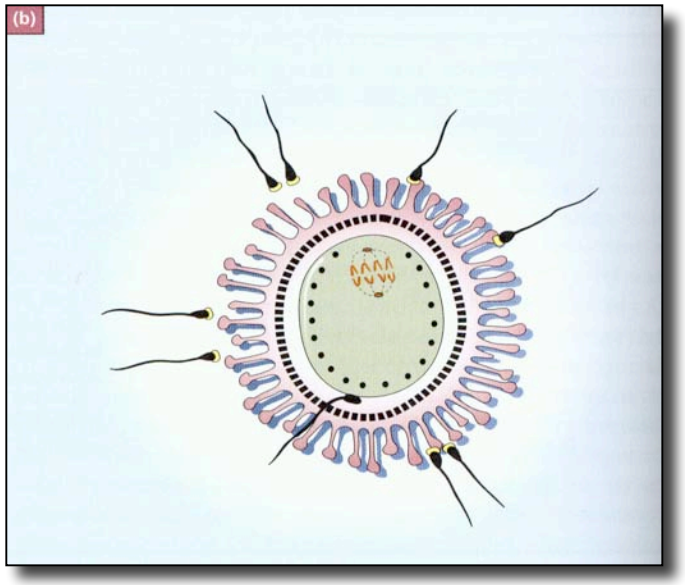
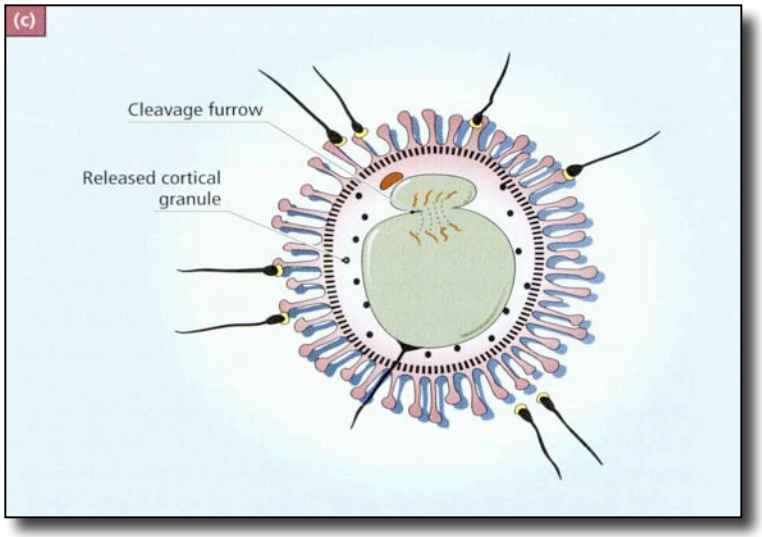
Fertilization

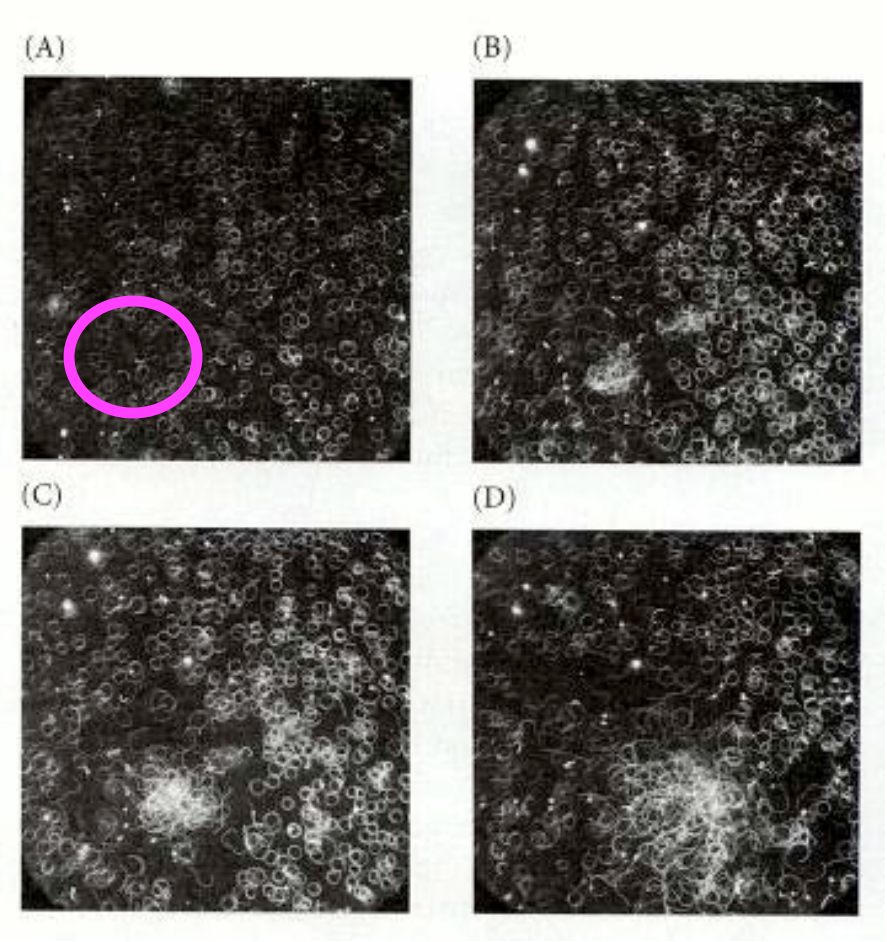


1. Chemical signaling
2. Sperm transport



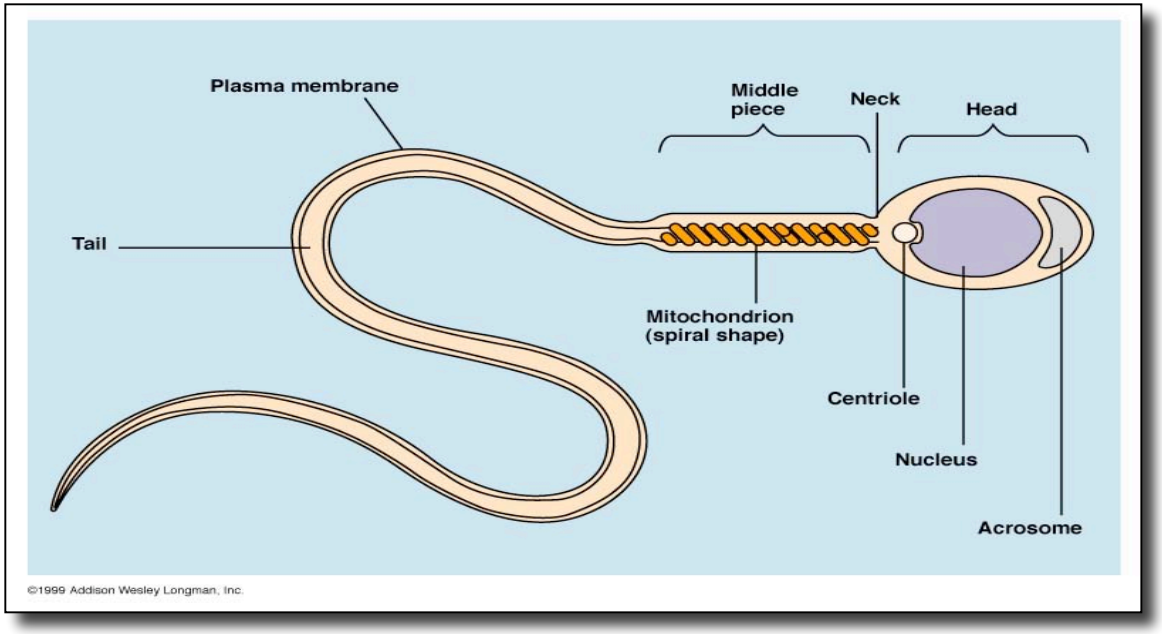
Sperm guidance: non-mammal

- Sperm Attraction - External
  - 1. Egg attracts sperm by chemotaxis
  - 2. Common in marine organisms
    - not well studied in mammals
    - chemotaxis is species specific

