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,

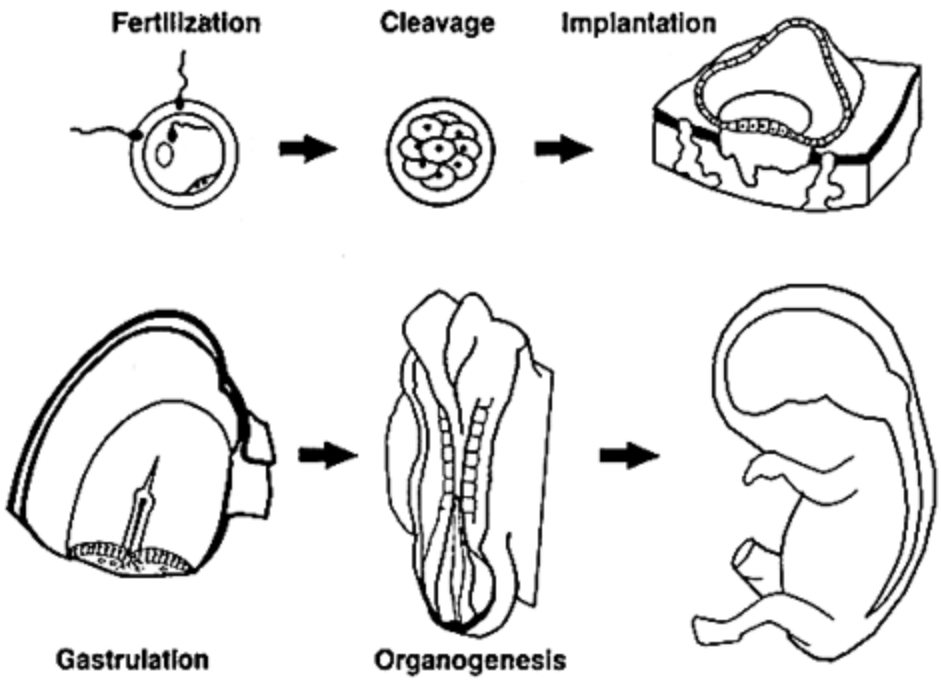
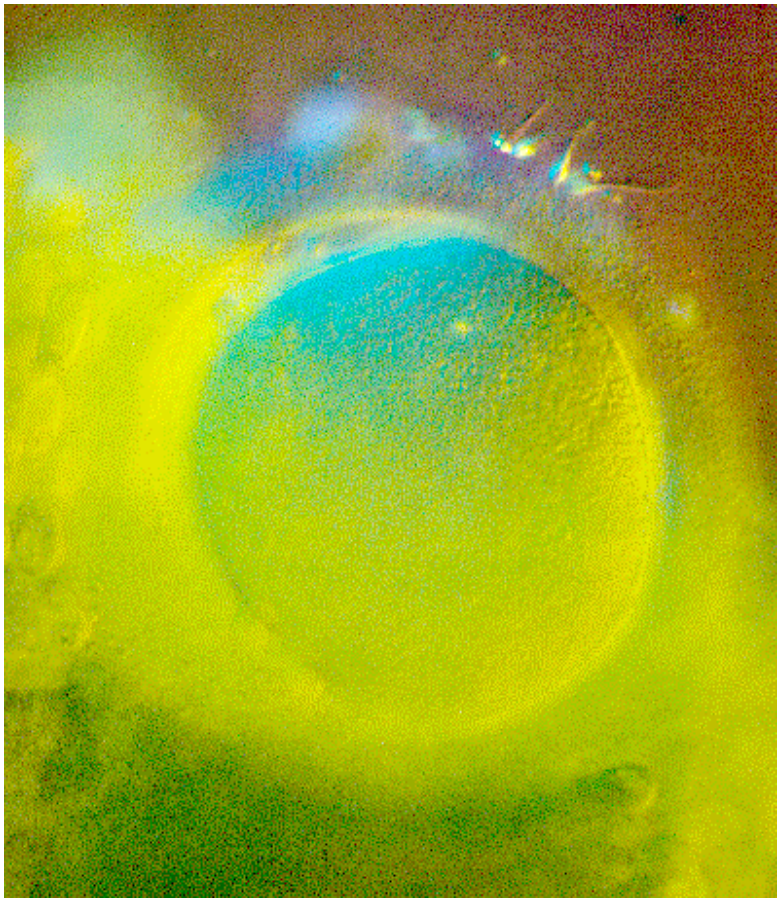


