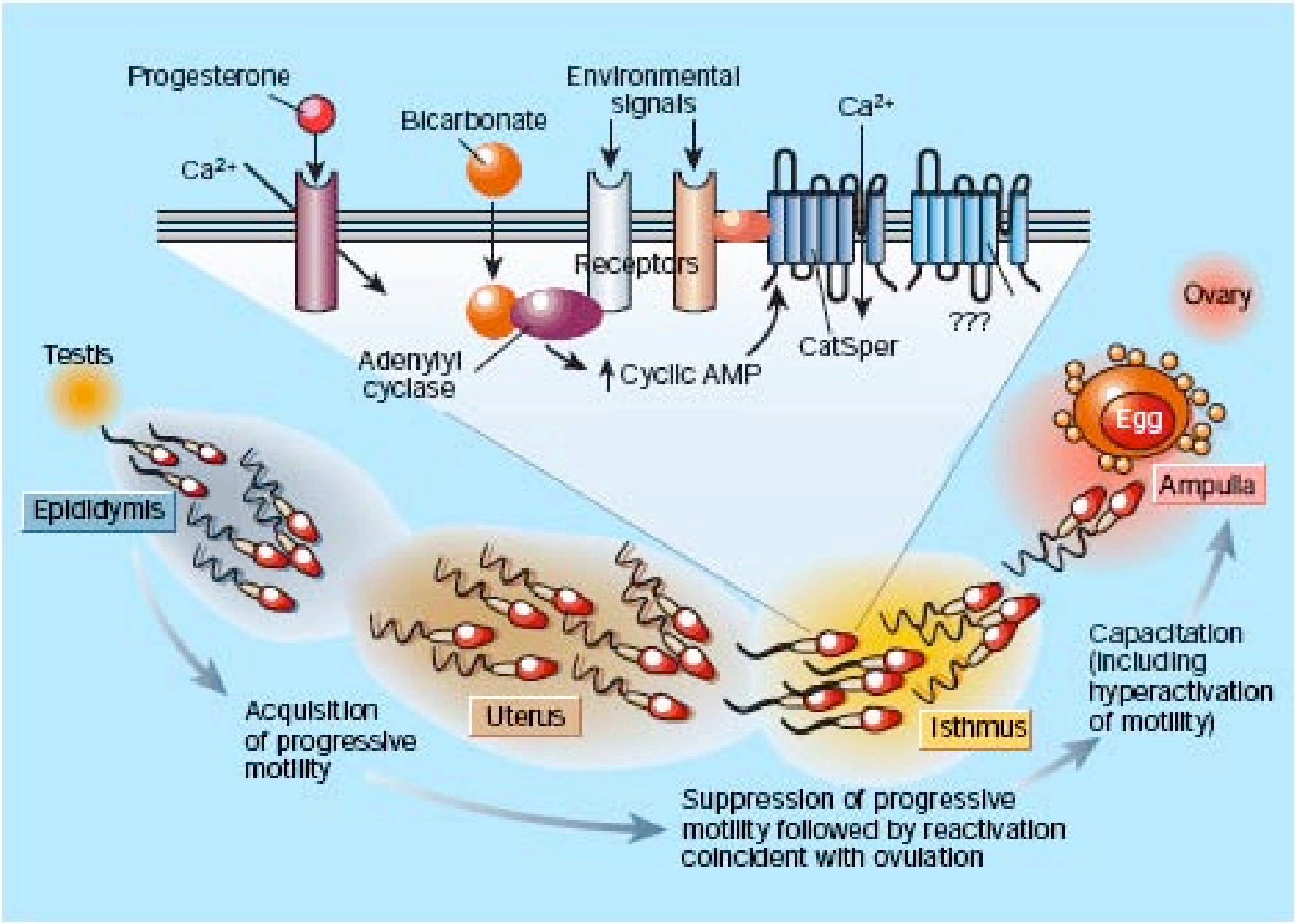
# Why Swim?

- Swimming seems to keep sperm up in 'current'
  - If they don't swim many attach to epithelium
  - Don't make it thru cervical mucus
- Swimming allows them to penetrate the zona & cumulus
  - CatSper- mice sperm can only fertilize eggs with cellular matrix removed



# To Swim or Not to Swim

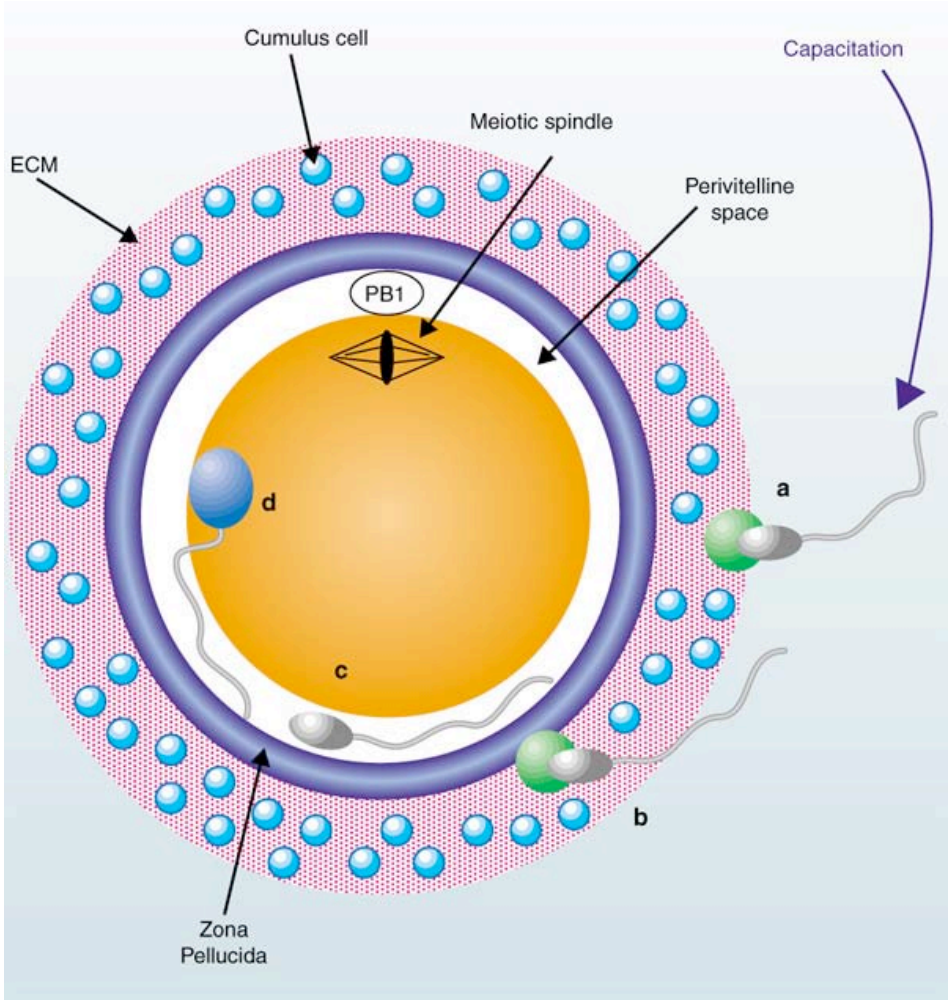
- Sperm can not 'swim' as they leave the testis - they are immobile
- Acquire 'progressive motility' in the epididymis
  - Don't move in epididymis
  - Swim with ejaculation
  - Requires $Ca^{++}$ to function
  - Requires CatSper membrane channel
    - CatSper-deficient mice sperm swim 1/3 of normal




# CatSper and other ion channel(s)? regulate sperm motility

Garbers Nature 413:580 (2001)

