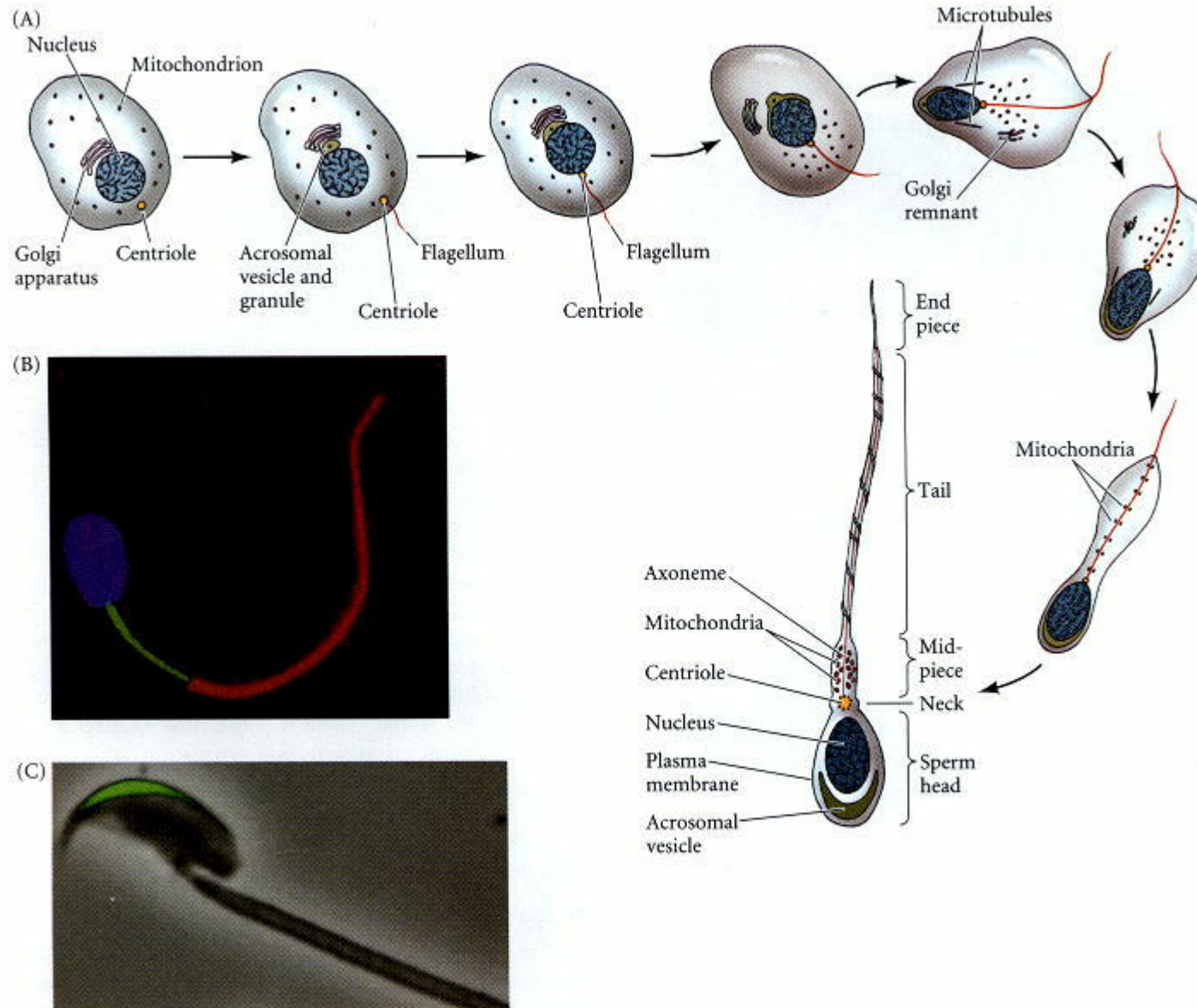


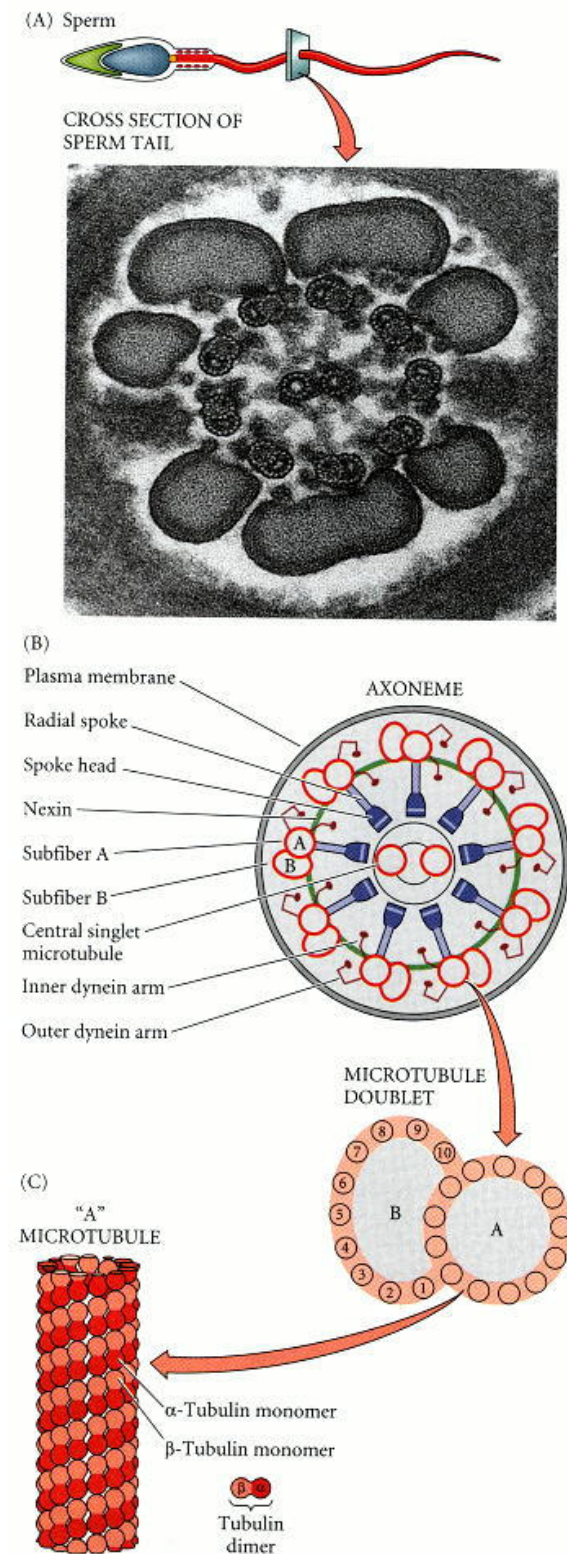
Fertilization

Sperm Development



Sperm Flagella

- Microtubule doublets slide past each other powered by dynein arms
- Dynein arms hydrolyze ATP



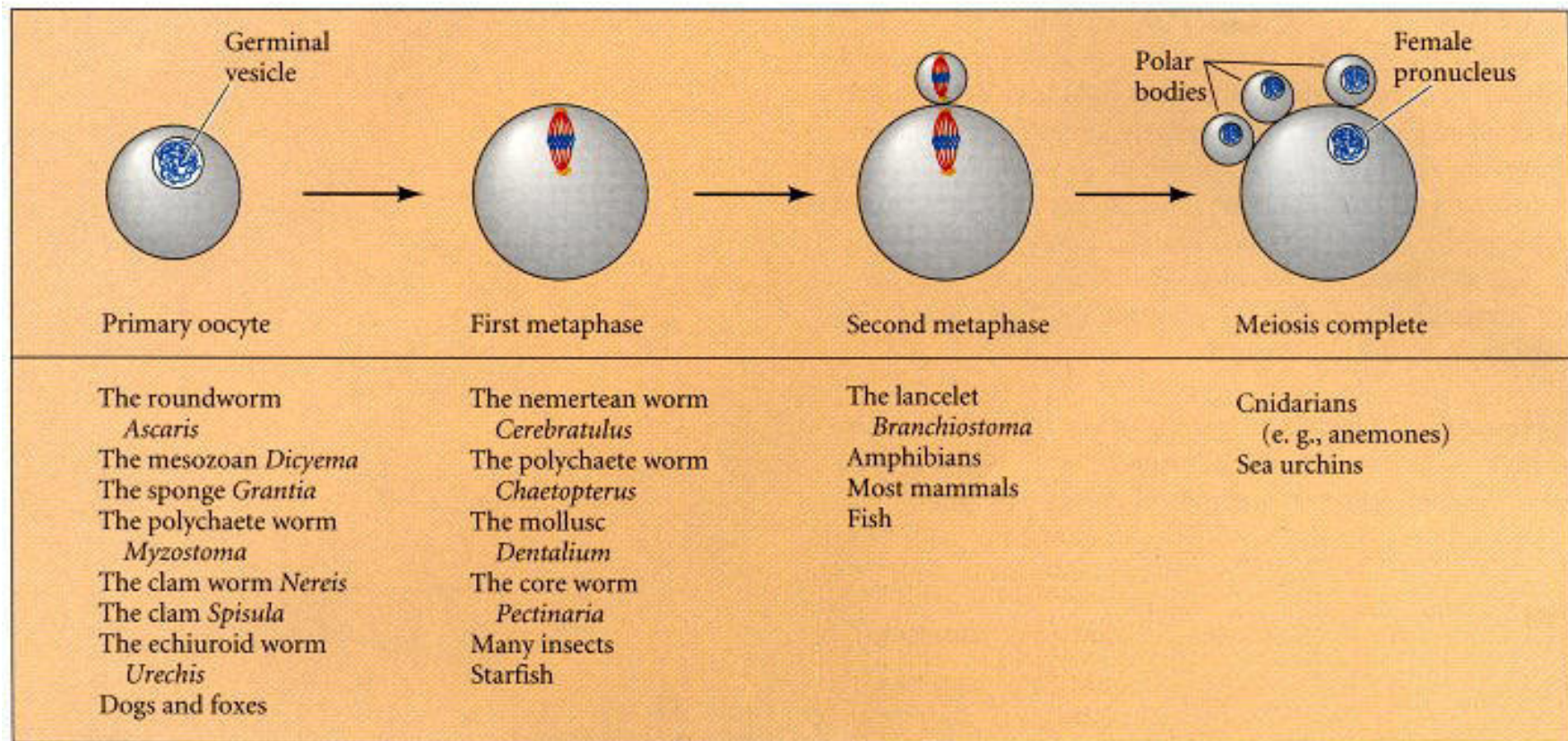
Acrosome

- Modified secretory vesicle derived from Golgi apparatus
- Sea Urchin
 - Globular actin found between nucleus and acrosome
 - Forms acrosomal process

Egg

- Filled with materials necessary for developing embryo
 - Proteins
 - Ribosomes and tRNA
 - mRNA
 - Morphogenic factors
 - Protective chemicals

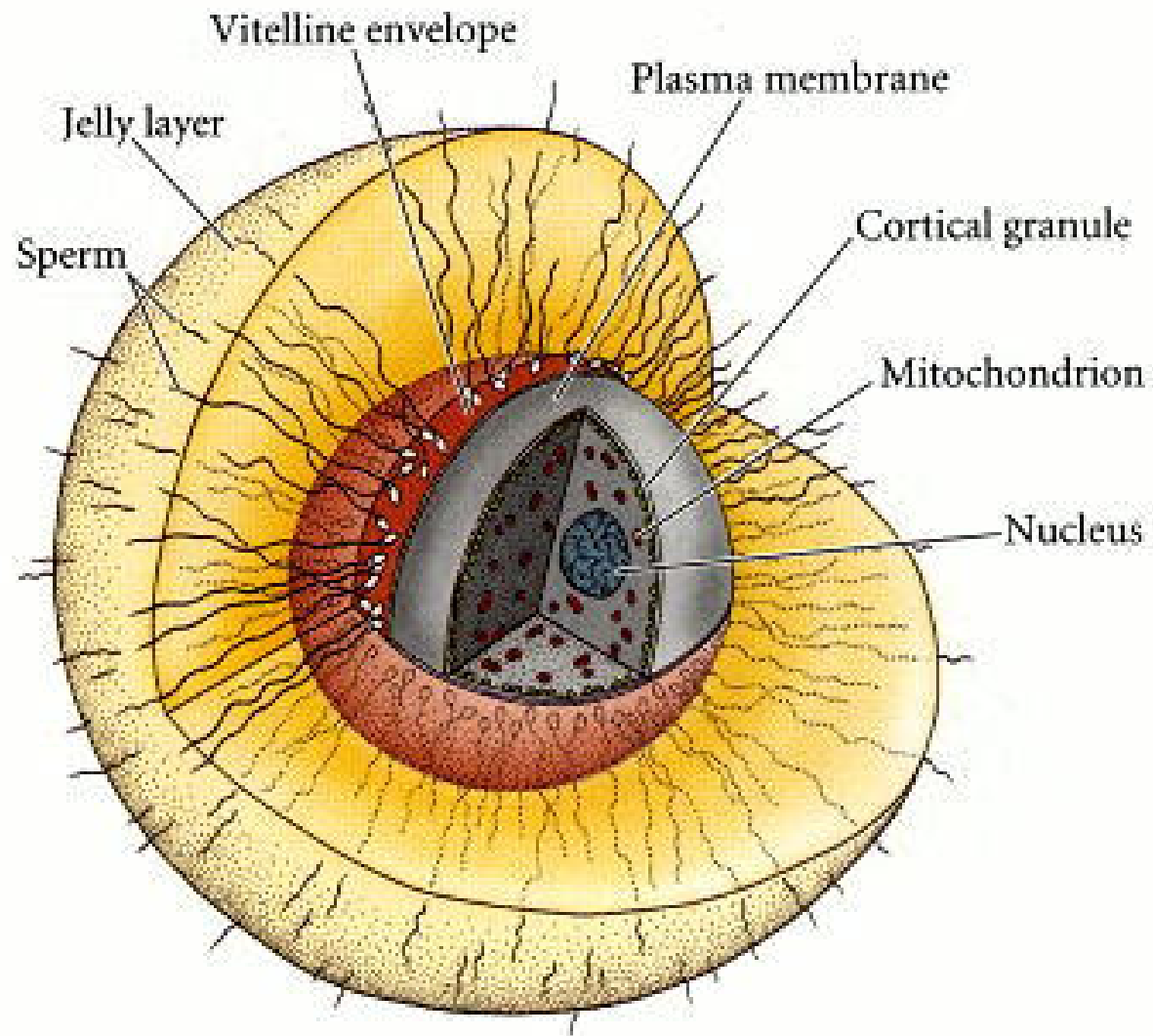
Egg Stage During Sperm Entry



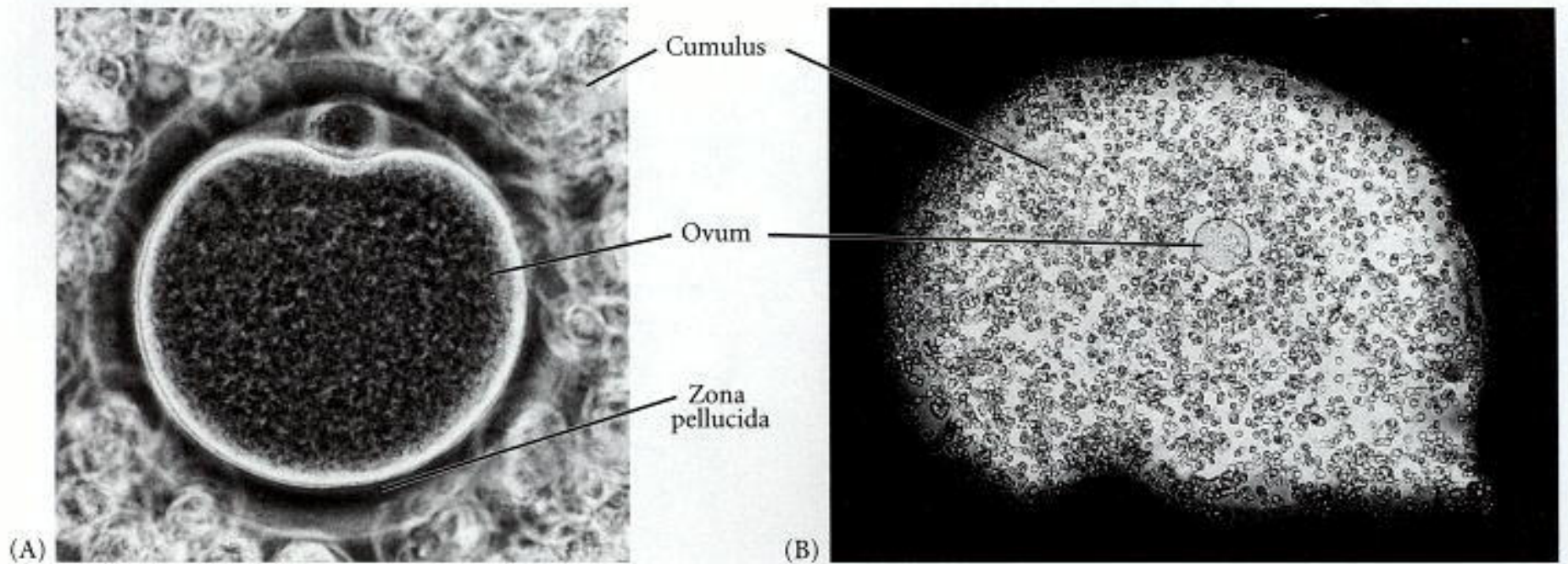
Extracellular Components

- Egg jelly (some organism)
- Vitelline envelope
 - Sperm-egg recognition (species specific)
- Mammals
 - Zona pellucida (mammals)
 - Extracellular matrix between membrane and vitelline envelope
 - Cumulus cells and corona radiata
 - Follicle cells that ovulate with egg

