Pathophysiology of Disease - Reproduction

• Human Reproduction and Development
  – Embryonic and Fetal Development
  – Sexual Differentiation
  – Production of gametes

Break

• Reproductive Toxicology
• Developmental Toxicology
5 Milestones of Human Development

• **Fertilization** The formation of the fertilized zygote by union of sperm and oocyte

• **Cleavage** Rapid set of cell divisions that increase the cell number without actual growth in size

• **Implantation** The invasion of the embryo into the maternal uterus

• **Gastrulation** The movement of cells that creates the basic body plan

• **Organogenesis** The process by which individual organs arise,
Fertilization
Figure 3-1. Acrosome reaction and a sperm penetrating an oocyte. The area outlined in A is detailed in B. 1. Sperm during capacitation, a period of conditioning that occurs in the female reproductive tract. 2. Sperm undergoing the acrosome reaction during which perforations form in the acrosome. 3. Sperm digesting a path through the zona pellucida by the action of enzymes released from the acrosome. 4. Sperm after entering the cytoplasm of the oocyte. Note that the plasma membranes of the sperm and oocyte have fused and that the head and tail of the sperm enter the oocyte.
2 Cells
4 Cells
Blastocyst
Figure 3-4. Drawings illustrating cleavage of the zygote and formation of the blastocyst. A to D show various stages of cleavage. The period of the morula begins at the 12- to 16-cell stage and ends when the blastocyst forms. E and F are sections of blastocysts. The zona pellucida disappears by the late blastocyst stage (5 days). The polar bodies shown in A are small, nonfunctional cells that soon degenerate. Cleavage and formation of the morula occur as the dividing zygote passes along the uterine tube. Blastocyst formation normally occurs in the uterus. Although cleavage increases the number of blastomeres, note that each of the daughter cells is smaller than the parent cell. As a result, there is no increase in the size of the developing embryo until the zona pellucida degenerates. The blastocyst then enlarges considerably. The embryoblast gives rise to the tissues and organs of the embryo.
Implantation

trophoblast (surface layer of cells)
inner cell mass

blastocyst
(uterine cavity)

endometrium
Week 1

**Fertilization** is complete within 24 hours of ovulation

Steps include:

- Passage of sperm through the corona radiata of the oocyte
- Penetration of the zona pellucida
- Fusion of the plasma membranes of the oocyte and sperm
- Completion of the second meiotic division of the oocyte and formation of the female pronucleus
- Formation of the male pronucleus
- Breakdown of pronuclear membranes and condensation of the chromosomes and arrangement for mitotic cell division
**Week 1**

**Cleavage** of the zygote after fertilization leads to compaction of the ball of cells into an inner cell mass (embryoblast) and the outer cell mass (trophoblast) marking formation of the blastocyst at approximately 4 days after fertilization.

**Trophoblast** – Thin outer cell layer that gives rise to the embryonic part of the placenta.

**Embryoblast**- a group of centrally located cells (blastomeres) that give rise to the embryo.
Week 1

- Zona Pellucida degenerates
- 6 days after fertilization the blastocyst attaches to the endometrial epithelium
- Trophoblast proliferates and differentiates into 2 cell layers:
  - **Cytotrophoblast** - mitotically active cells that produce new trophoblastic cells to increase the mass of the syncytiotrophoblast
  - **Syncytiotrophoblast** - rapidly expanding mass of cells that produce human chorionic gonadotropin (hCG). Fingerlike processes of the syncytiotrophoblast extend through the endometrial epithelium and invade the connective tissue
Week 2

**Implantation** is completed

Embryoblast becomes a bilaminar embryonic disc composed of:

**Epiblast**- thick layer of cells compose the floor of the amniotic cavity

**Hypoblast**-small cells adjacent to the exocoelomic cavity

Extraembryonic structures that develop in the second week include: amniotic cavity, amnion, yolk sac, connecting stalk and chorionic sac.
Figure 4-4. Origin of embryonic tissues. The colors in the boxes correspond to those used in drawings of sections of conceptuses.
Week 2

Endometrial connective tissue cells give rise to decidual cells which provide an immunologically privileged site for the conceptus.

Primordia of the uteroplacental circulation are forming 

Day 14 Precordal plate-the future site of the mouth is formed from endodermal cells
3 Weeks

- **Gastrulation** - Differentiation of 3 germ layers and axial orientation is established

- Appearance of primitive streak from migrating epiblast cells

- Development of the notochord which defines the axis of the embryo and indicates the future site of the vertebral bodies

- Beginning of angiogenesis, end of week blood is circulating and heart beats at day 21 or 22.
Week 3

3 Layers of the Trilaminar Embryonic Disc:

**Ectoderm** - epidermis, central and peripheral nervous system, and retina

**Endoderm** - epithelial linings of respiratory passages and GI tract, glandular cells of the liver and pancreas

**Mesoderm** - smooth muscular coats, connective tissues, vessels and most of the cardiovascular system, blood cells, bone marrow, skeleton, striated muscle, reproductive and excretory organs
Figure 6-4. Schematic drawing illustrating the derivatives of the three germ layers: ectoderm, endoderm, and mesoderm. Cells from these layers make contributions to the formation of different tissues and organs; for example, the endoderm forms the epithelial lining of the gastrointestinal tract and the mesoderm gives rise to connective tissues and muscles.
Week 3

**Primative Streak** – results from the proliferation and migration of epiblast cells to the median plane of the embryonic disc. The streak will elongate and form the primative node on the cranial end.

**Primative groove develops in the streak**

**Notochord** is formed by mesenchymal cells migrating from the primative node and pit.

1. Defines primordial axis of the embryo
2. Serves as the bases for development of the axial skeleton
3. Indicates the future site of the vertebral bodies
Neurulation
formation of the neural plant and neural folds and closing of the the folds to form the neural tube

day 16

embryonic disk

amnion

yolk sac

primitive streak
thickened band of cells; marks the onset of gastrulation

day 21

cell migrations, tissue folding, other events bring about the formation of a neural tube (forerunner of brain and spinal cord)

day 23

many somites formed; these are forerunners of most of axial skeleton and muscles
Week 3

**Somites** – develop from mesoderm on each side of the neural tube and ultimately give rise to the axial skeleton, associated musculature and adjacent dermis of the skin.