FIGURE 18-2. Milestones in development.

Julia A. McMillan, Catherine D. DeAngelis, Ralph D. Feigin, Joseph B. Warshaw, Oski's Pediatrics: Principles and Practice

Fertilization



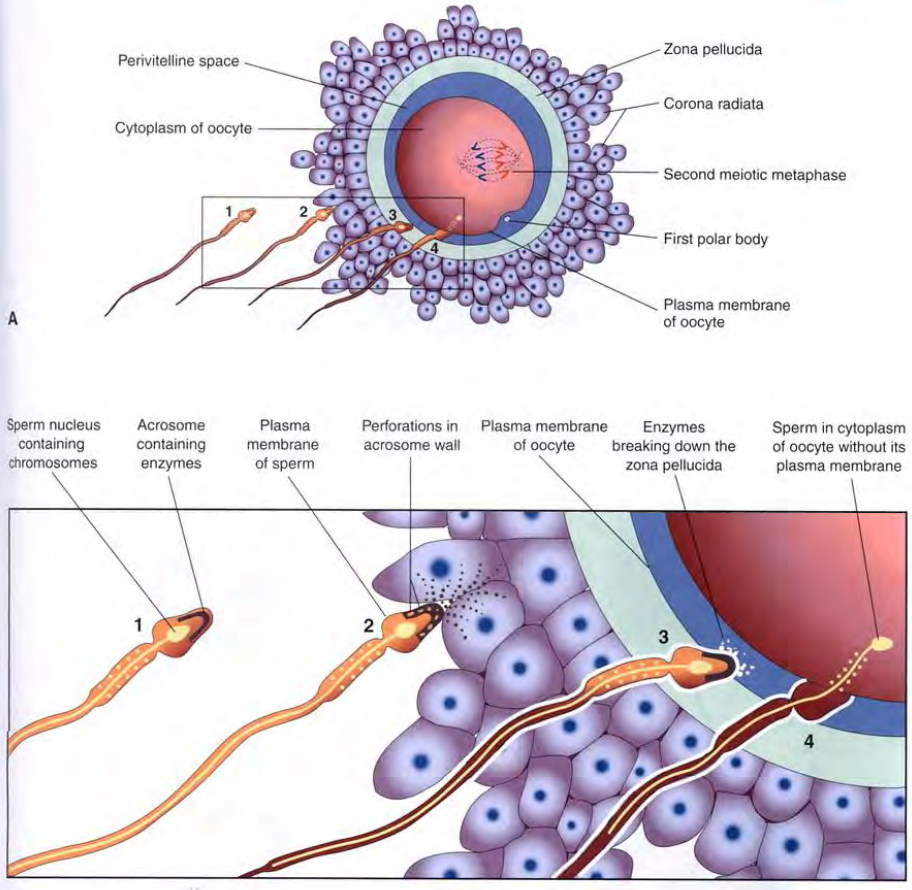
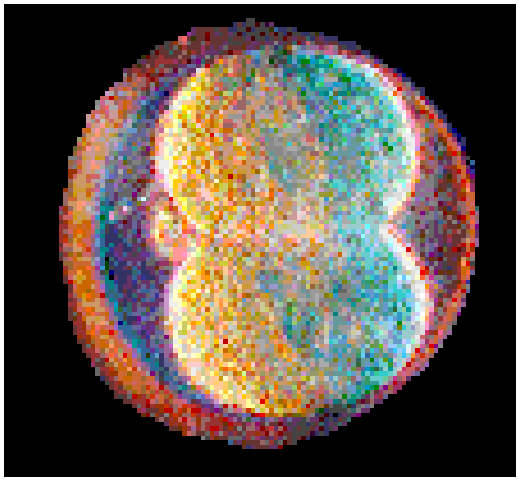
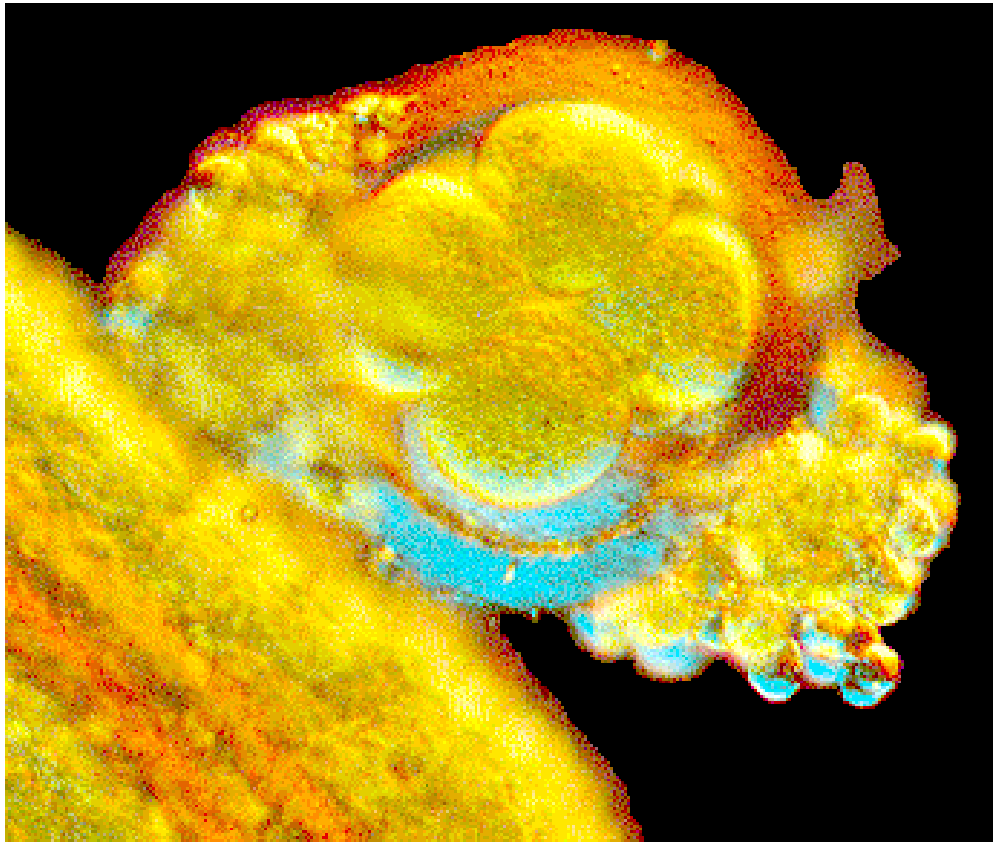


Figure 3 – 1. Acrosome reaction and a sperm penetrating an oocyte. The area outlined in *A* is detailed in *B*. *1*, Sperm during capacitation, a period of conditioning that occurs in the female reproductive tract. *2*, Sperm undergoing the acrosome reaction during which perforations form in the acrosome. *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.