Sea Urchin Egg



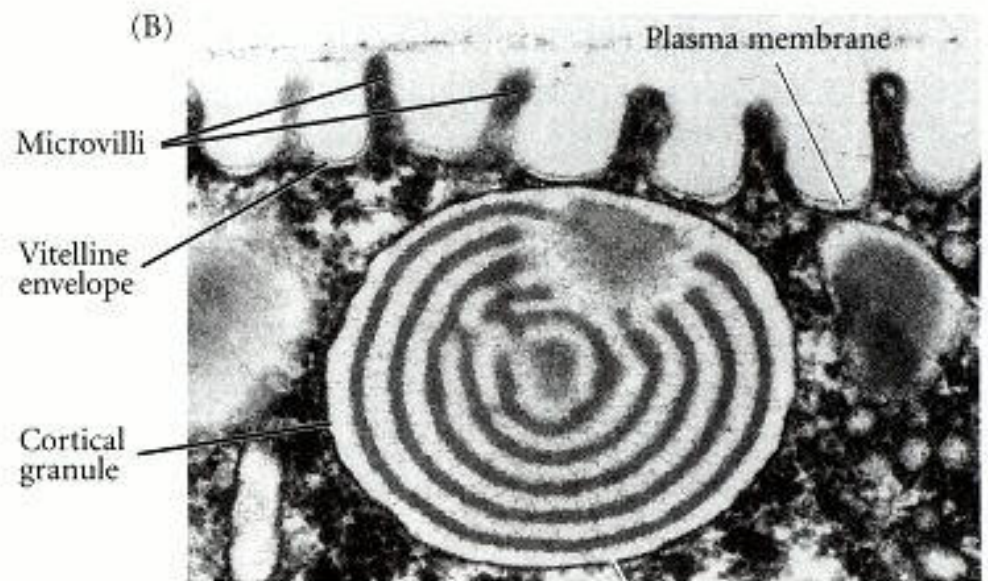
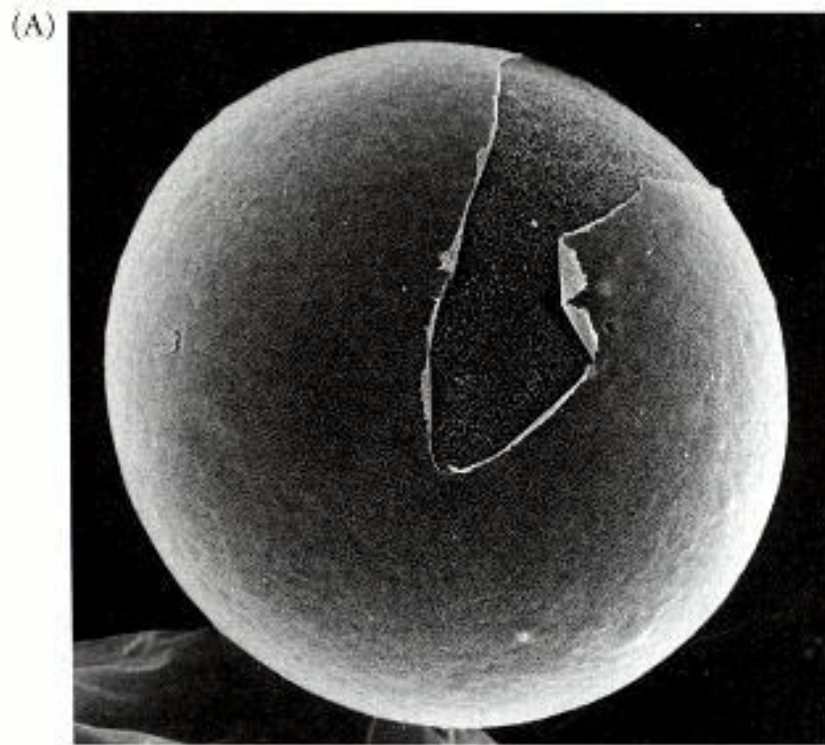
Mammalian Egg



Intracellular Components

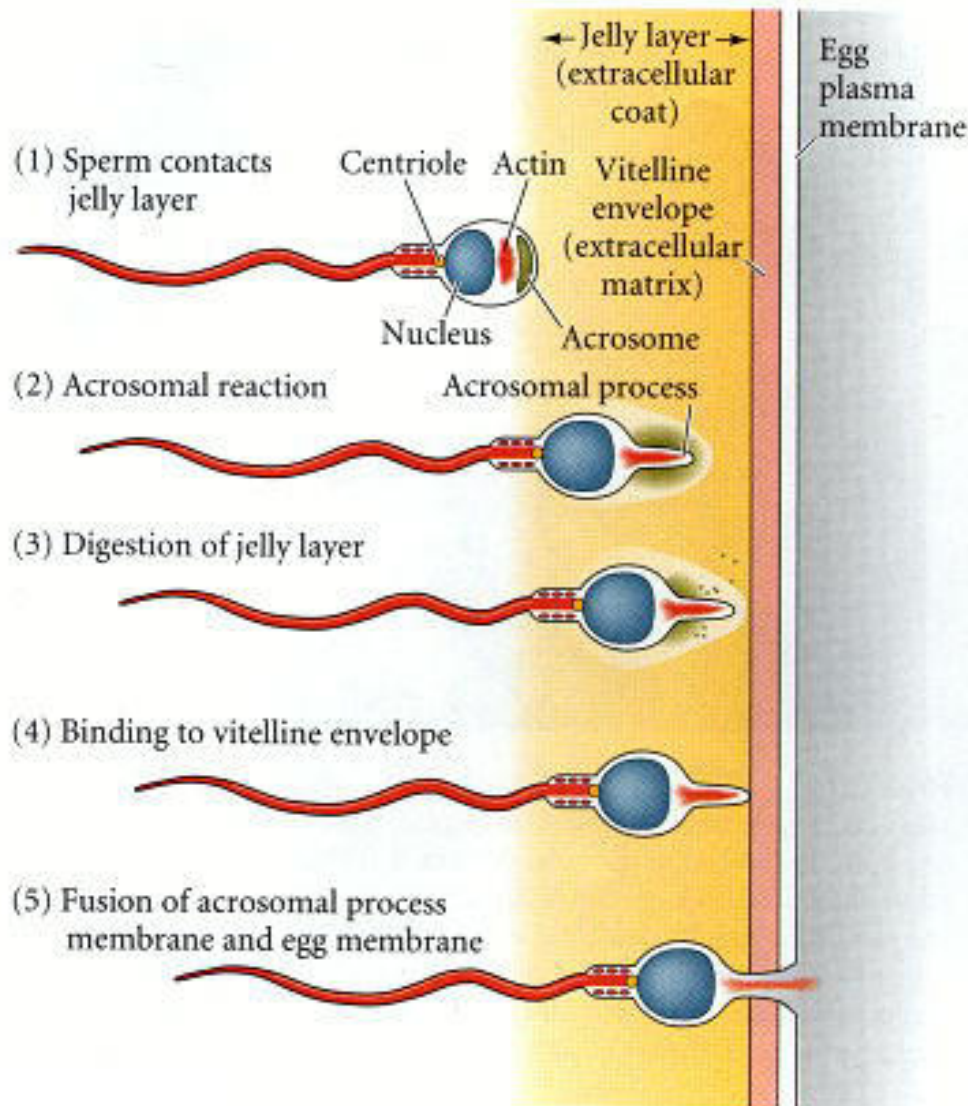
- Cortex
 - Gel-like, stiff cytoplasm
 - Contains globular actin (homologous to actin in sperm)
 - Cortical granules
 - Golgi derived (like acrosome)
 - Prevents polyspermy
 - Proteolytic enzymes
 - Mucopolysaccharides
 - Hylanin and adhesive glycoproteins

Sea Urchin Egg

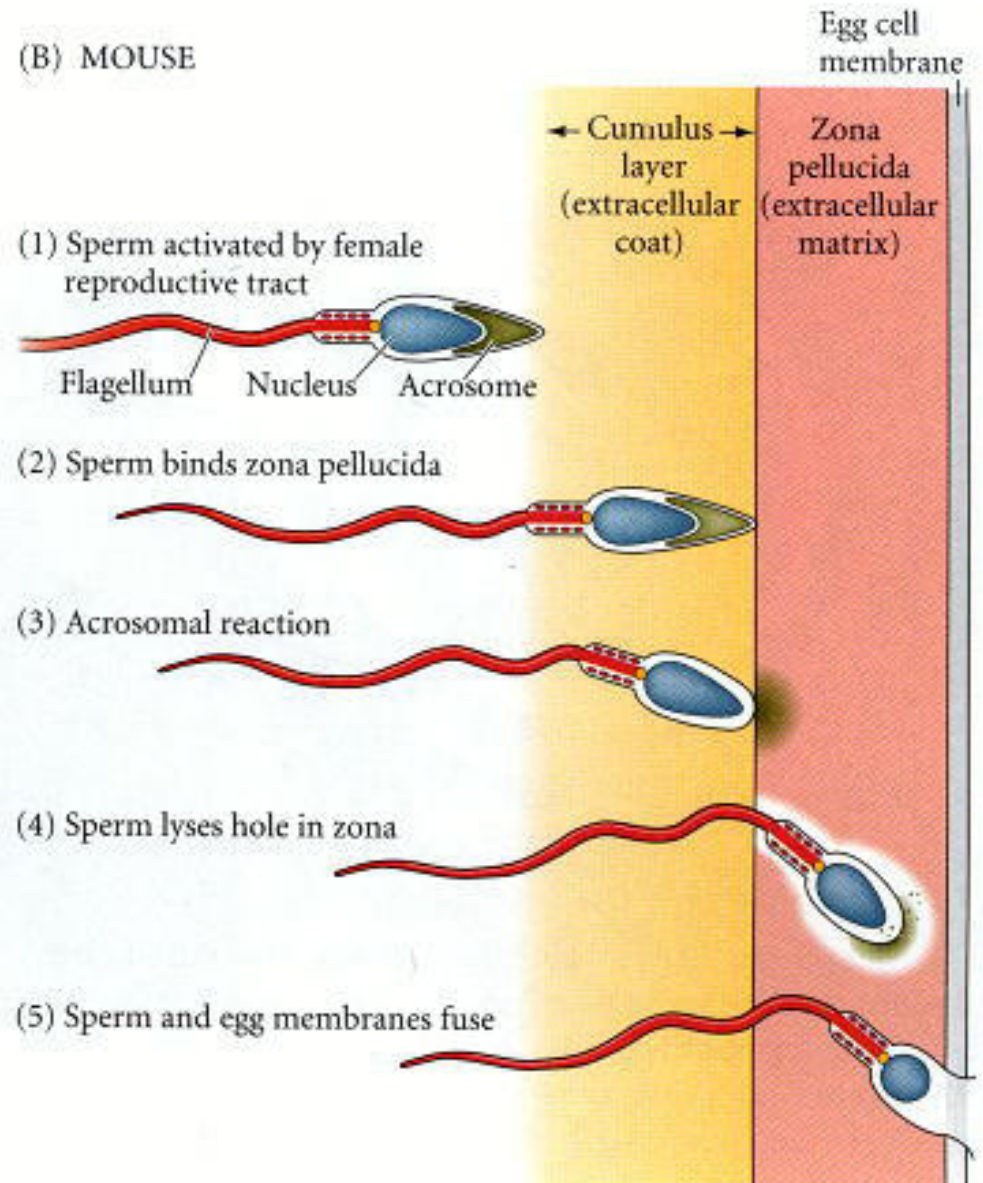


Fusion of Egg and Sperm

(A) SEA URCHIN



(B) MOUSE



Summary of Egg Sperm Recognition

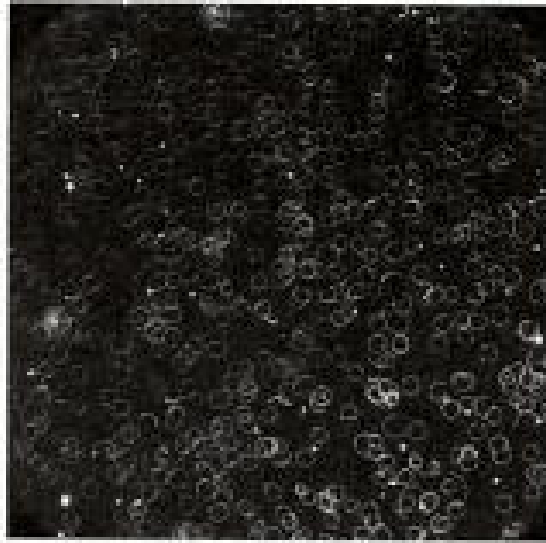
- 1) Chemotaxis
- 2) Acrosomal reaction
- 3) Binding
- 4) Passage through egg envelope
- 5) Fusion of membranes

Chemotaxis

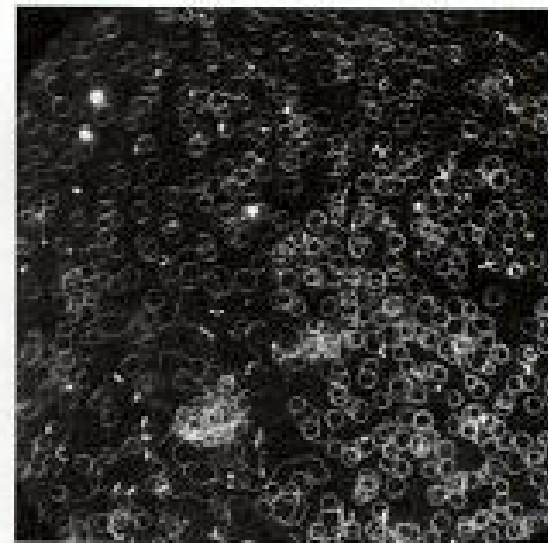
- Sea Urchin
 - Resact or speract – peptides released by egg
 - Sperm have resact or speract receptors
 - Species specific
 - Binding causes increase in cGMP
 - cGMP dependent calcium channel
 - Calcium ions enter sperm from water
 - Activates turning response
 - Activates sperm respiration and mobility

Sperm Chemotaxis in Sea Urchin

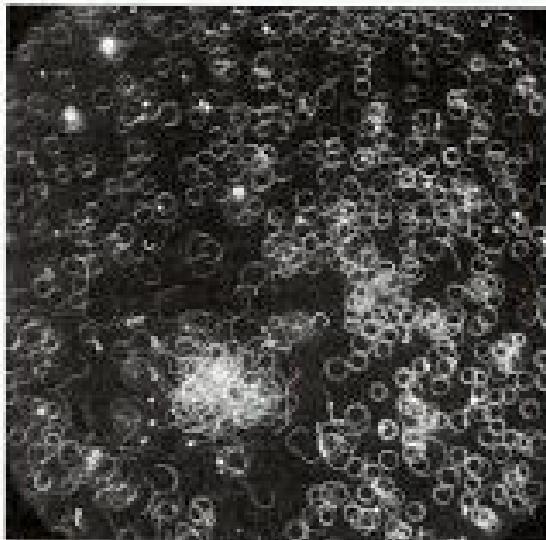
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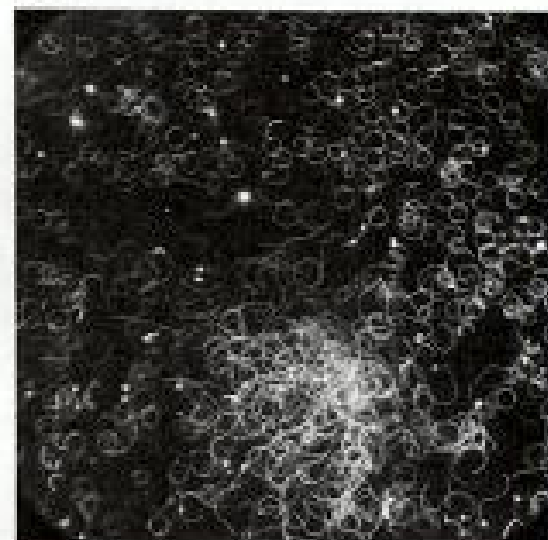
(B)



(C)



