Barry Quotation

It is a well-documented fact that guys will not ask for directions. This is a biological thing. This is why it takes several million sperm cells ... to locate a female egg, despite the fact that the egg is, relative to them, the size of Wisconsin.

Dave Barry

Fertilization title slide

Fertilization:

A cell-cell interaction between
two highly specialized cell types

Figure 7.1 ***The Human Infant Preformed in the Sperm, as Depicted by Nicolas Hartsoeker (1694)***

The nature of conception has been a matter for speculation for a long time…

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Gametes & Gametogenesis

Sperm & Egg - Similarities & Differences

Compare:

- similar size (initially - diploid, undifferentiated)
- undergo meiosis to create haploid cells

Contrast:

- male gametes - 4 haploid sperm / meiosis
- female gametes - 1 haploid egg / meiosis

Spermatogenesis

Oogenesis

Sperm & Egg - Similarities & Differences

Sperm - ‘stripped-down’ cell

Egg grows larger - huge ‘storehouse’

Figure 7.2(3) ***The Mammalian Sperm***

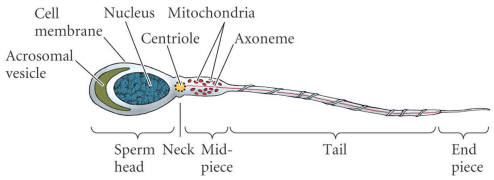
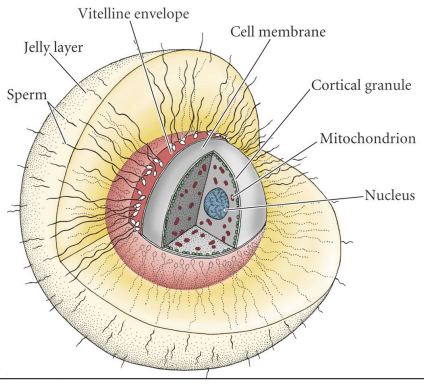


TABLE 19.2 **Cellular components stored in the mature oocyte of *Xenopus laevis***

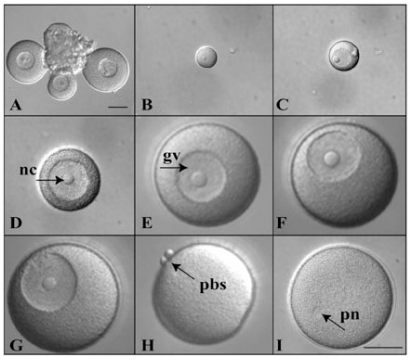
Component	Approximate excess over amount in larval cells
Mitochondria	100,000
RNA polymerases	60,000–100,000
DNA polymerases	100,000
Ribosomes	200,000
tRNA	10,000
Histones	15,000
Deoxyribonucleoside triphosphates	2,500

Source: After Laskey 1979.

Figure 7.4 ***Structure of the Sea Urchin Egg at Fertilization***



Sea Urchin Oogenesis



Gametes & Gametogenesis slide

Sea Urchin Fertilization

Sperm Acrosome Reaction

Sperm-Egg fusion

Egg 'blocks to polyspermy'

