Lecture 3

Germ Cells and Sex
The ovary of sow.

The ovary of mare.

The ovary of cow.

The ovary of ewe.
The ovary.

A generalized vertebrate ovary.

(Wilt and Hake, Ch 2, 2004)
The functions of accessory and supporting cells

- Producing of hormones
- Making layers surrounding the egg
- Aiding in release of the mature egg from the ovary
- Selectively \textit{importing} molecules from the circulation into the oocyte
No growth in most embryos until feeding occurring

Oogonium

Oogenesis

The orderly stockpiling of all the materials needed for development
Meiosis during oogenesis.

(Wilt and Hake, Ch 2, 2004)
Meiosis during oogenesis.

- Germ cells’ own biosynthetic capacities
- Oocyte importing yolk and other materials
- DNA replication

**First Meiotic Division**
1. DNA doubles
2. Homologous chromosomes pair and crossover occurs
3. Duplicated chromosomes are distributed to daughter cells

(Wilt and Hake, Ch 2, 2004)
Meiosis during oogenesis.

(Wilt and Hake, Ch 2, 2004)
Meiosis during oogenesis.

Second Meiotic Division
1. No DNA synthesis
2. Duplicated chromatids separate and are distributed to daughter cells (which are haploid)

(Wilt and Hake, Ch 2, 2004)
Meiosis during oogenesis.

(Wilt and Hake, Ch 2, 2004)
Meiosis during oogenesis.

(Wilt and Hake, Ch 2, 2004)
Meiosis produces haploid cells

Meiosis reduces the number of chromosomes from the diploid to the haploid number.

(Wolpert, Ch12, 2002)
Oogenesis in mammals

After the germ cells that form oocytes enter the embryonic ovary, they divide mitotically a few times and then enter the prophase of the first meiotic division. No further cell multiplication occurs, but the oocyte increases 100-fold mass.

(Wolpert, Ch12, 2002)
Terminology

- Oogonium
- Primary oocyte
- Secondary oocyte
- Polar body
- Egg / ootide
The structure of eggs.

(Wilt and Hake, Ch 2, 2004)
The unfertilized egg of *Xenopus*

The fertilized egg of zebra fish
The oocyte (A) and egg (B) of hen.
The cumulus oocyte complex (COC, A) and denuded oocyte (B) of mouse.
Sagittal section of testis illustrating segments of parenchymal tissue.

Transverse section through a seminiferous tubule

A
Indifferent cell
Spermatogonium

Spermatids

Secondary spermatocyte

Secondary spermatocyte

Primary spermatocyte

Supporting cell

Connective-tissue wall

Spermatogonium

Spermatozoa

Secondary spermatocyte

Primary spermatocyte

X-chromosome

Primary spermatocyte

Spermatogonium

B
Seminiferous tubule

C
A portion of a seminiferous tubule showing the relationship of the germ cells to the adjacent Sertoli cells.

(Pathways to Pregnancy and Parturition, 1st ed., 1997)
The structure of a vertebrate seminiferous tubule.

(Wilt and Hake, Ch 2, 2004)
Germ cells that develop into sperm enter the embryonic testis and become arrested at the $G_1$ stage of the cell cycle. After birth, they begin to divide mitotically again, forming a population of stem cell (spermatogonia).

(Wolpert, Ch12, 2002)
Comparing a spermatogonium and a spermatozoon.

(Wilt and Hake, Ch 2, 2004)
Nucleus
Mitochondria
Golgi apparatus

Proximal centriole
Distal centriole

Acrosomic vesicle
Acrosomic granule

Neck
Flagellum

Histone proteins
Proamines

Post nuclear cap
Mid piece
Structural diagram of spermatozoa

- Head
  - Post nuclear cap
  - Acrosome
  - Nucleus
  - Implantation region, containing proximal centriole

- Tail
  - Mid-piece
  - Main-piece
  - End-piece
Terminology

- Spermatogonia
- Primary spermatocyte
- Secondary spermatocyte
- Spermatid
- Spermatozoon
- Capacitation

Spermatogenesis
- spermatocytogenesis
- spermiogenesis
- spermiation
Sperm activation.