**Intraembryonic coelom** (body cavity)- that divides the lateral mesoderm into as two spaces the somatic (body wall) and splanchnic or visceral (gut wall).

**Cardiovascular system** begins to develop in the beginning of the 3\textsuperscript{rd} week with angiogenesis 

Embryonic blood vessels form and differentiate into the muscular and connective tissue of the vessels

Paired cardiogenic heart tubes form and fuse to create the primordial heart tube. By the end of the 3\textsuperscript{rd} week blood is circulating and the heart begins to beat on day 21-22.
Weeks 4-8 Organogenic Period

• Development of all major organ systems occur in the organogenic period

• Upper limb buds begin to show differentiation of elbows and large hand plates

• Digital rays-primordia of digits

• Embryo begins to show spontaneous movement

• External ear begins to develop

• Retinal Pigment forms- eye become obvious
4 weeks

- Forebrain produces prominent elevation of the head
- Upper limb buds are small swelling
- Lens placodes - future site of the lenses of the eyes
- Lower limb buds appear by the end of the week
- Characteristic C-shape, and tail like caudal eminence
Figure 6-5. A and B. Drawings of dorsal views of embryos early in the fourth week showing 8 and 12 somites, respectively. C, D, and E. Lateral views of older embryos showing 16, 27, and 33 somites, respectively. The rostral neuropore is normally closed by 25 to 26 days, and the caudal neuropore is usually closed by the end of the fourth week.
**22 days**

Figure 6 - A. Dorsal view of a five-somite embryo at Carnegie stage 10, about 22 days. Observe the neural folds and neural groove. The neural folds in the cranial region have thickened to form the primordium of the brain. B. Drawing indicating the structures shown in A. Most of the amniotic and chorionic sacs have been cut away to expose the embryo. C. Dorsal view of a 10-somite embryo at Carnegie stage 10, about 23 days. The neural folds have fused opposite the somites to form the neural tube (primordium of the spinal cord in this region). The neural tube is in open communication with the amniotic cavity at the cranial and caudal ends through the rostral and caudal neuropores, respectively. D. Diagram indicating the structures shown in C.
24 days

Figure 6-7. A, Dorsal view of a 13-somite embryo at Carnegie stage 11, about 24 days. The rostral neuropore is closing, but the caudal neuropore is wide open. B, Drawing indicating the structures shown in A. The embryo is curved because of folding at the cranial and caudal ends.
26 days

Figure 6 - B. Lateral view of a 27-zooms embryo at Carnegie stage 12, about 26 days. The embryo is very curved, especially its long tail-like caudal eminence. Observe the lens placode (primordium of the lens of the eye) and the otic pit, indicating early development of the internal ear. B. Drawing indicating the structures shown in A. The neural tube is closed, and three pairs of pharyngeal arches are present. (A, from Nishimura H, Semba H, Tanemura T, Tanaka O. Prenatal Development of the Human with Special Reference to Craniofacial Structures: An Atlas. Washington, DC, National Institutes of Health, 1977.)

28 days

Figure 6 - B. Lateral view of an embryo at Carnegie stage 13, about 28 days. The primordial heart is large, and its division into a primordial atrium and ventricle is visible. The neural tube and caudal eminence are evident. B. Drawing indicating the structures shown in A. The embryo has a characteristic C-shaped curvature, four pharyngeal arches, and upper and lower limb buds. (A, from Nishimura H, Semba H, Tanemura T, Tanaka O. Prenatal Development of the Human with Special Reference to Craniofacial Structures: An Atlas. Washington, DC, National Institutes of Health, 1977.)
5 Weeks

- Growth of the head, rapid development of the brain and facial prominence
- Upper limb buds are paddle shaped
- Lower limb buds are flipper-like
32 days
(5 weeks)

Figure 6-10. A, Lateral view of an embryo at Carnegie stage 14, about 32 days. The second pharyngeal arch has overgrown the third arch, forming a depression known as the cervical sinus. The mesonephric ridge indicates the site of the mesonephric kidney, an interim kidney (see Chapter 14). B, Drawing indicating the structures shown in A. The upper limb buds are paddle-shaped, whereas the lower limb buds are flappedlike. (A, From Nishimura H, Semba H, Tanimura T, Tanaka O: Prenatal Development of the Human with Special Reference to Craniofacial Structures: An Atlas. Washington, DC, National Institutes of Health, 1977.)
6 Weeks

- Upper limb buds begin to show differentiation of elbows and large hand plates
- Digital rays-primordia of digits
- Embryo begins to show spontaneous movement
- External ear begins to develop
- Retinal Pigment forms-eye become obvious
Figure 6-11. A, Lateral view of an embryo at Carnegie stage 17, about 42 days. Digital rays are visible in the hand plate, indicating the future site of the digits (fingers). B, Drawing indicating the structures shown in A. The eye, auricular hillocks, and external acoustic meatus are now clearly discernible.
7 Weeks

- Limbs undergo considerable change; fingers partially separate.
- Intestines enter extraembryonic coelum in proximal part of the umbilical cord.
8 Weeks

• Final week of embryonic development
• Digits of the hands are separated but webbed
• Notches visible between digits of the feet
• Tail-like caudal eminence is present but small
56 days
(End of Week 8)

Figure 6-12. A, Lateral view of an embryo at Carnegie stage 23, about 56 days. The embryo now has a distinctly human appearance. B, Drawing indicating the structures shown in A. The scalp vascular plexus is reduced and the caudal eminence has disappeared. (A, From Nishimura H, Semb H, Tanimura T, Tanaka O: Prenatal Development of the Human with Special Reference to Craniofacial Structures: An Atlas. Washington, DC, National Institutes of Health, 1977.)
Landmarks of Organogenesis:
1. Formation of the neural plate and its folding into the neural tube

2. Migration of the neural crest cells to multiple locations within the embryo and their differentiation into various cell types