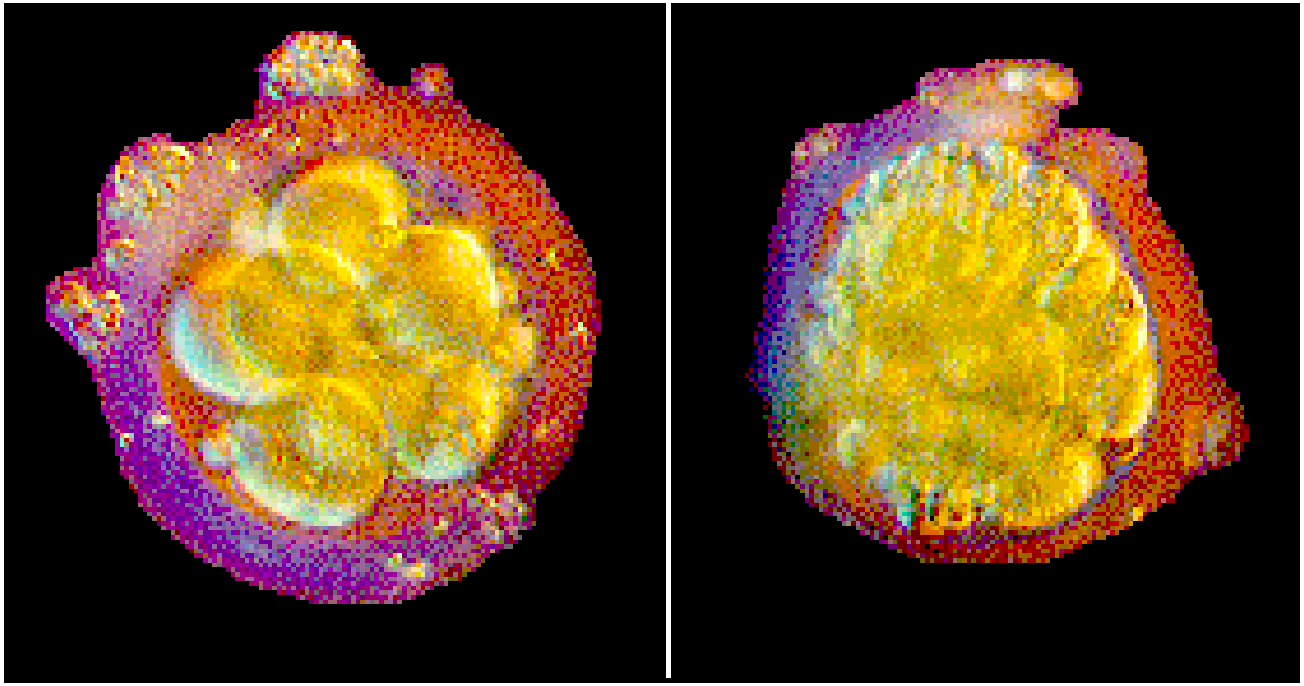
2 Cells



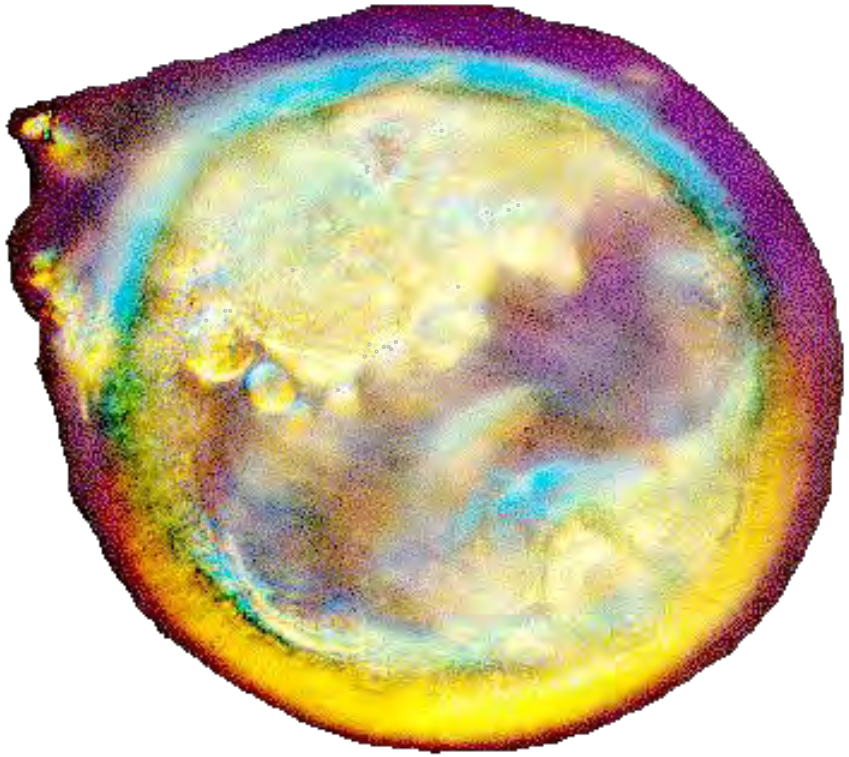
4 Cells



16 Cells



Blastocyst



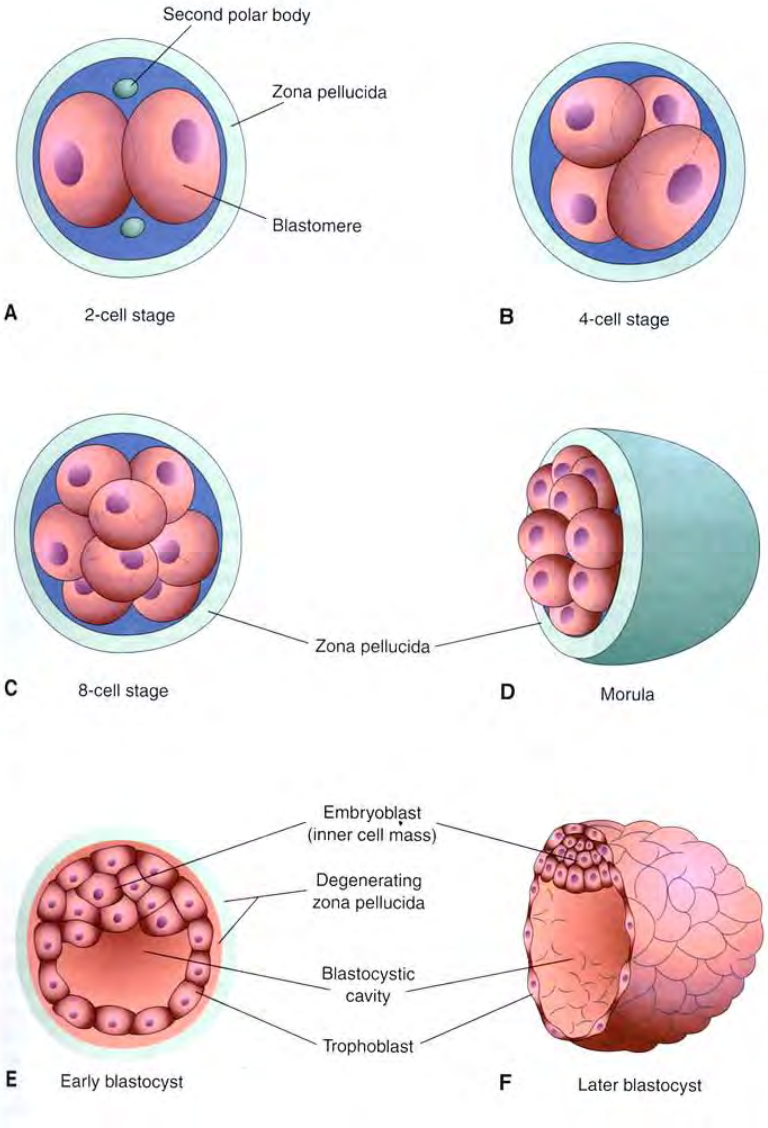
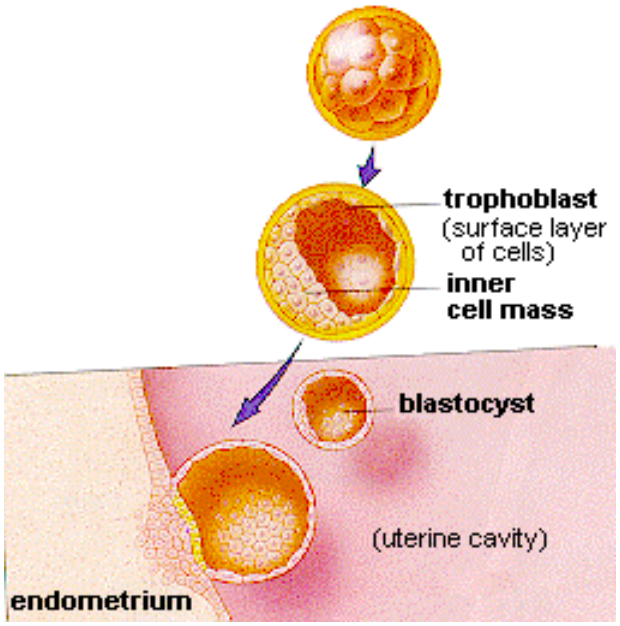
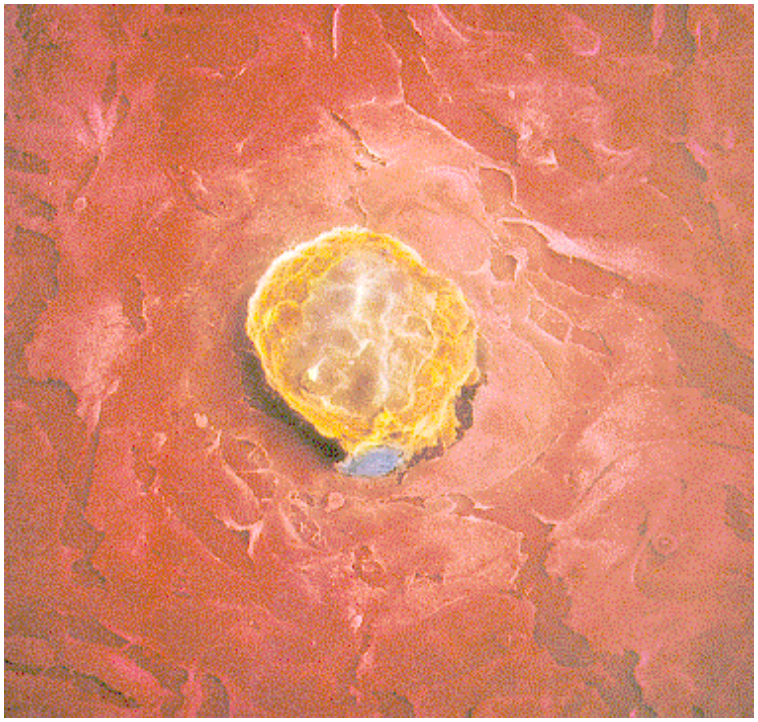


Figure 3-4. Drawings illustrating cleavage of the zygote and formation of the blastocyst. *A* to *D* show various stages of cleavage. The period of the morula begins at the 12- to 16-cell stage and ends when the blastocyst forms. *E* and *F* are sections of blastocysts. The zona pellucida disappears 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 cells. 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





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 ooctye 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