# Capacitation and Acrosome Reaction



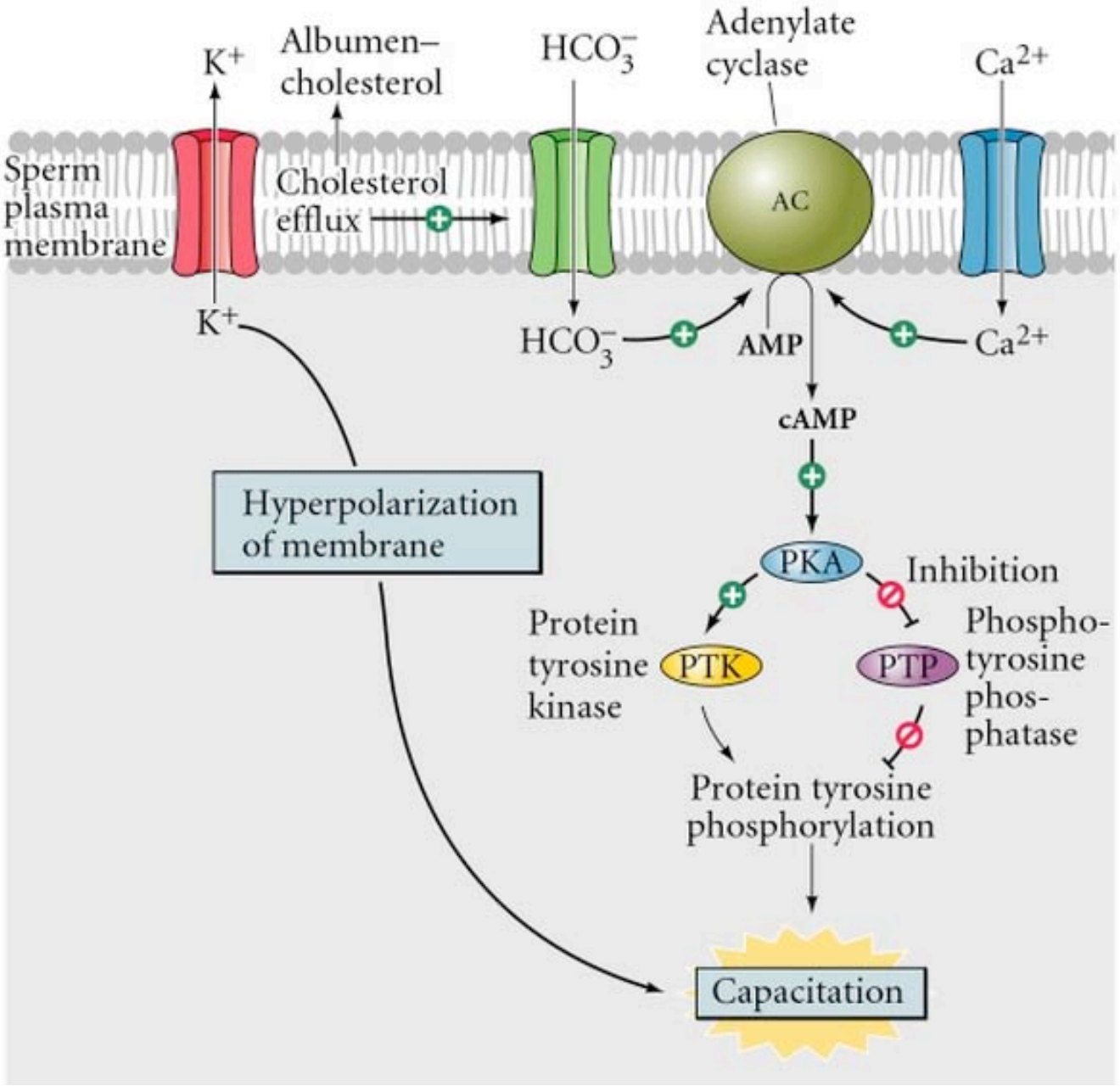
# Capacitation

- newly ejaculated sperm can not undergo acrosome reaction required for fertilization
  - maturational process in female tract called CAPACITATION
  - requirements vary among species

# Observations

- Capacitation is a change in the makeup of the sperm membrane
  - Observed decrease in cholesterol / phospholipid ratio
    - Cholesterol efflux
    - Removal of cholesterol by sterol-binding proteins
  - Array of sperm membrane proteins phosphorylated
    - Tyrosine phosphorylated - cAMP dependent
    - Adenylyl cyclase bicarbonate sensitive
  - Elevation in intracellular pH and bicarbonate conc
  - Removal of a number of glucosylphosphatidylinositol (GPI) – anchored proteins

Evans and Florman Nature 2002


K+
Albumen–
cholesterol
HCO3−
Adenylate
cyclase
Ca2+
Sperm
plasma
membrane
Cholesterol
efflux
AC
K+
HCO3−
AMP
Ca2+
cAMP
Hyperpolarization
of membrane
PKA
Inhibition
Protein
tyrosine
kinase
PTK
PTP
Phospho-
tyrosine
phos-
phatase
Protein tyrosine
phosphorylation
Capacitation

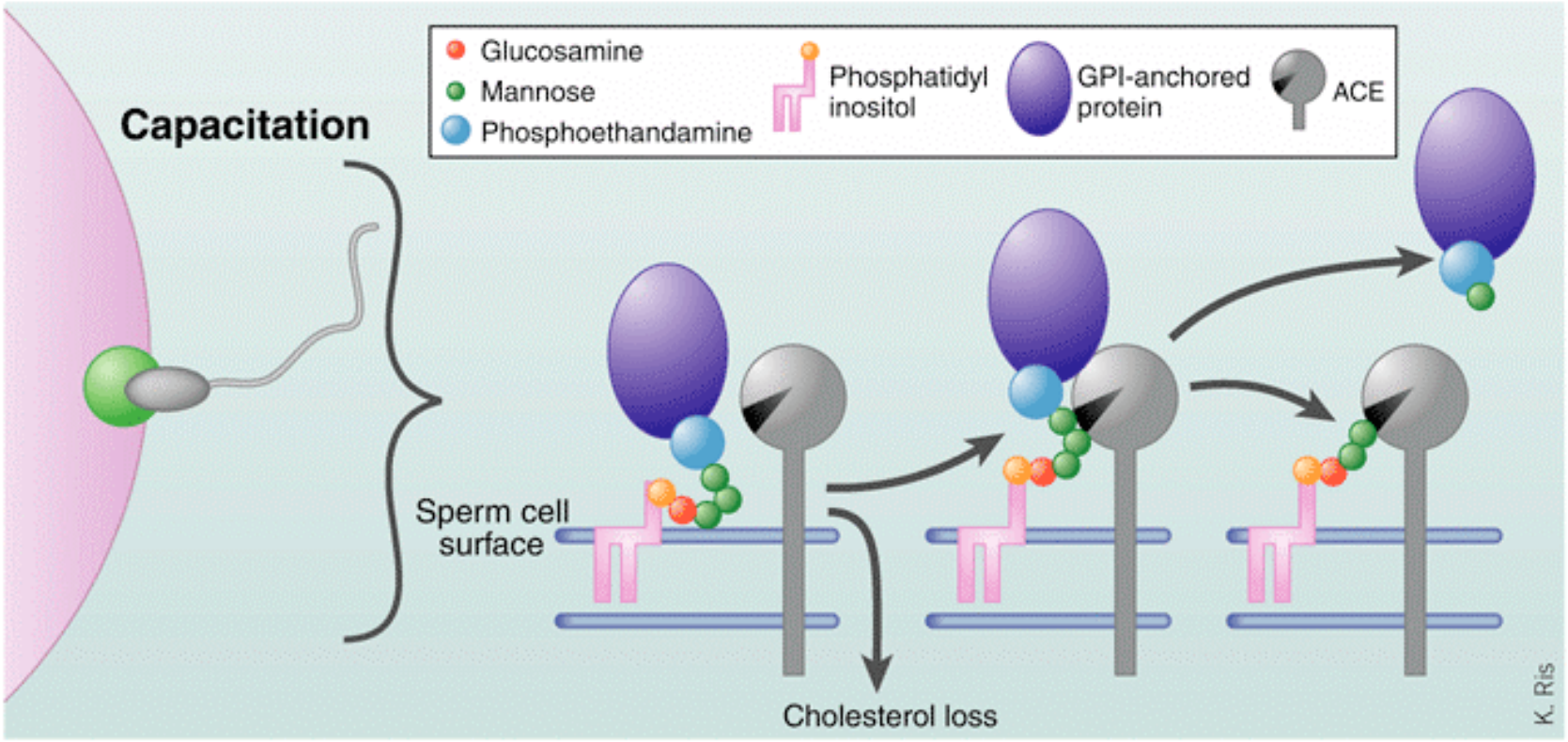
# Angiotensin-converting enzyme (ACE)

- Key regulator of blood pressure
  - Cleaves small peptides
    - Angiotensin I & bradykinin
  - peptidase
- Two forms
  - Somatic (150-180 kDa)
  - Testicular (100-110 kDa)

# ACE in the Testes

- ACE has GPI-anchored protein releasing activity
  - Mammals
    - >200 cell surface proteins with various functions
    - Anchored to membrane by covalently bonded GPI moiety
    - GPI = glycosylphosphatidylinositol
  - ACE has GPIase
    - (GPI-anchored protein releasing activity)

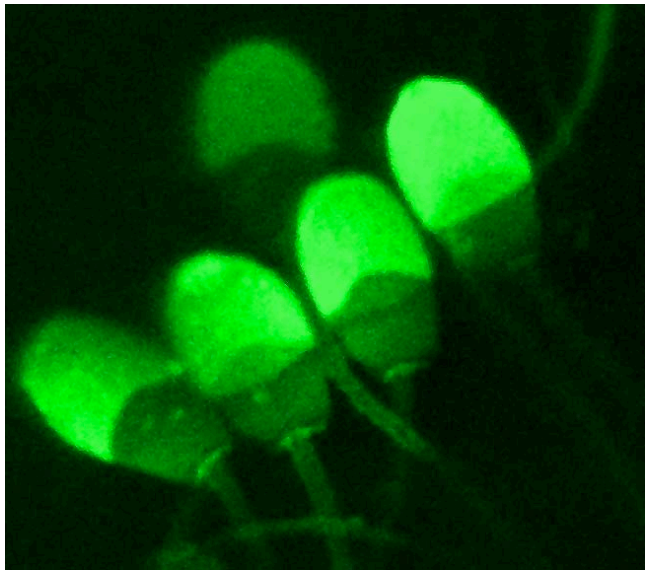
# ACE and Capacitation




Mayor Nature Struc Molec Bio 12:107 (2005)

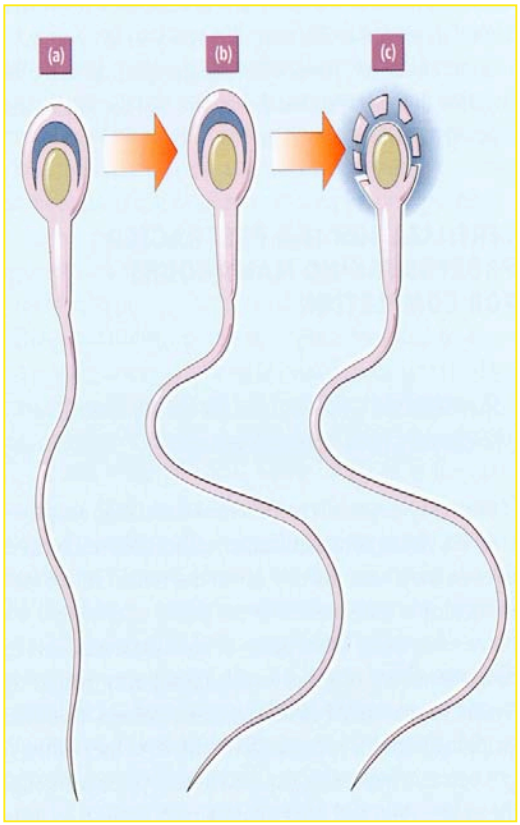
# Acrosome Reaction

- 2nd in series of egg/sperm interactions
- in most organisms
- - two components
  - a. acrosomal vesicle rupture
  - b. extension of acrosomal process