3. Segregation of the paraxial somites

4. Arrangement of the 3 germ layers into specialized structures (limbs, eyes, ears..)
<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>Figure Reference</th>
<th>Carnegie Stage</th>
<th>No. of Somites</th>
<th>Length (mm)*</th>
<th>Main External Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-21</td>
<td>6-1A, 6-2A</td>
<td>9</td>
<td>1-3</td>
<td>1.5-3.0</td>
<td>Flat embryonic disc. Deep neural groove and prominent neural folds. Otic to three pairs of somites present. Head fold evident.</td>
</tr>
<tr>
<td>22-23</td>
<td>6-5A, 6-6A, C</td>
<td>10</td>
<td>4-12</td>
<td>2.0-3.5</td>
<td>Embryos straight or slightly curved. Neural tube forming and formed opposite somites, but widely open at rostral and caudal neuropores. First two pairs of pharyngeal arches visible.</td>
</tr>
<tr>
<td>24-25</td>
<td>6-5C, 6-7A</td>
<td>11</td>
<td>13-20</td>
<td>2.5-4.5</td>
<td>Embryos curved into head and tail folds. Rostral neuropore closing. Otic placodes present. Optic vesicles formed.</td>
</tr>
<tr>
<td>31-32</td>
<td>6-10A</td>
<td>14</td>
<td>†️</td>
<td>5.0-7.0</td>
<td>Upper limbs are paddle-shaped. Lens pits and nasal pits visible. Optic cup present.</td>
</tr>
<tr>
<td>37-40</td>
<td>16</td>
<td></td>
<td></td>
<td>8.0-11.0</td>
<td>Foot plates formed. Pigment visible in retina. Auricular hillocks developing.</td>
</tr>
<tr>
<td>47-49</td>
<td>19</td>
<td></td>
<td></td>
<td>16.0-18.0</td>
<td>Limbs extend ventrally. Trunk elongating and straightening. Mid gut herniation prominent.</td>
</tr>
<tr>
<td>50-51</td>
<td>20</td>
<td></td>
<td></td>
<td>18.0-22.0</td>
<td>Upper limbs longer and bent at elbows. Fingers distinct but webbed. Notches between the digital rays in the feet. Scalp vascular plexus appears.</td>
</tr>
<tr>
<td>52-53</td>
<td>21</td>
<td></td>
<td></td>
<td>22.0-24.0</td>
<td>Hands and feet approach each other. Fingers are free and longer. Toes distinct but webbed. Stubby tail present.</td>
</tr>
<tr>
<td>54-55</td>
<td>22</td>
<td></td>
<td></td>
<td>23.0-28.0</td>
<td>Toes fuse and longer. Eyelids and auricles of external ears more developed.</td>
</tr>
<tr>
<td>56</td>
<td>6-12</td>
<td>23</td>
<td></td>
<td>27.0-31.0</td>
<td>Head more rounded and shows human characteristics. External genitalia still have sexless appearance. Distinct bulge still present in umbilical cord, caused by herniation of intestines. Caudal eminence ('tail') has disappeared.</td>
</tr>
</tbody>
</table>

*The embryonic lengths indicate the usual range. In stages 9 and 10, the measurement is greatest length (GL); in subsequent stages crown-rump (CR) measurements are given.

Fetal Period (day 56 to Birth)

- Characterized by tissue differentiation, growth, and physiologic maturation
- Exposures during this period are likely to result in effects on growth and functional maturation
3 months or 16 Weeks
<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>CR Length (mm)*</th>
<th>Foot Length (mm)*</th>
<th>Fetal Weight (gm)†</th>
<th>Main External Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preivable Fetuses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>7</td>
<td>8</td>
<td>Eyelids closing or closed. Head round. External genitalia still not distinguishable as male or female. Intestines in umbilical cord.</td>
</tr>
<tr>
<td>10</td>
<td>61</td>
<td>9</td>
<td>14</td>
<td>Intestine in abdomen. Early fingernail development.</td>
</tr>
<tr>
<td>12</td>
<td>87</td>
<td>14</td>
<td>45</td>
<td>Sex distinguishable externally. Well-defined neck.</td>
</tr>
<tr>
<td>14</td>
<td>120</td>
<td>20</td>
<td>110</td>
<td>Head erect. Lower limbs well developed. Early toenail development.</td>
</tr>
<tr>
<td>16</td>
<td>140</td>
<td>27</td>
<td>200</td>
<td>Ears stand out from head.</td>
</tr>
<tr>
<td>18</td>
<td>160</td>
<td>33</td>
<td>320</td>
<td>Vernix caseosa covers skin. Quickening (signs of life felt by mothers).</td>
</tr>
<tr>
<td>20</td>
<td>180</td>
<td>39</td>
<td>460</td>
<td>Head and body hair (lanugo) visible.</td>
</tr>
<tr>
<td><strong>Viable Fetuses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>210</td>
<td>45</td>
<td>630</td>
<td>Skin wrinkled and red.</td>
</tr>
<tr>
<td>24</td>
<td>230</td>
<td>50</td>
<td>820</td>
<td>Fingernails present. Lean body.</td>
</tr>
<tr>
<td>26</td>
<td>250</td>
<td>55</td>
<td>1000</td>
<td>Eyes partially open. Eyelashes present.</td>
</tr>
<tr>
<td>28</td>
<td>270</td>
<td>59</td>
<td>1300</td>
<td>Eyes open. Good head of hair. Skin slightly wrinkled.</td>
</tr>
<tr>
<td>30</td>
<td>280</td>
<td>63</td>
<td>1700</td>
<td>Toenails present. Body filling out. Testes descending.</td>
</tr>
<tr>
<td>32</td>
<td>300</td>
<td>68</td>
<td>2100</td>
<td>Fingernails extend to fingertips, Skin smooth.</td>
</tr>
<tr>
<td>38</td>
<td>360</td>
<td>83</td>
<td>3400</td>
<td>Prominent chest; breasts protrude. Testes in scrotum or palpable in inguinal canals. Fingernails extend beyond fingertips.</td>
</tr>
</tbody>
</table>

*These measurements are averages and so may not apply to specific cases; dimensional variations increase with age.
†These weights refer to fetuses that have been fixed for about 2 weeks in 10% formalin. Fresh specimens usually weigh about 5% less.
‡There is no sharp limit of development, age, or weight at which a fetus automatically becomes viable or beyond which survival is ensured, but experience has shown that it is uncommon for a baby to survive if its weight is less than 500 gm or its fertilization age or developmental age is less than 22 weeks. Even fetuses born during the 26- to 28-week period have difficulty surviving, mainly because the respiratory and central nervous systems are not completely differentiated. The term abortion refers to all pregnancies that terminate before the period of viability.
TIMETABLE OF HUMAN PRENATAL DEVELOPMENT
1 TO 9 WEEKS

1. Stage 1: Fertilization
2. Stage 2 begins: Zygote divides
3. Zona pellucida
4. Stage 3 begins: Early blastocyst
5. Trophoblast
6. Stage 4: Implantation begins
7. Stage 5 begins
8. 2-week stage: Amniotic cavity
9. Lacunae appear in syncytiotrophoblast
10. Cytotrophoblast
11. Maternal blood lacunar network
12. Extraembryonic mesoderm
13. Stage 6 begins
14. Connecting stalk

Anatomy:
- Amnion
- Bilaminar disc
- Embryonic disc
- Prechordal plate
- Primary villi
- Coelom
- Eroded gland
- Lacunar network
Stage 7 begins

Trilaminar embryo

Amnion

Migration of cells from primitive streak.