Fig. 7.8(1) ***Events Leading to Fusion of Egg & Sperm Plasma Membranes***

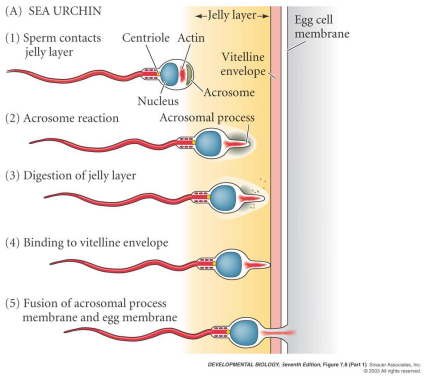


Figure 7.10(1) ***Acrosome Reaction in Sea Urchin Sperm***

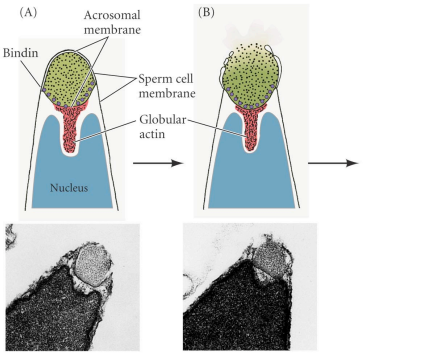
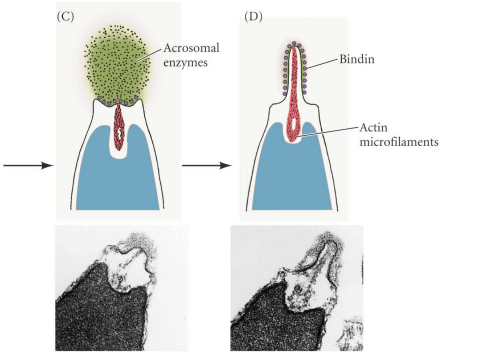


Figure 7.10(2) ***Acrosome Reaction in Sea Urchin Sperm***



Acrosome Reaction

Role of Ca^{++} in the Acrosome Reaction

Contact of sperm with jelly coat causes Ca^{++} entry
[crosses membrane from seawater to sperm cytoplasm]

Ca^{++} triggers acrosomal vesicle fusion.

Experiment

Control: Sperm in normal SW ➳ No Ac Rxn

Normal SW + A23187 ➳ Ac Rxn

Ca^{++}-free SW + A23187 ➳ No Ac Rxn

Conclusion: Ca^{++} from SW triggers Ac Rxn

Figure 7.19(1) ***The Entry of Sperm into Sea Urchin Eggs***

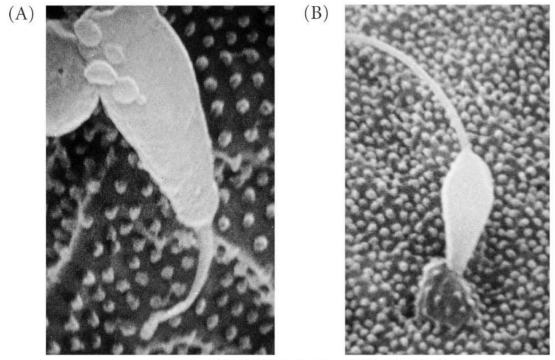


Figure 7.19(2) ***The Entry of Sperm into Sea Urchin Eggs***

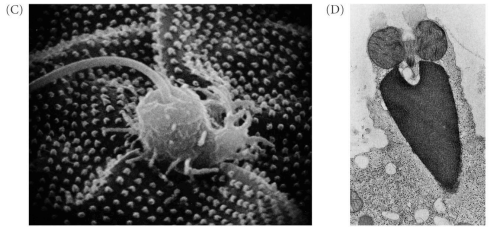


Figure 7.14 ***Species-Specific Binding of Acrosomal Process to Egg Surface in Sea Urchins***

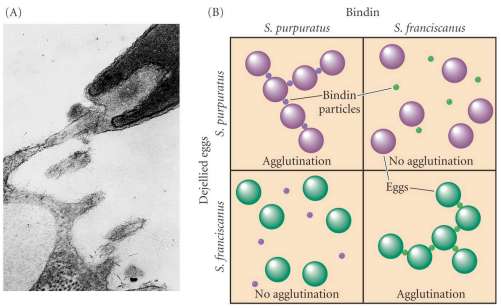


Figure 7.15(1) ***Localization of Bindin on the Acrosomal Process***

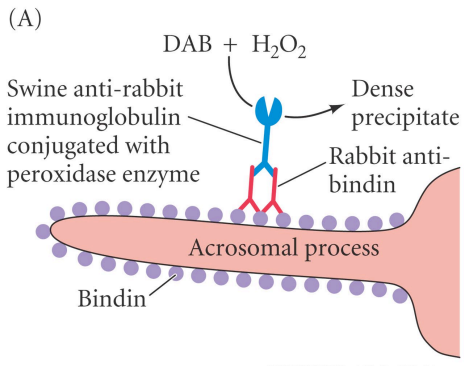


Figure 7.15(2) ***Localization of Bindin on the Acrosomal Process***

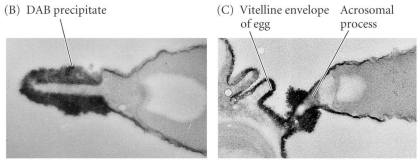
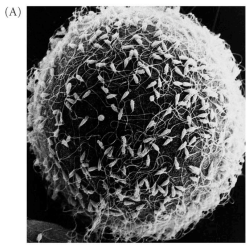


Figure 7.16 ***Bindin Receptors on the Egg***



SEM of egg with sperm bound to surface - bindin receptors limited

Sperm binding to beads coated with bindin receptor protein (note binding to acrosomal process)

Blocking Polyspermy in Sea Urchin

"Fast Block" - electrical, within seconds

"Slow Block" - structural, ~60 sec later

Fast Block to Polyspermy 1

Fast Block to Polyspermy

Binding of sperm to egg plasma membrane triggers Na^+ to cross membrane, causing electrical depolarization.