# Acrosome Reaction

- acrosome reaction activated in invertebrates or in vitro by:
  - a. soluble egg jelly or uterine factor
  - b. actual contact with egg plasma membrane
  - c. increasing Ca++ concentration of seawater

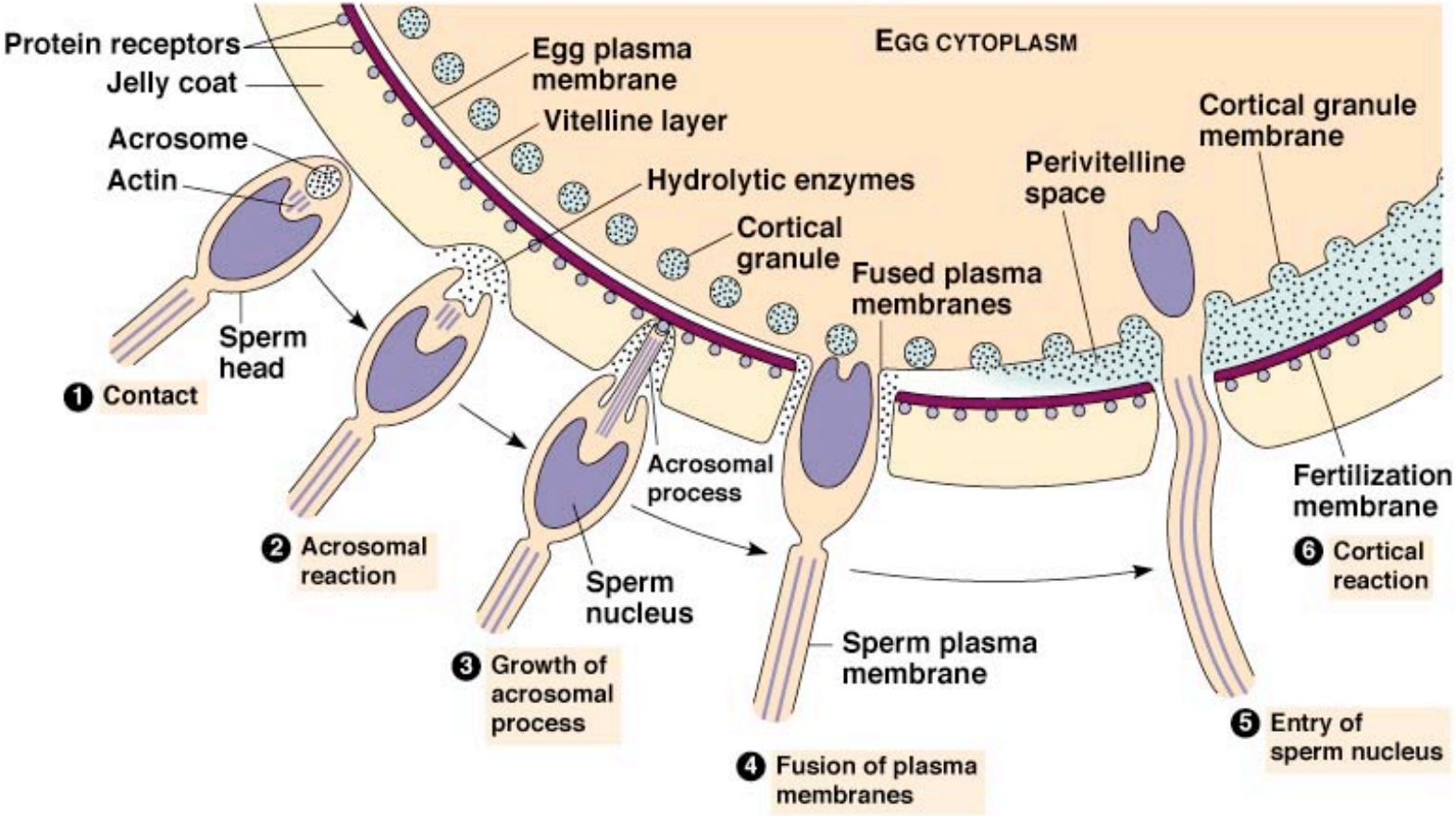


# Acrosome Sequence

- sequence of events in a sea urchin
  - a. contact with jelly
  - b. influx of Ca++
  - c1. exocytosis of acrosomal vesicle
  - d1. release of lytic enzymes and exposure of bindins
  - c2. efflux of H+ and influx of Na+
  - d2. intracellular increase in pH
  - e2. actin polymerization
  - f2. extension of acrosomal process

# Fertilization

# Acrosome Reaction



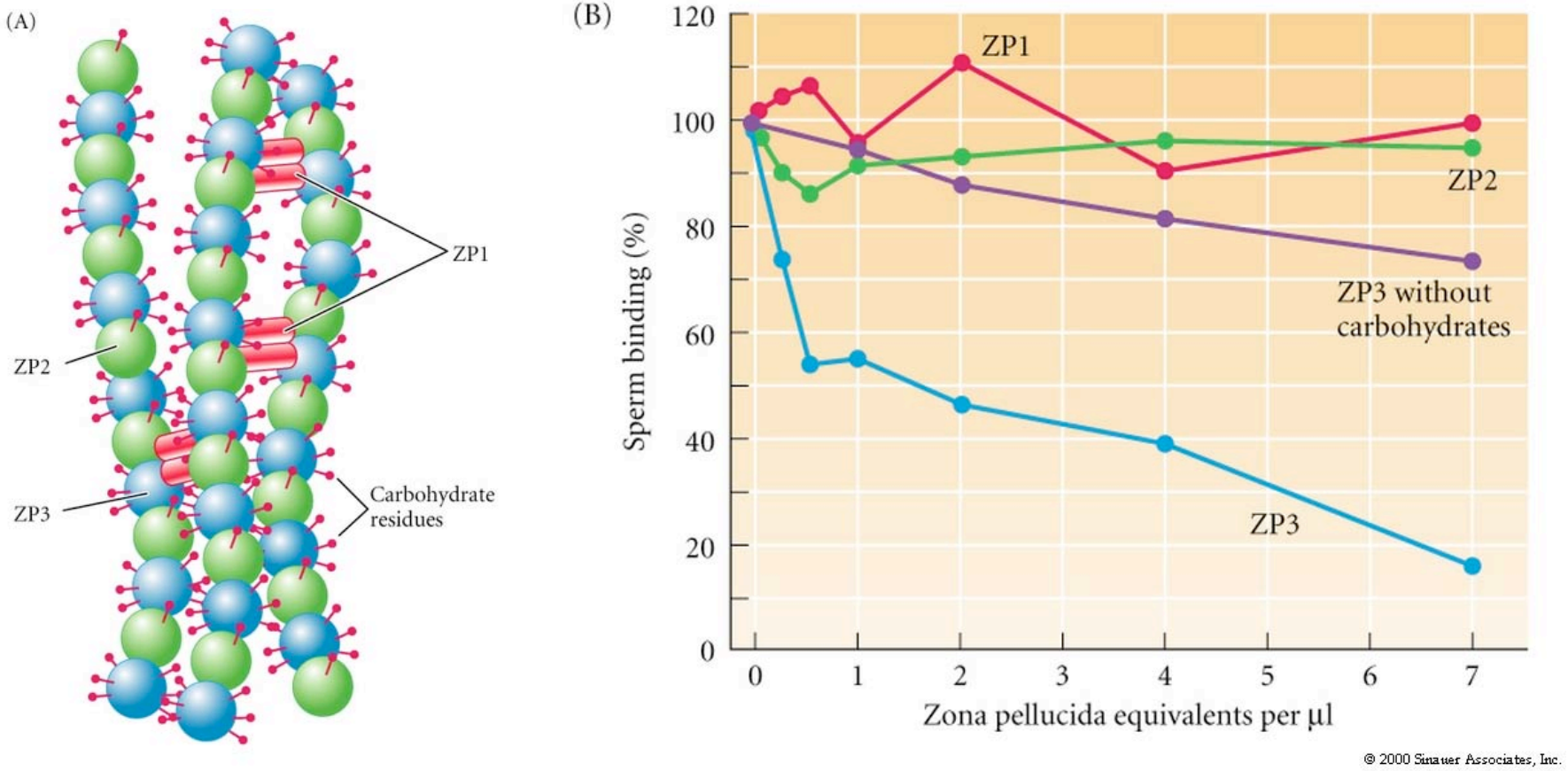
# Mammals

- in mammals capacitation prerequisite step for sperm binding to zona pellucida and acrosome reaction