Sea urchin

(Wilt and Hake, Ch 2, 2004)
The cortical reaction of fertilization in sea urchin.

(Wilt and Hake, Ch 2, 2004)
The sperm has been activated and the acrosome approaches the egg surface.

(Wilt and Hake, Ch 2, 2004)
The acrosome and egg plasma membrane have come into contact.

The ligand-receptor interaction occurred.

(Wilt and Hake, Ch 2, 2004)
The sperm head is fusing with the egg plasma membrane.

(Wilt and Hake, Ch 2, 2004)
The sperm nucleus has entered the egg cytoplasm.

(Wilt and Hake, Ch 2, 2004)
Fertilization of a mammalian egg

(Wolpert, Ch12, 2002)
Egg activation caused by changes in membrane potential.

(Wilt and Hake, Ch 2, 2004)
Egg activation by a calcium ionophore compared with fertilization.

There is a rapid increase in respiration after exposure to A23187.

(Wilt and Hake, Ch 2, 2004)
Calcium wave at fertilization

A series of images showing an intracellular calcium wave at fertilization in a sea urchin egg. 5-10 μm/sec

(Wolpert, Ch12, 2002)
Ca\textsuperscript{++} at Fertilization

Fertilization of Sea urchin
G protein signaling through phospholipid hydrolysis.

(Wilt and Hake, Ch 2, 2004)
Egg activation by a calcium ionophore compared with fertilization.

DNA synthesis in the female pronucleus is activated after exposure to ionophore.

(Wilt and Hake, Ch 2, 2004)
Profile of maturation-promoting factor (MPF) activity in early *Xenopus* development

(Wolpert, Ch12, 2002)
The electrical block to polyspermy.

(Wilt and Hake, Ch 2, 2004)
A time line of events in the fertilization reaction in sea urchins.

(Wilt and Hake, Ch 2, 2004)
Sperm acrosome reaction
Change in ion conductance
Increase in nitric oxide
Ca$^{2+}$ release, H$^+$ extrusion
Cortical granule exocytosis

Increase in respiration
Intracellular redistribution of enzymes
Syngamy

Time (min)

2

Increase in protein synthesis
Polyadenylation of mRNA

25

Pronuclear fusion
DNA synthesis

90

Mitosis

(Wilt and Hake, Ch 2, 2004)
Egg and sperm interactions at fertilization

- Fertilization involves cell-surface interactions between egg and sperm.
- The sperm has to pass several barriers to enter the egg.
- In mammalian eggs, the sperm first passes through a layer of cumulus cells embedded in hyaluronic acid aided by the hyaluronidase activity on its surface.
- The 2nd layer is the zona pellucida, a layer of glycoproteins.
- The acrosomal reaction (release of enzymes in the sperm head) is mediated by interaction of the ZP3 species-specific receptor and adhesion molecules in the sperm head.
Fertilization requires cell surface interaction

- The acrosome releases acrosin (a protease) and an acetylglucosaminidase which degrades glycoproteins.
- The sperm surface proteins (i.e. fertilin) that can bind the egg's surface are exposed during the acrosomal reaction.
- Fertilin binds an intergrin-like receptor of the egg plasma membrane to initiate sperm-egg fusion.
- In some invertebrates (i.e. sea urchins), an actin driven acrosomal projection allows the sperm and egg to touch.
- Changes in the egg membrane at fertilization block polyspermy so that only one sperm enters an egg.
- Enzymes are released that prevent other sperm from binding to the zona pellucida.
**Fertilization in mammals**

- Hyaluronidase activity on surface of mammalian sperm
  - Sperm penetrates cumulus layer
  - Zona pellucida elicits acrosomal reaction in sperm
  - Enzymes released from acrosome sperm head
  - Sperm plasma membrane fuses with egg plasma membrane
  - Calcium waves
  - Cortical reaction provides block to polyspermy
  - Sperm nucleus delivered into cytoplasm
  - Egg completes meiosis and development is initiated
Fertilization and activation of development

- At fertilization a number of events occur to activate development (such as increase in protein synthesis, structural changes [cortical rotation]).
- Main events are the completion of meiosis, fusion of the nuclei to form a diploid zygotic genome and the start of mitosis.
- Fertilization causes a calcium wave and egg activation.
- The sharp increase in calcium initiates the cell cycle by acting upon proteins that control the cell cycle.
- The *Xenopus* egg is kept in metaphase II by maturation-promoting factor (MPF), a complex including cyclin.
- The calcium wave activates a kinase that leads to cyclin degradation, the end of meiosis and nucleus fusion.
Paternal and maternal genomes are both required for normal mouse development.