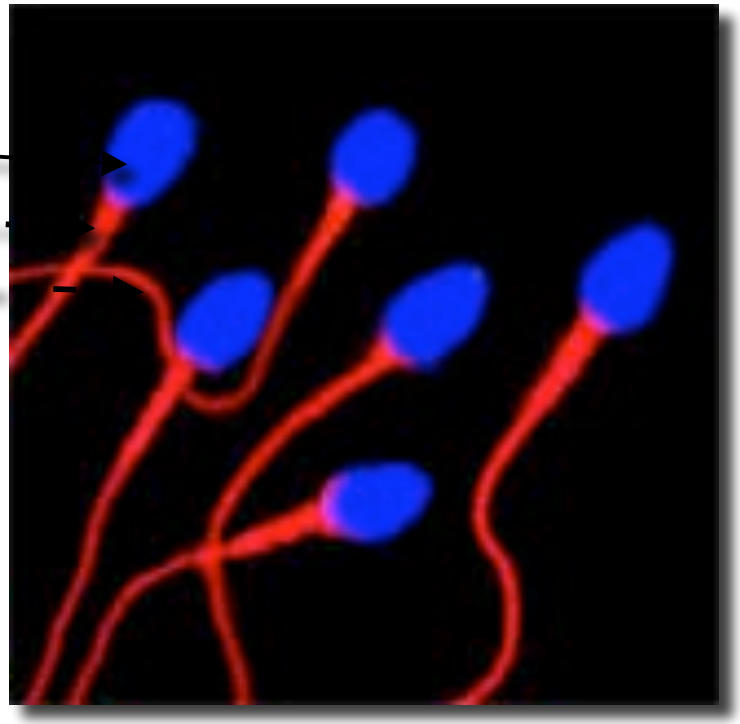


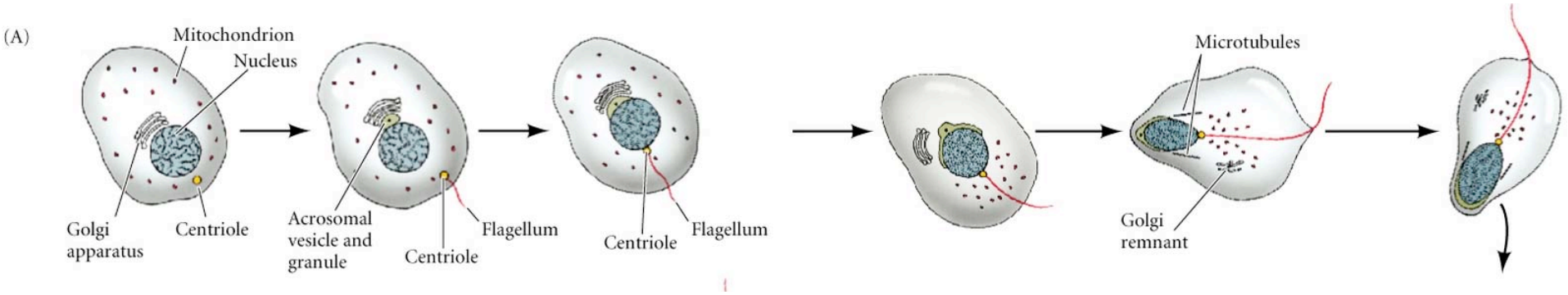
Ward G E, Brokaw C J, Garbers D L, Vacquier V D. Chemotaxis of *Arbacia punctulata* spermatozoa to resact, a peptide from the egg jelly layer. *J. Cell Biol.* 1985; 101: 2324–2329. [PubMed]

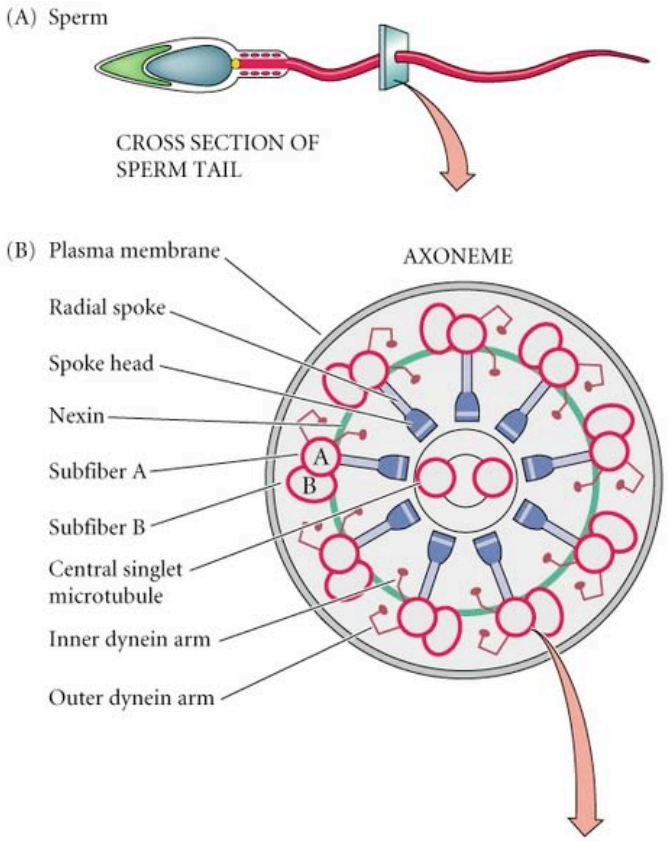
Sperm Anatomy

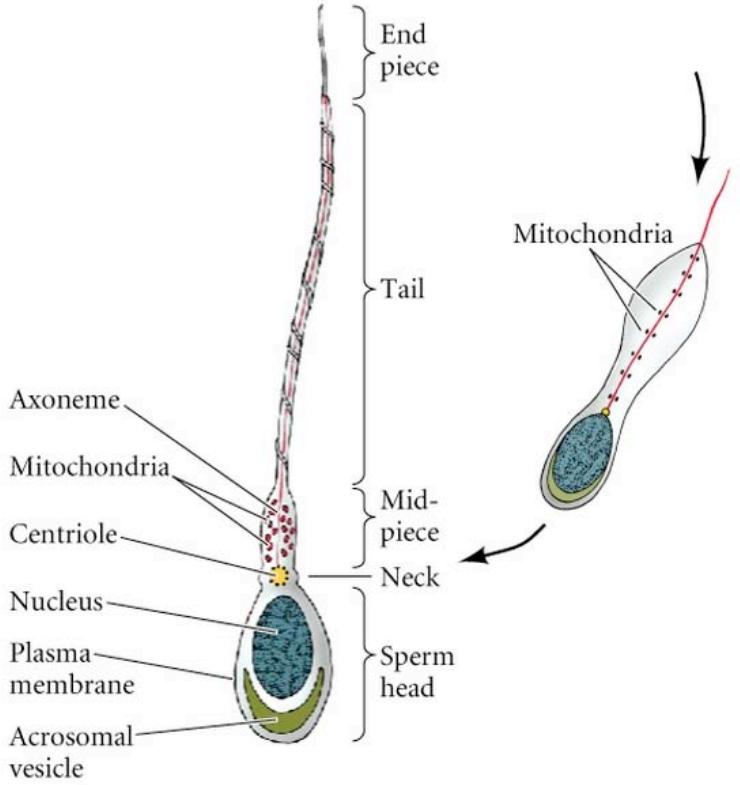


- Sperm has
  - head
  - mid piece
  - tail
- 3 key components
  - nucleus
  - propulsion system
  - sac of enzymes to penetrate egg