# Mouse

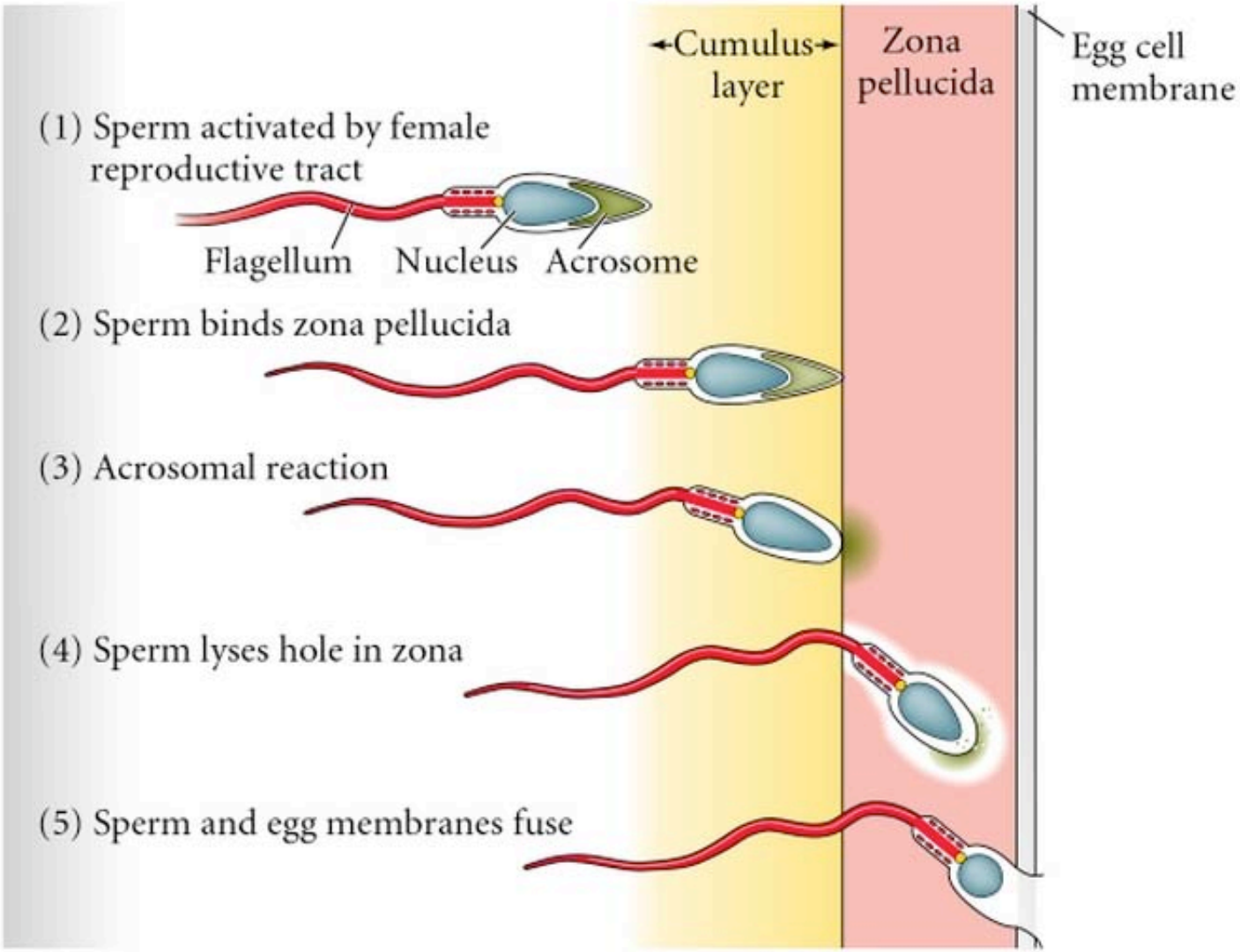
- acrosome reaction occurs after binding of sperm and egg
  - a. sperm binds with zona pellucida (ZP)
  - b. ZP composed of 3 proteins (ZP1, ZP2, ZP3)
  - c. ZP has specific protein that binds to ZP3 receptor on sperm
    - ZP3 - 83kD glycoprotein
      - initiates acrosome reaction
      - has lysins as described above

# ZP3 & Sperm Binding




Bliel and Wassarman, 1980; Florman and Wassarman, 1985

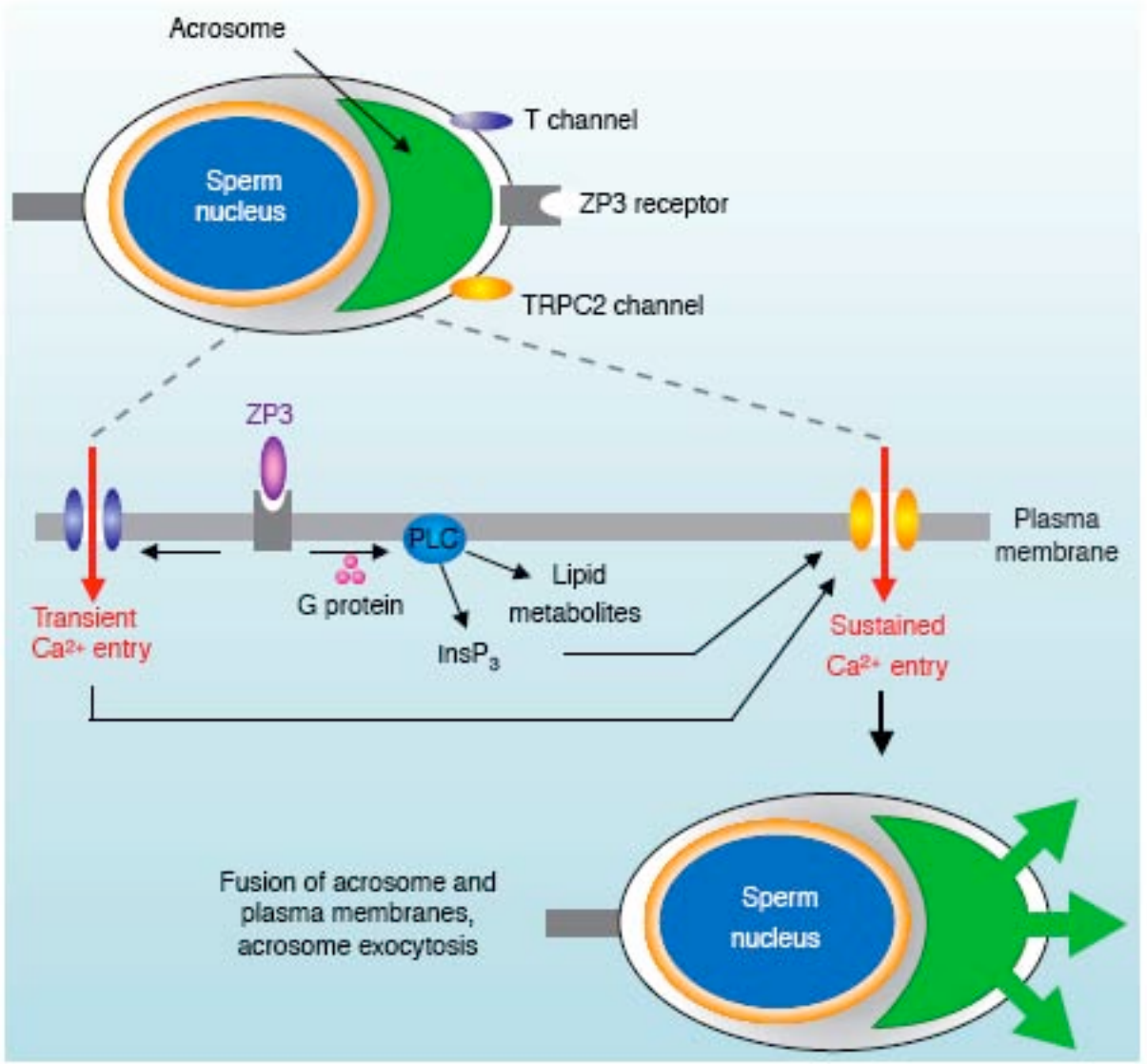
(B) MOUSE



# Recognition in Mammalian Sperm

- a. Sperm adhesion to the ZP based on protein-carbohydrate recognition
- b. N-acetylglalactosaminyl linkages from ZP3 with sperm receptor
- c. Sperm receptor still being characterized
- d. detected in
  - mouse, G. pig and human to date
- e. binding to ZP3 triggers acrosome reaction
  - Opens T-type low voltage activated $Ca^{++}$ channels
  - Activates G proteins which induces sustained Ca influx and exocytosis