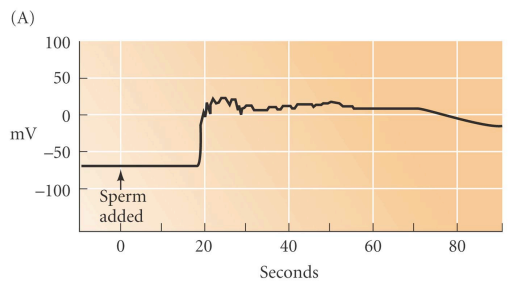
All cells have membrane potential, about -70 mV

Na entry causes shift to +20 mV

Potential begins return to normal after ~ 1 min

By ~ 1 min, slow block (cortical reaction) starts

Figure 7.22(1) ***Membrane Potential of Sea Urchin Eggs Before & After Fertilization***



Electrode in egg during fertilization shows potential changes

Fast Block to Polyspermy

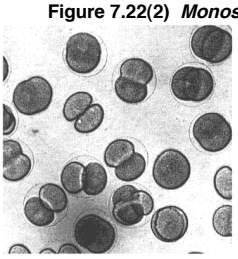
Experiment: alter Na^+ concentration in SW

Control: Normal Na^+-SW blocks polyspermy

Reduced Na^+ in SW allows more polyspermy

Figure 7.22(2) ***Monospermy vs. Polyspermy***

(B)



(C)

	Na^+ (m*M*)	Percentage of polyspermic eggs
normal seawater	490	22*
	360	26
	120	97
	50	100

*Experimental conditions (high sperm conc) allow some polyspermy even in normal seawater

Fast Block to Polyspermy

Experiment: use electrode to control egg membrane potential

Positive membrane potential blocks sperm entry, even in Na^+-free SW (so no Na^+ entry)

Negative membrane potential allows sperm entry, despite Na^+ crossing membrane

Conclusion: although Na^+ entry normally causes the depolarization, Na^+ entry itself is not essential for the fast block.

Slow Block to Polyspermy 1

Slow Block to Polyspermy

The Cortical Reaction causes the vitelline membrane to be released from the surface of the egg, raising up to form the fertilization envelope.

This makes a permanent, physical block to further sperm entry.