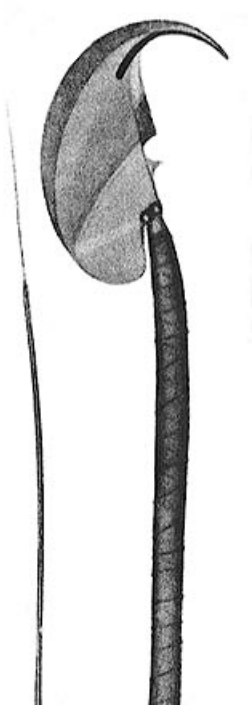




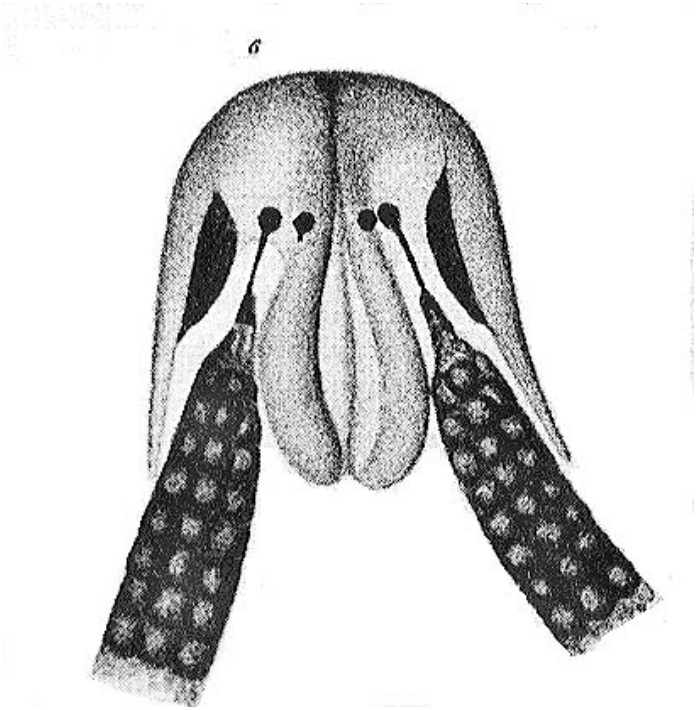




Sperm Morphology

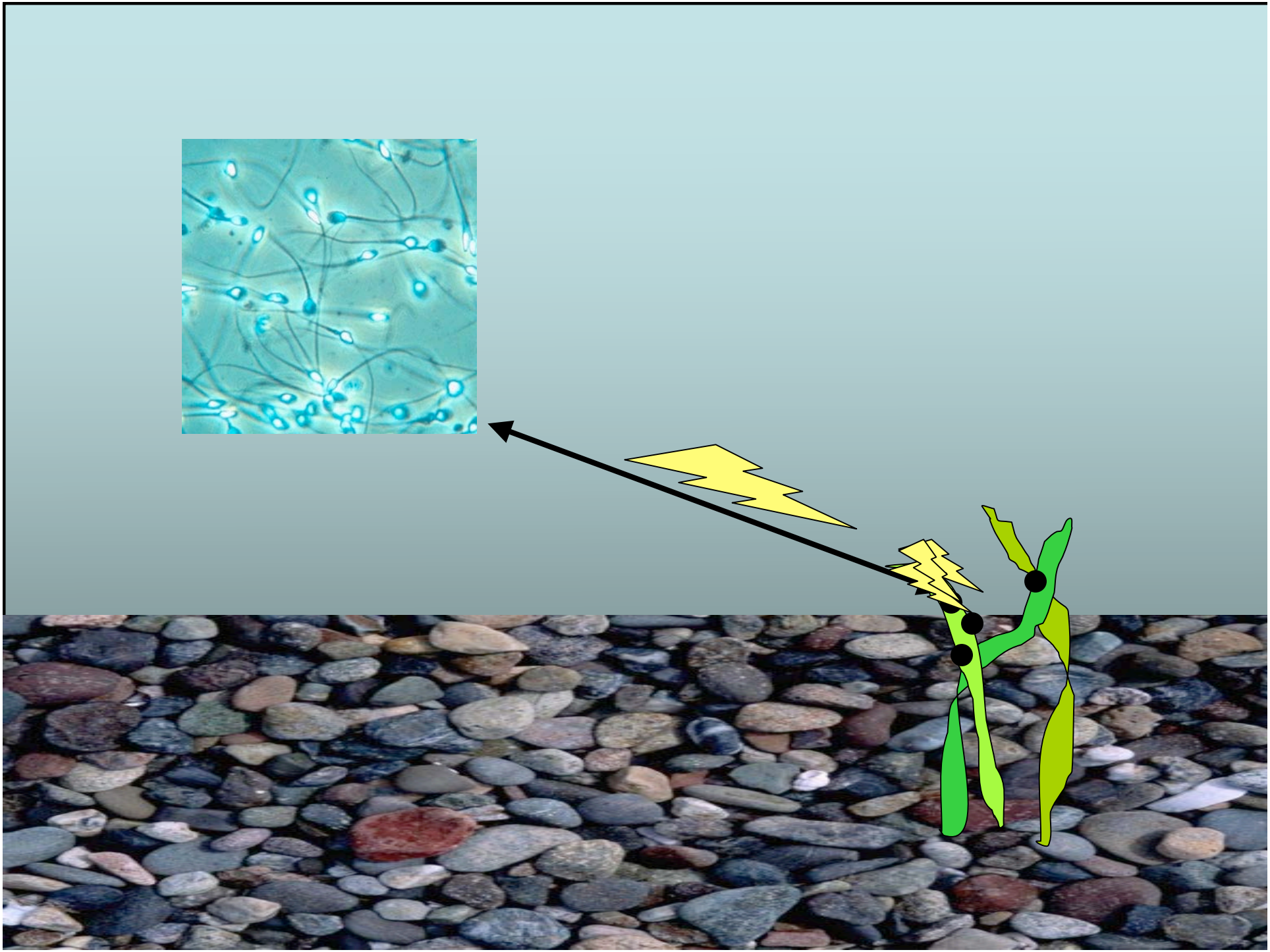


Mouse



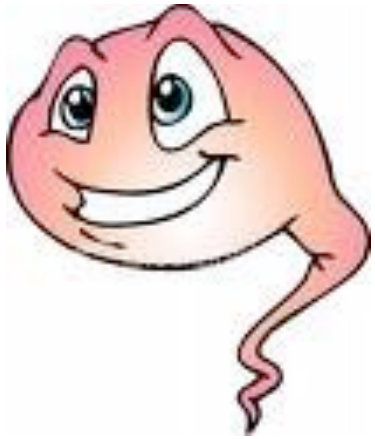
Opossum

- Vary in shape
- Heads contain
  - Condensed DNA
  - Acrosome
    - Highly modified lysosome
  - Proteins

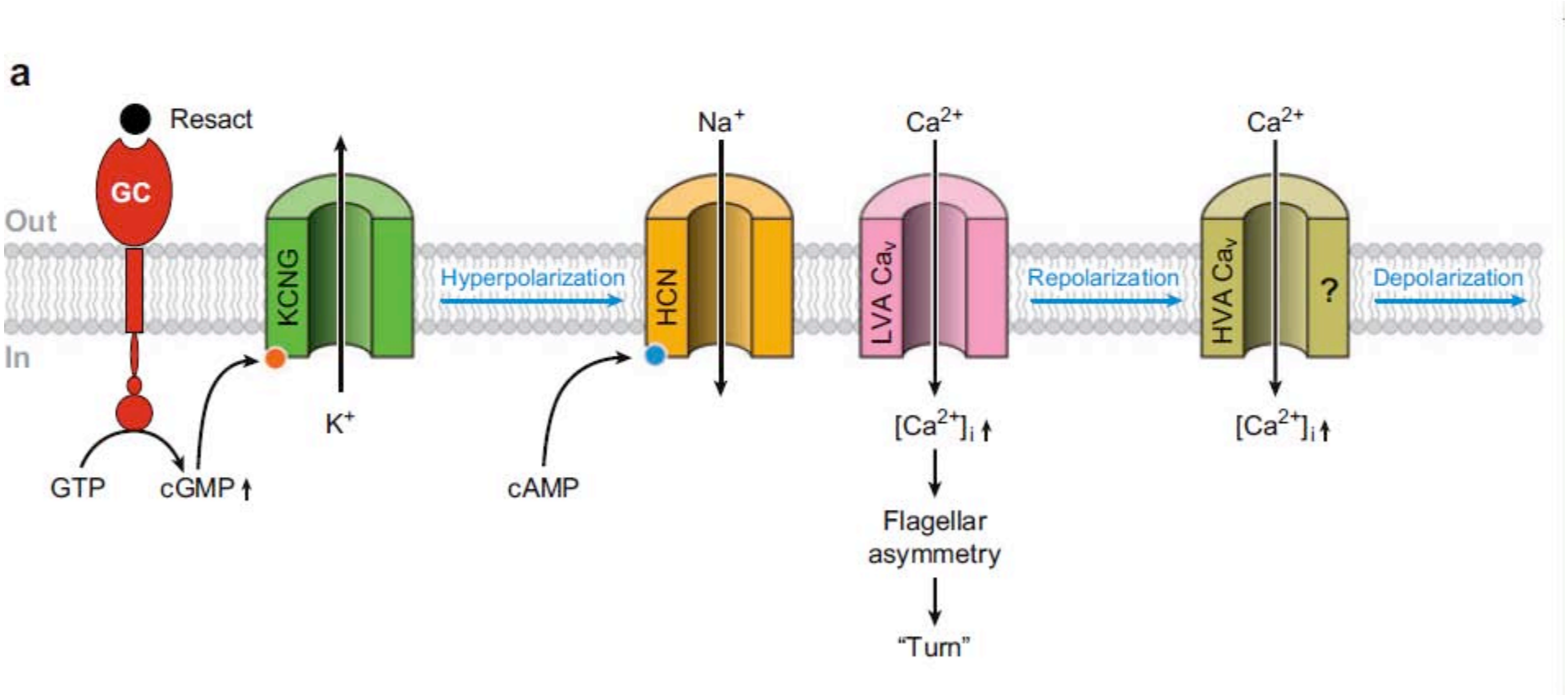


Sperm sensitivity to chemo-attractant

- Femtomolar concentrations
- 14,000 - 1,000,000 receptors per sperm
- “works” up to micromolar concentrations
- Highly species specific