(D)



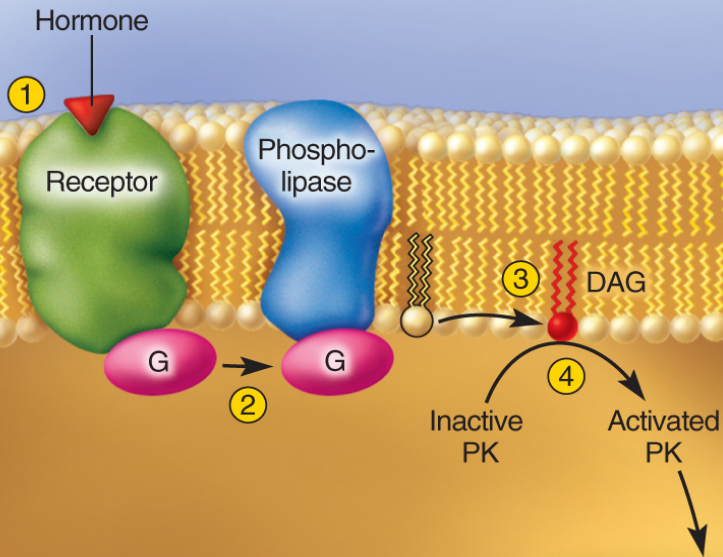
Acrosome Reaction in Sea Urchin

- Sea Urchin
 - Sperm cell surface receptors bind to egg jelly polysaccharides
 - Polysaccharides are species specific
 - Prevents cross species fertilization

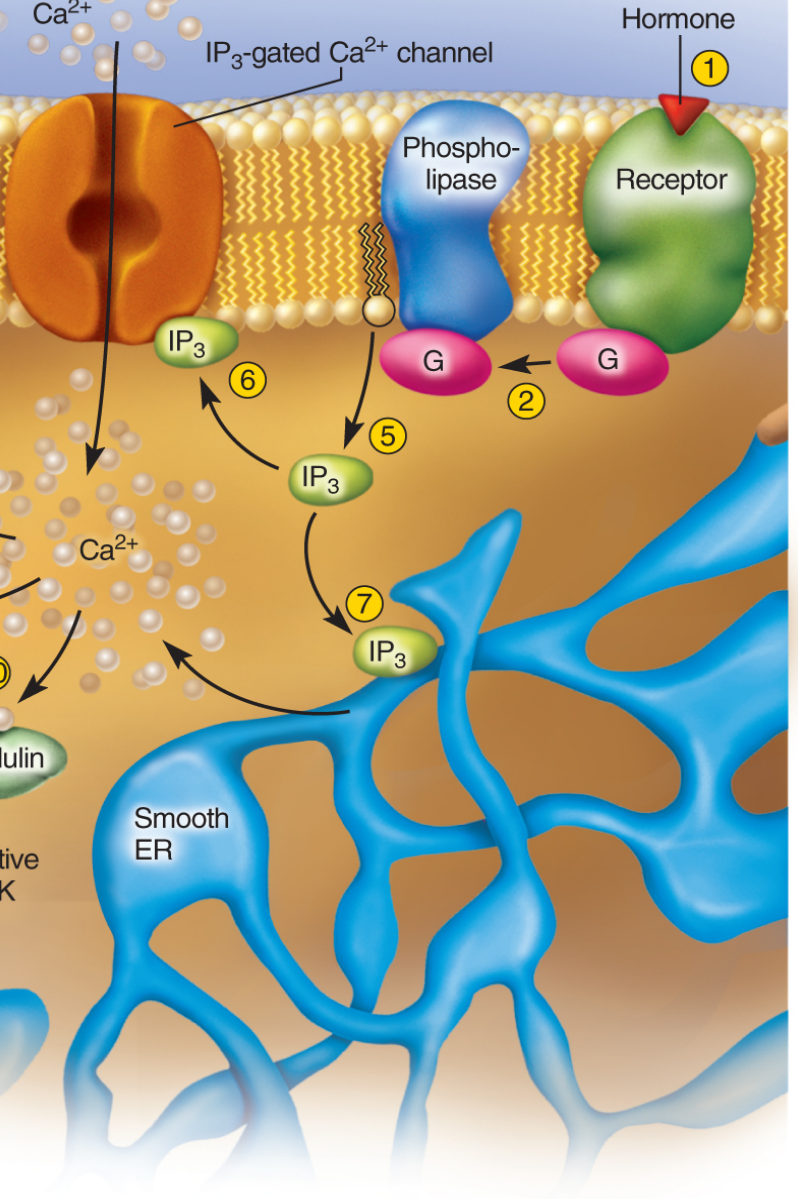
Activation in Sea Urchin

- Open calcium transport channel
- Activates sodium ion (in), hydrogen ion (out) pump
- Activates phospholipase (triggers IP_3 second messenger)

Diacylglycerol (DAG) pathway



Inositol triphosphate (IP_3) pathway



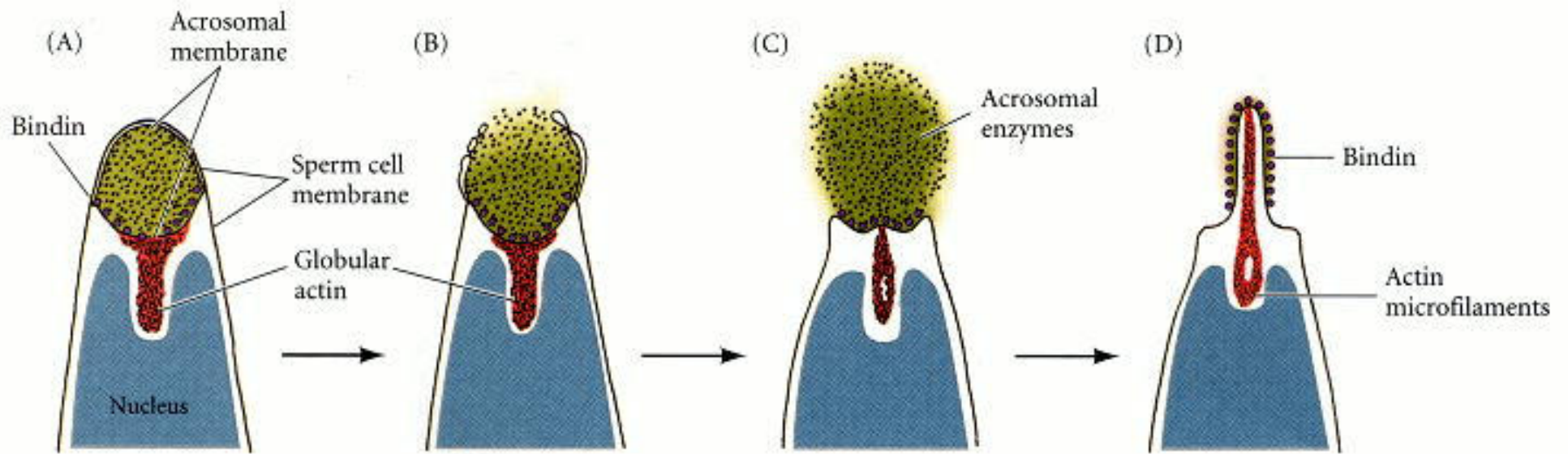
Hormones

ADH
TRH
OT
LHRH
Catecholamines

Key

DAG Diacylglycerol
G G protein
 IP_3 Inositol triphosphate
PK Protein kinase

Acrosome Reaction in Sea Urchin



Acrosome Reaction in Sea Urchin

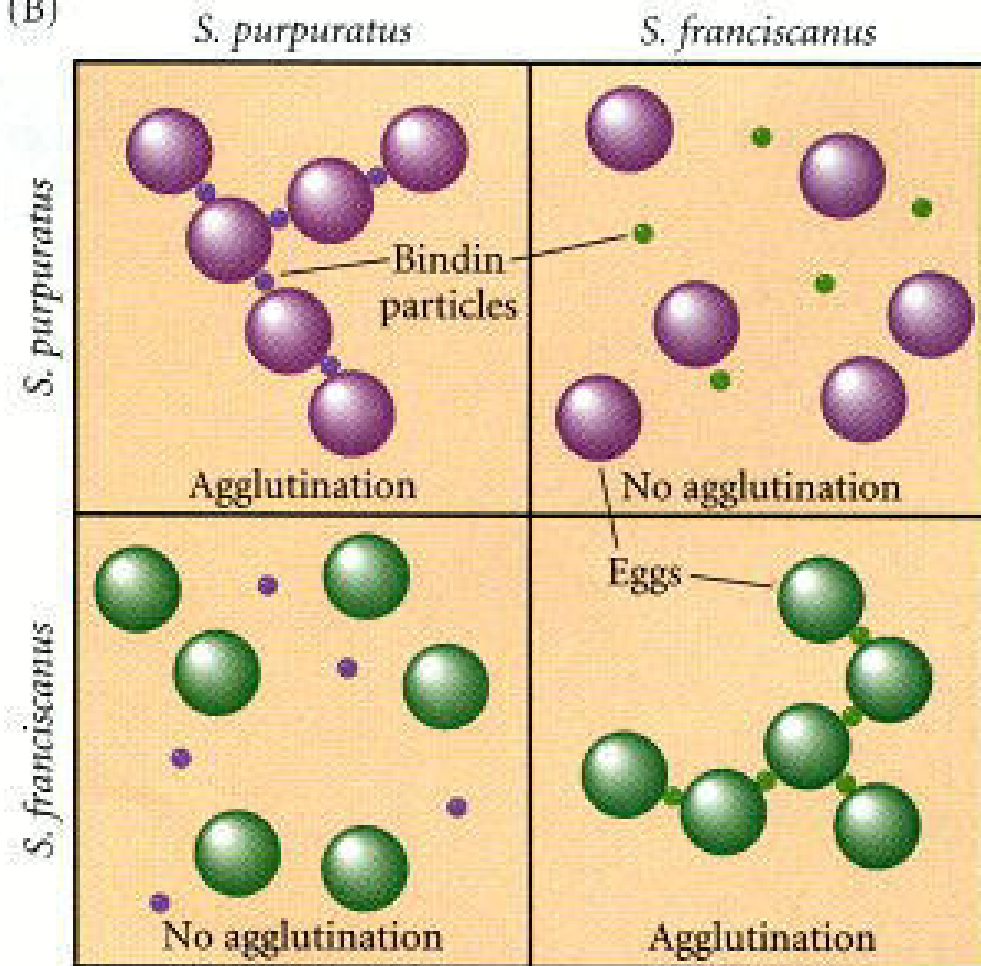
- High calcium causes:
 - Fusion of acrosome to cell membrane
 - Contents are dumped out
 - Acrosomal process formation
 - Calcium dependent RhoB causes polymerization of globular actin
 - Acrosome process extends toward egg membrane
 - Bindin proteins exposed

Acrosome Process is Species Specific Sea Urchin

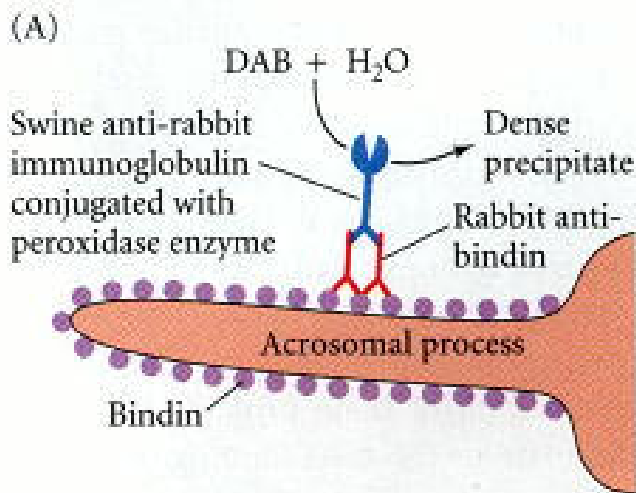
(A)



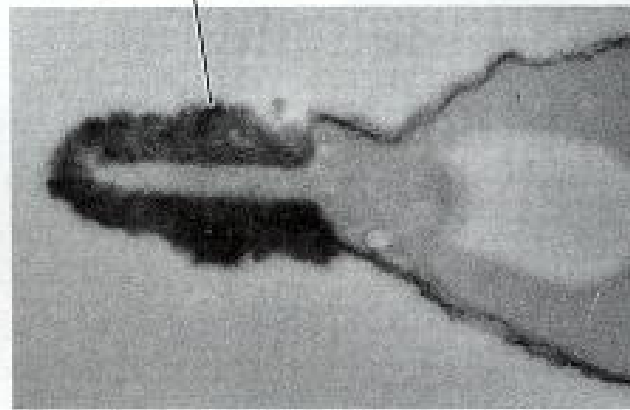
(B)