Figure 7.24(1) ***Cortical Granule Exocytosis***

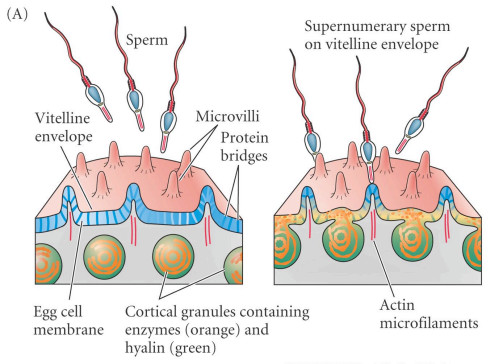


Figure 7.24(2) ***Cortical Granule Exocytosis***

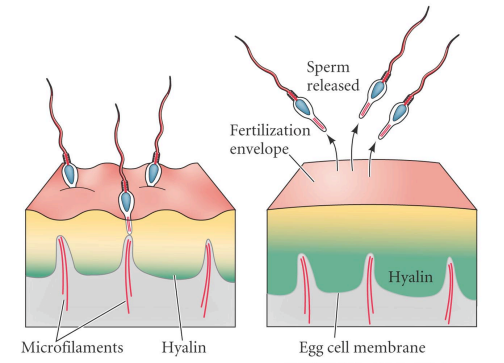


Figure 7.6(1) ***The Sea Urchin Egg Cell Surface***

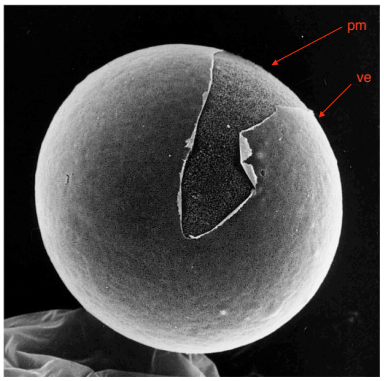
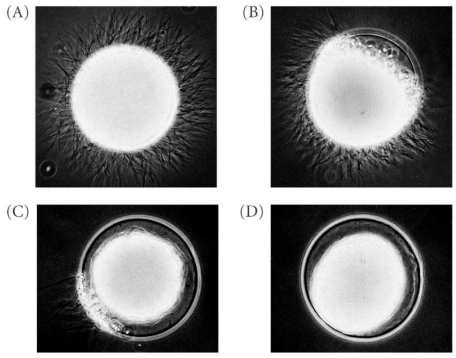


Figure 7.23 ***Formation of the Fertilization Envelope and Removal of Excess Sperm***



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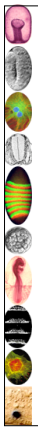

Slow Block to Polyspermy 2

Slow Block to Polyspermy

Cortical Reaction - fusion of cortical vesicles (aka granules) with the plasma membrane (pm)

Contents of cortical vesicles released into the space between pm & vitelline envelope (ve)

Slow Block to Polyspermy

Cortical Granule contents:

Proteases - digest connections between pm & ve, digest sperm receptors

Peroxidases - cross-link proteins in vm to toughen, ve becomes fertilization envelope

Mucopolysaccharides - draw water osmotically into space between pm & ve

Hyaline - forms protective and structural layer around the zygote

Figure 7.6(2) ***The Sea Urchin Egg Cell Surface***

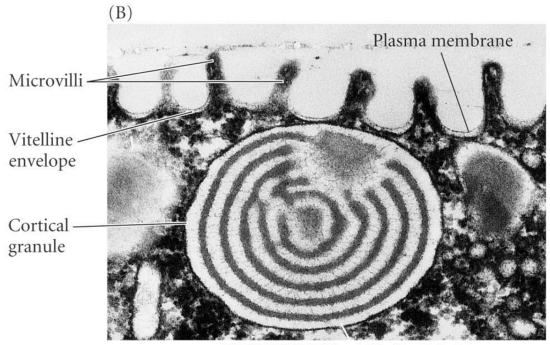

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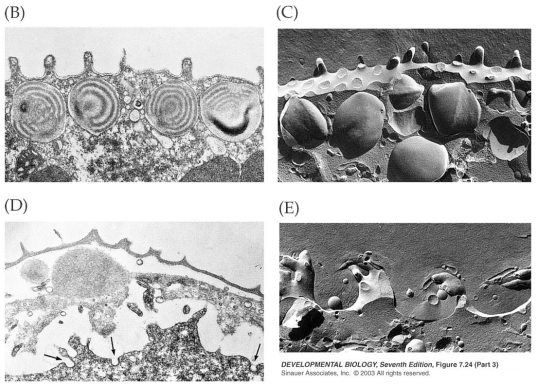
Figure 7.24(3) ***Cortical Granule Exocytosis - TEM and Freeze-fracture SEM***



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Slow Block to Polyspermy

Cortical Reaction - fusion of cortical vesicles (aka granules) with the plasma membrane (pm)

Sperm triggers increase in cytoplasmic Ca^{++}

Experiment

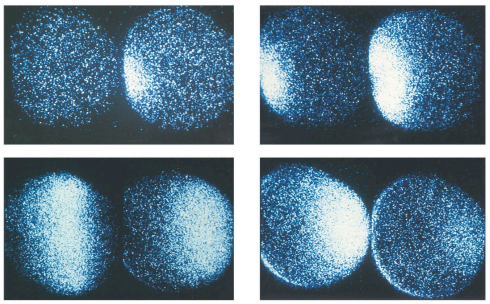
Eggs in Normal SW ➳ No Cortical Rxn

Normal SW + A23187 ➳ Cortical Rxn

Ca^{++}-free SW + A23187 ➳ Cortical Rxn

Conclusion: Ca^{++} for CR comes from inside egg

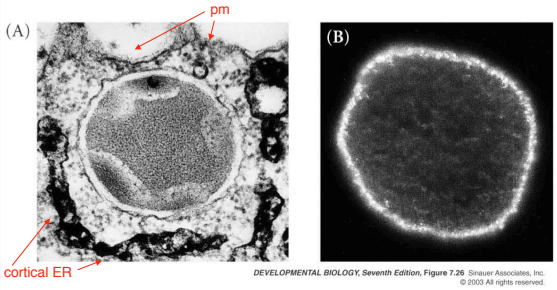
Figure 7.25 ***Wave of Calcium Release across Sea Urchin Eggs During Fertilization***



