What are the challenges of fertilization?

1. distance
2. timing
3. species specificity
4. approach and fusion with egg
5. egg activation (release from quiescent/arrested state)
6. prevention of polyspermy

video clip - ascidian fertilization
- represents all of the challenges listed above

1. Distance
   - sperm must travel to egg
   - fresh water - osmotic changes
     - fish, frogs - fertilize in close proximity to eggs
     - some fish sperm - live <20 seconds
   - sea water - osmotic adjustments not a problem
   - broadcast spawners (coelenterates, sea urchin, some ocean fish)
     - dicey proposition - water motion, no internal energy source (sperm) = short effective life span
     - coordinated spawning
     - sperm/eggs in very large numbers (sea urchin - 4 x 10^8 eggs; 10^{12} sperm)

   - internal or (quasi-internal) fertilization - fewer gametes/greater chance of success
     - sperm have readily available energy source, so can survive longer
     - antioxidants - keep cells healthy

   - sperm are motile (most species)
   - however, in many species, female plays vital role in sperm transport
     - e.g. mammals (rabbits) sperm transported in "mucus channels"
       (mucopolysaccharides) to ampulla of oviduct (near ovary)
   - uterine muscular activity aids transport
   - sperm may swim to egg once they are in the vicinity
   - sperm may use motility primarily to get through egg outer layers egg

2. Timing - e.g. both sperm and eggs must be ready for fertilization
   (NOTE - neither gamete is fully mature coming from gonad)

   - general - gametogenesis controlled at endocrine level
- **spawning/mating** generally controlled by combination of **environmental and endocrine cues**

**specific** (i.e. short-term) - both sperm and egg have limited life span

even in highly coordinated spawning/mating, allowances for biological variation

environmental influences on timing - e.g.

- sperm are generally **static (quiescent)** until spawning/mating
- sea urchin sperm **activated** upon entry into sea water (ionic/electrical)
- mammalian sperm - fairly quiescent in **epididymus**; activated upon entry into cervical media (etc.)

**sperm capacitation** (final maturation - mammals, generally) in female reproductive tract

- involves removal/modification of sperm surface proteins, possibly restructuring of membranes
- capacitation can be accomplished by incubation in various media

3. **Species specificity** - deal with below; chemotaxis, sperm-egg binding

4. **Approach and fusion with egg**

**SLIDES**

**chemotaxis**

- sperm from most species are guided to vicinity of egg by chemicals produced by the egg

  - e.g. **resact** (sea urchin *Arbacia punctulata*; not stimulate other species)

  (3. **Species specificity**)

  - resact triggers Ca²⁺ changes in tail; results in changes of flagellar angle
  - 14 amino acid peptide
  - NOTE - small molecule (easily diffused); readily available in eggs (portion of egg jelly); easy to modify (i.e. evolution of species specificity)

  - mammalian chemoattractant hypothesized

In order to fertilize, sperm must get through outer layers (including cells) of egg

- **egg jelly** (sea urchin, amphibians)
- **vitelline envelope** (e.g. urchins); **zona pellucida** (mammals)
- **granulosa cells** (mammals)
- **plasma membrane** (all)

**Acrosome reaction - sperm-egg fusion**

- acrosome contents - lysosomal enzymes
  (NOTE - acrosome is Golgi-derivative)
- acrosome reaction - vesicularization of membranes; release of lysosomal components
- great amount of variation in AR among species

**sea urchin** - acrosome reaction
- AR triggered be jelly coat components (e.g. fucose sulfate polymer)
- receptor in sperm head binds; transient rise in $\text{Ca}^{2+}$
- acrosome contents digest hole through jelly coat
- **acrosomal process** - **globular actin** below acrosomal membrane polymerizes into microfilaments
  - acrosomal process puts sperm membrane in contact with vitelline envelope
    - sperm protein - **bindin** - binds to egg receptor (not identified yet)
      - adhesion
    - different species have different bindins (**3. Species specificity**)
      - bindin = one of the fastest evolving molecules (**3. Species specificity**)
- acrosomal enzymes digest vitelline envelope, allow contact of sperm and egg plasma membranes
- membranes fuse
- **fertilization cone** forms from egg membrane
  - egg **globular actin** forms microfilaments - pushes plasma membrane out around sperm head
- sperm drawn into egg
  - mechanisms of drawing sperm head in are unclear

**mammals** (e.g. mouse)
- granulosa cells - matrix = **hyaluronic acid**
- **hyaluronidase** on sperm head (possibly redistributed by capacitation) dissolves matrix between granulosa cells (motility drives sperm in?)
- sperm bind to specific **zona pellucida** molecules (**3. Species specificity**)
  - ZP1, ZP2, ZP3
    - sperm bind to ZP3
      - sperm receptor - ?
- acrosome reaction - in granulosa (rabbits); upon contact with ZP (mice)
  - **transient rise in intracellular** $\text{Ca}^{2+}$
- sperm moves into **perivitelline space** (between ZP and plasma membrane)
- sperm plasma membrane proteins exposed or modified by AR bind to egg membrane
- contact with egg plasma membrane - **fusion**
- sperm is drawn into egg

5. Egg activation
- $\text{Ca}^{2+}$ transients
- activation of **protein kinase C** (PKC)
- increase in **metabolism**
- **DNA replication**, **protein synthesis**
- resumption of **meiosis**
- fertilization stimulates quiescent egg to reenter cell cycle; begin development

- **mechanism of egg activation?**
  - plasma membrane contact or membrane fusion
  - evidence for both
  - may be species differences

- all mechanisms involve **Ca\(^{2+}\) transients** - brief oscillations of intracellular calcium transients - waves of calcium released from *internal (ER) stores*
  release triggered by **PIP\(_2\) (phosphatidylinositol bisphosphate) pathway**

**VIDEO - CALCIUM WAVE**
**SLIDES**

- **protein kinase C** involved in many reactions

- release from **meiotic arrest**
  - most amphibians and mammals arrested at **second metaphase of meiosis**
  - meiosis prevented from completion by high **M-phase promoting factor (MPF)**
  - also by high **mos protein (proto-oncogene)**
  - **Ca\(^{2+}\) activates kinase activity, reduces MPF and mos protein degraded**
  - **meiosis resumes; polar body formation**
  - **pronuclei fuse**

6. **Prevention of polyspermy**
   fast block (ionic - depolarization)
   slow block (cortical reaction)

**fast block** (urchins, frogs, **not most mammals**)
- resting potential depends upon maintenance of unequal intra- and extracellular ionic content (Na/K ATPase)
- most cells - **resting potential = -70 mV** (negative inside)
- sperm can fuse to membranes with -70 mV, but not positive therefore, no more binding in depolarized membrane
- depolarization achieved by opening ion channels
  - different ions
    - sea urchin - Na\(^+\)
    - frog - Cl\(^-\)
- membranes **repolarize** within about 1 minute

**slow block**
- **cortical reaction** - begins soon after sperm -egg contact; complete ~ 1 min triggered by PKC (mammals) or by **Ca\(^{2+}\)** (urchins)
- **cortical granules** (located in cortex)
exocytosis into perivitelline space

- cortical granule contents
  1. form **hyaline layer**
     - protease cleave proteins that tether vitelline membrane
     - add polysaccharides - attract water
     - hyaline layer lifts vitelline envelope off of plasma membrane
  2. **peroxidases** harden vitelline envelope - **fertilization envelope**
  3. modify sperm receptors - no more binding

- Mammals - **zona reaction** - similar to cortical reaction