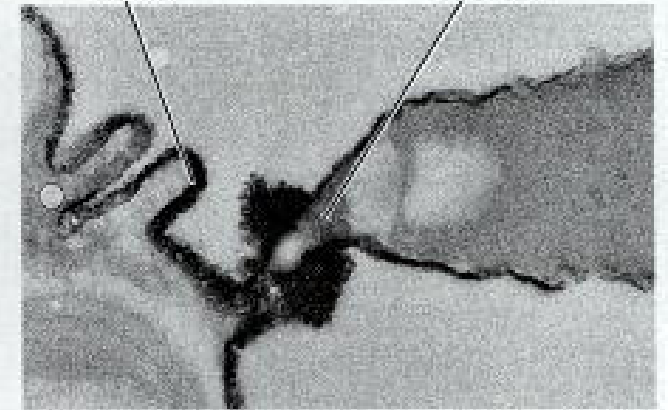
Bindin Localization in Sea Urchin



(B) DAB precipitate

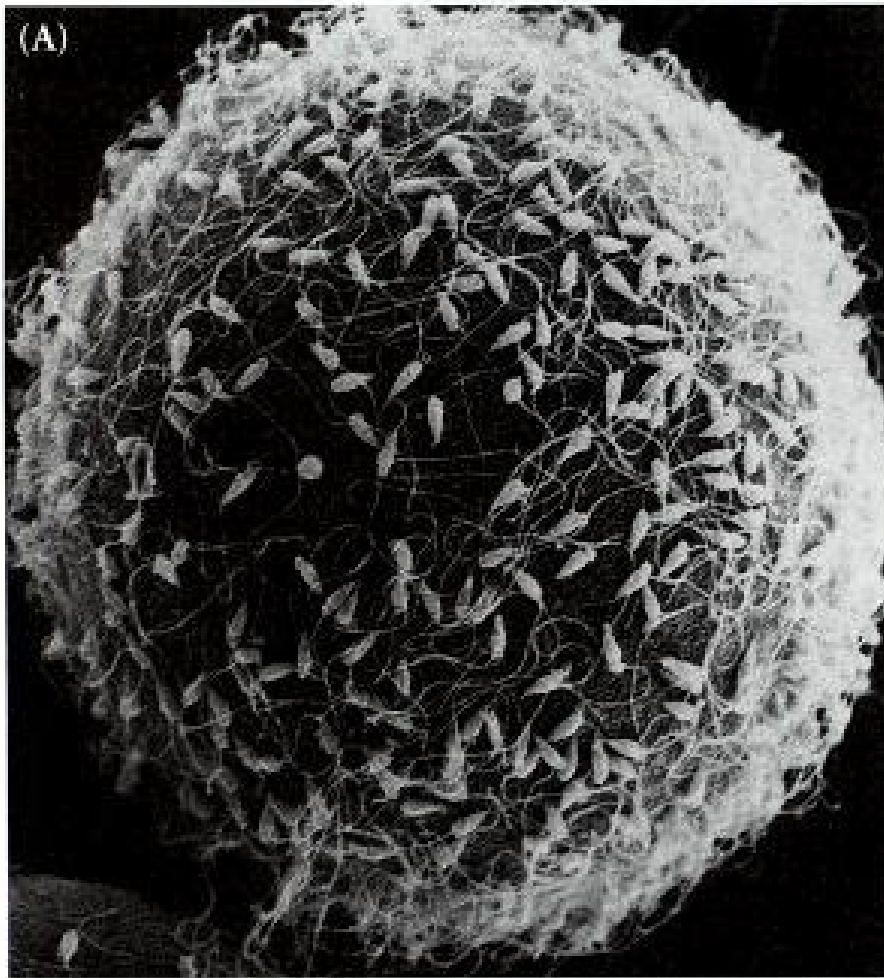


(C) Vitelline membrane of egg Acrosomal process



Bindin Receptors (EBR1) on Egg in Sea Urchin

- EBR1 limiting factor in sperm binding

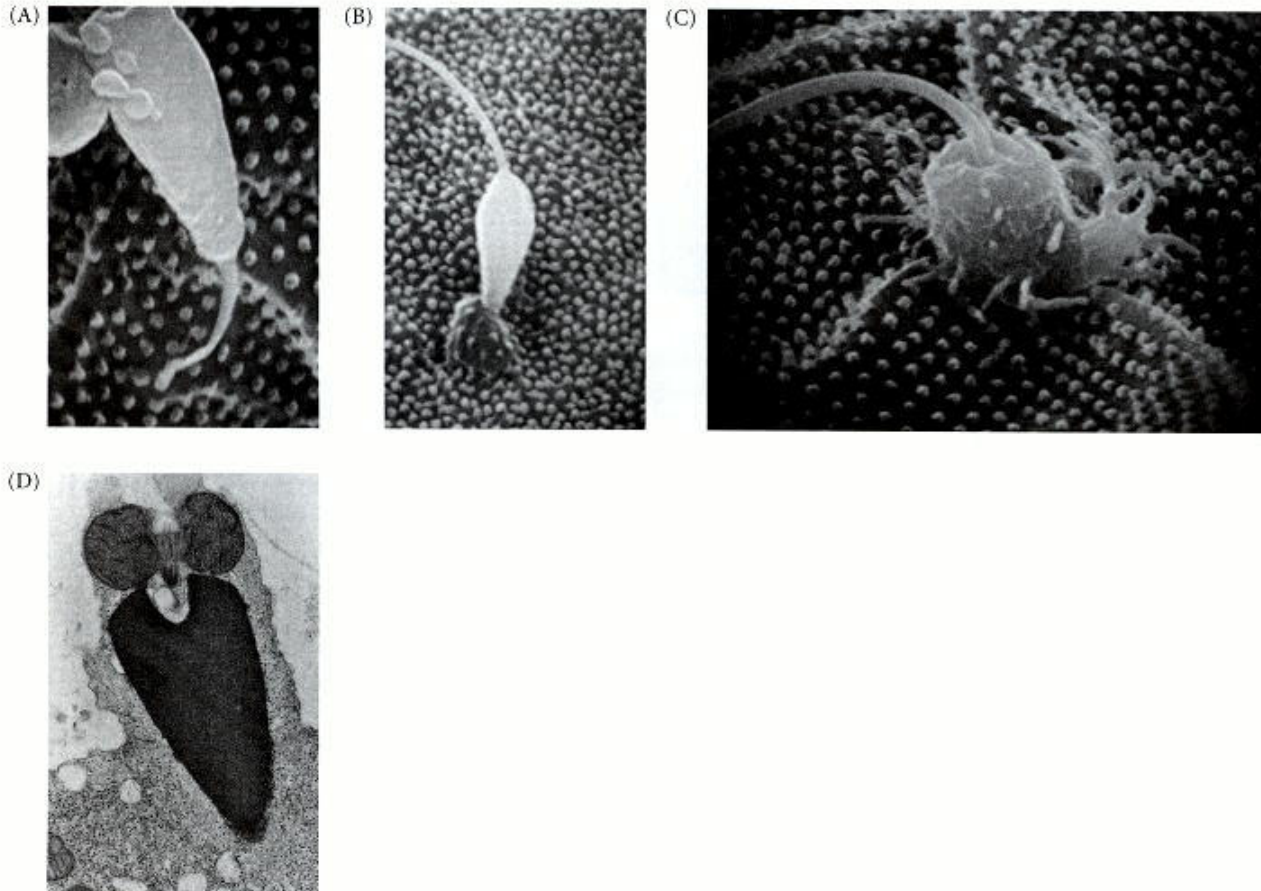


(B)



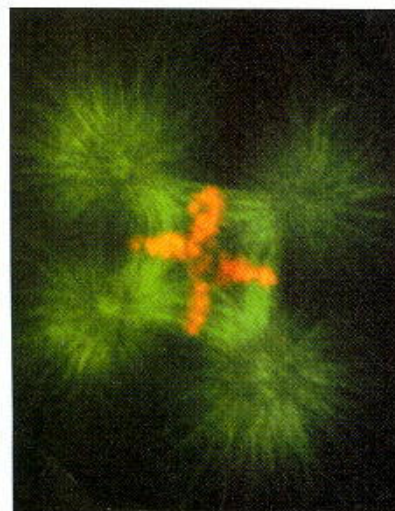
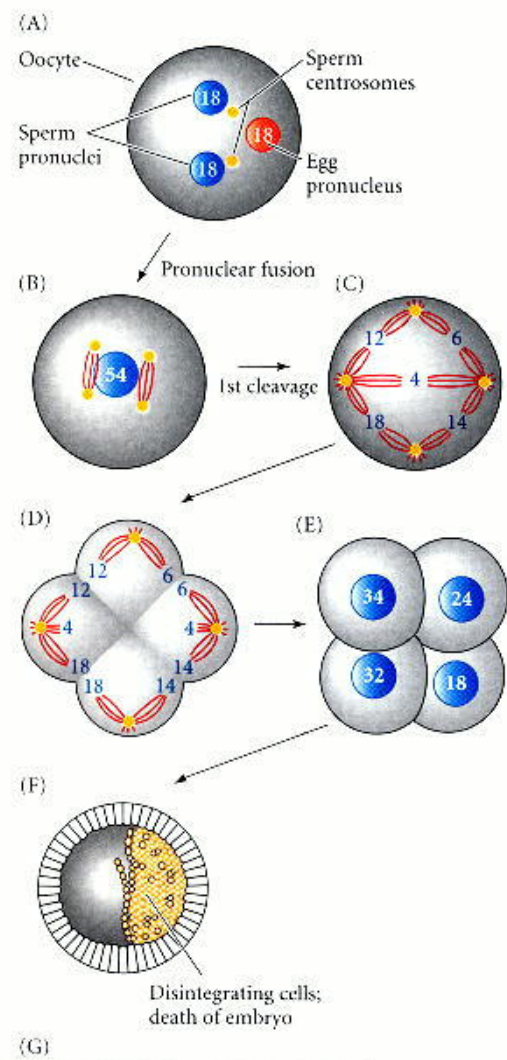
Fusion of Sperm and Egg Membranes (Sea Urchin)

- Egg produces fertilization cone
 - Globular actin polymerizes

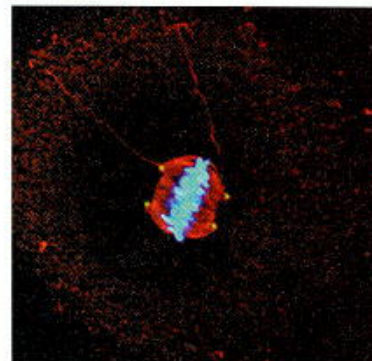


Polyspermy

- When more than one sperm fertilized egg
 - Polyploid nucleus (two sperm enter – triploid)
 - More than two centrioles (multiple cleavage planes)
 - Causes mess! Embryo does not develop



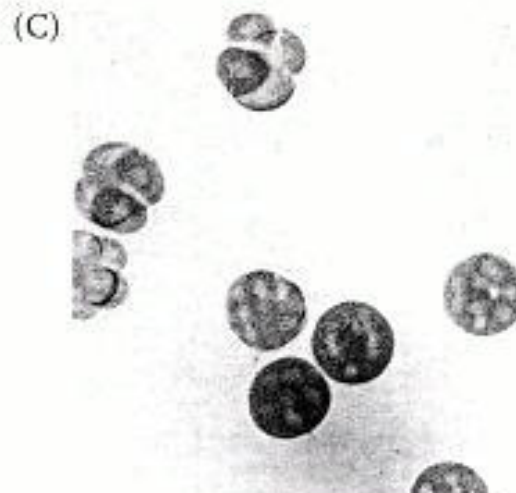
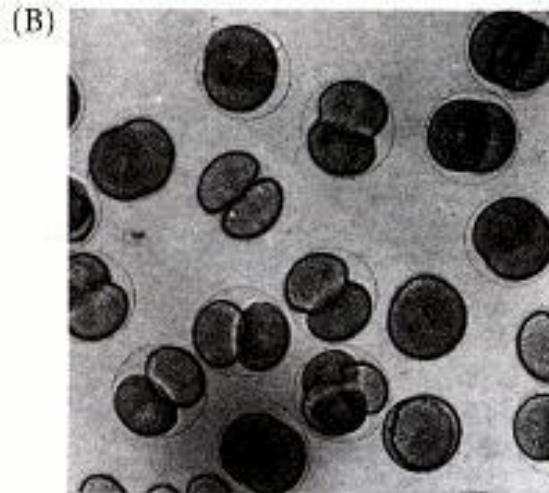
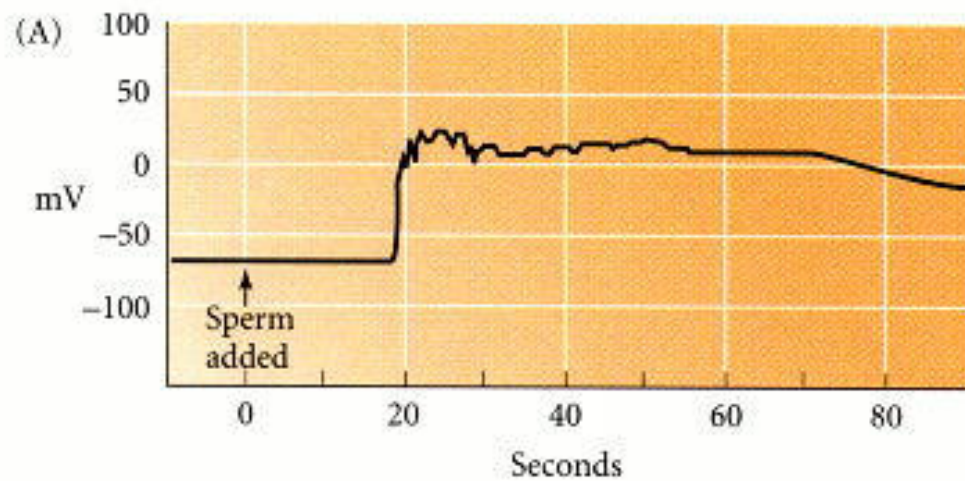
(H)



Fast Block – Sea Urchins

- Change in membrane potential blocks sperm
 - Egg -70 mV
 - Inside negative
 - High Na^+ outside egg (sea water)
 - Binding of sperm causes influx of Na^+
 - Potential changes to +20 mV
 - Sperm cannot fuse at this membrane potential
 - Short lived
 - Slow block needed (cortical reaction)

Fast Block – Sea Urchins



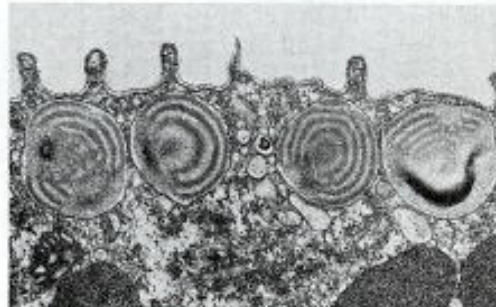
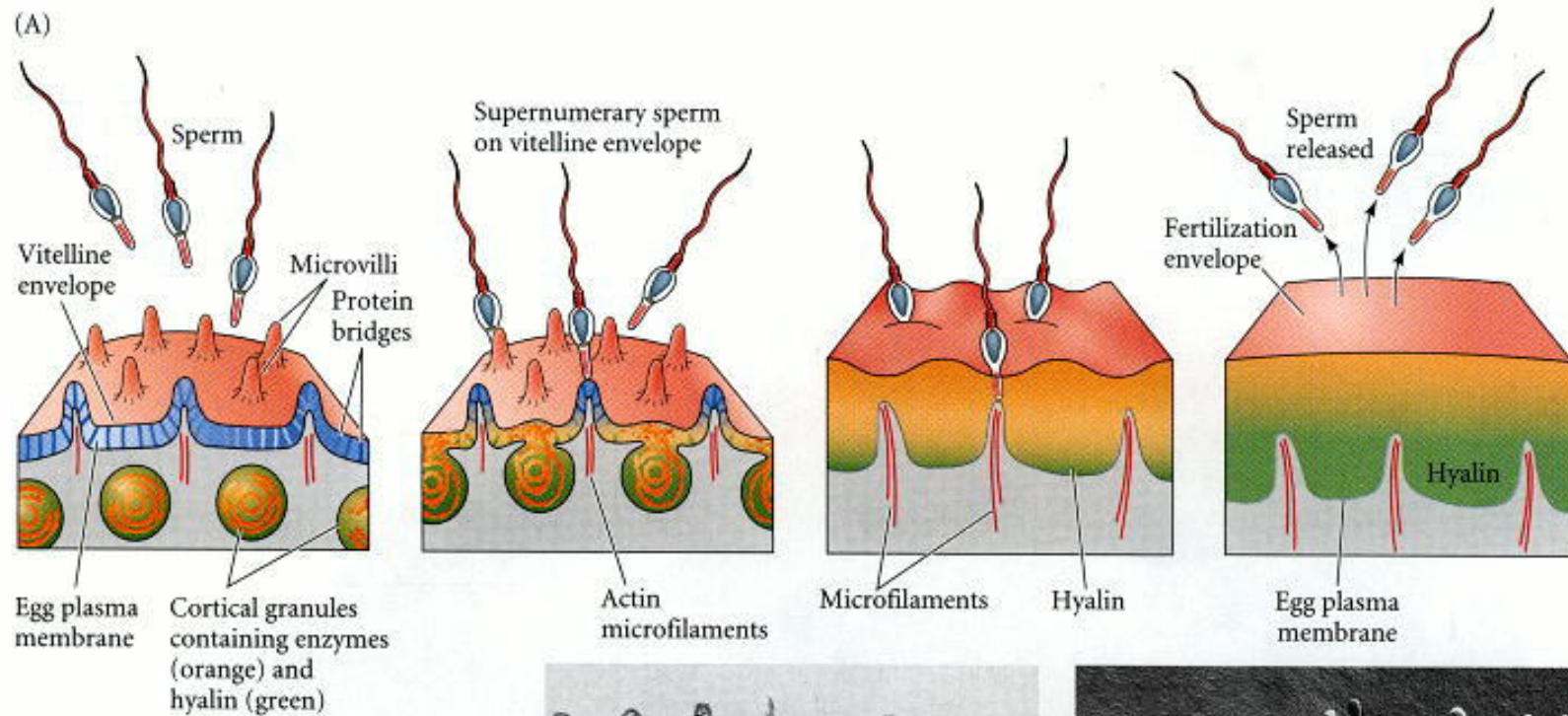
(D)

Na ⁺ (mM)	Percentage of polyspermic eggs
490	22
360	26
120	97
50	100

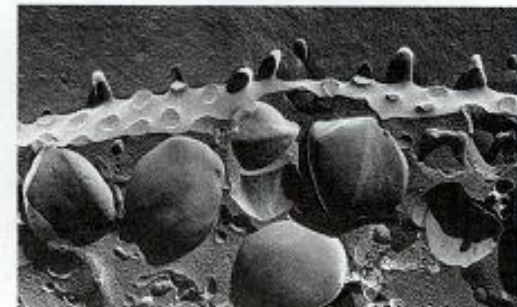
Slow Block – Sea Urchin

- AKA – cortical reaction
- Calcium from endoplasmic reticulum is released
- Cortical granules fuse with plasma membrane
- Cortical granule contents dumped out
 - Serine protease – cuts binding connections
 - Mucopolysaccharides – osmotically swells
 - Peroxidase – crosslinks extracellular proteins

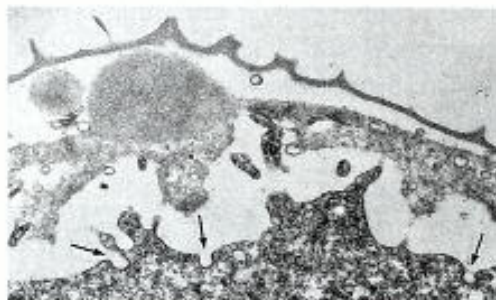
Cortical Granule Exocytosis – Sea Urchin



(B)



(C)