Dye (injected into egg) fluoresces in presence of Ca^{++}

Fig. 7.26 ***ER Surrounding Cortical Granules in Sea Urchin Eggs***

Ca^{++} for CR likely comes from ER at the cortex, just below the egg plasma membrane.

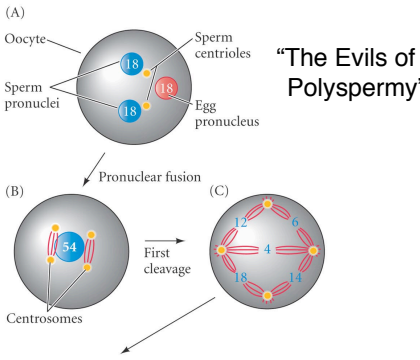


TEM showing cortical granule and stained ER at surface of egg

Calcium channels in cortical ER of whole egg

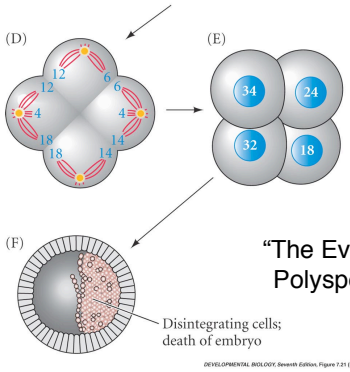
The Evils of Polyspermy

Fig. 7.21(1) ***Aberrant Development in a Dispermic Sea Urchin Egg***



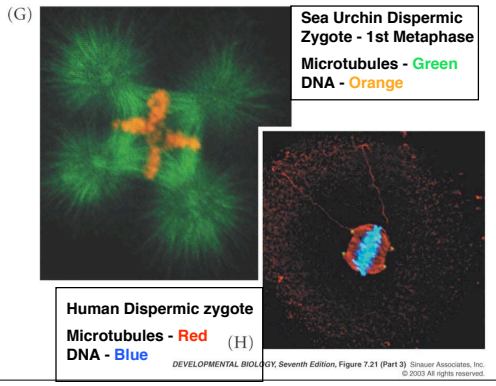
"The Evils of Polyspermy"

Fig. 7.21(2) ***Aberrant Development in a Dispermic Sea Urchin Egg***



"The Evils of Polyspermy"