Primrose streak

Primitive streak

Arrows indicate migration of mesenchymal cells.

Stage 8 begins

Neural plate

Neural groove

Somite

Primitive node

Primitive streak

Length: 1.5 mm

Stage 9 begins

Brain

Neural groove

Somite

Primitive streak

Thyroid gland begins to develop.

Stage 10 begins

Rostral neuropore

Heart bulge begins to beat

Heart

Primordia of eye and ear present.

Caudal neuropore

Neural folds fusing.

Stage 11 begins

Rostral neuropore

Heart bulge

Rostral neuropore closes

2 pairs of pharyngeal arches

Stage 12 begins

Otic pit

Upper limb bud

CRL = crown-rump length.

Stage 13 begins

Site of otic (ear) pit

Fore brain

Branchial arches

CRL : 4.0 mm

Stage 14 begins

Developing eye

Nasal pit

Primitive mouth

Stage 15 begins

Eye

Upper limb bud

CRL : 7.0 mm

Stage 16 begins

Large head

Ear

Eye

Foot plate

CRL : 9.0 mm

Stage 17 begins

External acoustic meatus

Digital rays

Digital rays

Ear

CRL : 13.0 mm

Stage 18 begins

Stage 19 begins

Stage 20 begins

Stage 21 begins

Neural groove

First pairs of somites

CRL : 5.0 mm

Stage 22 begins

Heart begins to beat

CRL : 10.0 mm

Stage 23 begins

Stage 24 begins

Stage 25 begins

Stage 26 begins

Stage 27 begins

CRL : 8.0 mm

Stage 28 begins

Stage 29 begins

Stage 30 begins

Stage 31 begins

Stage 32 begins

Stage 33 begins

Stage 34 begins

Stage 35 begins

Stage 36 begins

Stage 37 begins

Stage 38 begins

Stage 39 begins

Stage 40 begins

Stage 41 begins

Stage 42 begins

Oral and nasal cavities confluent.

CRL : 9.0 mm

CRL : 10.0 mm

CRL : 13.0 mm
TIMETABLE OF HUMAN PREGNATAL DEVELOPMENT
7 to 38 weeks

AGE (weeks)

43  Actual size

44  Stage 18 begins

45  Head large but chin poorly formed. Grooves between digital rays indicate fingers.

46  Wall of uterus Uterine cavity

47  Amniotic sac Smooth chorion

48  Genital tubercle External ear

49  Eyelid Wrist, fingers fused

50  Upper limbs longer and bent at elbows. Fingers distinct but webbed.

51  Eye Ear

52  Stage 21 begins

53  Large forehead

54  Genital tubercle Urethral groove

55  Anus or

56  Stage 23

57  Beginning of fetal period.

58  Eye Ear

59  Placenta

60  Genitalia Phallus

61  Urogenital fold Labioscrotal fold

62  Perineum

63  Genitalia Phallus

64  Face has human profile. Note growth of chin compared to day 44.

65  Cleft lip

66  Ears still lower than normal.

67  Clitoris Labium minus

68  Urogenital groove Labium majus

69  Genitalia have or characteristics but still not fully formed.

70  Glans penis Urethral groove Scrotum

CRL: 16 mm

CRL: 18 mm

CRL: 30 mm

CRL: 45 mm

CRL: 50 mm

CRL: 61 mm
Eleventh Week to Full Term
Sexual Differentiation

Figure 2. Sex differentiation in humans. The presence of a male testis that secretes both testosterone (T) and anti-Müllerian hormone (AMH) results in the induction of the Wolffian duct into the future vas deferens, epididymis, and seminal vesicles and the regression of the müllerian duct, respectively. In females, the fetal ovary does not secrete either of these substances, so the Wolffian duct regresses, whereas the Müllerian duct gives rise to the fallopian tubes (oviduct), uterus, and the upper portion of the vagina. Androgens (including testosterone) have a key role in the development of the male genital tract but are not the only signaling pathways involved. For a sperm and oocyte to meet as one, millions of spermatocytes move the seminiferous tubules of the testis to mature in the epididymis before traveling through the vas deferens and entering to enter the female, where they traverse the vagina, cervix and uterus before typically encountering a single ovary in one of the fallopian tubes. Surgical contraception involves blocking this pathway by removing segments of the hamnav deferens (that is, vasectomy) in a man or both fallopian tubes (that is, tubal ligature) in a woman.

Matzuk et al review 2008
Schematic of Sex Determination

**Males**

SOX9 + FGF9 maintain levels for males
Loss of either SOX9 or FGF9 causes male to female differentiation
Wnt 4 is repressed by FGF9 in testis
SRY represses Rsypo1

**Females**

Rsypo1 encodes a secreted protein to amplify Wnt4
Wnt4 up regulation represses SOX9 and FGF9
Loss of Wnt4 or Rsypo1 causes female to male differentiation
Physiological Actions of Androgen

In utero:

- External genitalia development
- Wolffian Duct development

Pubertal:

- External genitalia
- Hair growth
- Linear growth
- Accessory sex organs
- Voice
- Pyche (aggressive attitudes, sexual potency)

Adult:

- Hair growth, baldness
- Phyche (sexual potency)
- Bone loss prevention
- Maintenance of spermatogenesis
- Muscle mass increase
- Initiation of spermatogenesis
Changes in plasma hormone concentrations during puberty in boys (top) and girls (bottom).

Stage 1 of puberty is preadolescence in both sexes.

In boys, stage 2 is characterized by beginning enlargement of the testes, stage 3 by penile enlargement, stage 4 by growth of the glans penis, and stage 5 by adult genitalia.