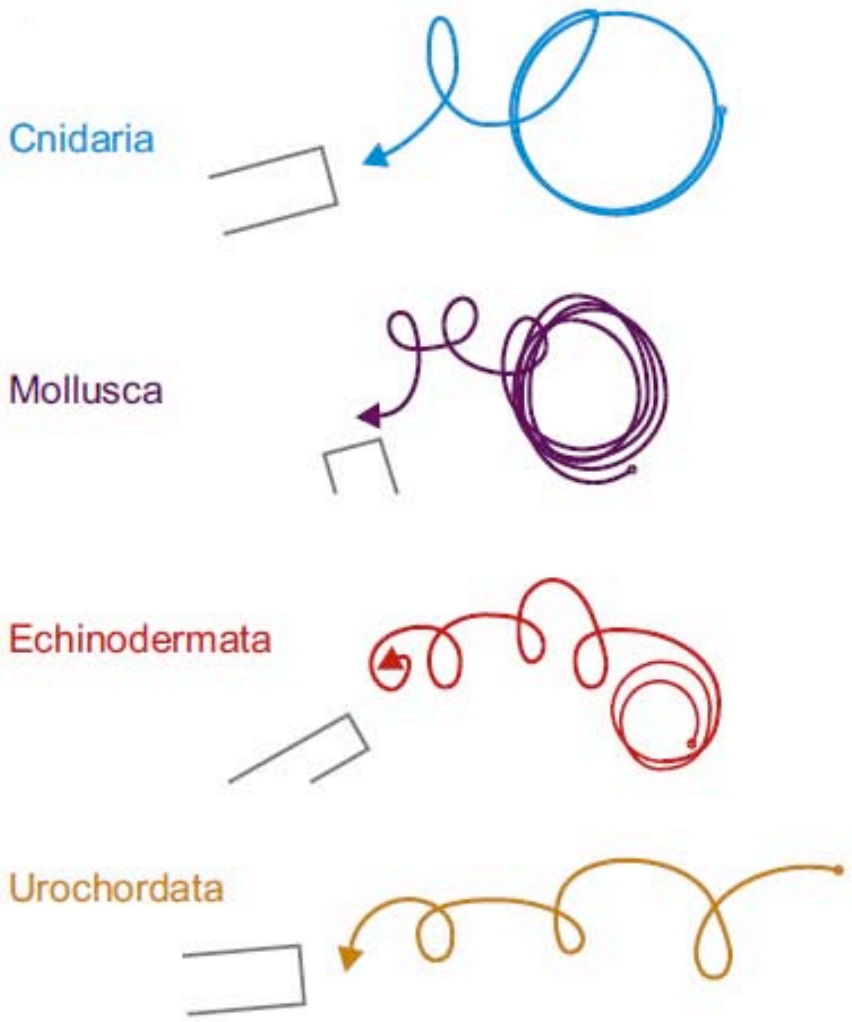


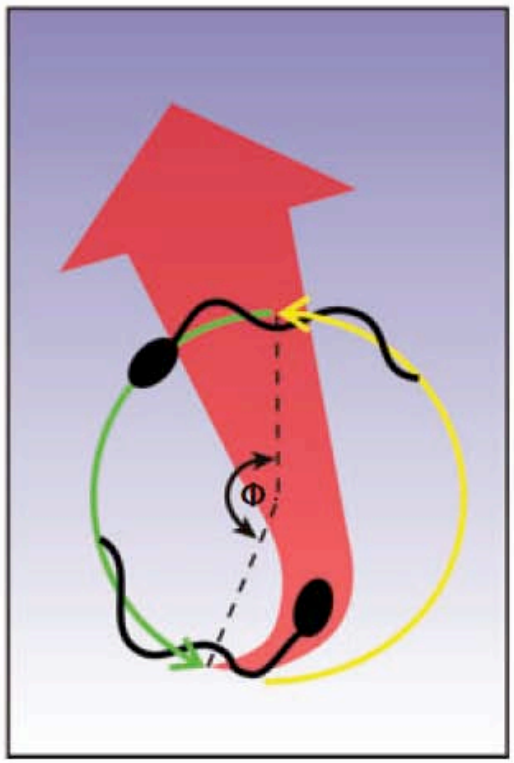
Brainless movement: mechanisms of chemotaxis in urchins



Kaupp et al., 2007; Annu. Rev. Physiol.

Sperm swimming patterns





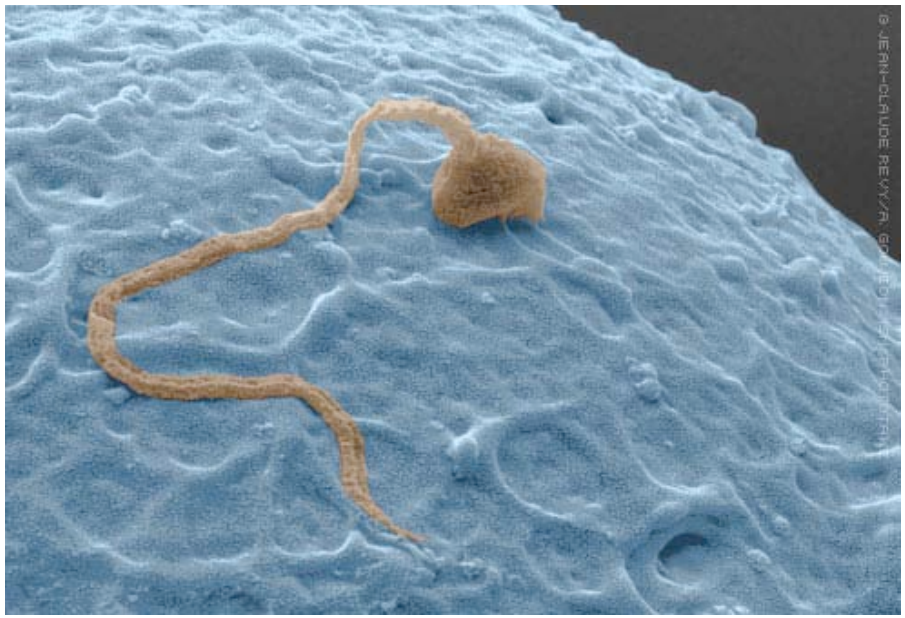
Kaupp et al., 2007; Annu. Rev. Physiol.