Figure 7.21(3) ***Aberrant Development in a Dispermic Zygotes***



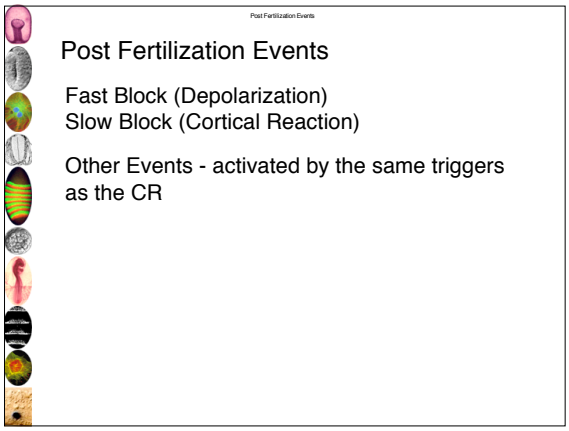


Figure 7.27(1) ***Postulated Pathway of Egg Activation in the Sea Urchin***

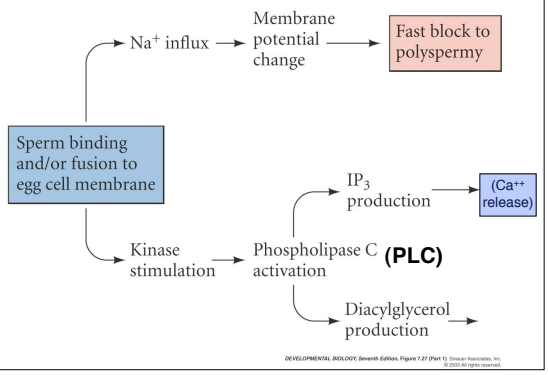


Figure 7.27(2) ***Postulated Pathway of Egg Activation in the Sea Urchin***

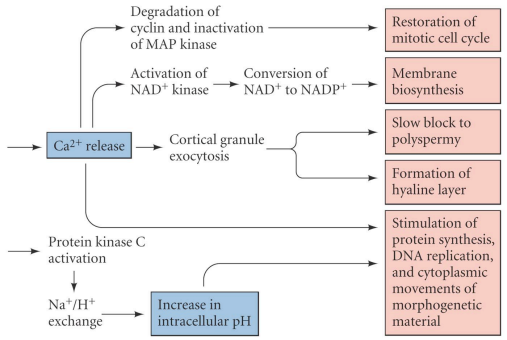


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TABLE 7.1 Events of sea urchin fertilization

Event	Approximate time postinsemination[a]
EARLY RESPONSES	
Sperm-egg binding	0 seconds
Fertilization potential rise (fast block to polyspermy)	within 1 sec
Sperm-egg membrane fusion	within 1 sec
Calcium increase first detected	10 sec
Cortical granule exocytosis (slow block to polyspermy)	15–60 sec
LATE RESPONSES	
Activation of NAD kinase	starts at 1 min
Increase in $NADP^+$ and NADPH	starts at 1 min
Increase in O_2 consumption	starts at 1 min
Sperm entry	1–2 min
Acid efflux	1–5 min
Increase in pH (remains high)	1–5 min
Sperm chromatin decondensation	2–12 min
Sperm nucleus migration to egg center	2–12 min
Egg nucleus migration to sperm nucleus	5–10 min
Activation of protein synthesis	starts at 5–10 min
Activation of amino acid transport	starts at 5–10 min
Initiation of DNA synthesis	20–40 min
Mitosis	60–80 min
First cleavage	85–95 min

Main sources: Whitaker and Steinhardt 1985; Mohri et al. 1995.

[a]Approximate times based on data from *S. purpuratus* (15–17°C), *L. pictus* (16–18°C), *A. punctulata* (18–20°C), and *L. variegatus* (22–24°C). The timing of events within the first minute is best known for *Lytechinus variegatus*, so times are listed for that species.

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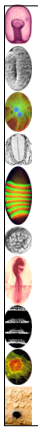


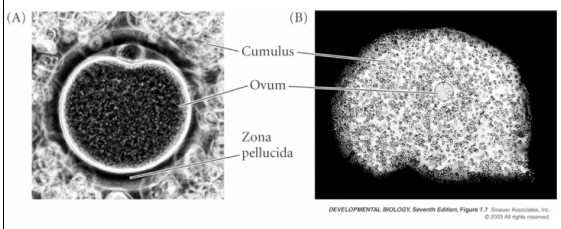
Mammalian Fertilization

Mammalian Fertilization

mainly studied in rodents (mice especially)

Compare & contrast with sea urchin fertilization

Figure 7.7 ***Hamster Eggs Immediately Before Fertilization***



Zona pellucida functions similarly to sea urchin jelly coat and vit. env.

Fig. 7.8(2) ***Events Leading to Fusion of Egg & Sperm Plasma Membranes***

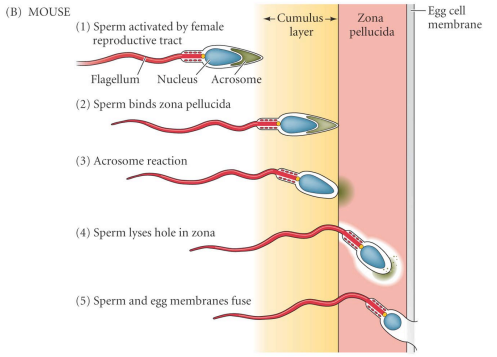
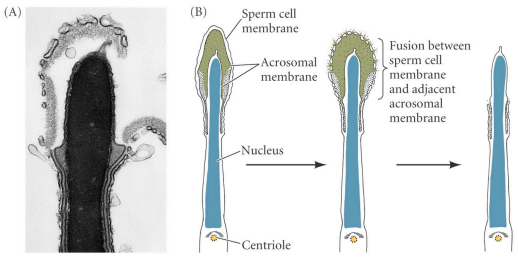