In girls, stage 2 is characterized by breast buds, stage 3 by elevation and enlargement of the breasts, stage 4 by projection of the areolas, stage 5 adult breasts.


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Reproductive Hormone Signaling

Figure 3: Neuronal circuits control pituitary and gonadal function. The hypothalamus, which has a number of nuclei and pathways that affect reproductive behavior, secretes a key decapeptide, GnRH, that binds to its receptor, GnRHR, on the gonadotropes and is involved in induction of sexual maturity through its regulation of the synthesis and secretion of the pituitary gonadotropins FSH and LH. Kisspeptin (KISS1), secreted from neurons whose cell bodies are located in the anteroventral periventricular (AVPV) and arcuate (ARC) nuclei of the hypothalamus, signals through its receptor (KISS1R) to regulate pulsatile secretion of GnRH from additional hypothalamic neurons and thus affects the pathway at a higher level. FSH and LH have key roles on the gonads in both sexes, being involved in folliculogenesis, ovulation and steroidogenesis in females while functioning in gonadal growth, steroidogenesis and spermatogenesis in males. During pregnancy, human chorionic gonadotropin (hCG) production from the early placenta takes over the role of LH, stimulating the ovarian corpus luteum to produce progesterone, which, in turn, stimulates the uterus and maintains pregnancy. Equally important are a number of peptide (for example, inhibin (INH) and steroidogenic (that is, estradiol and testosterone) feedback systems from the gonads to the pituitary and hypothalamus. Multiple mutations in this axis have been identified in humans and mice (Supplementary Tables 1 and 2).
Gametogenesis

4 haploid sperm are formed from 1 primary spermatocyte

Sperm cell content is highly refined and specialized for movement

Puberty

1 mature oocyte is produced from the maturation of a primary oocyte

Completed just before ovulation

Oocyte is the largest human cell - retaining cytoplasm, RNA and ribosomes for protein synthesis after fertilization.

Completed after fertilization

Prophase of the 1st meiotic division until puberty

Figure 2 - S. Normal Gametogenesis, or conversion of germ cells into gametes. The drawings compare spermatogenesis and oogenesis. Oogonia are not shown in this figure because they differentiate into primary oocytes before birth. The chromosome complement of the germ cells is shown at each stage. The number designates the total number of chromosomes, including the sex chromosome(s) (shown after the comma). Note: (1) Following the two meiotic divisions, the diploid number of chromosomes, 46, is reduced to the haploid number, 23; (2) four sperms form from one primary spermatocyte; whereas only one mature oocyte results from maturation of a primary oocyte; (3) the cytoplasm is conserved during oogenesis to form one large cell, the mature oocyte. The polar bodies are small nonfunctional cells that eventually degenerate.
**Cell Divisions During Human Spermatogenesis**

**Spermatogenesis:** Production of round haploid spermatids from diploid cells

**Spermiogenesis:** Production of haploid spermatogonia with condensed nucleus, acrosomal cap, and tail

**Spermiation:** Release of spermatozoan into lumen of seminiferous tubule
Kinetics of Spermatogenesis

<table>
<thead>
<tr>
<th>Species</th>
<th>Time to complete spermatogenesis</th>
<th>Duration of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>70 day</td>
<td>16 days</td>
</tr>
<tr>
<td>Rat</td>
<td>48 days</td>
<td>12 days</td>
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</tbody>
</table>
Hormonal Regulation of Spermatogenesis

**Blood-Testis Barrier**

- Protects spermatogenic cells from exposure to:
  - a. immune cells
  - b. toxins
  - c. pathogens

- Barrier is maintained by tight junctions between Sertoli cells.

- Undifferentiated and differentiating spermatogonia in the basement membrane are exposed to circulating factors, while adlumenal differentiating spermatocytes and spermatids are protected.

- Leydig cells are in the interstitial space
Sperm production: 800 million/day or 5 million/10 min

Regulation of Sperm Output:
1. Sertoli cell number
2. Germ cell survival
   a. apoptosis
   b. toxic chemicals
   c. nutrition
   d. hormonal insufficiency

~40% of infertility of couples is attributed to the male.

Normal Sperm Count Criteria by WHO
>20 million sperm/mL
75% viability
50% forward mobility
At least 30% normal shape and form
Is Fertility Declining?

Figure 1. Mean sperm density in 101 studies published 1934–1996 and simple regression line.

Figure 2. Interactive regression model for mean sperm density by year and geographic region, after controlling for proven fertility, abstinence time, age, specimen collection method, method of counting sperm, whether the study was included by Carlsen et al. (4), and interaction of region and study year.

Swan, 2000
Human Follicle Development

<table>
<thead>
<tr>
<th>Follicular Status</th>
<th>Stage &amp; Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Follicle</td>
<td>Oogonium, Fetus → Mitosis</td>
</tr>
<tr>
<td>Primordial Follicle</td>
<td>Primary Oocyte, at Birth</td>
</tr>
<tr>
<td></td>
<td>Melosis, or Before, Melosis</td>
</tr>
<tr>
<td></td>
<td>Arrest (Diploctene)</td>
</tr>
<tr>
<td>Primary Follicle</td>
<td>Primary Oocyte, After Birth</td>
</tr>
<tr>
<td></td>
<td>Melosis Arrest (Diploctene)</td>
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<tr>
<td>Secondary Follicle</td>
<td>Secondary Oocyte, After Start</td>
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<td>Melosis II</td>
</tr>
<tr>
<td>Tertiary Follicle</td>
<td>Secondary Oocyte</td>
</tr>
</tbody>
</table>

Changes in Human Germ Cell Number

- Birth: 7 months
- Puberty: 400,000
- Menopause: (400-500 oocytes)

Spermatogenesis:

- Spermatogonum
- Primary spermatocyte
- Secondary spermatocytes
- Normal sperm

Oocytes:

- Oogonium
- Primary Oocyte
- Secondary Oocyte
- Tertiary Oocyte
Ovulatory Cycle
Reproductive & Developmental Toxicology
Reproductive Toxicology

The study of pharmacokinetics, mechanisms, pathogenesis and outcome following exposure to agents or conditions leading to abnormal reproductive capacity or reproductive organ development

Endocrine Disruptors
Agents that adversely alter the endocrine system in humans or wildlife
Impact of Environment on Development

- Developmental origins of health and disease

- Originally described by Barker in 1995 as the impact of intrauterine maternal malnutrition leading to cardiovascular and metabolic disorders in adult offspring

- Endocrine disrupting chemicals with effects in adult offspring (insecticides, fungicides, PCB, PBB, dioxins…).
Epigenetics

- First described by C. Waddington (1940s) to describe the developmental program where genes determine individual phenotype and internal and external environmental cues are also taken into consideration “beyond or above genetics”

- Currently describes the study of mitotically and meiotically heritable changes in gene function ie expression without changing DNA sequence

Molecular Mechanisms:
- DNA methylation (first described as X inactivation)
- Post-translational modifications of histone proteins

- Recently non-coding RNAs (miRNA) have been identified to have normal biological (potentially pathophysiologial) roles in reproduction and development.
Endocrine System

• Hormone systems including glands, hormones made and released into the blood stream from these glands, and receptors in organs and tissues that recognize and respond to hormones.