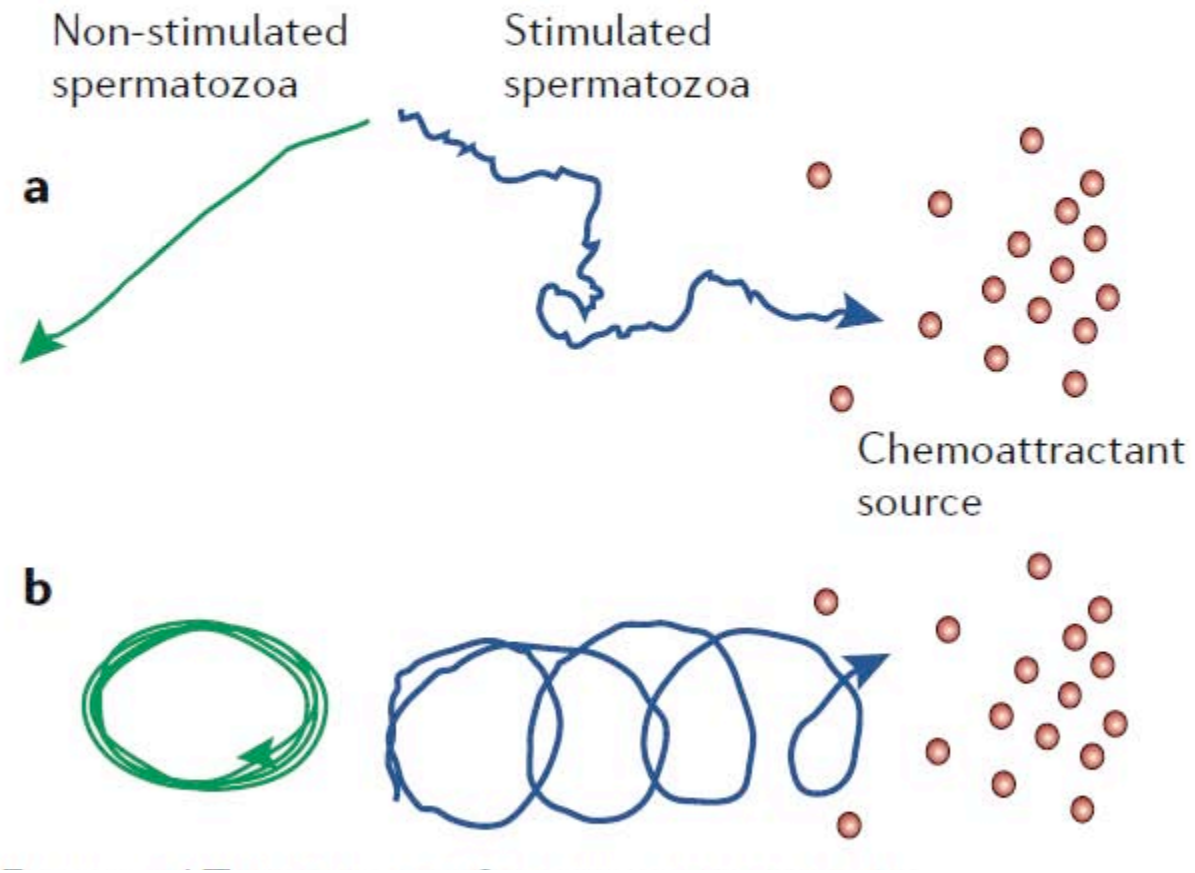
Sperm guidance in mammals

- “Competitive race” model
- Chemotaxis and thermotaxis

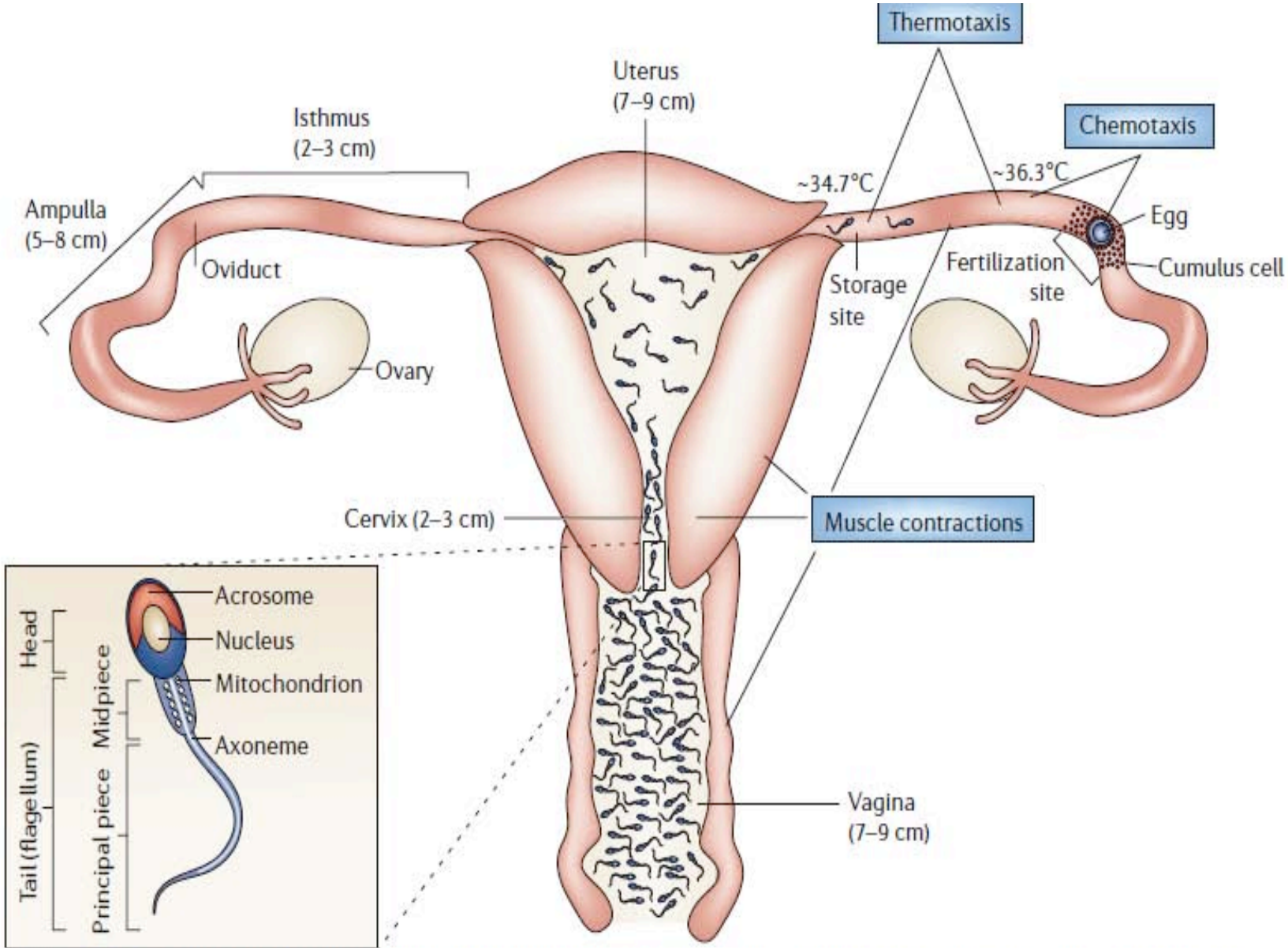
Chemotaxis in mammals

- Many types of chemoattractants
  - Progesterone
- Over 30 chemoattractant receptors
- Far more complex than that of marine invertebrates



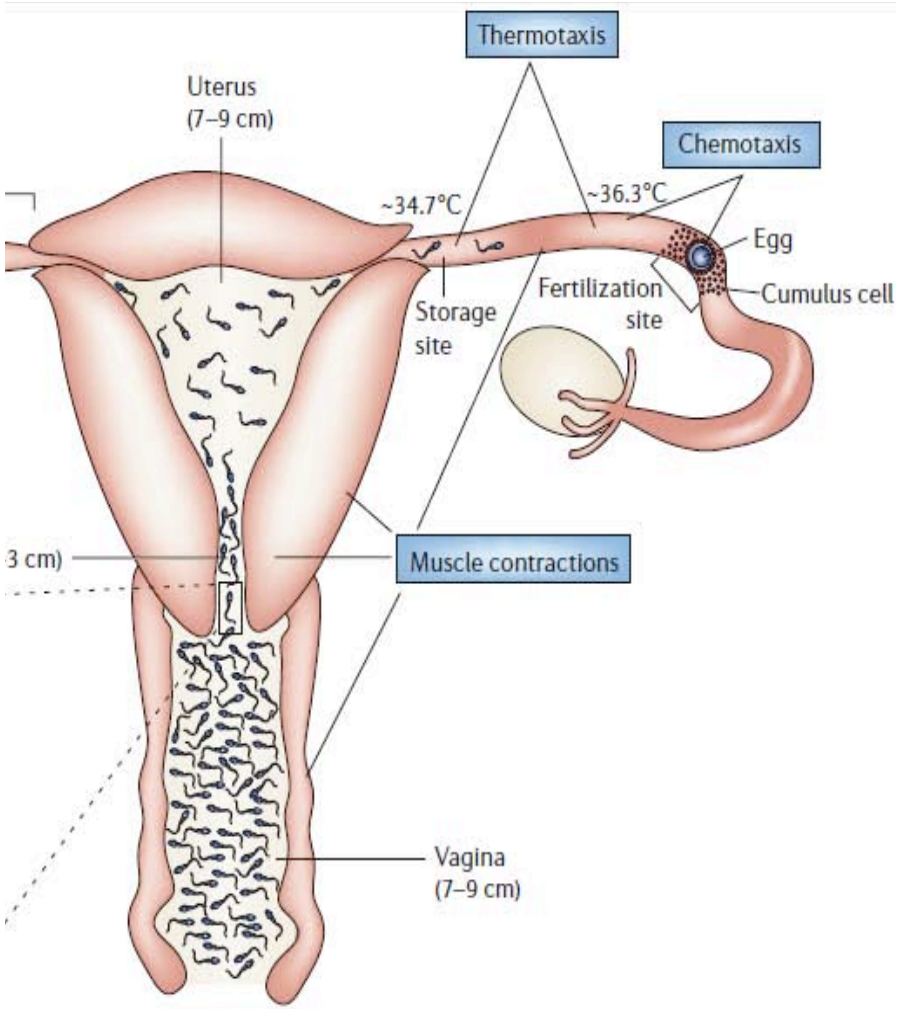


Eisenbach and Giojalas, 2006, Nature



Eisenbach and Giojalas, 2006, Nature

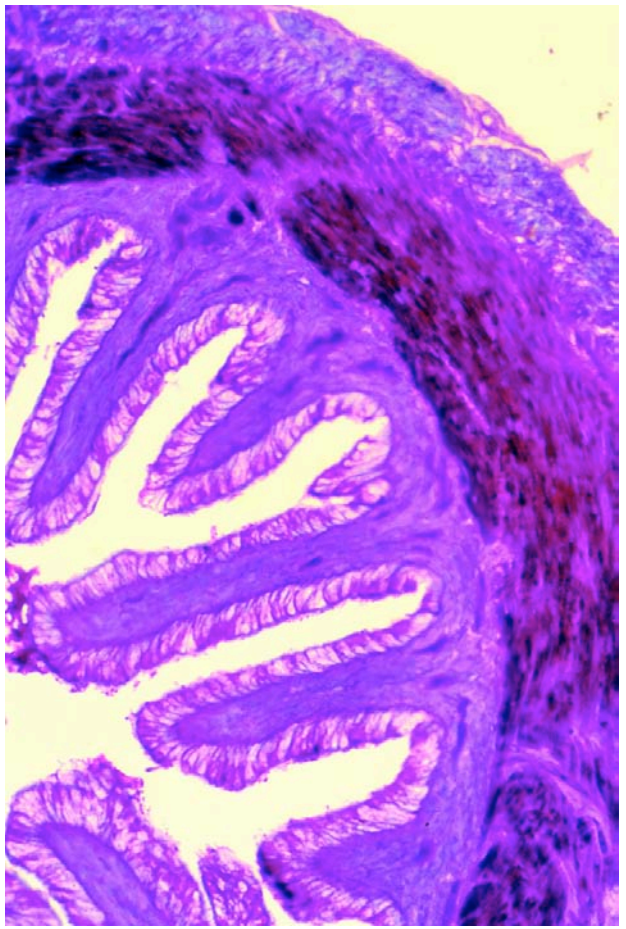
Sperm transport



- 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?

Why Swim?