Figure 7.11 ***Acrosome Reaction in Hamster Sperm***



Acrosome reaction is less dramatic (no process forms)

Mammalian Fertilization

Proteins in the zona pellucida promote fertilization

ZP proteins - trigger acrosome reaction (ZP3)
initial sperm binding (ZP3)
post-AR sperm binding (ZP2)

ZP proteins are extracellular matrix glycoproteins

Figure 7.17(2) ***Mouse ZP3, the Zona Protein that Binds Sperm***

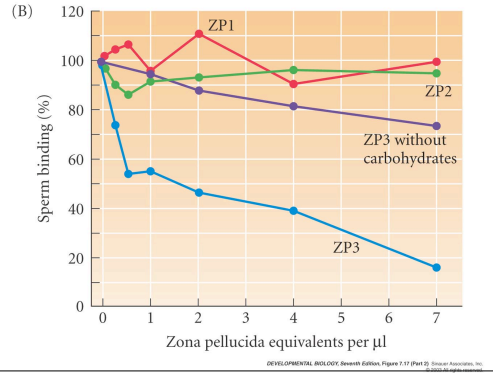
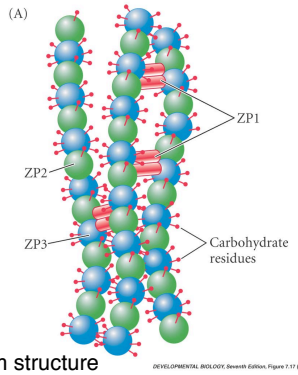
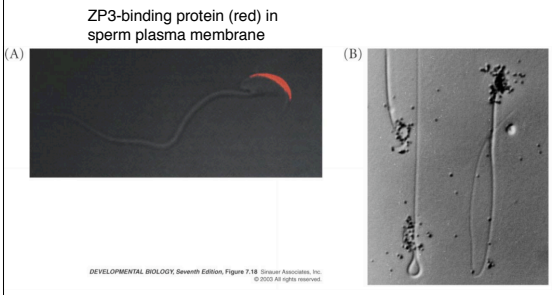


Figure 7.17(1) ***Mouse ZP3, the Zona Protein that Binds Sperm***



ZP proteins form structure

Figure 7.18 ***Sperm ZP3-Binding Proteins at the Zona Pellucida***



Radioactively-labeled ZP3 binds to mouse sperm

Figure 7.20(1) ***Entry of Sperm into Golden Hamster Egg***

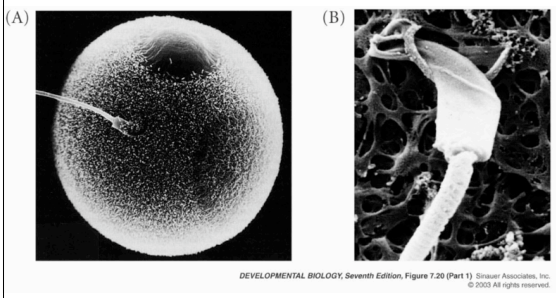


Figure 7.20(2) ***Entry of Sperm into Golden Hamster Egg***

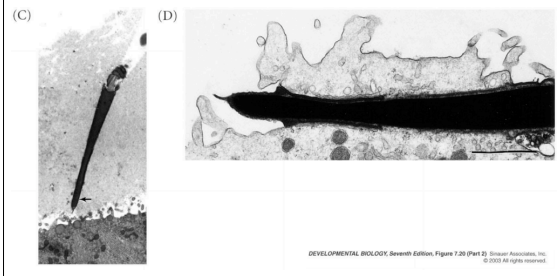
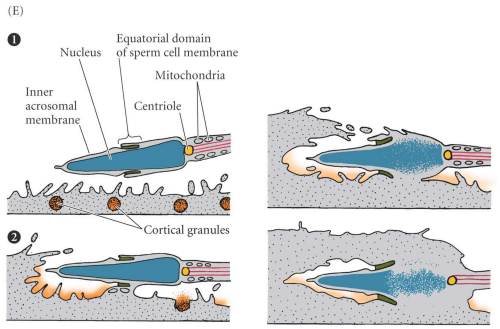
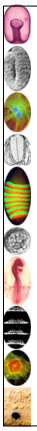