# Acrosome Reaction



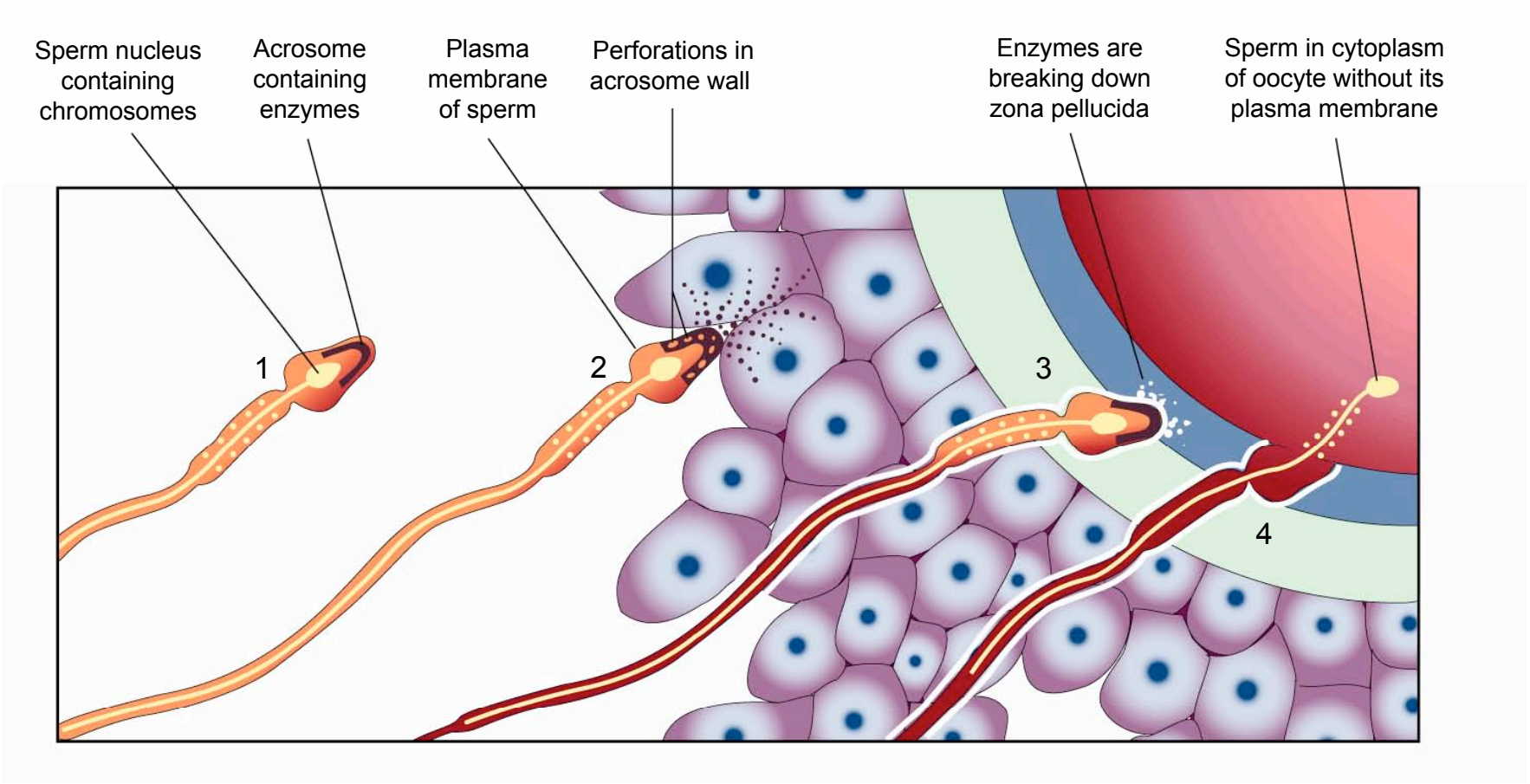

Evans and Florman Nature 2002

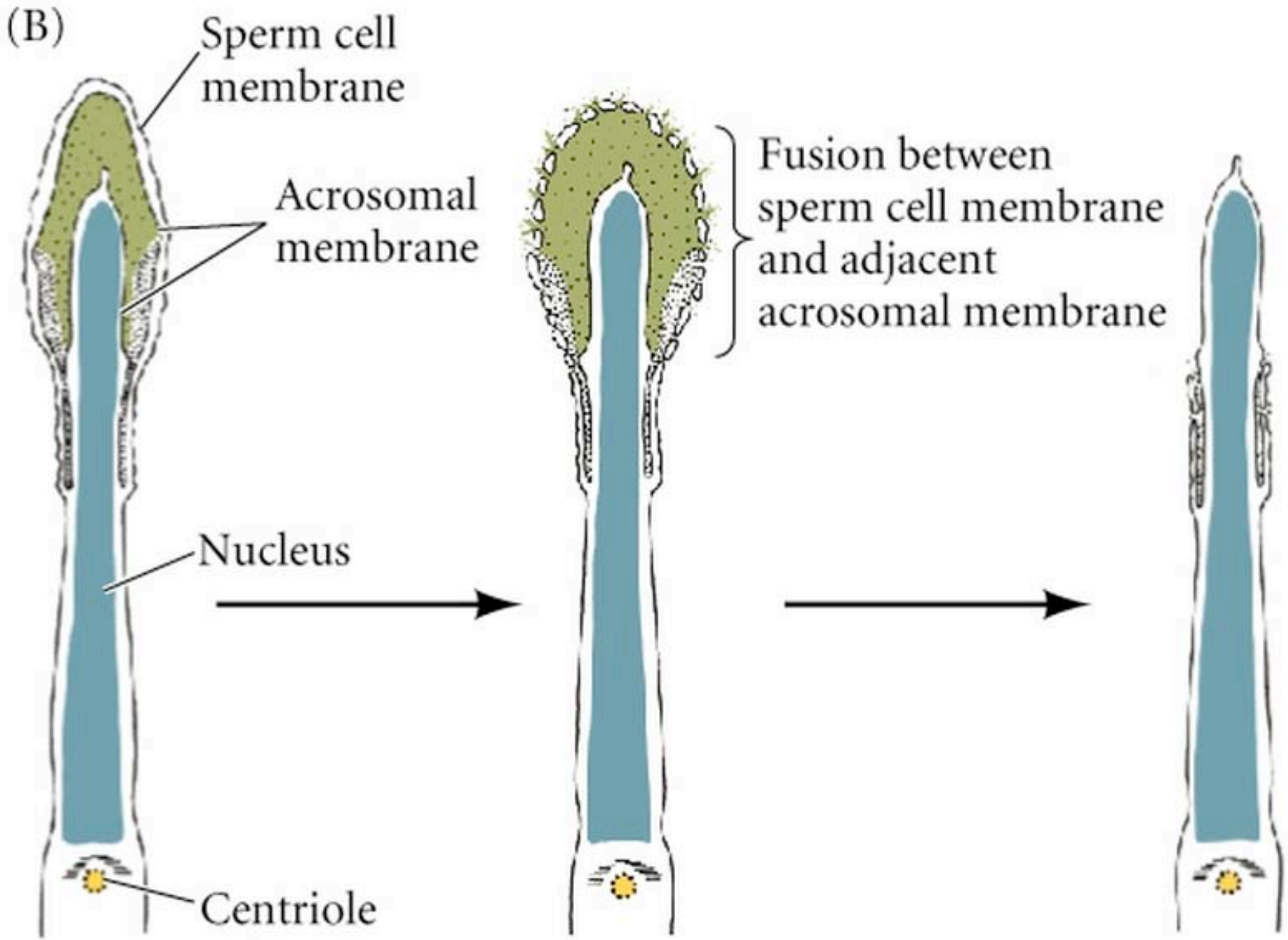
Nature Cell Biology & Nature Medicine www.nature.com/fertility

# Human

- acrosome reaction begins distant from egg at corona radiata
- sperm releases enzymes:
  - Hyaluronidase
    - hydrolyzes hyaluronic acid of extracellular matrix
  - Corona-dispersing enzyme
  - Acrosin
    - AA composition like trypsin
    - must be activated to work by glycoprotien in female reproductive tract

# Human Acrosome Reaction

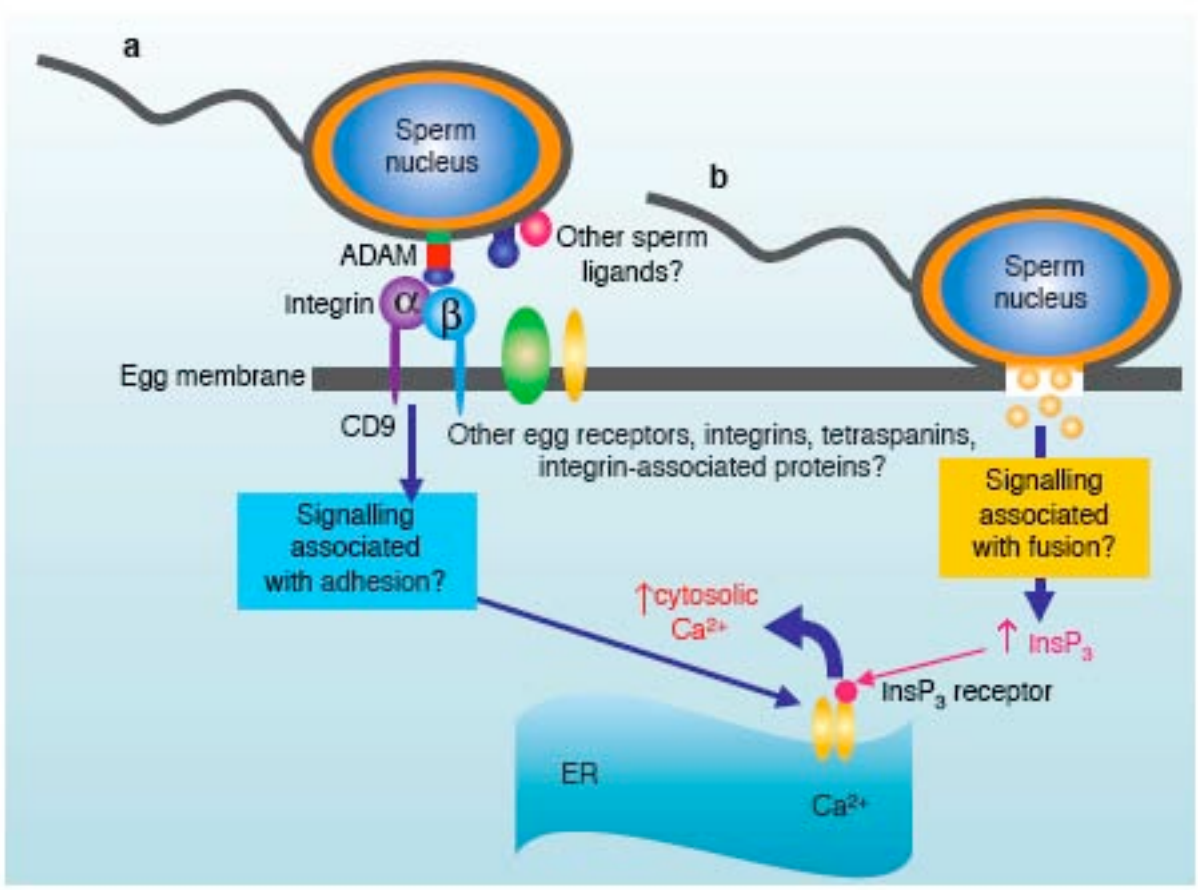




# Sperm Egg Adhesion

- After penetration of ZP
  - Sperm adhere to and fuse with egg plasma membrane
  - Involves proteins on sperm
    - Fertilin-$\alpha$
      - disintegrin and metalloprotease domain 1 or ADAM1
    - Fertilin-$\beta$ (ADAM2)
    - cyritestin (ADAM3)
    - CRISP1 (cysteine-rich secretory protein 1)
  - All demonstrated important by antobody knockout studies
  - Interact with oocyte membrane integrin family proteins