• Major constituents of the endocrine sys.
  – Ovaries, testes, pituitary, thyroid and adrenal glands
  – > 50 hormones have been identified in humans and other vertebrates
Processes under Endocrine Regulation

- Growth and function of the reproductive system
- Metabolism and blood sugar regulation
- Development of brain and nervous system
- Fundamentally all biological processes from conception through old age
Endocrine Disruptor Screening Program

Focuses on estrogen, androgen and thyroid hormones

**Estrogens**  - produced primarily in ovaries
  - targets female sexual development and reproduction

**Androgens**  - Testosterone produced in testes
  - targets male sex characteristics and reproduction
Mechanism of Endocrine Disruption

• Mimic a natural hormone
• Block the effects of a hormone from a receptor
• Stimulate or inhibit the endocrine system to cause over or under production of hormones
Endocrine-Disrupting Chemicals

Diethylstilbestrol (DES)- synthetic estrogen

• Prescribed (1940-1970s) to ~5 million women to block spontaneous abortion and promote fetal growth

• A case-controlled study found an association with DES exposure in the 1st trimester- prior to 18th week induced genital tract anomalies in offspring

• Risk of clear cell adenocarcinomas of the vagina and cervix ~0.14-1.4/1000 exposed pregnancies

• Also found high incidence of male reproductive effects including epididymal cysts, low semen volume and quality
Endocrine-Disrupting Chemicals

Diethylstilbestrol (DES)

Classic example of the potential for a developmental/reproductive toxin to produce latent and devastating manifestations.
Potential Genetic Targets for Male Fertility

Figure 5: Mouse models of male reproductive defects provide new insights into the causes of male infertility. This figure, updated from Matzuk and Lam	extsuperscript{2}, reveals the genes known today that influence testicular and sperm function in the mouse. The genes highlighted in red are in black, with new genes identified since then and others not shown previously in blue. Communication between each cell type compartment within the testis (spermatogonia, interstitial cells, and blood vessels, as well as between individual cell types (germ cells, Sertoli cells, peritubular myoid cells, Leydig cells) is necessary for proper development and function. It is noteworthy that the genes fall into specific categories of function, such as those involved in signal transduction, homologous recombination, or energy production. Gene targeting in the mouse models has provided new insights into potential etiologies of male infertility (see Supplementary Table 2). OT, oligospermatogenesis; OR, oligozoospermia; OR, oligospermia; Morph., morphology defects; M., mobility defects.
Potential Genetic Targets for Female Fertility

Mouse models with female fertility defects

Ovulation
CEBP-β, IL6ST, LFNG, LHR, NOS3, NRIP1, NR2C2, OA1D1, PEDSA1, PDE4D, PTGS2, PR, SIRT1, SRB1, SULT1E1, TRP73, YBX2

Preovulatory follicle
ADAMTS1, IMP42L, INH-α, IFR5, ER-α, ER-β, SH2B1, SOD1, SOX3, TNFRSF1A

Cumulus expansion
AMBP, AREG, BMP15, BMPR1B, EREG, GDF9, PTGER2, PTX3, TNFAIP6

Maternal effect
CSF2, DNMT10, DNMT3L, DPPA3, HSF1, NRIP1, NPM2, PAD6, UBE2A, ZAP1, ZFP36L2

Fertilization
BSG, CD9, CD81, PI, PLCB1, ZP1, ZP2, ZP3

Implantation and uterine
ACSL4, CBS, CENPB, CSF1, CYP40, DLGAP5, FKBP4, HMX3, HHFA1, HOXA10, HOXA11, IL11RA, LHX1, LEF1, LPAR3, NCOA1, NDPP, OVOL1, PAX8, PR, PTGS2, TEKT1, WNT7A

Post-implantation and post-partum
ABCA1, BAG4L1, BMS, CSF1, DAZAP1, FOXB1, FZD4, GDI1, HSF1, INHBB, NR2C2, OXT, OXTR, SHOX5A1

Steroidogenesis
ARNTL, CYP11A, CYP19A1, CYP40, DHCR24, NR5A2, STAR, VDR

Embryonic germ cell
BCL2, BCL2L2, BMP4, BMP8B, DAZL, FST, GJA1, GLP1, KIT, KITL, NOS3, PRDM1, SMAD1, SMAD5, STRA8, TCF21, TIAL1, WT1, WNT4, ZFX

Central
ACVR2A, AIP, AIRE, CDK4, CDKN1C, CC-α, CPE, CRTC1, DFR2, FSH-β, EGR1, GGT1, GHR, GNRH, GNRHR, INSL3, KISS1, KISS1R, LEP, LEPR, LH-β, OTX1, NPR2, POULF1, PROP1, STAT3, TGF-β1, TSHβ, TKT

Meiotic and DNA repair
ATM, BRWD1, CDC25B, CDK2, CKS2, CNBP1, CPEB1, DMC1, ERCC1, ERCC2, FANCA, FANC, FANC, FANCC, FANCD1, FANCL, FANM2, GA4, GPR3, HS2SF2, MEI1, MO, MLH1, MLH3, MSH4, MSH5, NBN, NOS3, PALI2, REC8, SGOL2, SMC1B, SPC11, SYCP1, SYCP2, SYCP3, TOP5, TRIP13, UBB, UBR2
Developmental Toxicology

The study of pharmacokinetics, mechanisms, pathogenesis and outcome following exposure to agents or conditions leading to abnormal development