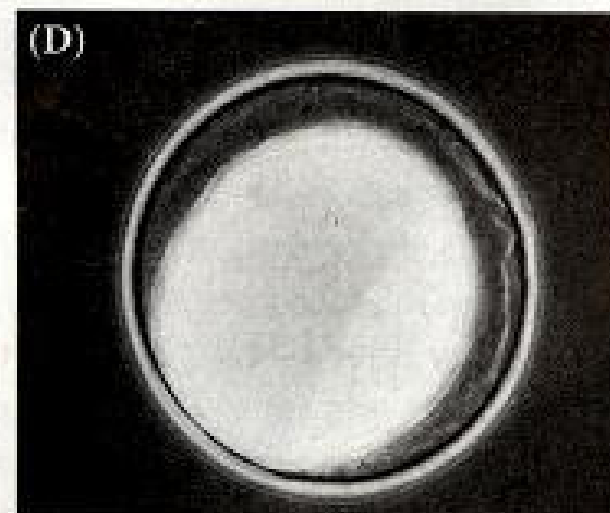
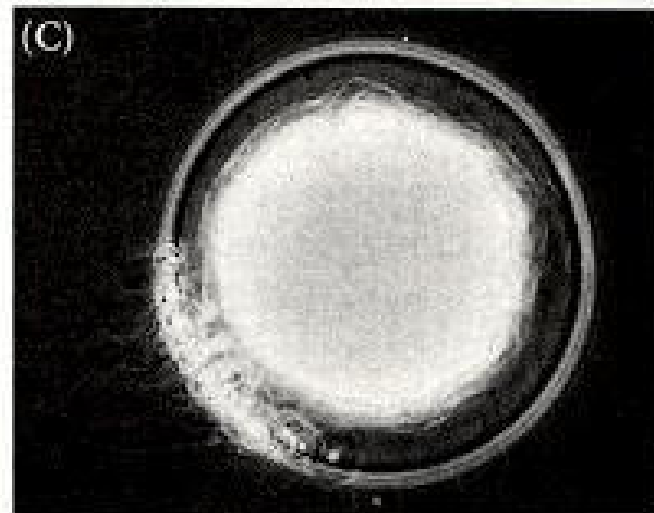
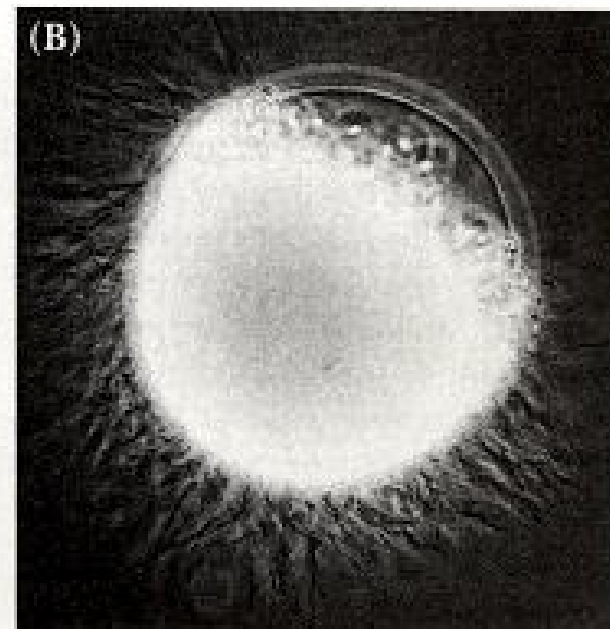
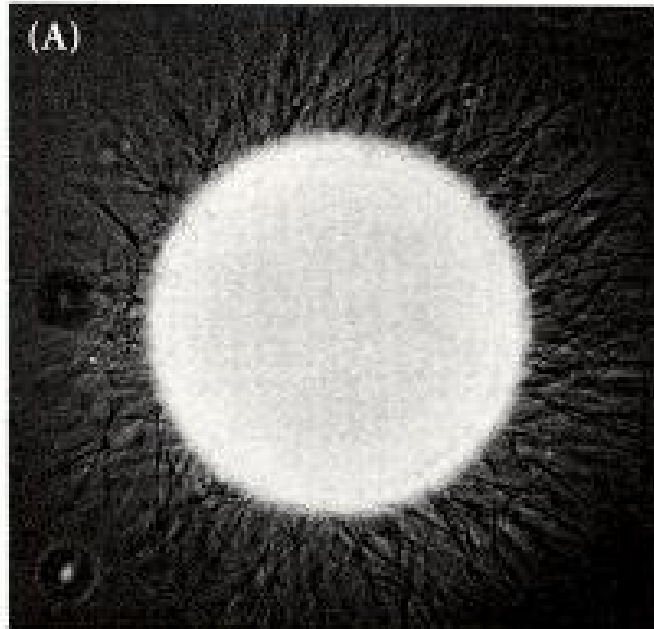


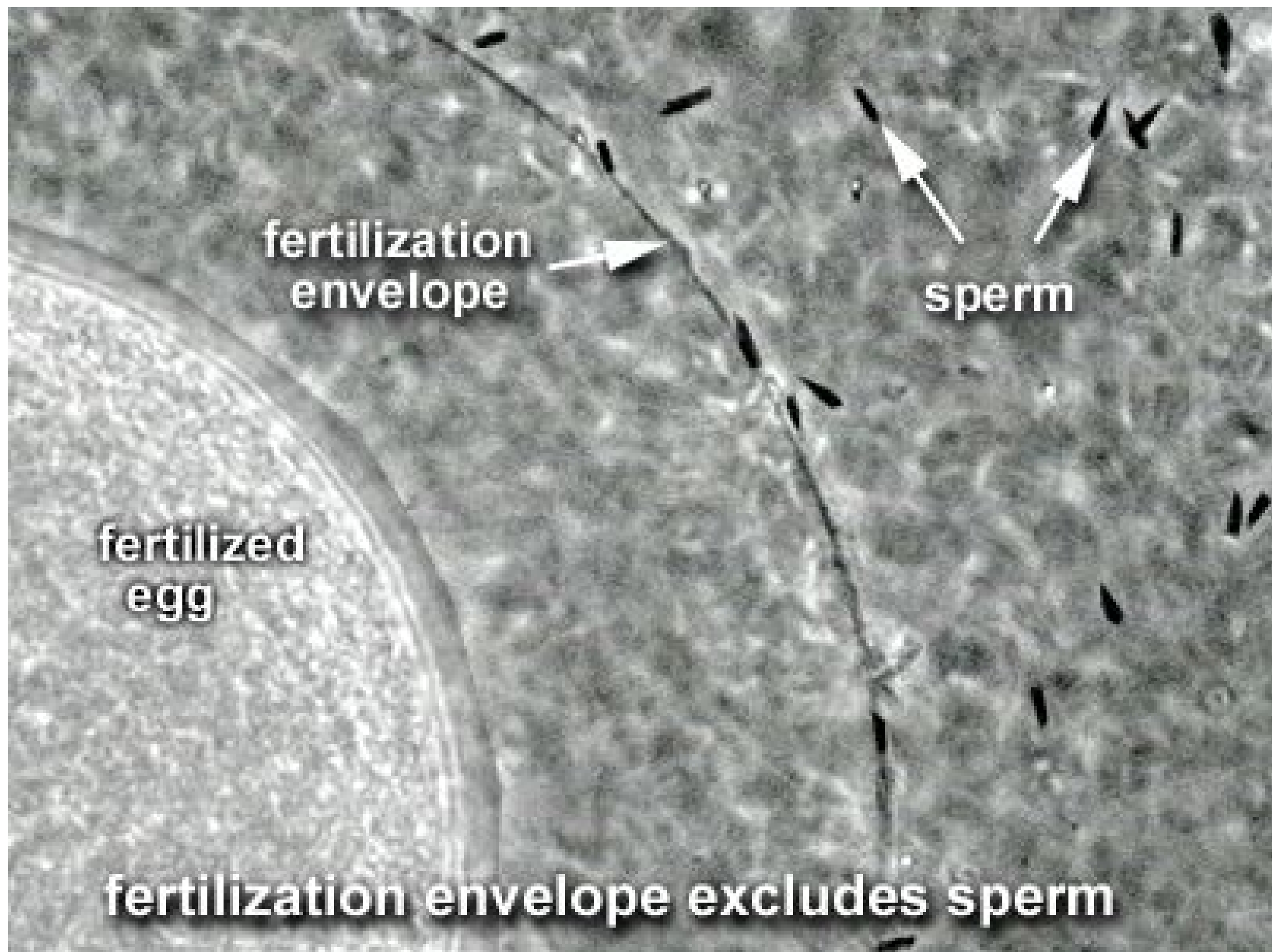
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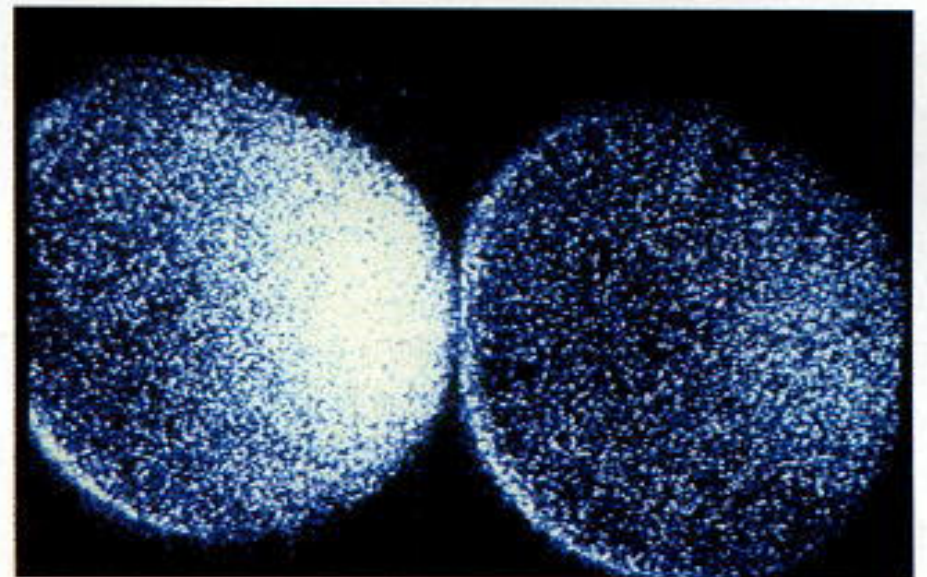
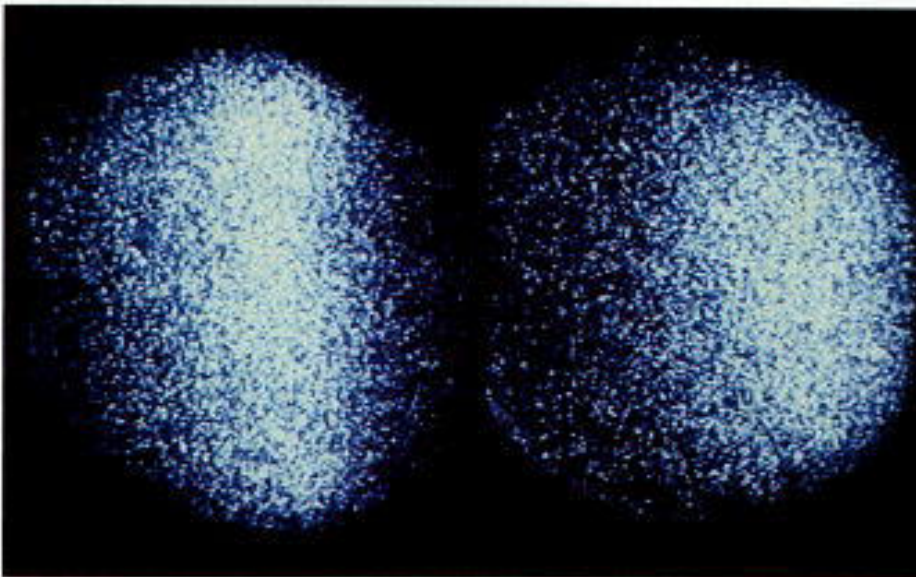
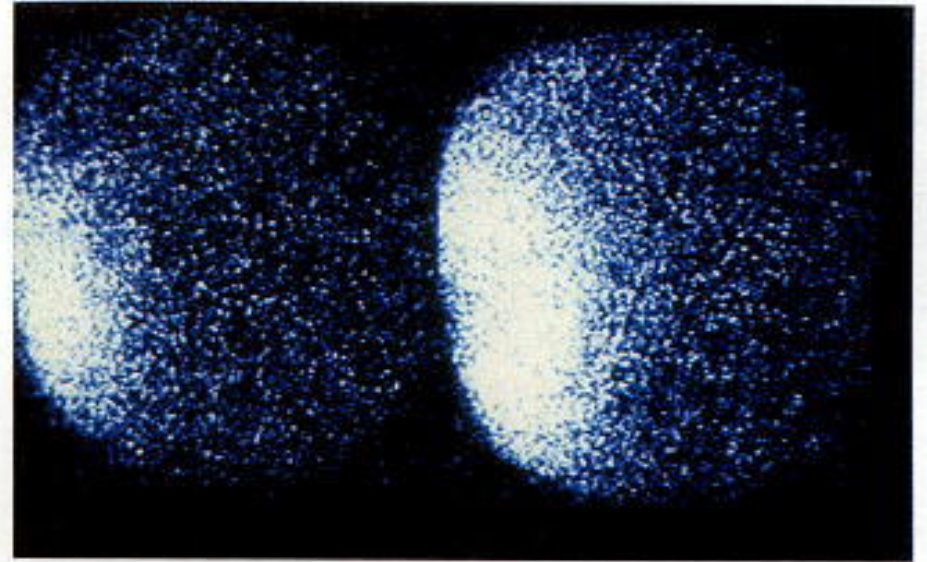
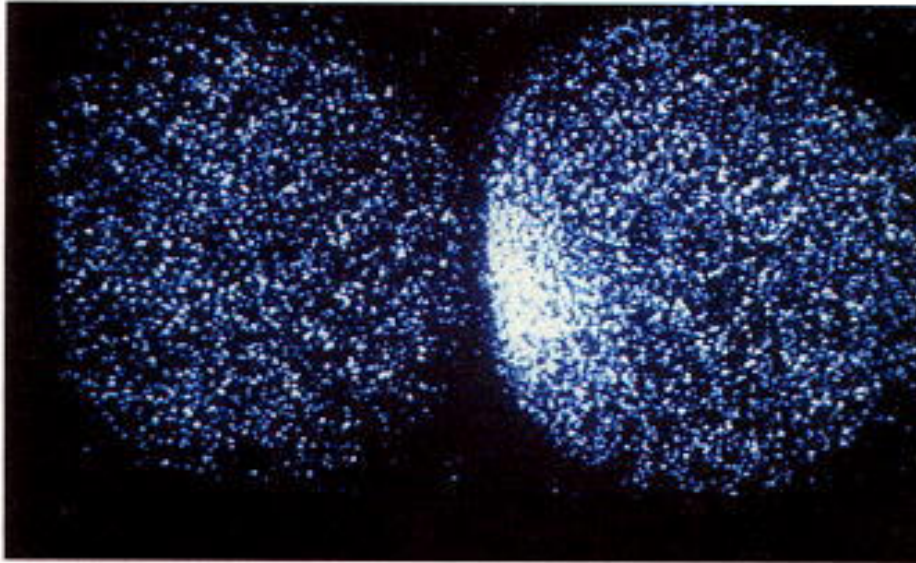
(E)

Fertilization Envelope – Sea Urchin





Calcium Release – Sea Urchin

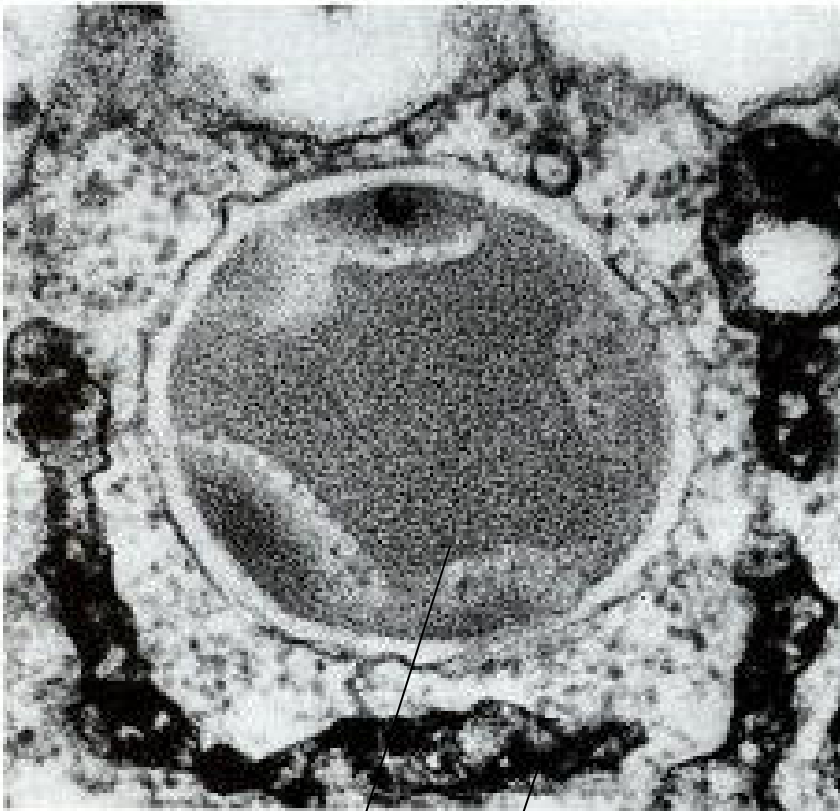


Sea Urchin Egg Activation

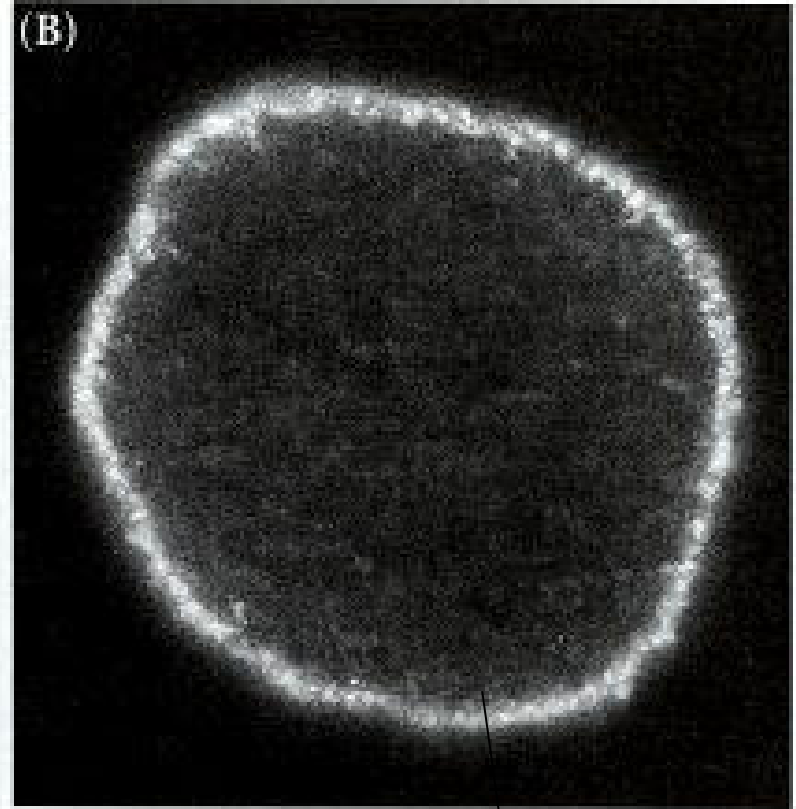
- Before fertilization sea urchin egg is dormant
 - No transcription
 - No translation
 - Low metabolic activity
 - Low cellular respiration
- Fertilization removes blocks to these biochemical function