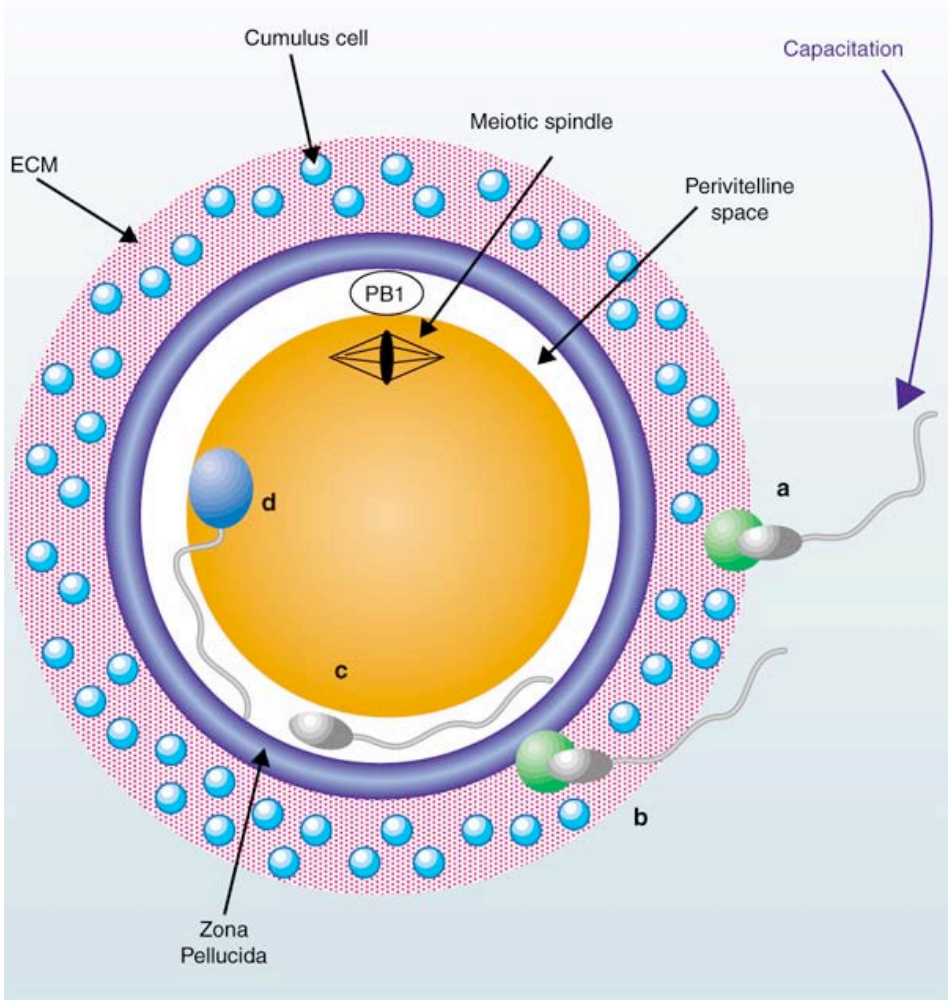
- Swimming seems to keep sperm up in ‘current’
  - If they don’t swim many attach to epithelium
  - Don’t make it thru cervical mucus
- Swimming allows them to penetrate the zona & cumulus
  - CatSper- mice sperm can only fertilize eggs with cellular matrix removed



To Swim or Not to Swim

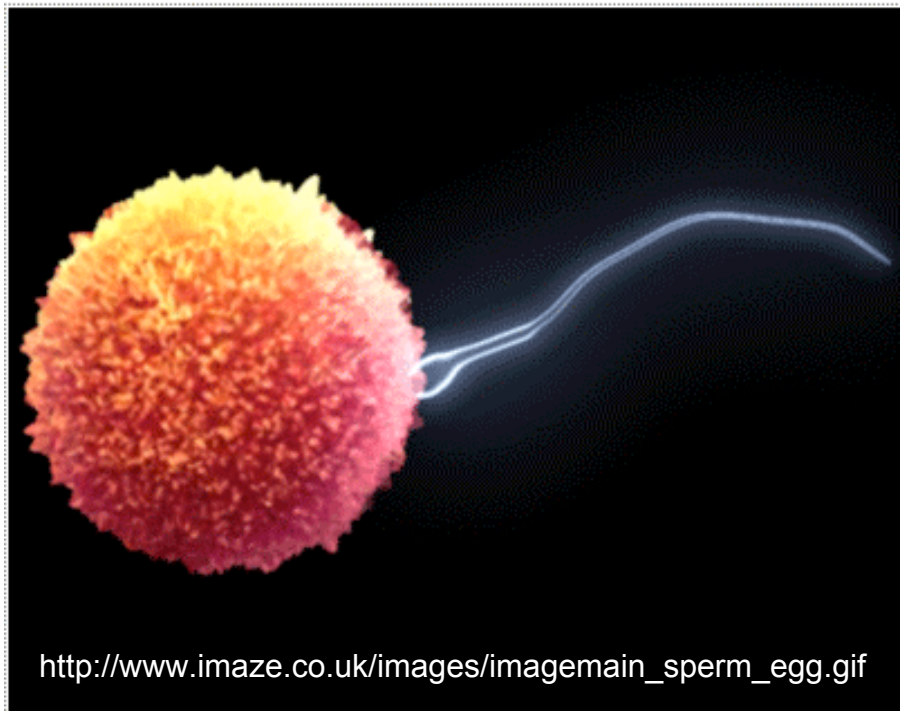
- Sperm can not 'swim' as they leave the testis - they are immobile
- Acquire 'progressive motility' in the epididymis
  - Don't move in epididymis
  - Swim with ejaculation
  - Requires Ca^{++} to function
  - Requires CatSper membrane channel
    - CatSper-deficient mice sperm swim 1/3 of normal

Capacitation and Acrosome Reaction



Capacitation

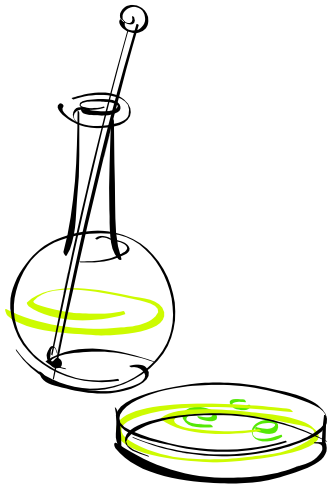
- Newly ejaculated sperm can not undergo acrosome reaction required for fertilization
  - maturational process in female tract called CAPACITATION
  - requirements vary among species
  - we still don't know the exact mechanisms involved in capacitation



http://www.imaze.co.uk/images/imagemain_sperm_egg.gif

Observations

- Capacitation is a change in the makeup of the sperm membrane
- In-vitro media includes:
  - Energy substrate
  - $NaHCO_3$
  - Ca^{2+}
  - Low K^+
  - Isoosmotic concentrations of Na^+



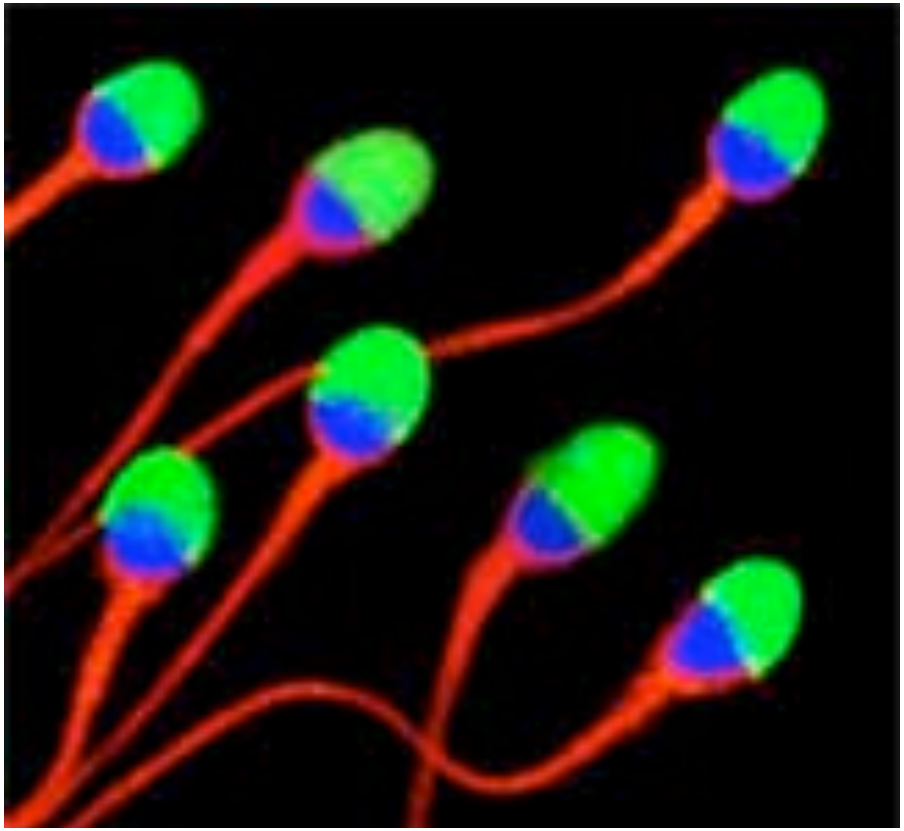
- Capacitation correlates with
  - Cholesterol efflux from the sperm plasma membrane
  - Increase in membrane permeability to Ca^{2+}
  - Increase in tyrosine phosphorylation of several proteins
  - Removal of a number of glucosylphosphatidylinositol (GPI) – anchored proteins

Evans and Florman Nature 2002
Breibart et al., Molecular and Cellular Endocrinology, 2010