Teratology

The study of abnormal development or structural birth defects, as a descriptive science
Teratology

The probability of a malformation being produced by a teratogen depends upon:

1. The dose of the agent
2. The stage at which the embryo was exposed
3. Genotype of the embryo and mother
Mechanisms Developmental Toxicology

1. Mutations
2. Chromosomal Breaks
3. Altered Mitosis
4. Altered Nucleic Acid Integrity or Function
5. Diminished Supplies of Precursors or Substrates
6. Decreased Energy Supplies
7. Altered Membrane Characteristics
8. Osmolar Imbalance
9. Enzyme Inhibition
Outcomes of these Mechanisms

1. Reduced Cell Proliferation
2. Cell Death
3. Altered Cell-Cell Interactions
4. Reduced Biosynthesis
5. Inhibition of Morphogenetic movements
6. Mechanical Disruption of Developing Structures
## Stages in Human Development

<table>
<thead>
<tr>
<th>Week</th>
<th>Age of Embryo (in weeks)</th>
<th>Fetal Period (in weeks)</th>
<th>Full Term</th>
<th>Major Morphological Abnormalities</th>
<th>Functional Defects and Minor Morphological Abnormalities</th>
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<tr>
<td>1</td>
<td>dividing zygote, implantation and gastrulation</td>
<td>CNS</td>
<td>heart</td>
<td>not susceptible to teratogens</td>
<td>prenatal death</td>
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</table>
Window of Susceptibility to Teratogens

- Identify critical time-points with heightened sensitivity to an exposure
- These windows may encompass young children and pregnant women
- Actual windows may be much smaller timeframes during development
Example of Windows of Susceptibility – Retinoic Acid Exposure in the Hamster

Dose used was maternal LD$_{50}$

Total incidence of malformations lower prior to organogenesis but increases to 100% by gestational day 73/4.

Taken from Shenefe, 1972
Retinoids

• Excess vitamin A causes malformations in face, limbs, heart, central nervous system, and skeleton

• Teratogenicity of retinoids have been demonstrated in both humans and animals

• One mechanism that has been identified for its action relates to nuclear receptor activation and altered transcriptional events

• Even with known teratogenic effects, 13-cis-retinoic acid (Acutane) was marketed in 1982. The introduction included very strong warnings regarding pregnancy and physician and patient education programs to avoid exposure of pregnant women.
Causes of Spontaneous Mutations in Newborns

<table>
<thead>
<tr>
<th>Cause</th>
<th>Number $^b$</th>
<th>Percent of total</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic causes</td>
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<tr>
<td>Chromosome abnormalities</td>
<td>157 (45)</td>
<td>10.1</td>
<td>Trisomies, deletions</td>
</tr>
<tr>
<td>Single mutant genes</td>
<td>48</td>
<td>3.1</td>
<td>Chondrodystrophies</td>
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<tr>
<td>Familial</td>
<td>225 (3)</td>
<td>14.5</td>
<td>Renal agenesis</td>
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<tr>
<td>Multifactorial inheritance</td>
<td>356 (23)</td>
<td>23.0</td>
<td>Anencephaly, some heart defects</td>
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<td>Teratogens</td>
<td>49</td>
<td>3.2</td>
<td>Infants of diabetic mothers</td>
</tr>
<tr>
<td>Uterine factors</td>
<td>39 (5)</td>
<td>2.5</td>
<td>Breech presentation</td>
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<tr>
<td>Twinning</td>
<td>6 (2)</td>
<td>0.4</td>
<td>Acardia, conjoinings</td>
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<tr>
<td>Unknown cause</td>
<td>669 (24)</td>
<td>43.2</td>
<td>Gastoschisis</td>
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<tr>
<td>Subtotals</td>
<td>1,549 (102)</td>
<td>100.0</td>
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<tr>
<td>Overall total births</td>
<td>69,227</td>
<td></td>
<td></td>
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</table>

$^a$Total frequency 2.2%.

$^b$Parentheses indicate therapeutic abortions.

Common Endpoints for Assessment of Developmental Toxicity Due to Environmental Agents

• Prenatal and Postnatal Death
• Structural Abnormalities (Malformation)
• Altered Growth
• Functionality Deficits
• Longer Term Reproductive Effects
Methods for Detection of Developmental Toxins

• Animal Testing
• Epidemiology
• Computer Based Modeling
• Cell based technologies
What has been tested?

• >90% of drugs approved between 1980 and 2000 have no human data on developmental toxicology. New drug approvals rely on animal testing data

• As of 2000, ~4100 chemicals have had teratogenicity testing with
  ❖ 66% found non-teratogenic
  ❖ 7% teratogenic in more than 1 species
  ❖ 18% teratogenic in most species tested
  ❖ 9% no definitive result

• In Humans only ~50 chemicals or conditions have been documented to alter prenatal development
<table>
<thead>
<tr>
<th>Drugs</th>
<th>Maternal conditions</th>
<th>Other exposures</th>
</tr>
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<tbody>
<tr>
<td>Aminopterin/methotrexate (Amethopterin)</td>
<td>Alcohol use</td>
<td>Chorionic villus sampling</td>
</tr>
<tr>
<td>Androgenic hormones</td>
<td>Insulin-dependent diabetes mellitus</td>
<td>Dilation and curettage</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>Iodide deficiency</td>
<td>Gasoline fumes (excessive)</td>
</tr>
<tr>
<td>Busulfan</td>
<td>Maternal phenylketonuria</td>
<td>Heat</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Myasthenia gravis</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Chlorobiphenyls</td>
<td>Smoking cigarettes or marijuana</td>
<td>Methyl isocyanate</td>
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<tr>
<td>Cocaine</td>
<td>Systemic lupus erythematosus</td>
<td>Methylene blue</td>
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<tr>
<td>Cyclophosphamide</td>
<td>Vitamin A deficiency</td>
<td>Toluene (excessive)</td>
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<tr>
<td>Diethylstilbestrol</td>
<td>Intrauterine infections</td>
<td>Trauma, blunt</td>
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<tr>
<td>Etretinate</td>
<td>Cytomegalovirus</td>
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<tr>
<td>Heroin/methadone</td>
<td>Herpes simplex</td>
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<tr>
<td>Iodide</td>
<td>Parvovirus</td>
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<tr>
<td>Isotretinoin (13-cis-retinoic acid)</td>
<td>Rubella</td>
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<td>Lithium</td>
<td>Syphilis</td>
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<td>Phenobarbital</td>
<td>Toxoplasmosis</td>
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<td>Phenytoin</td>
<td>Varicella</td>
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<td>Propylthiouracil</td>
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<td>Prostaglandin</td>
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<td>Tetracycline</td>
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<tr>
<td>Thalidomide</td>
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<td>Trimethadione/paramethadione</td>
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<td>Warfarin</td>
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<td><strong>Heavy metals</strong></td>
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<td>Lead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercury</td>
<td></td>
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<tr>
<td><strong>Radiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer therapy</td>
<td></td>
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</tr>
</tbody>
</table>
What are Normal Rates of Pregnancy Loss and Mutation in a Population?