Figure 7.20(3) ***Entry of Sperm into Golden Hamster Egg***





Post Fertilization Even 2ts

Post Fertilization Events

(Completion of meiosis)

Pronuclear fusion

Activation of egg metabolism

DNA synthesis (reactivate cell cycle - S phase)

Cytoplasmic rearrangement

Figure 7.5(1) ***Egg Maturation at the Time of Sperm Entry in Different Animal Species***

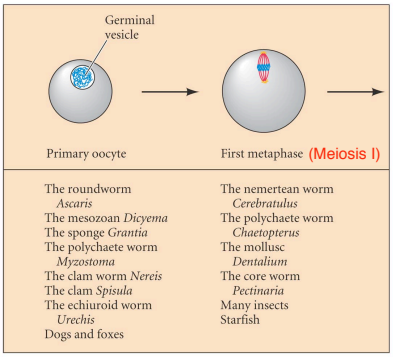


Figure 7.5(2) ***Egg Maturation at the Time of Sperm Entry in Different Animal Species***

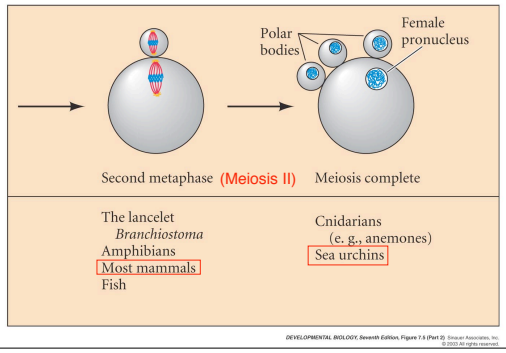


Fig. 7.31(1) ***Nuclear Events in Fertilization of the Sea Urchin***

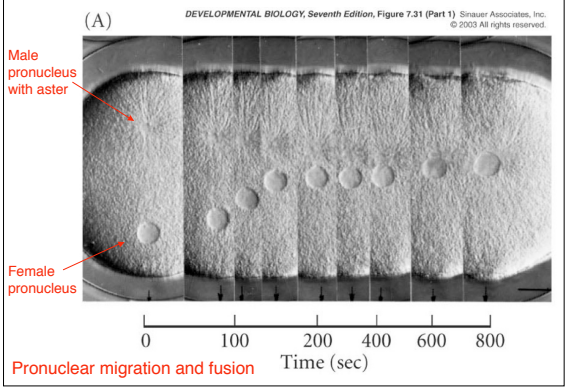


Fig. 7.31(2) ***Nuclear Events in Fertilization of the Sea Urchin***

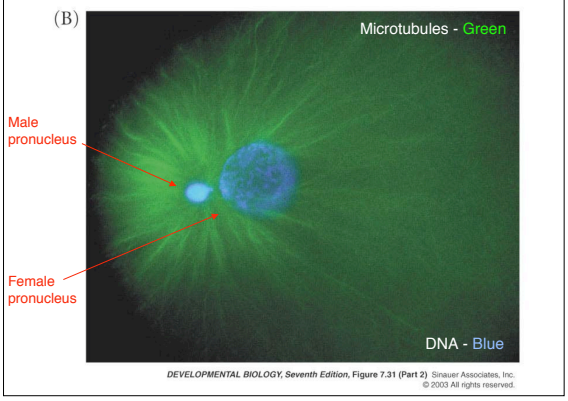


Figure 7.32 ***Pronuclear Movements During Human Fertilization***

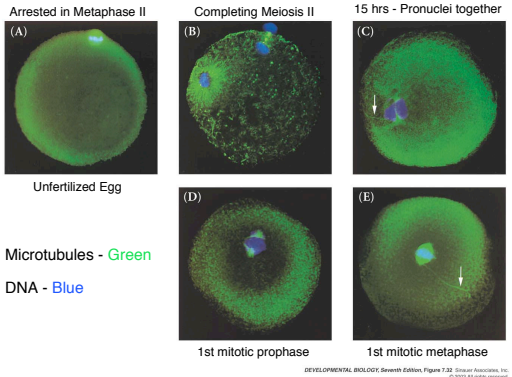


Fig. 7.33 ***Reorganization of Cytoplasm in the Newly Fertilized Frog Egg***