Cortical Granule

(A)



(B)

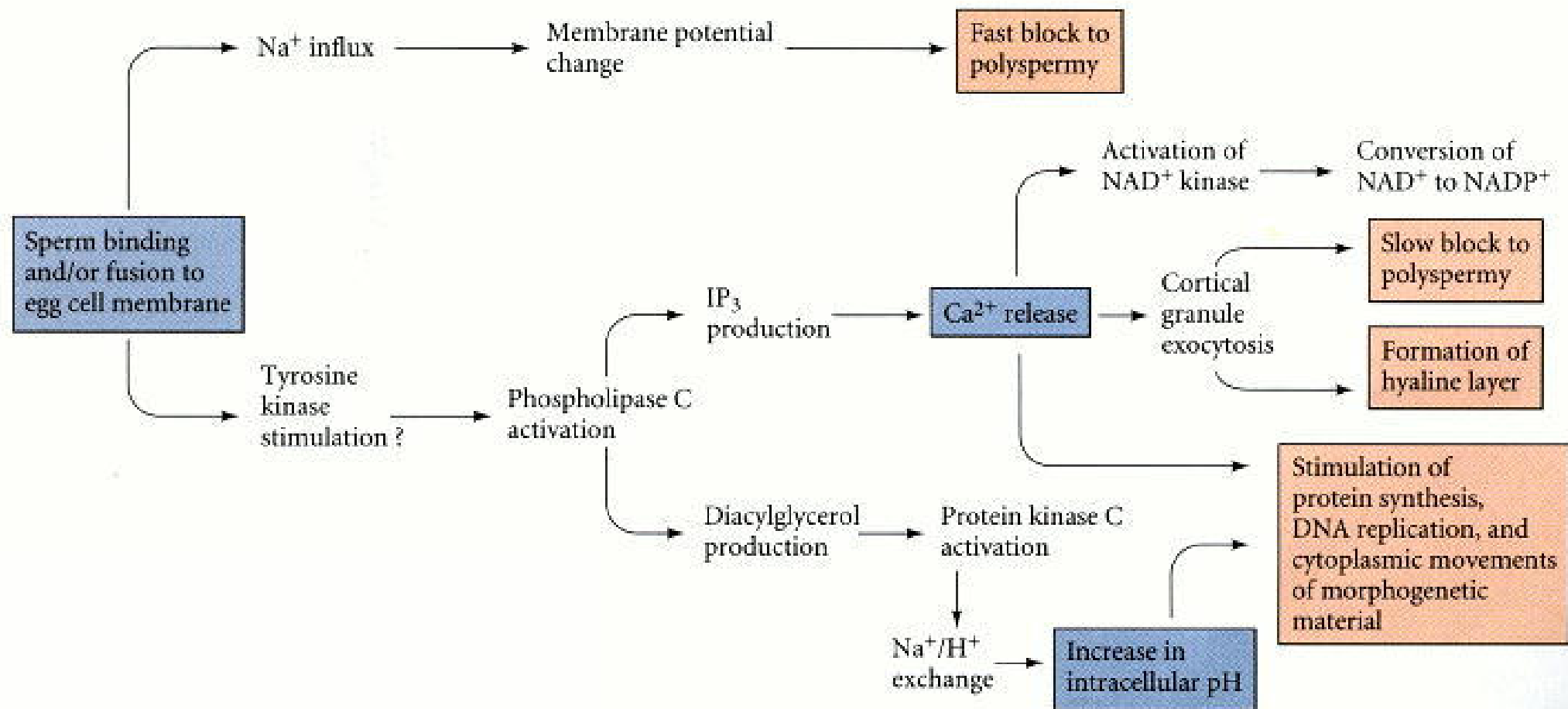


Cortical Granule

Endoplasmic Reticulum

Labeled Calcium Release Channels

Egg Activation Pathway



Is it possible to block the cortical reaction?

- EGTA
 - Chelates calcium (binds calcium)
 - Injecting EGTA into egg inhibits:
 - Cortical reaction
 - Change in membrane potential
 - Cell division

Is it possible to induce the cortical reaction without fertilization?

- Calcium ionophore A23187
 - Allows flow of calcium ions across membrane
 - Triggers cortical reaction without fertilization

Early Response

- Activated by Cortical granule/ Ca^{2+} release
 - Activates NAD^+ Kinase
 - Converts NAD^+ to NADP^+
 - NADP^+ required for phospholipid synthesis

Late Response

- Activation of DNA and Protein synthesis
 - Caused by increase in pH and Ca^{2+}

pH Increase – Late response

- Na^+/H^+ exchange
 - Na^+ in H^+ out
 - Increases pH inside of egg
 - Diacylglycerol pathway
 - Promotes DNA synthesis
 - Artificially increasing pH has same effect

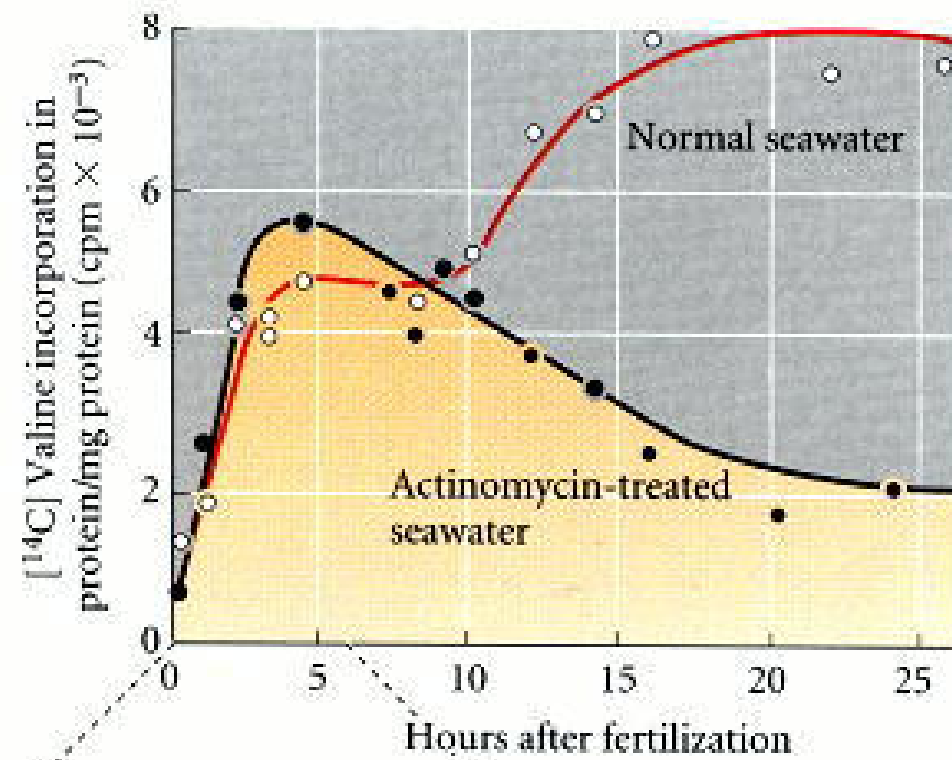
Ca²⁺ Increase – Late Response

- MAP kinase arrests egg cell cycle before S-phase (DNA synthesis)
 - Calcium inactivates MAP kinase
 - MAP kinase inactivation removes DNA synthesis block
 - DNA synthesis can proceed

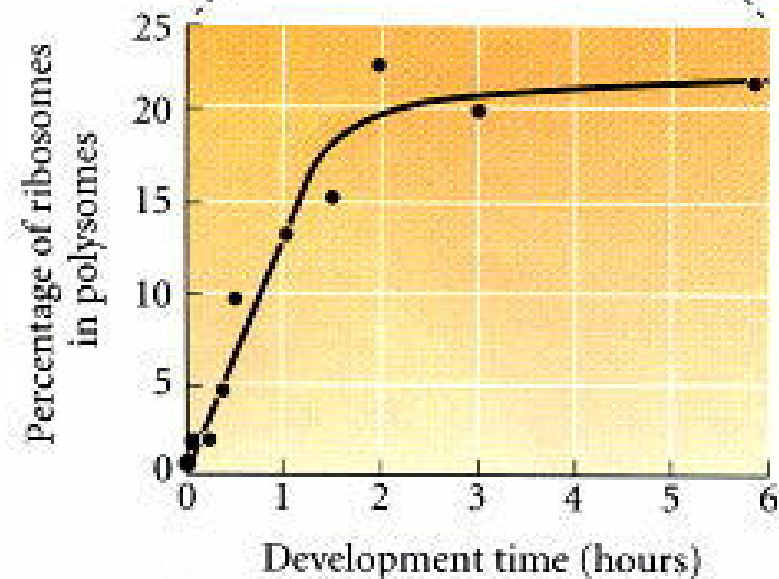
Activation of Translation After Fertilization

- Caused by increase in Ca^{2+} and pH
 - Protein synthesis starts with stored mRNA's
 - Actinomycin inhibits transcription

(A)



(B)



Why is stored mRNA not translated?

- Translation inhibitors bind mRNA
 - 4E-binding protein in sea urchins
 - Maskin in mice

What types of mRNA's are stored?

- mRNA's for early cleavage events
 - Histones – needed to form chromosomes
 - Tubulins – needed to form mitotic spindle
 - Actins – needed for cytokinesis
 - Morphogenetic factors – needed for embryo patterning

Cyclin B - mRNA

- Cyclin B required to pass cell cycle checkpoint
 - Blocked by 4E-binding protein
 - Cyclin B binds Cdk1 to form MPF (mitosis promoting factor)
 - Fertilization initiates mitosis

What does the sperm contribute?

- Haploid genome (half of the total chromosomes)
 - Initially very condensed
- Centrosome
 - Produces mitotic spindle
 - Most animals

What does the egg contribute?

- Haploid genome (half of the total chromosomes)
- Mitochondria
 - Mitochondrial DNA
- Cytoplasm
- Morphogenetic factors
- mRNA and proteins needed for cleavage
- Yolk - food

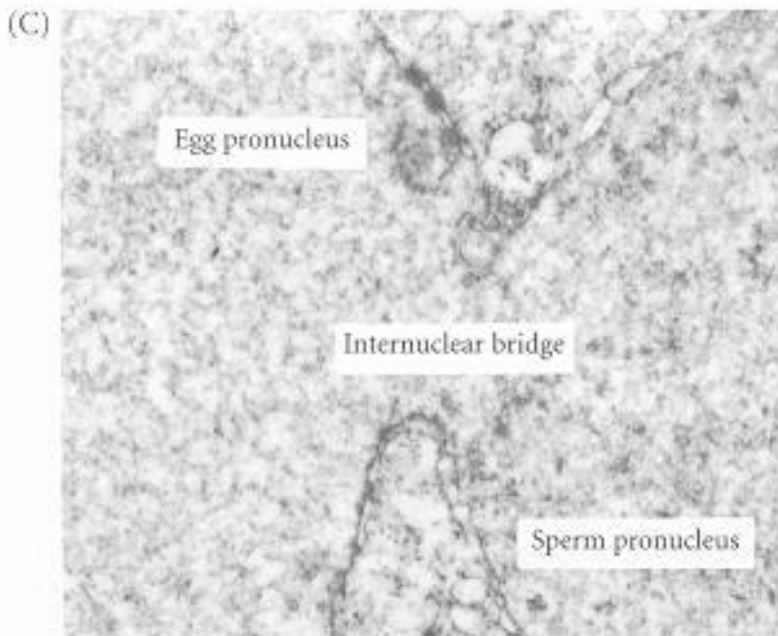
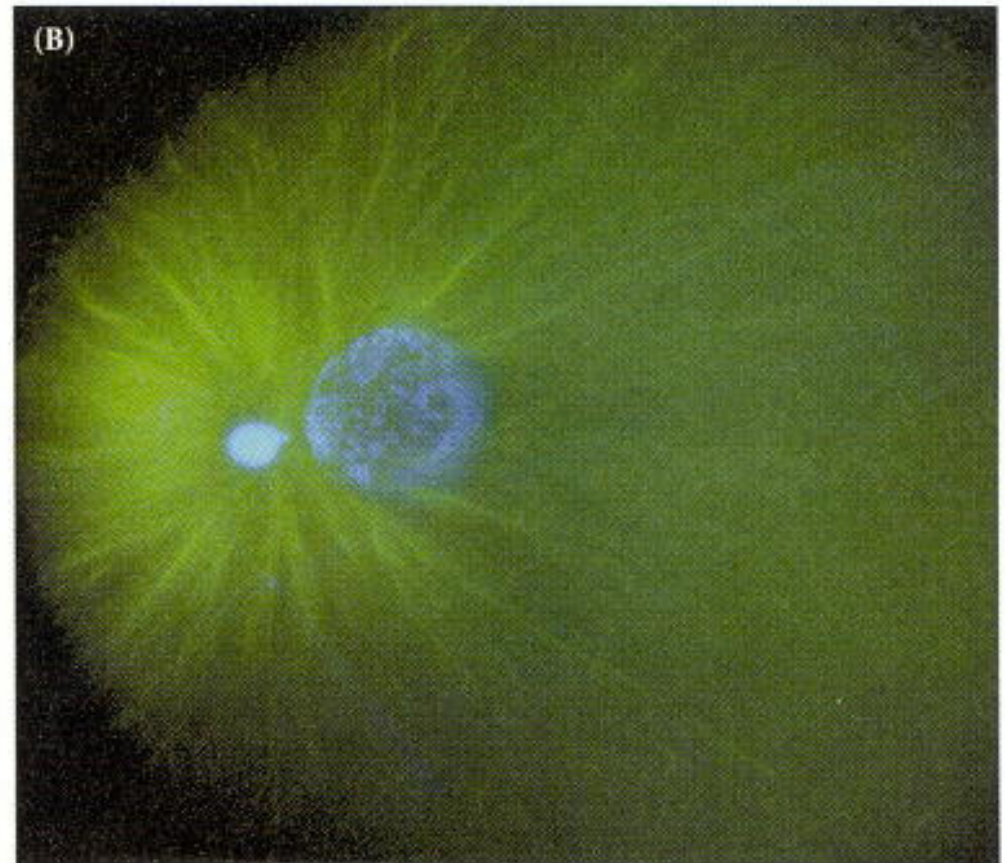
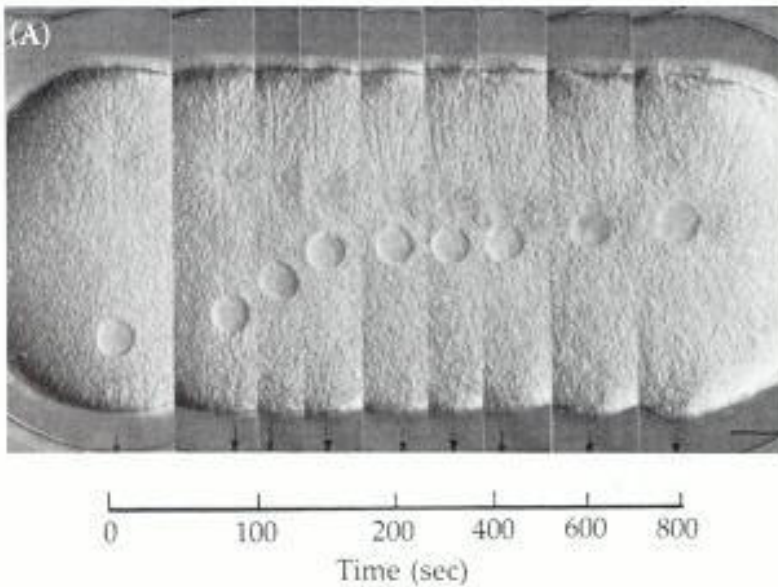
Fusion of Genetic Material

- Sperm and egg pronuclei must migrate toward each other and fuse.
- Both sets of chromosomes become active.