- Post-implantation loss ~31%
- Major birth defects 2-3% increasing to 6-7% at 1 year of age
- Minor birth defects ~14%
  - Low birth weight 7%
  - Infant Mortality prior to 1 yr. 1.4%
  - Abnormal neurological function ~16%
Causes of Birth Defects

• 15-25% Known genetic transmission
• 4% Maternal conditions
• 3% Maternal infections
• 1-2% Deformations due to mechanical problems (umbilical cord amputations)
• 1% Chemical or other environmental exposure
• 65% Unknown Etiologies
Genetic and fetal factors
Species, race, gender
Congenital anomalies
Chromosomal disorders
Fetal hormones (insulin, corticosteroids, thyroid hormone, androgens)
Growth factors (insulin-like growth factors I and II, epidermal growth factor, transforming growth factor–alpha)

Maternal uterine environment
Uterine and placental anatomy
Uteroplacental function
Human placental lactogen
Substrate fluxes and transfer
Uterine blood flow
Maternal systemic disease

Macroenvironment
Infectious agents (STORCH)
Diet and nutrition
Social and emotional stress
Drugs and smoking
Teratogens and toxins
Altitude and temperature
Ionizing radiation

STORCH, syphilis, toxoplasmosis, rubella, cytomegalovirus, herpesvirus.
Principles of Teratology/Developmental Toxicology

Susceptibility to agents depends upon:

- Genotype
- Developmental Stage at Exposure
- Agents mechanism of action on developing cells and tissues and presence of mechanisms of protection
- Access of the agent to developing tissues
Major Regulator of Access of Agents to a Embryo/Fetus

Placenta:

- Regulates blood flow
- Transport barrier
- Metabolizes chemicals
3 Major Determinants of Placental Transfer

1. Type of placentation
2. Physicochemical properties of the agent
3. Rate of placental metabolism

Mechanisms of Transfer

1. Passive diffusion
2. Facilitated transport
3. Active transport
Modifiers of the Rate of Transfer

1. Lipid solubility
2. Molecular weight
3. Protein binding
4. Transfer mechanism
5. Degree of ionization
6. Placental metabolism
7. Dose
How are/have Human Developmental Toxins Detected?

1. Epidemiology
   a. Easiest with rare but obvious outcomes
   b. Large groups with well defined exposures

2. Strong animal data combined with case-reports

3. Strong animal data with mechanistic information that would pertain to humans
Figure 17-3. Drawings illustrating development of the limbs (32–56 days).
## Thalidomide

Incidence of limb malformations in Hamburg, West Germany

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940-1959</td>
<td>No cases of reduced long bones of limbs</td>
</tr>
<tr>
<td>1959</td>
<td>1 case</td>
</tr>
<tr>
<td>1960</td>
<td>30 cases</td>
</tr>
<tr>
<td>1961</td>
<td>154 cases</td>
</tr>
</tbody>
</table>

In 1961 Lenz and McBride identify the sedative Thalidomide as the causative agent.
Thalidomide

Associated Defects:
1. Absence of limbs or reduced long bones in limbs
2. Congenital heart disease
3. Ocular, intestinal and renal anomalies
4. Malformations of the external and inner ear

Uses of Drug: Sedative (sleep aid, treatment of nausea and vomiting in pregnancy

Drug withdrawn in Nov 1961

5850 malformed infants were identified worldwide.
Thalidomide

Animal Testing Followed:

1. Very complex series of responses found in animals

2. At least 19 laboratory species tested. Malformations found in some rats, no effect in hamsters or most mice.

3. Several rabbit strain and eight of nine primate species duplicated the human response

4. Mechanisms of toxicity not yet determined

5. Period of Susceptibility 20-36 days post-fertilization (key period of limb development)
Thalidomide

Hypothesized Mechanisms of Teratogenesis:

1. Effect on angiogenesis
2. Integrin regulation
3. Oxidative DNA damage
4. TNFα inhibition
5. Effects on glutathione and redox status
Maternal Factors Affecting Development

1. Genetics
2. Disease
3. Nutrition
4. Stress
5. Placental Toxicity
6. Maternal Toxicity

Figure 10-7. Interrelationships between maternal susceptibility factors, metabolism, induction of maternal physiologic or functional alterations, placental transfer and toxicity, and developmental toxicity.

A developmental toxicant can cause abnormal development through any one or a combination of these pathways. Maternal susceptibility factors determine the predisposition of the mother to respond to a toxic insult, and the maternal effects listed can adversely affect the developing conceptus. Most chemicals traverse the placenta in some form, and the placenta can also be a target for toxicity. In most cases, developmental toxicity is probably mediated through a combination of these pathways.
Specific Agents – Teratogenic Potential in Humans

- **Anticonvulsants**
  - Hydantoin- syndrome seen in ~10% of exposed babies, digital and nail hypoplasia, depressed nasal bridge, mental retardation, slight increase congenital heart disease
  - Valproic acid ~1% increased risk of neural tube defects
  - Carbamazepine ~1% increased risk of neural tube defects
  - Phenobarbital – no increased risk when used along

- **Antimicrobial Agents**
  - Sulfanoamides- hazard to newborn not teratogen
  - Aminoglycosides- potential for hearing loss

- **Progestogens**- no association with limb anomalies or CV defects as previously reported

- **Accutane**-1st trimester fetal malformations including microcephaly, ear abnormalities, cardiac defects, CNS lesions

- **Corticosteroids**- No evidence of teratogenic effects in humans, palatal teratogen in rodents

- **Psychotropic Drugs**
  - Litium possible slight increase in Ebstein’s anomaly