A normal biparental embryo has contributions from both the paternal and maternal nucleus in the zygote after fertilization.

(Wolpert, Ch 12, 2002)
Imprinting of genes controlling embryonic growth

In mouse embryos, the paternal gene for insulin-like growth factor 2 (igf-2) is on, but the gene on maternal chromosome is off. In contrast, the igf-2r gene is on in the maternal genome, and off in the paternal genome. The product of this gene tends to inhibit growth, as it is involved in degrading IGF-2. H19 may regulate igf-2.

(Wolpert, Ch12, 2002)
Sex determination
Sex determination in humans

The inset shows a diagrammatic representation of the banding in the chromosomes, which are regions of increased chromatin concentration.

(Wolpert, Ch12, 2002)
Determination of sexual phenotype

• The early male and female embryos are very similar and differentially develop in latter stages.

• Even in some vertebrates, gender is not always chromosome dependent (i.e. temperature at which the alligator embryo develops determines gender, and some fish can change sex depending on environment).

• In mammals, sex-determining region on the Y chromosome (Sry), once known as the testes-determining factor encodes a transcription factor that specifies maleness.

• Translocation of Sry region to the X results in XX males and Sry alone injected into XX mouse eggs produce males.
Sex reversal in humans due to chromosomal exchange

At meiosis in male germ cells, the X and Y chromosome paired up (central panel), and there is often crossing over of the distal region (blue cross), which does not affect sexual development (left panel).

On rare occasions, crossing over involves a larger segment that includes the SRY gene (red cross), so that the X chromosome now carries this male-determining gene (right panel).

(Wolpert, Ch12, 2002)
Determination of sexual phenotype in mammals

• Mammalian sexual phenotype is regulated by gonadal hormones.

• All mammals begin development as gender neutral, the presence of the Y chromosome induces testis development that produce hormones that switch the development of somatic tissues into the male pathway.

• This means that the sex of only the gonads is genetically determined but the rest of the cells are neutral (whatever their chromosome complement is) and their fate depends upon hormones.

• The mesonephros (embryonic kidney) contributes to both male and female reproductive organs.
In the final stage of migration, the cells move from the gut tube into the genital ridge, via the dorsal mesentery.
Development of the gonads and related structures in mammals

Top panel: early in development, there is no difference between males and females in the structures. The future gonads lie adjacent to the mesonephros.

Bottom panels: after testes develop in the male, their secretion of Müllerian-inhibitory substance result in degeneration of the Müllerian duct by programmed cell death whereas the Wolffian duct becomes the vas deferens.

(Wolpert, Ch12, 2002)
Wolffian & Müllerian duct determination

- Wolffian & Müllerian ducts differentiate in different ways depending upon gender.
- On the sides of the mesonephros are the Wolffian ducts and Müllerian ducts that open into the cloaca.
- In females (in the absence of the testes), the Müllerian ducts develop into the oviducts (Fallopian tubes) and the Wolffian ducts degenerate.
- In males, the Wolffian duct becomes the vas deferens.
- The genital region differentiates after gonad development with the action of the gonadal hormones.
Development of the genitalia in humans

At an early embryonic stage, the genitalia are the same in males and females (top). After testis formation in males, the phallus and the genital fold give rise to penis, whereas in females they give rise to the clitoris and the labia minus. The genital swelling forms the scrotum in males and the labia majus in females.

(Wolpert, Ch12, 2002)
Environmental signals specify germ cell sex in mammals

Migrating germ cells, whether XX or XY, enter meiotic prophase and start developing as oocytes unless they are in a testis.

(Wolpert, Ch12, 2002)