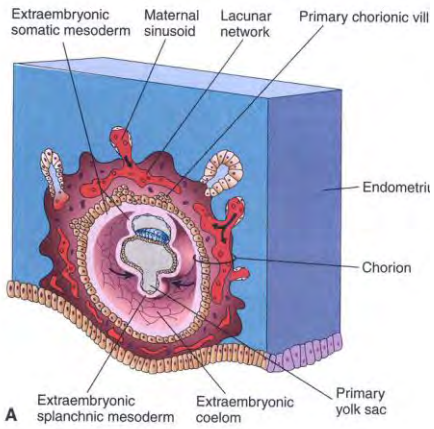
Week 1

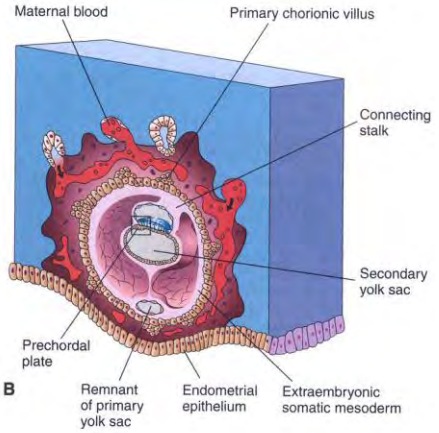
- Zona Pellucida degenerates
- 6 days after fertilization the blastocyst attaches to the endometrial epithelium
- Trophoblast proliferates and differentiates into 2 cell layers:

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





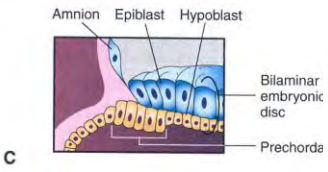


Figure 3–5. Drawings of sections through implanted human embryos, based mainly on Hertig et al., 1956. Observe: (1) the defect in the endometrial epithelium has disappeared; (2) a small secondary yolk sac has formed; (3) a large cavity, the extraembryonic coelom, now surrounds the yolk sac and amnion, except where the amnion is attached to the chorion by the connecting stalk; and (4) the extraembryonic coelom splits the extraembryonic mesoderm into two layers: extraembryonic somatic mesoderm lining the trophoblast and covering the amnion, and the extraembryonic splanchnic mesoderm around the yolk sac. *A*, 13 days, illustrating the decrease in relative size of the primary yolk sac and the early appearance of primary chorionic villi. *B*, 14 days, showing the newly formed secondary yolk sac and the location of the prechordal plate in its roof. *C*, Detail of the prechordal plate outlined in *B*.

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.