Pronuclei Migration

- Sperm chromosome decondense
 - Sperm histones replaced by egg histones
- Centrosome migrates to side of sperm pronucleus facing egg pronucleus
- Centrosome sends out microtubules
 - Integrate with egg microtubules
 - Migrate toward egg pronucleus
 - Pulls the two pronuclei together

Sperm pronucleus migrating toward egg pronucleus



Fusion of pronuclei forms zygote

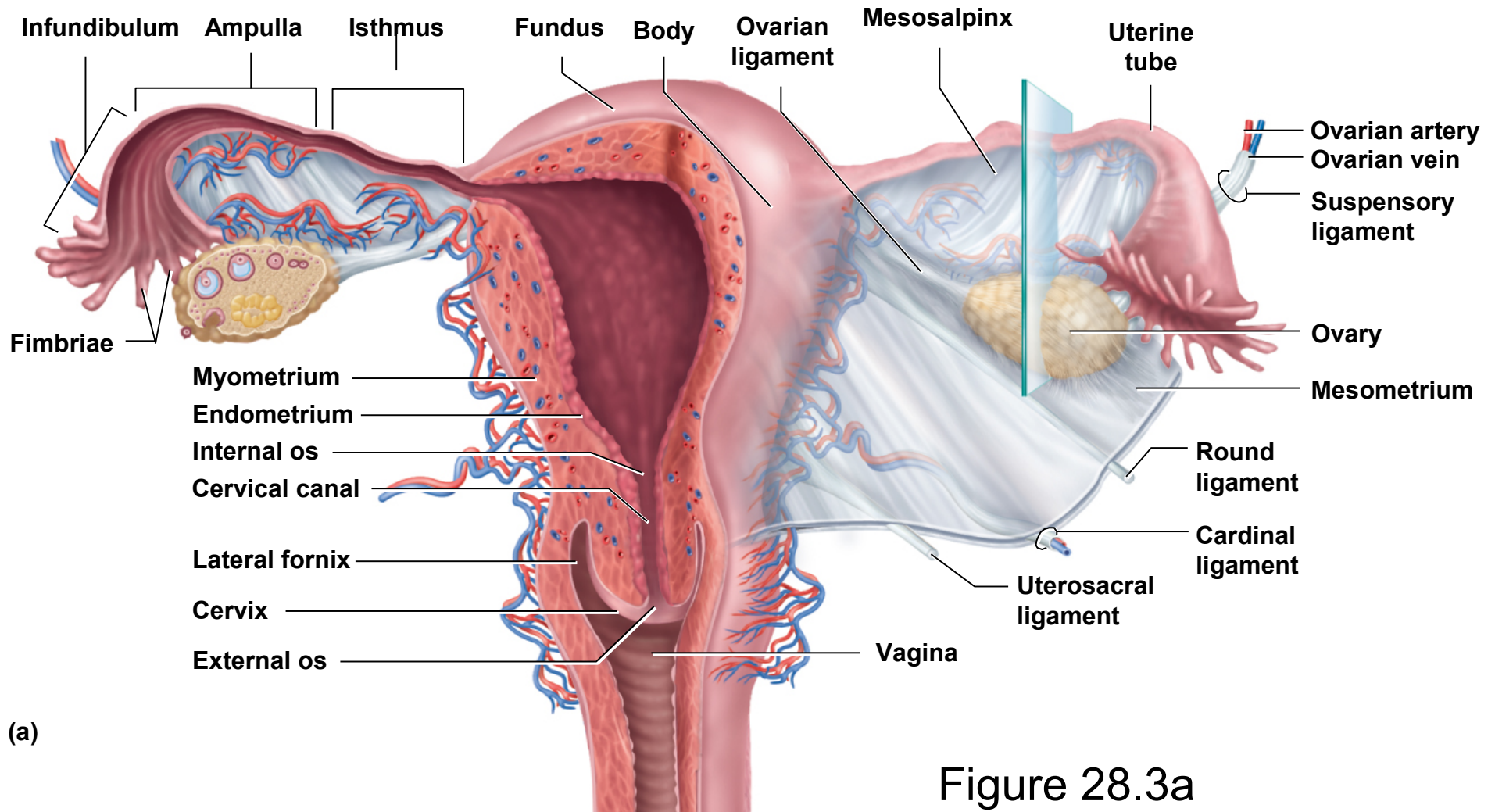
- Diploid nucleus
- DNA synthesis begins either just before fusion or after fusion

Mammalian Fertilization

- Internal fertilization
 - Occurs in the ampulla of the oviduct
 - Both sperm and egg must travel to this location
- Early cleavage stages occur in the oviduct before implantation into uterus

Uterus

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(a)

Figure 28.3a

How do oocytes migrate to oviduct?

- Oocytes ovulates off surface of ovary
 - Carries cumulus cells in an extracellular matrix
- Taken up by fimbriae at opening of oviduct
 - Ciliary beating draw in oocyte
- Combination of cilia and muscle contraction moves oocyte toward ampulla (site of fertilization)

How do sperm cells migrate to oviduct?

- Deposited into vagina near cervix
- Pass cervix, through uterus, into isthmus of oviduct then finally into the ampulla of oviduct
- Flagellar motion not sufficient to transport sperm to destination
 - Muscular contraction of uterus
 - Flagella most important inside oviduct

Sperm capacitation

- Capacitation – final maturation of sperm
 - Completed during trip to oviduct
 - Unblocks acrosome reaction

What are the advantages of capacitation?

- Longer time viable sperm are present.
- Increased chance of sperm meeting egg.

What changes during capacitation?

- Cholesterol removed
 - Rafting of receptor proteins on anterior end.
- Cell surface proteins and carbohydrates change
 - Unmask receptor proteins
- Efflux of potassium ions
 - Inside of sperm becomes more negative
 - Allows easier influx of Ca^{2+}
- Protein phosphorylation
 - Activates receptor proteins
- Plasma membrane and outer acrosomal membrane fuse

What is the fate of uncapacitated sperm?

- Bind to oviduct membrane in isthmus
- Slows down capacitation
- Increases longevity of sperm

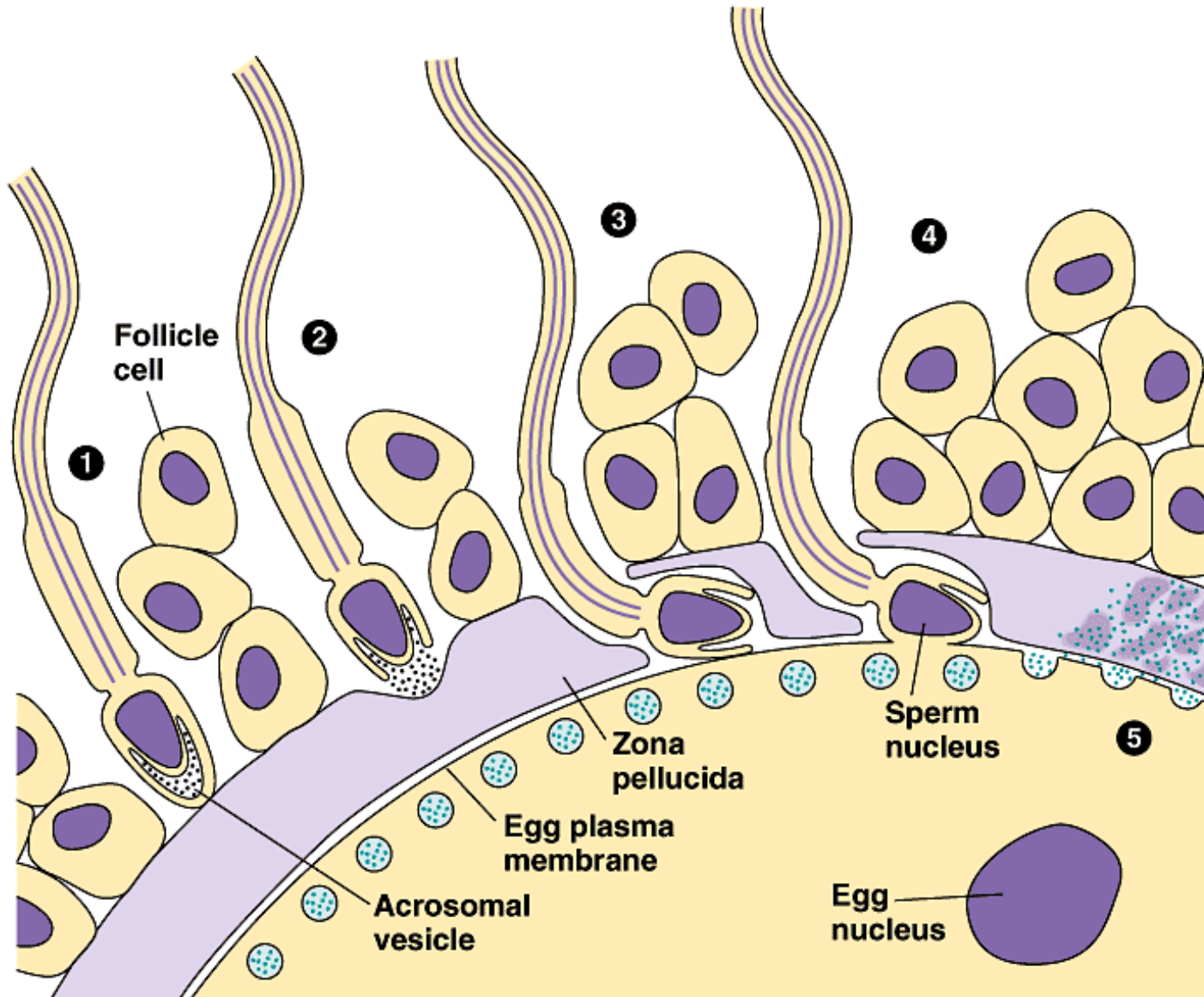
Thermotaxis of sperm

- Capacitated sperm move toward warmer region of oviduct.
 - Sense temperature gradient.
 - Ampulla 2°C warmer than isthmus.

Chemotaxis of Sperm

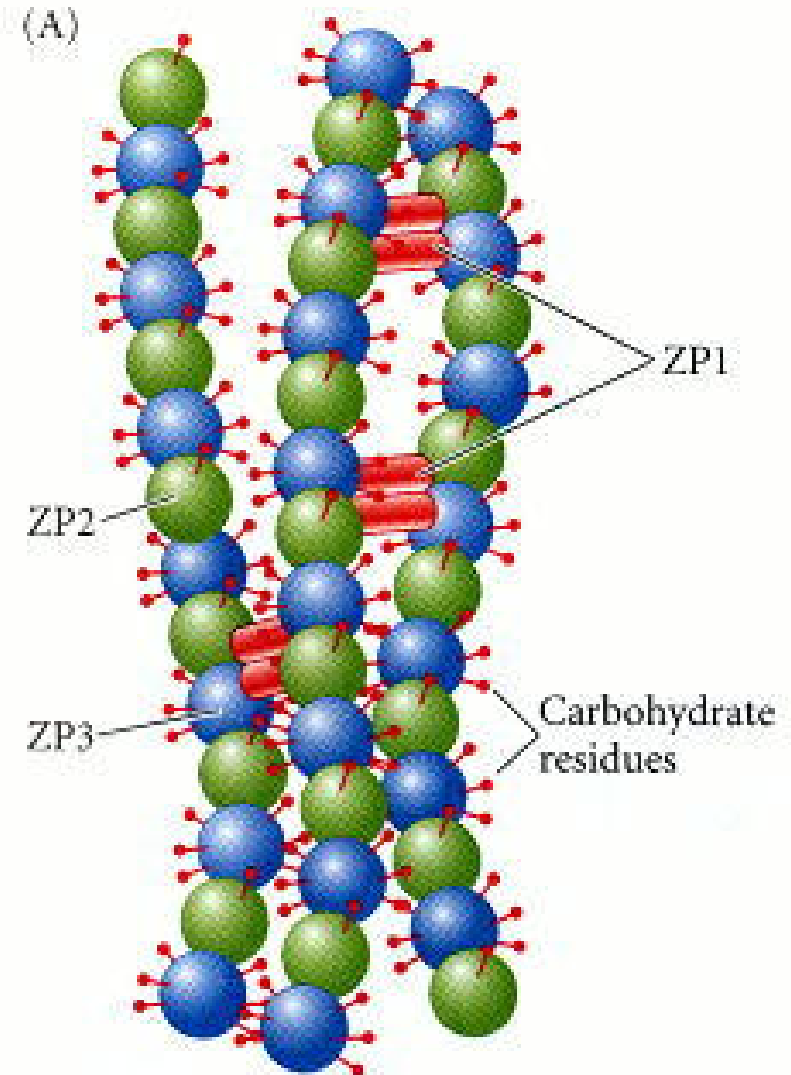
- Oocyte and cumulus cells secrete sperm chemotactic factors.
- Capacitated sperm move toward areas with higher level of chemotactic factors.

Mammalian Fertilization



Zona Pellucida

- Made of:
 - Three crosslinked glycoproteins
 - Polymer of ZP2 and ZP3 crosslinked by ZP1
 - Peripheral proteins
- Bind sperm and initiates acrosomal reaction



Early Stages in Sperm Binding

- 1) Weak interaction with peripheral proteins.
- 2) SED1 protein on side of sperm head binds to zona protein complex.
 - Antibodies against SED1 blocks fertilization.

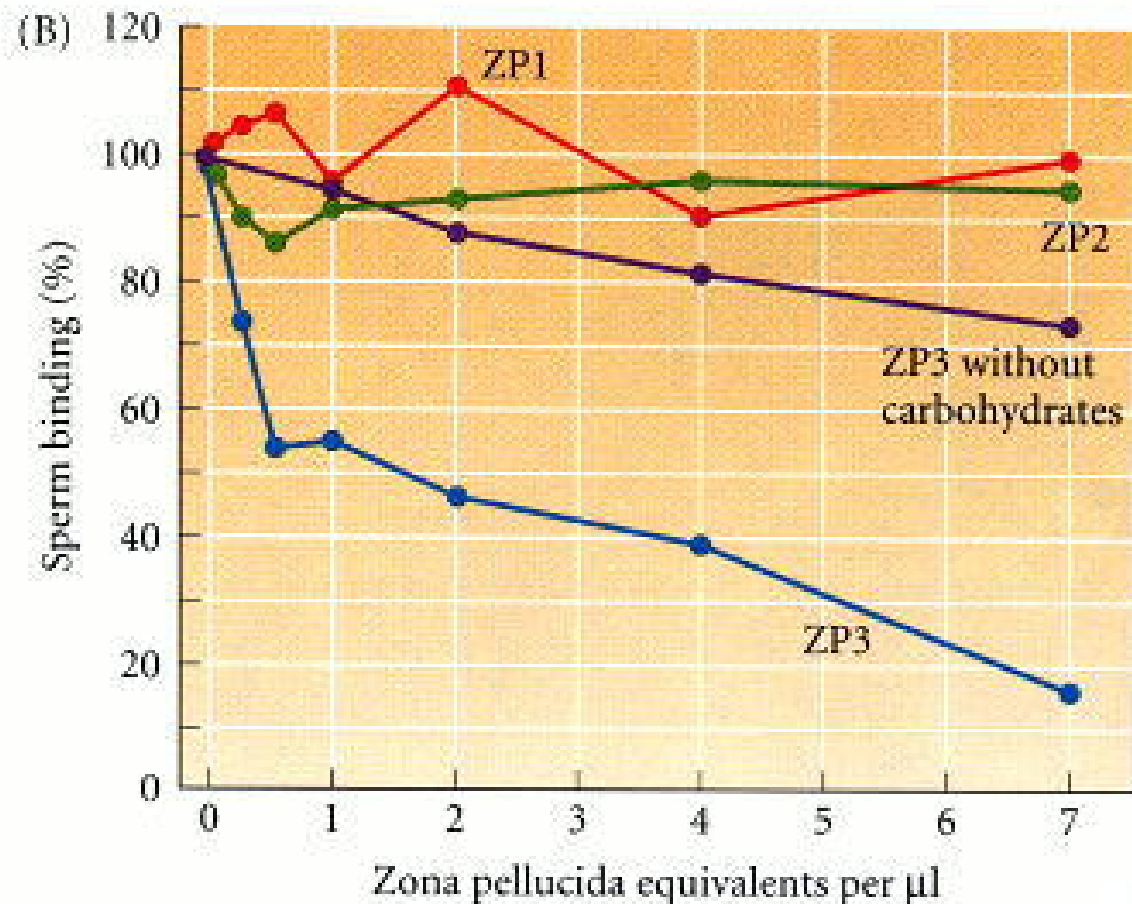
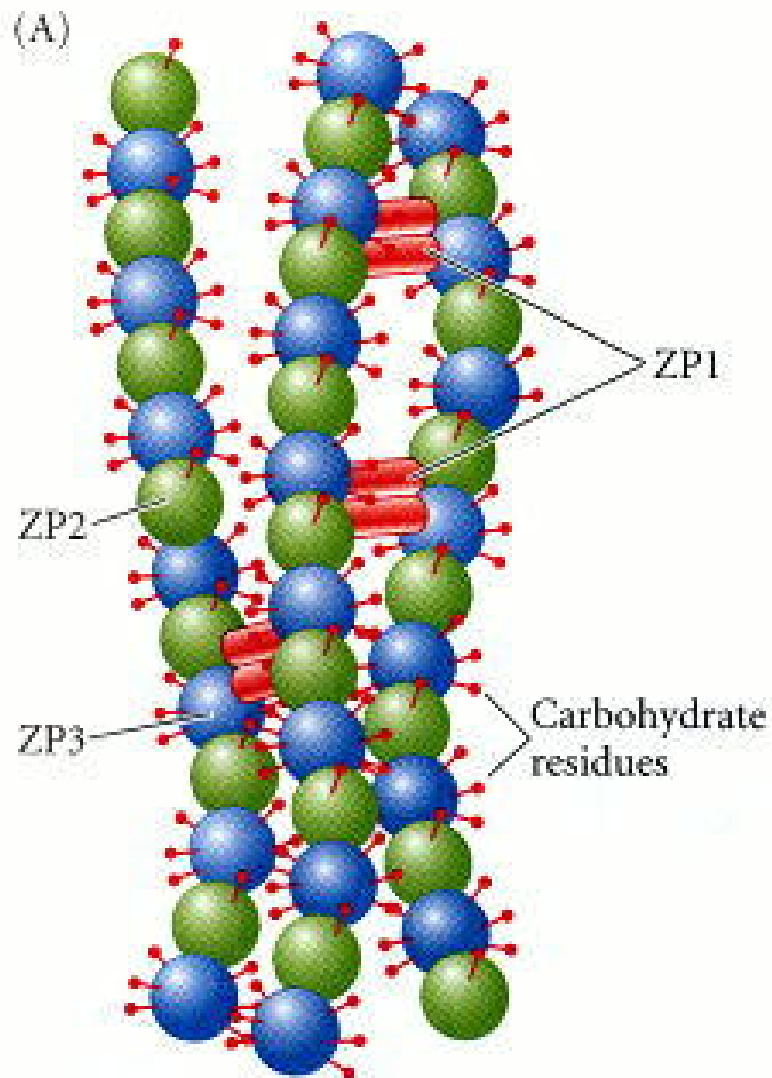
Final Stages in Sperm Binding