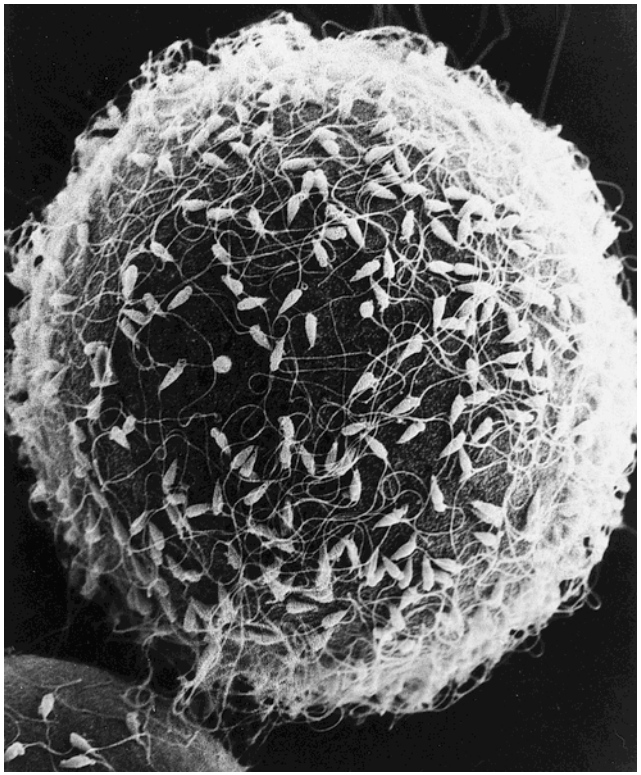
# Sperm-Egg Adhesion




Evans and Florman Nature 2002

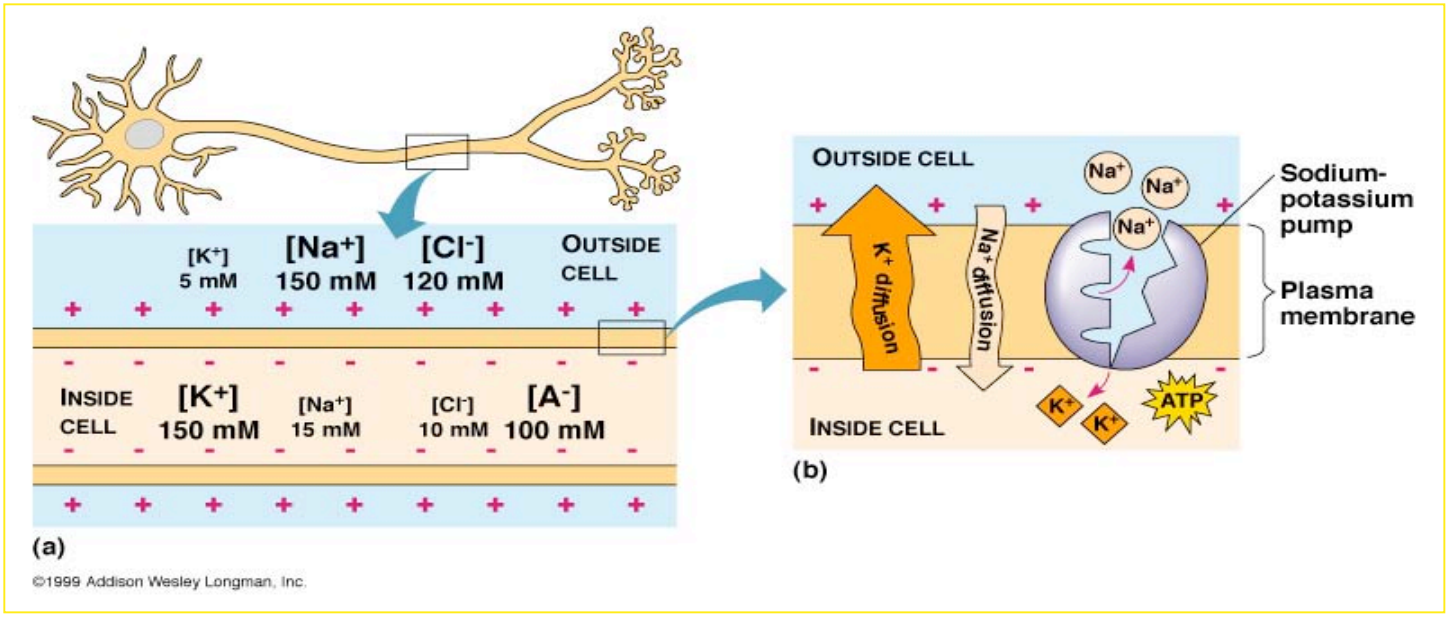
# Polyspermy

- Want only one sperm to bind with egg
  - Most species
- But…..
  - 100s will be in area around egg
  - How do you block polyspermy?



# Blocking Polyspermy

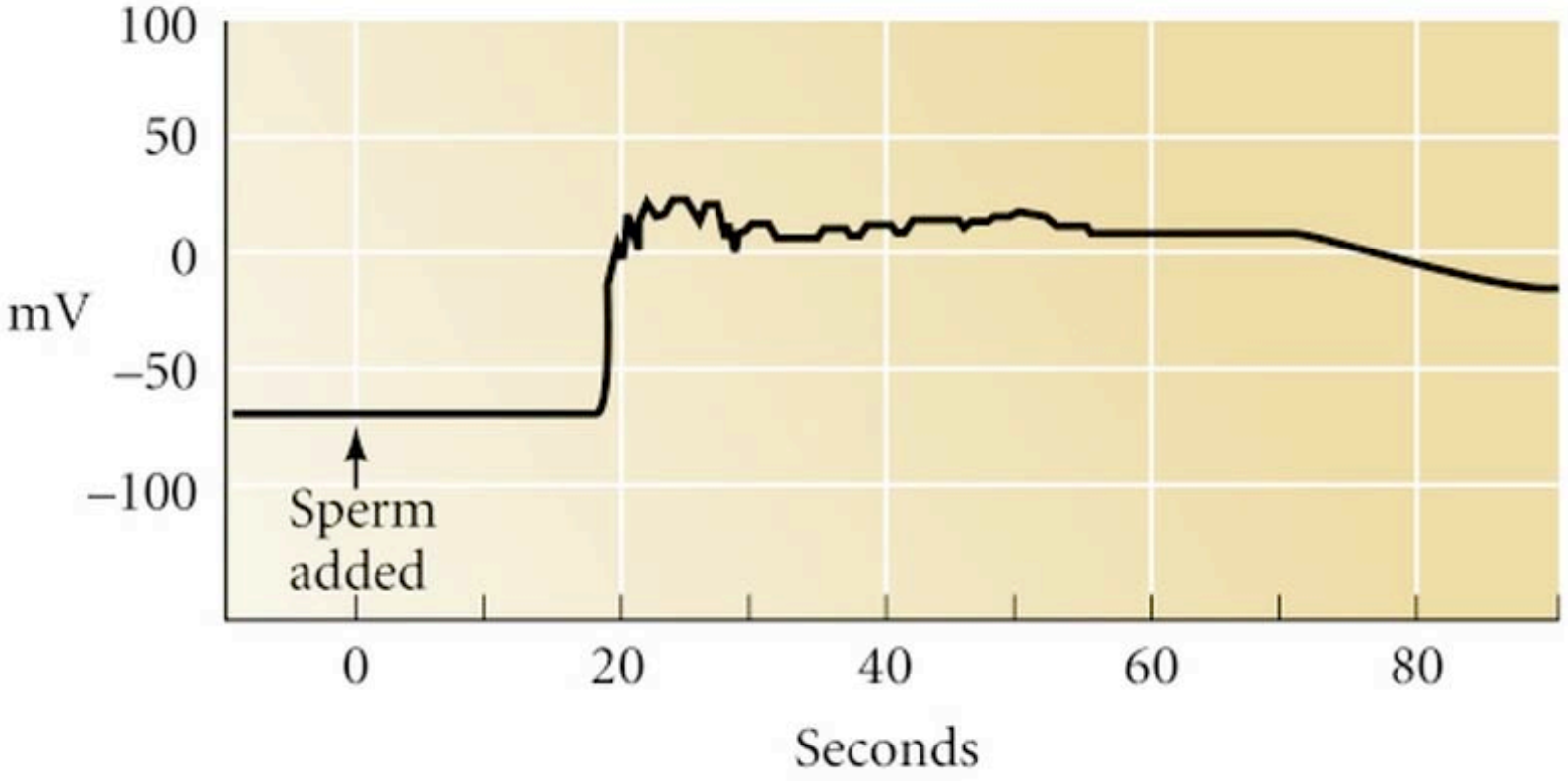
- Fast Block
  - Involves changes in oocyte membrane polarity
  - Normal resting potential: -70mV



# Fast Block

- Fusion of sperm and egg ephemeral change in ion permeability (< 1min)
- Causes influx of $Na^+$ ions
- Loss of electrical potential
  - Can change from -70mV to +20 mV
- -10mV required for sperm/oocyte binding
- Thus - first sperm binds - others don't
- Effect lasts less than 1 min to several mins