Acrosome

Silver nitrate stain
and Giemsa

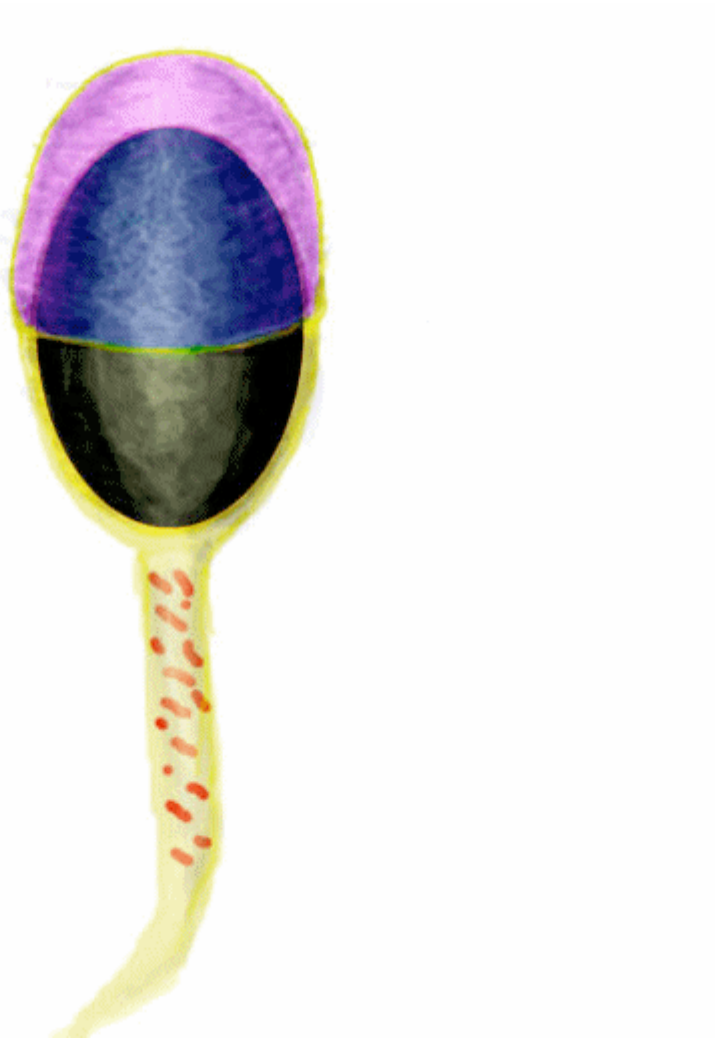
- Modified lysosome
  - Contains proteolytic enzymes



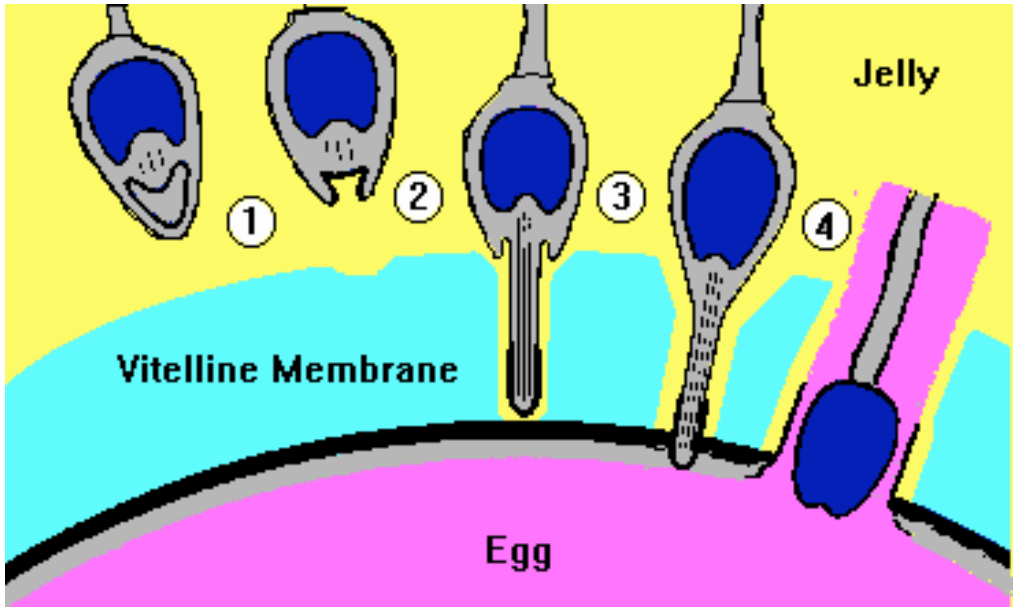
FITC lectin = green = acrosome
TOTO-3 iodide = blue = DNA
Nile red = membrane lipid = tail

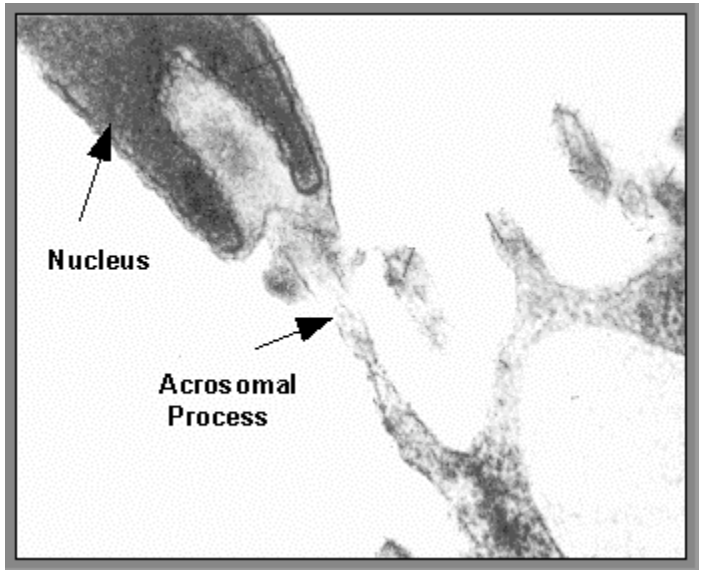
Acrosome Reaction

- Much of our knowledge comes from marine invertebrates
- Two components
  - a. acrosomal vesicle rupture
  - b. extension of acrosomal process