- 1) Sperm binds to ZP3
- 2) ZP3 is crosslinked to GalT on surface of sperm
- 3) Acrosome reaction is induced

ZP competition experiments

- Adding soluble ZP3 prevents fertilization
- ZP3 inactivated when carbohydrates are removed

ZP proteins and fertilization



Radioactive ZP3 binds to sperm head

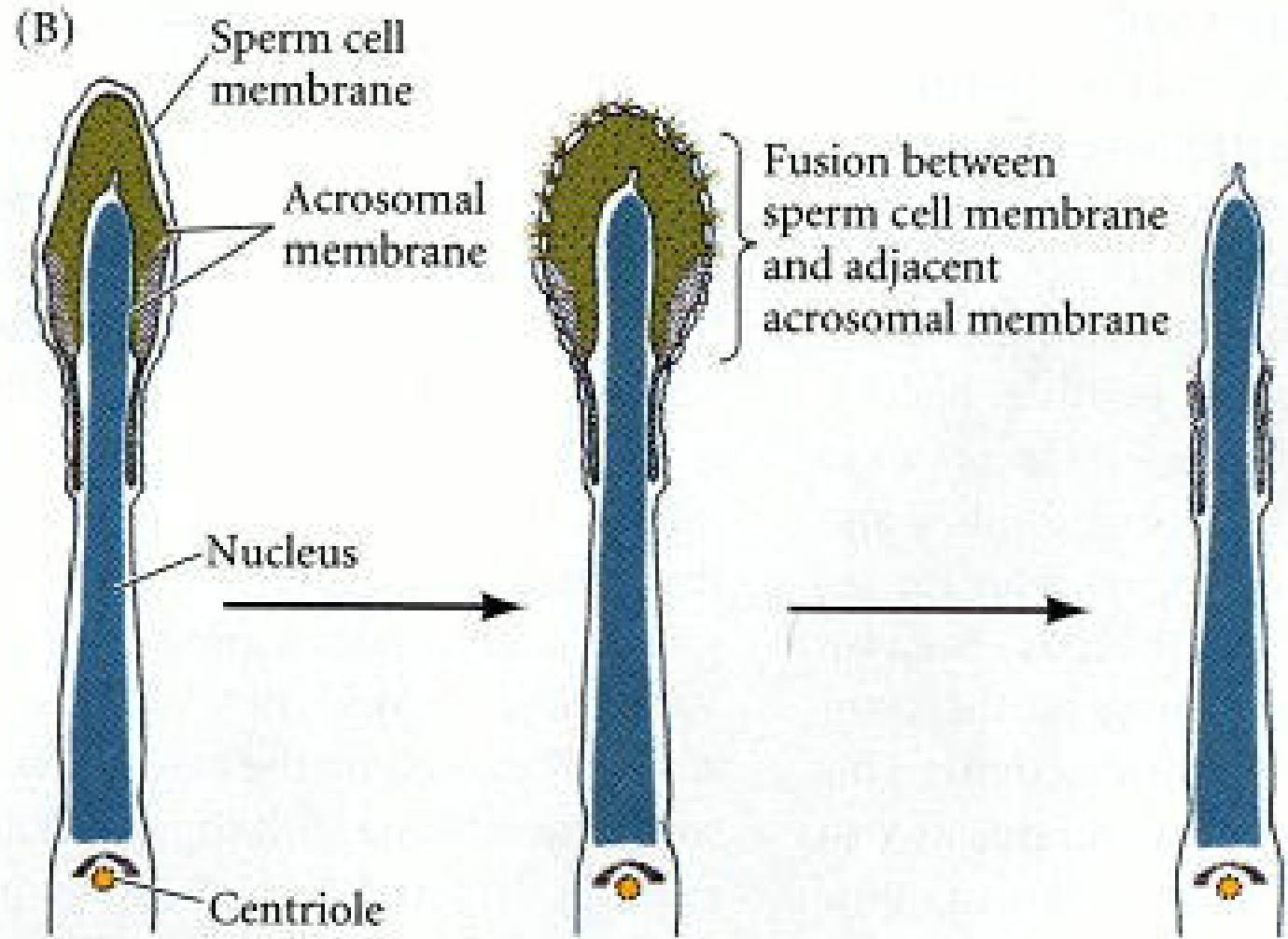
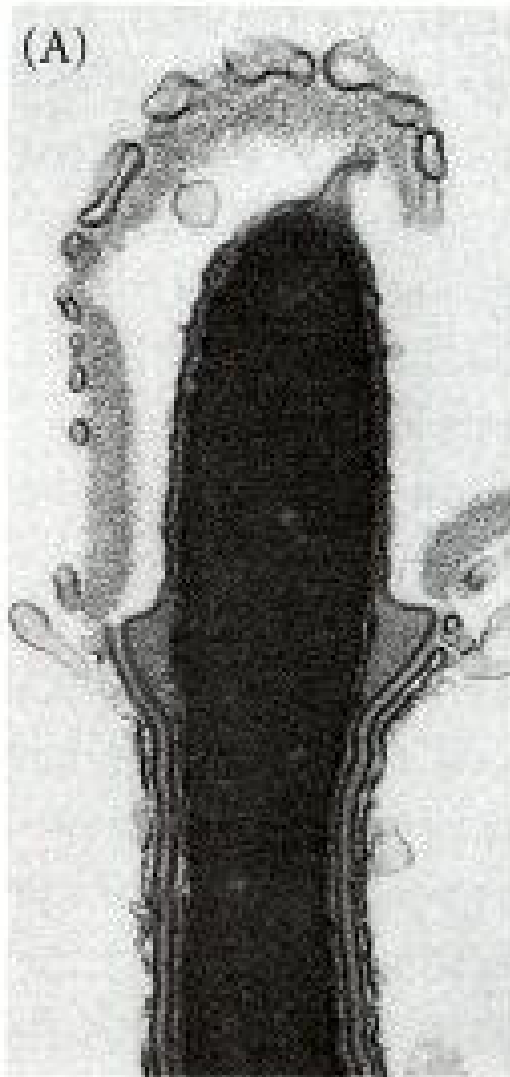
(B)



Induction of Acrosomal Reaction

- Crosslinking of sperm surface receptors by ZP3:
 - (1) Activates G proteins
 - (2) Opens calcium channels
 - (3) Exocytosis of acrosomal vesicle

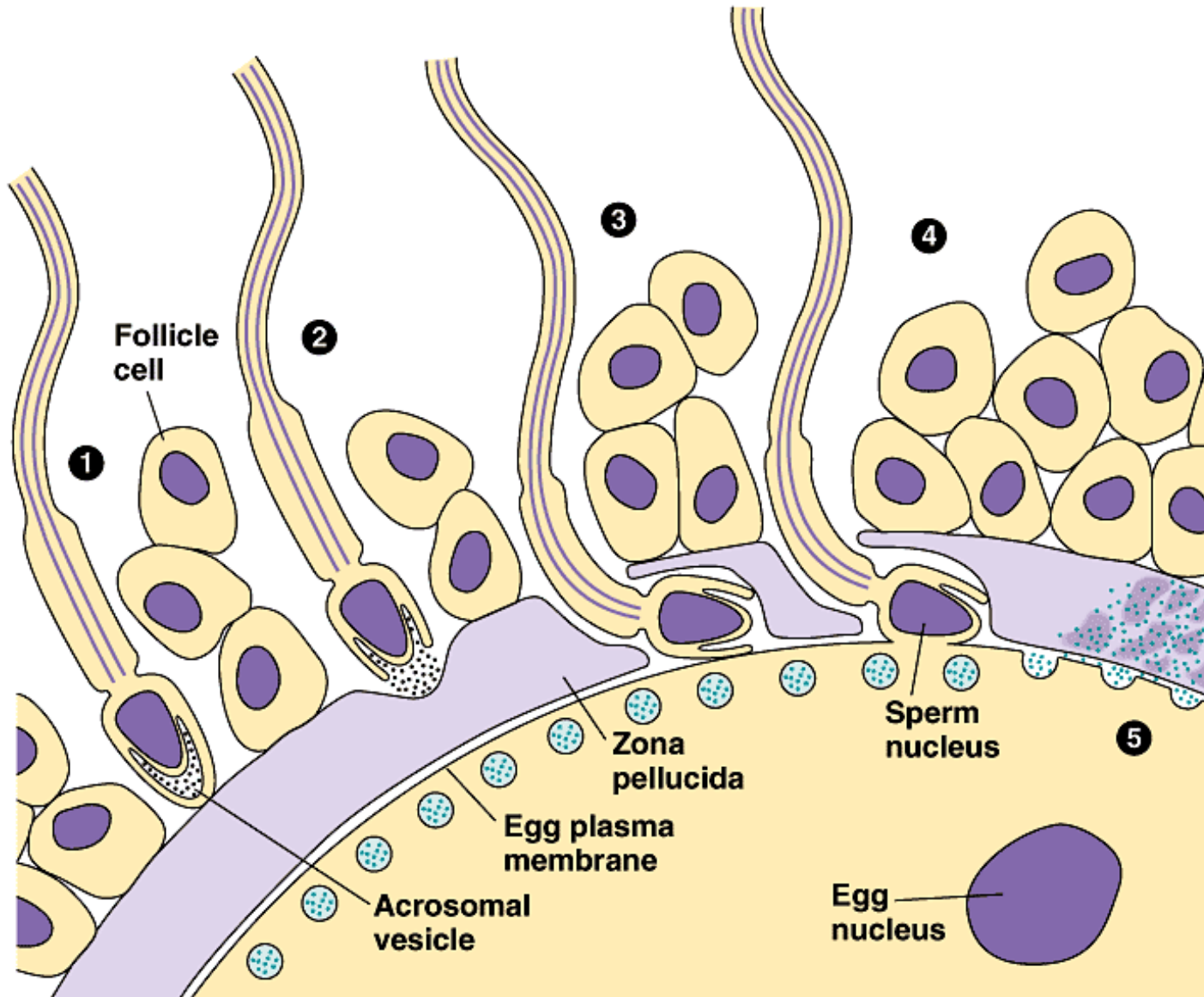
Acrosome Exocytosis



Acrosomal Reaction

- Proteases released – zona proteins digested
- ZP3 shed from sperm during acrosome reaction
 - Inner acrosomal membrane now at surface of cell
 - Binds to ZP2

Mammalian Fertilization



Gamete Membrane Fusion

- Possible mechanism
 - CD9 (integrin binding protein) on egg might interact with Izumo protein on sperm
 - CD9 or Izumo knockout mutations block binding

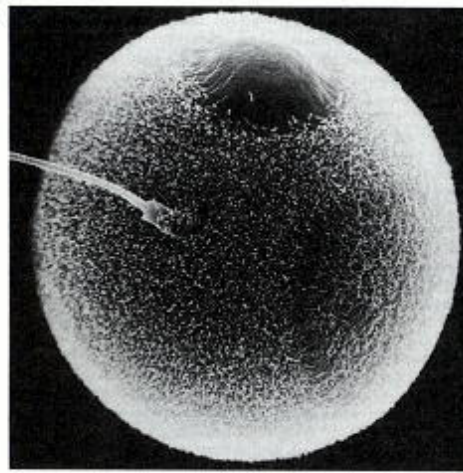
Prevention of Polyspermy

- No fertilization envelope in mammals
- Cortical granule reaction releases:
 - N-acetylglucosaminidase
 - Breaks connection between GalT on sperm and N-acetylglucosamine residue on ZP3 in zona pellucida
 - Protease
 - Cleave ZP2
 - Sperm not bound to membrane are released

Gamete Fusion

- Actin polymerization in egg forms microvilli that reach up to sperm.
- Binding starts at equatorial domain
- Sperm pronucleus, centrosome and mitochondria taken into egg.
 - Pronucleus fuses with egg pronucleus
 - Centrosome organizes microtubules
 - Sperm mitochondria are destroyed by egg

(A)



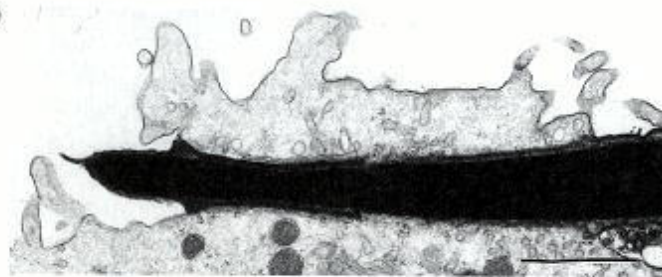
(B)



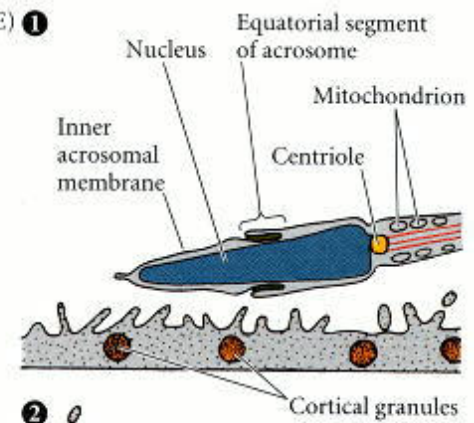
(C)



(D)



(E) 1



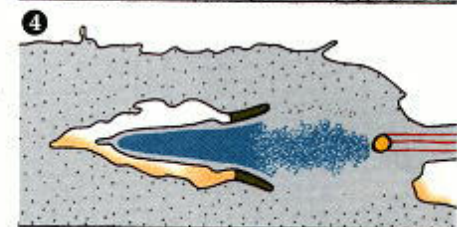
2



3



4



Fusion of Genetic Material

- Takes about 12 hours
 - Sperm DNA tightly bound by protamines
 - Compacted by disulfide bonds
 - Glutathione in egg breaks disulfide bonds
 - Releases grip on DNA
 - Oocyte arrested at metaphase II
 - Calcium activates kinase
 - Results in destruction of cyclin
 - Cell cycle resumes

DNA Synthesis

- Calcium inactivates MAP kinase
 - Activates DNA synthesis in sperm and egg pronuclei
 - Sperm centrosome form aster and pulls pronuclei together
 - Mitosis starts immediately before zygote nucleus forms

Pronuclear Movements