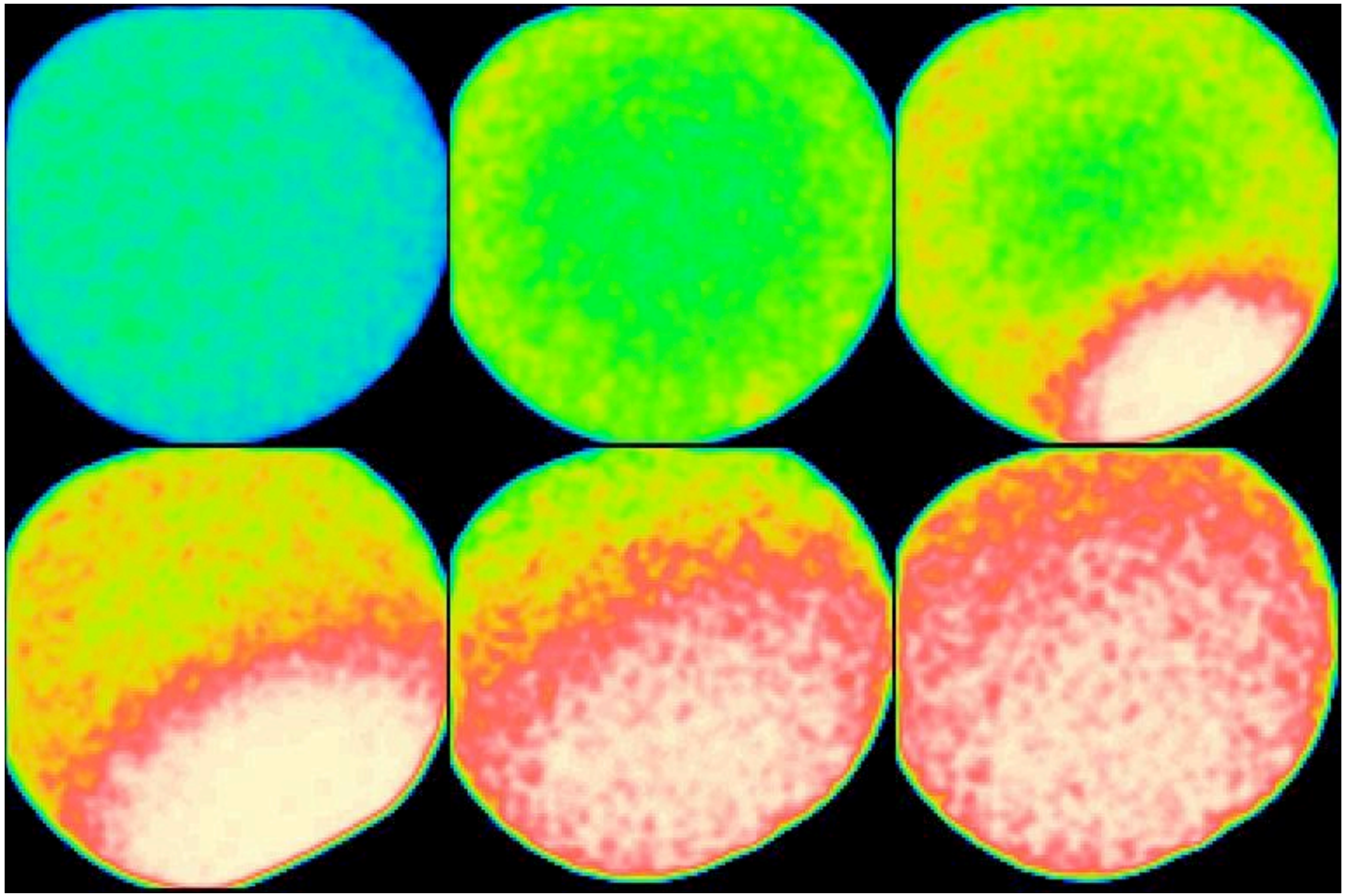
# Membrane Potential Change



# Slow Block

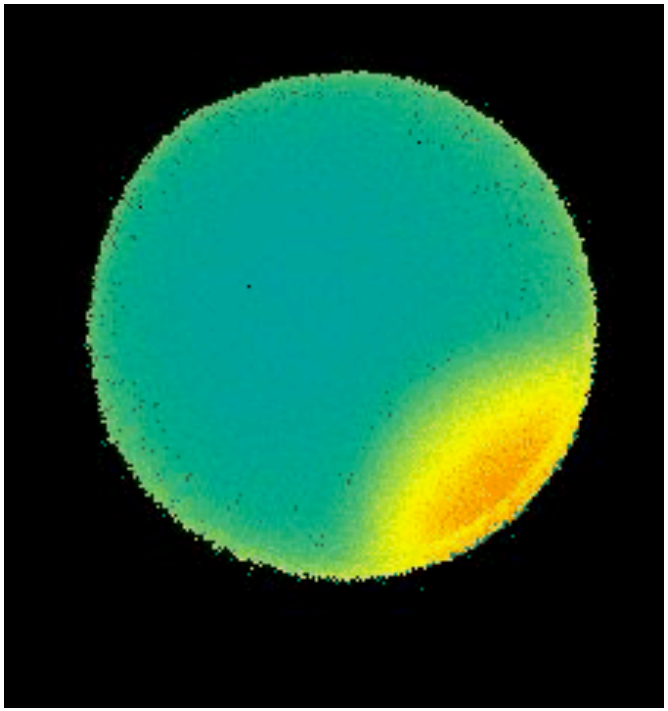
- Change in ion potential activates oocyte
  - Cytoplasmic calcium wave triggered
  - $Ca^{++}$ released from endoplasmic reticulum
- $Ca^{++}$ ions initiate cortical granules release
  - Homologous to acrosome in sperm
  - Contain glycosaminoglycans (GAGs) and proteases
  - 1000s in cortex of cytoplasm of egg in many species
  - $Ca^{++}$ initiates exocytosis