Acrosome Reaction

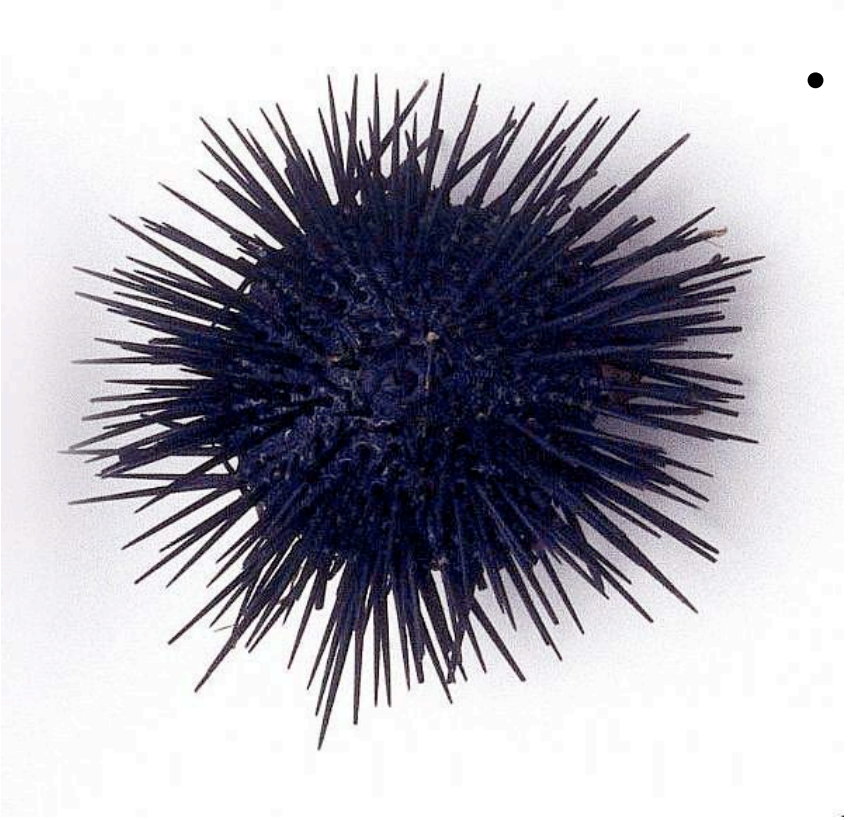




TEM

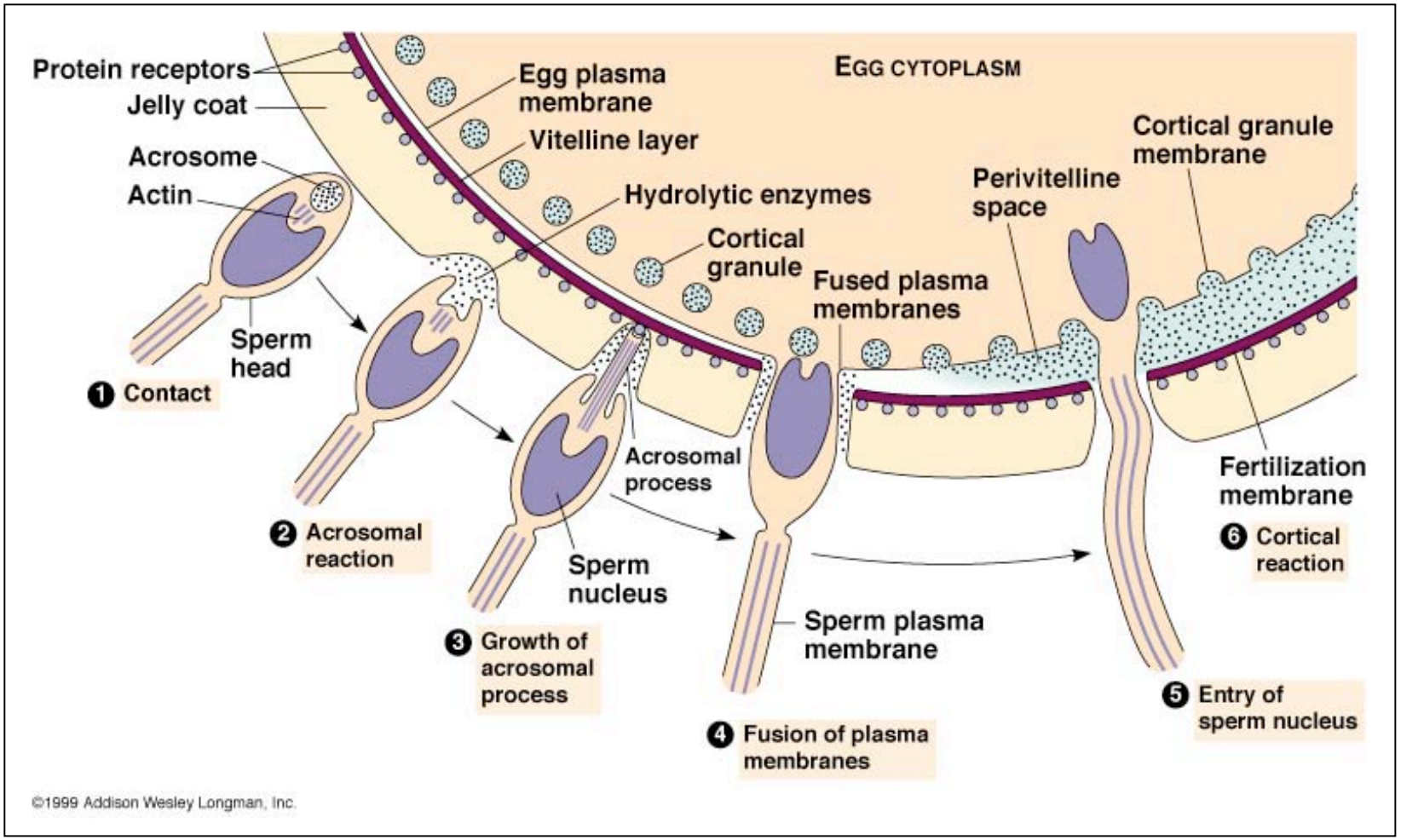
www.luc.edu/faculty/wwasser/dev/acrosome.htm

Acrosome Sequence



- sequence of events in a sea urchin
  - contact with jelly
  - influx of Ca++
  - release of lytic enzymes and exposure of bindins
  - efflux of H+ and influx of Na+
  - intracellular increase in pH
  - actin polymerization
  - extension of acrosomal process

Acrosome Reaction

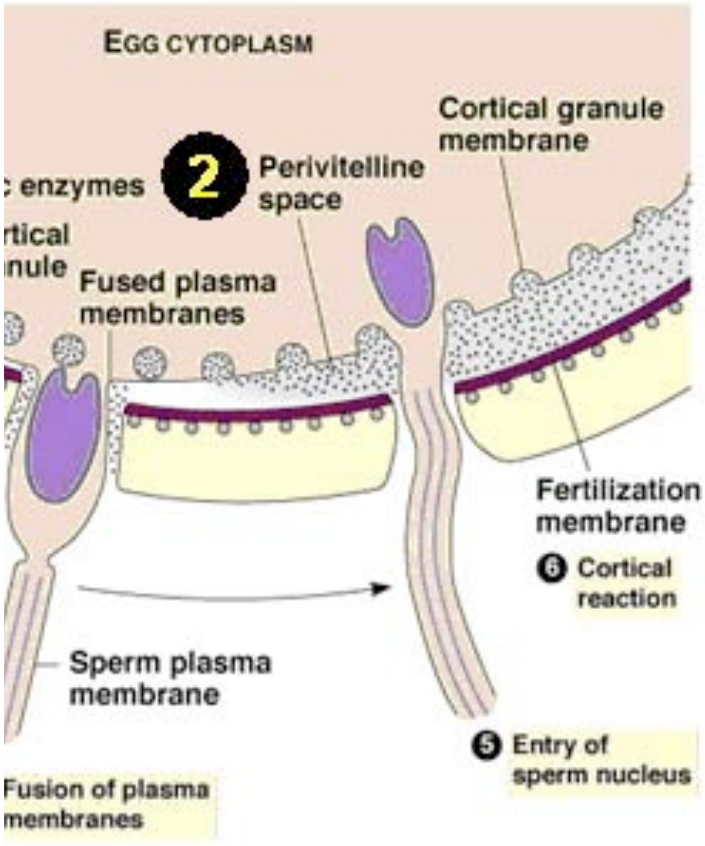


Fast block to polyspermy

- Change in electrical potential of the egg
  - Ionic concentration of the egg is different than its surroundings
  - Resting potential about -70mV
  - 1-3 seconds shifted to +20mV
  - Sperm cannot readily fuse with a membrane having a positive resting potential

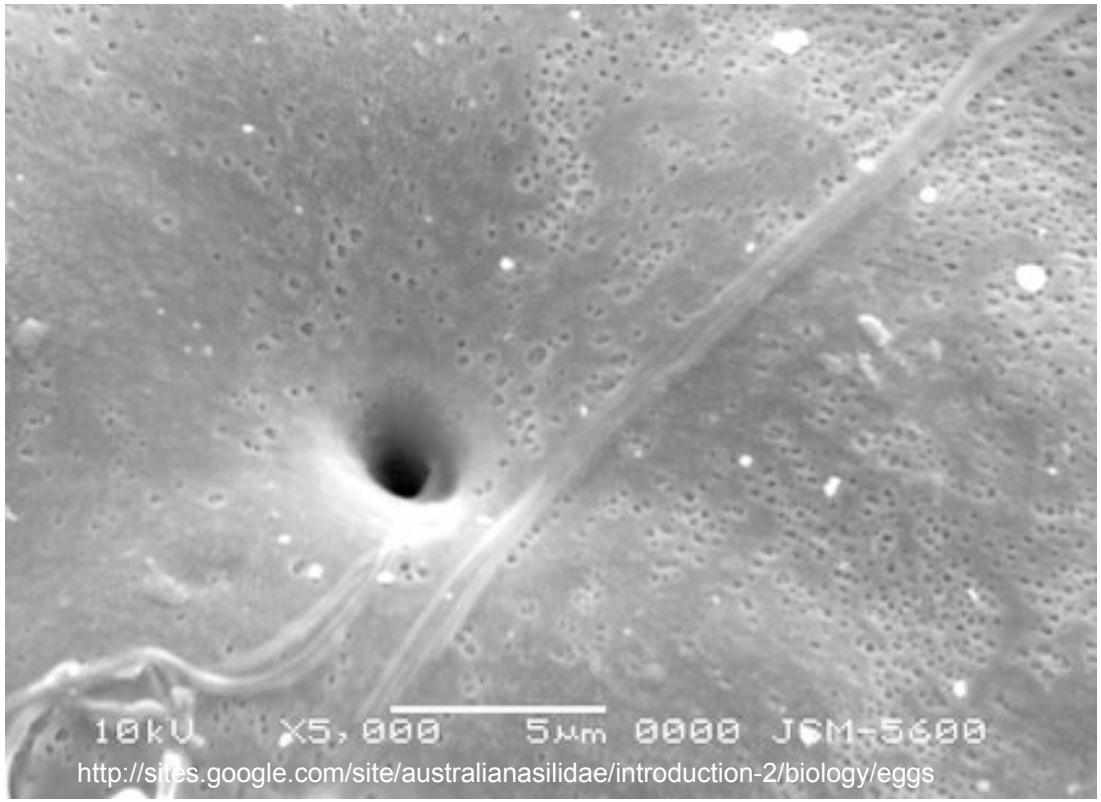
Slow block to polyspermy

- Cortical granule reaction
  - 4 proteins
    - **Proteases**
      - Clip off the binding receptor and anything attached to it
    - **Mucopolysaccharides**
      - Water influx
    - **Peroxidase enzyme**
      - Hardens the membrane
    - **Hyalin**
      - Provides support during cleavage (scaffolding)



Micropyle

– Some fish, amphibians



http://sites.google.com/site/australianasilidae/introduction-2/biology/eggs

Recognition in Mammalian Sperm

- Sperm adhesion to the ZP based on protein-carbohydrate recognition
- Sperm bind to ZP3
- “Tethers” the sperm
- Detected in
  - mouse, G. pig and human to date
- Binding to ZP3 triggers acrosome reaction
  - Involves a G-protein, calcium influx, rise in pH