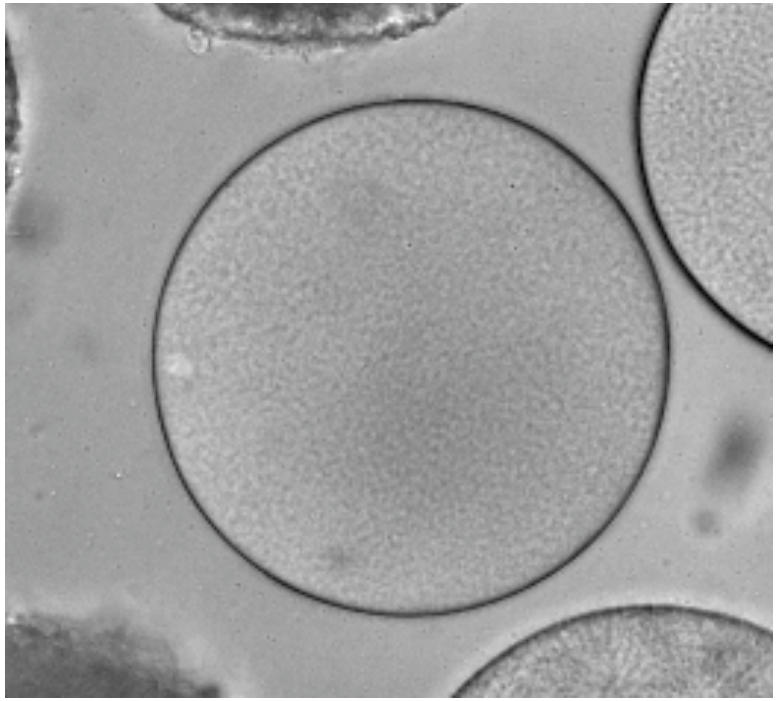
# Calcium Wave with Fertilization



http://petrus.ncl.ac.uk/

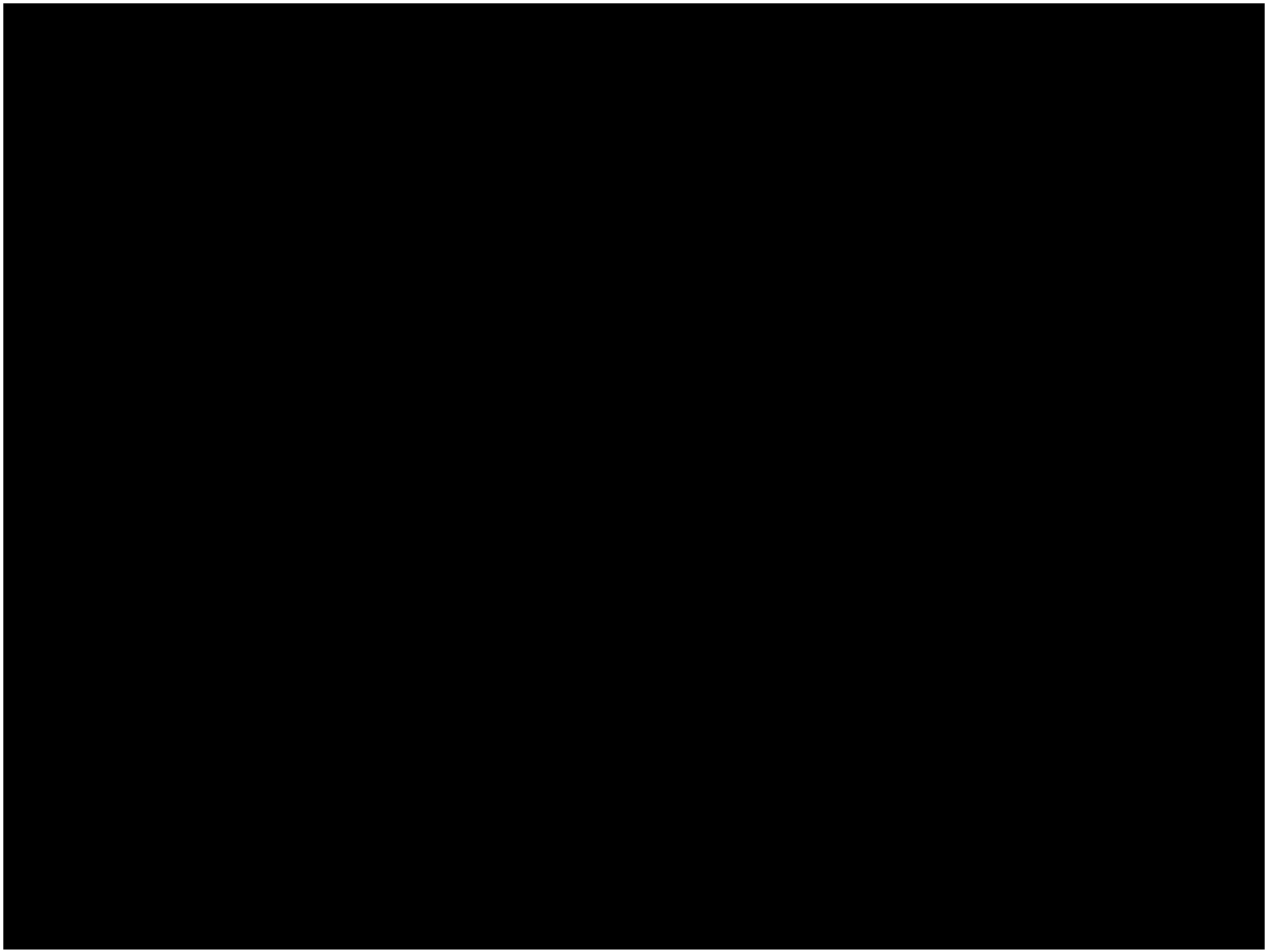
# Calcium Wave - Constriction



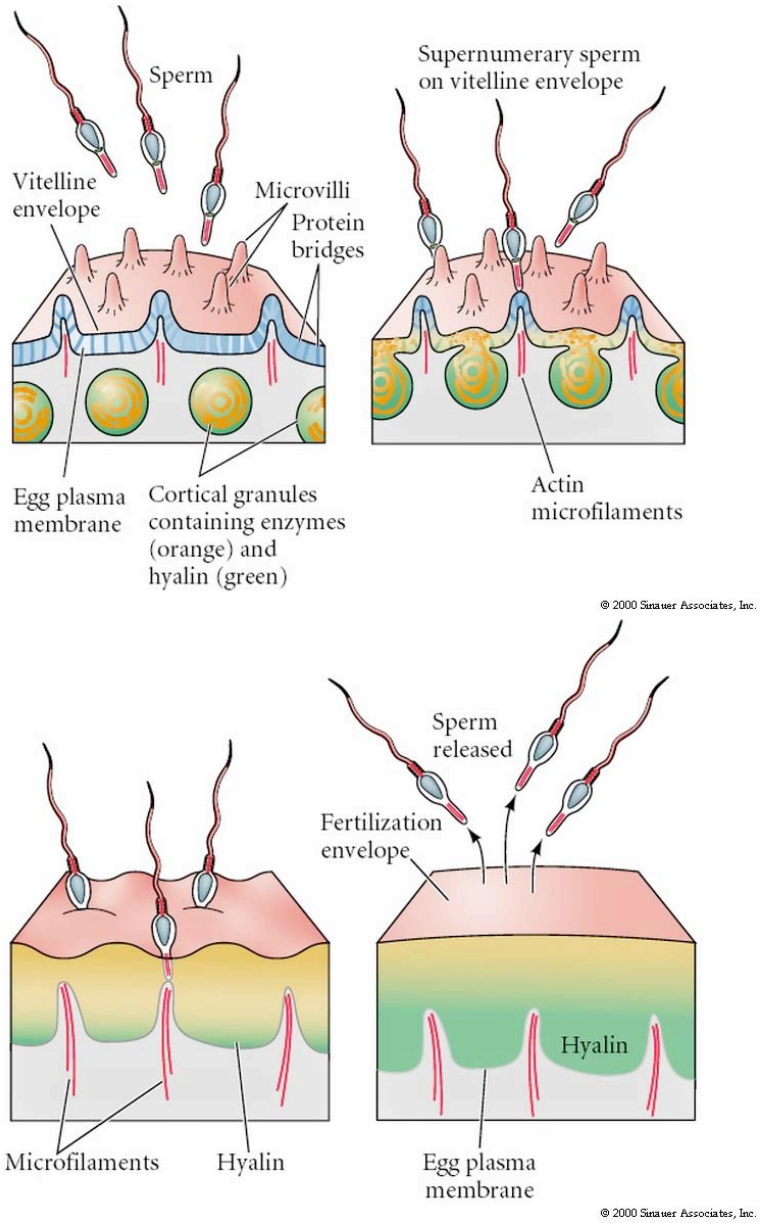


biodev.obs-vlfr.fr/recherche/dougall/

# Calcium and Fertilization

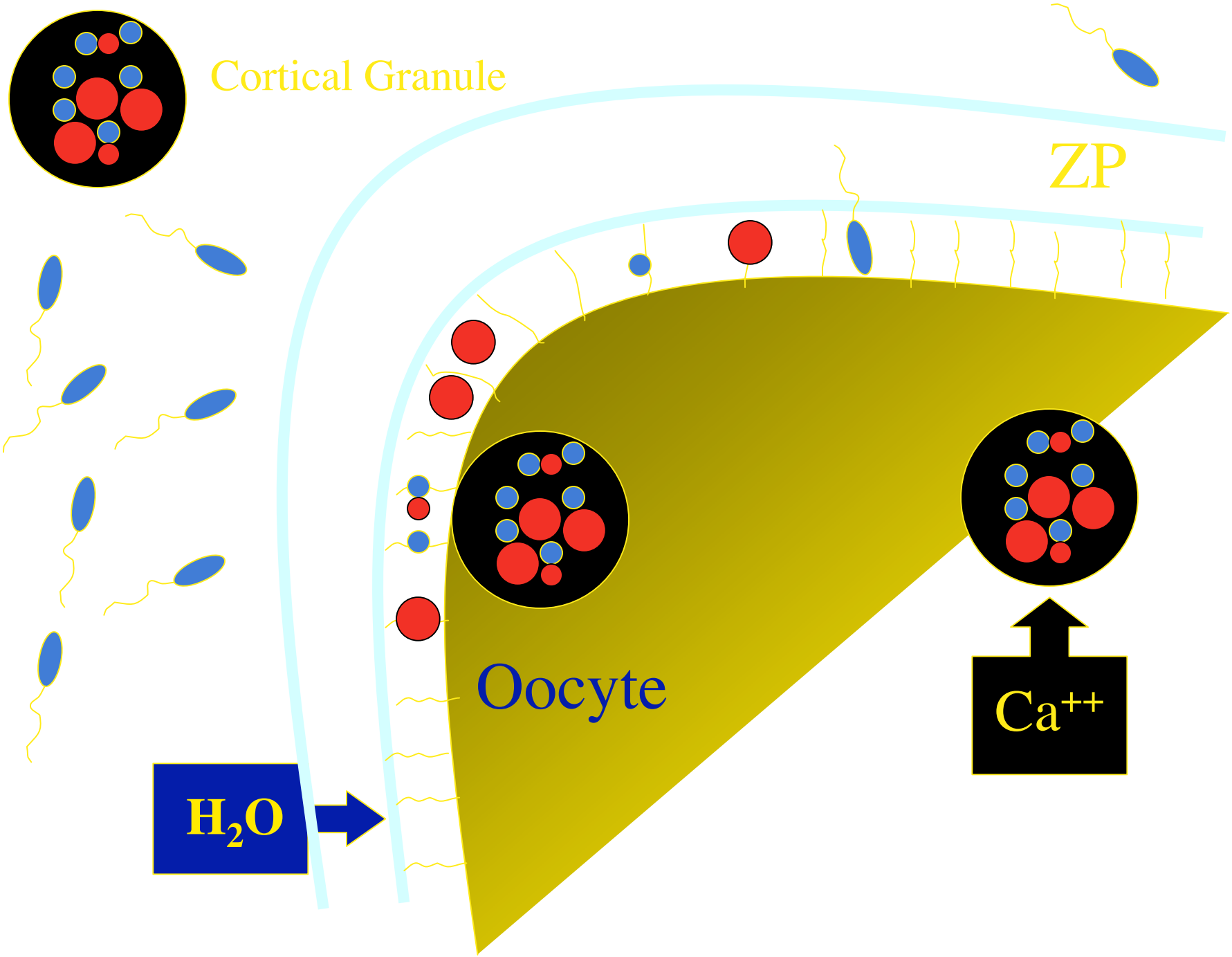


# Cortical Granule Reaction



# Cortical Granules Response

- $Ca^{++}$ stimulates exocytosis
- Proteases dissolve connection between ZP and oocyte membrane
- Other enzymes 'harden' ZP
  - Tyrosine-tyrosine cross linkages
- GAGs increase osmolarity of space between oocyte and ZP
  - Water floods into space raising ZP off oocyte


Cortical Granule
ZP
Oocyte
$H_2O$
$Ca^{++}$

# Natural Polyspermy

- Some species with large ova have physiological polyspermy
  - Chondrichthyans
  - Urodeles
  - Reptiles
  - Birds
- All but one sperm pronucleus blocked from fusing with female pronucleus

# First Mitosis - Zygote