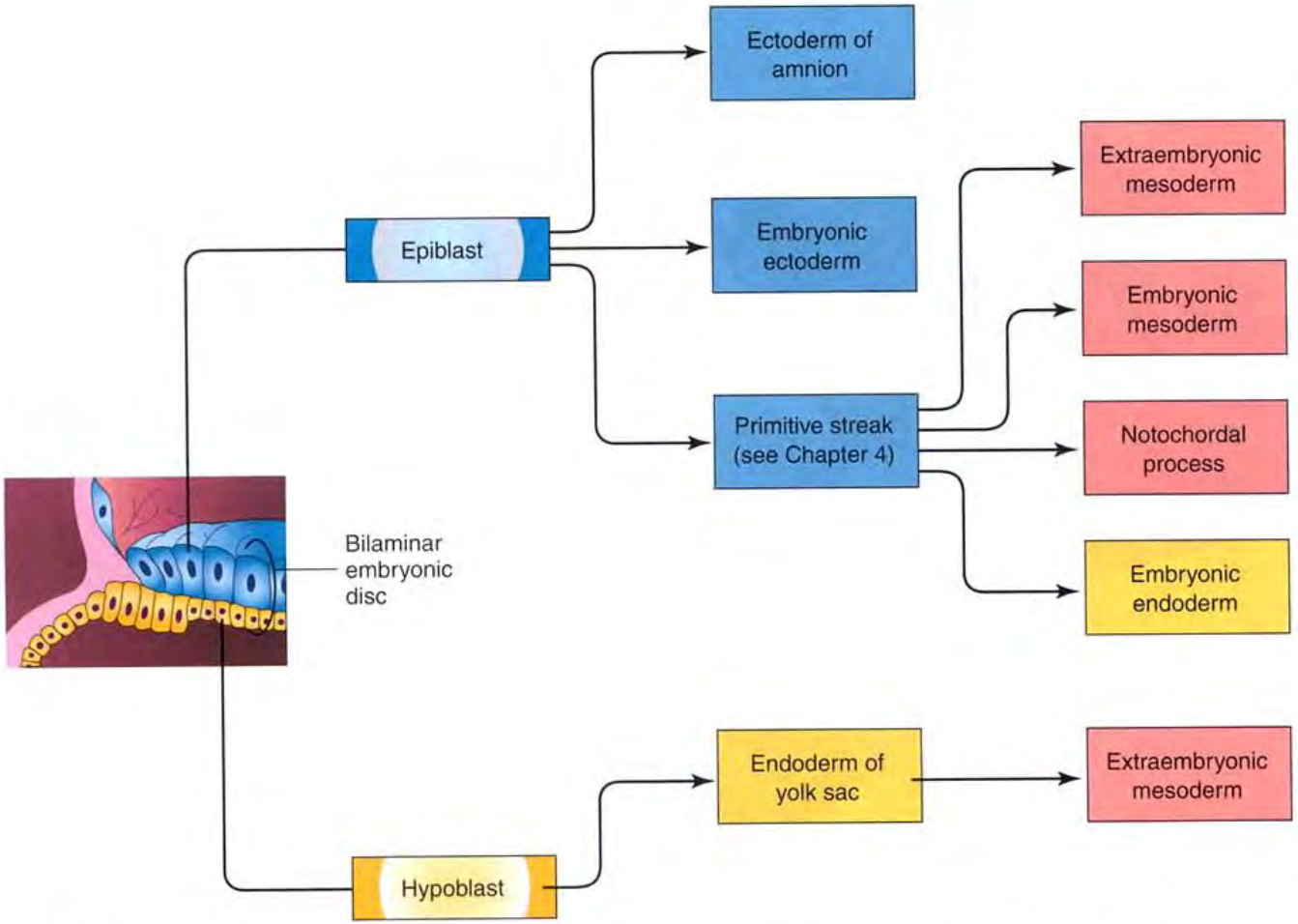


Figure 4 - 4. Origin of embryonic tissues. The colors in the boxes correspond to those used in drawings of sections of conceptuses.

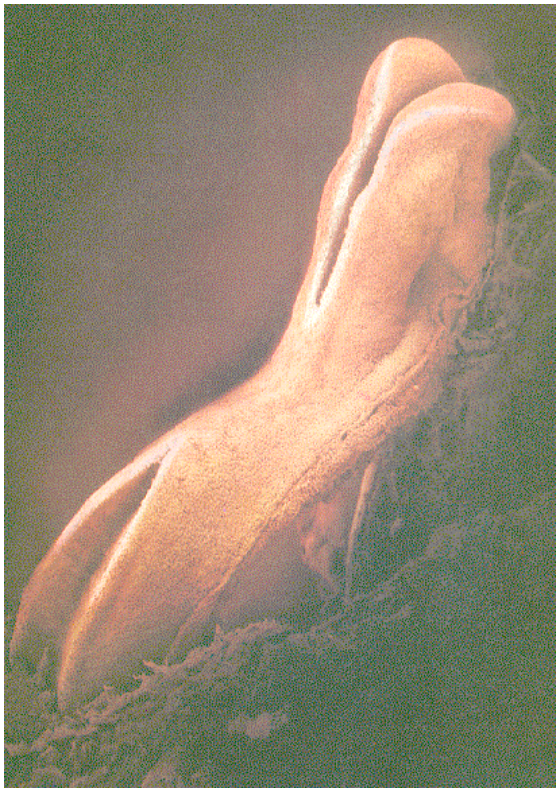
Week 2

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

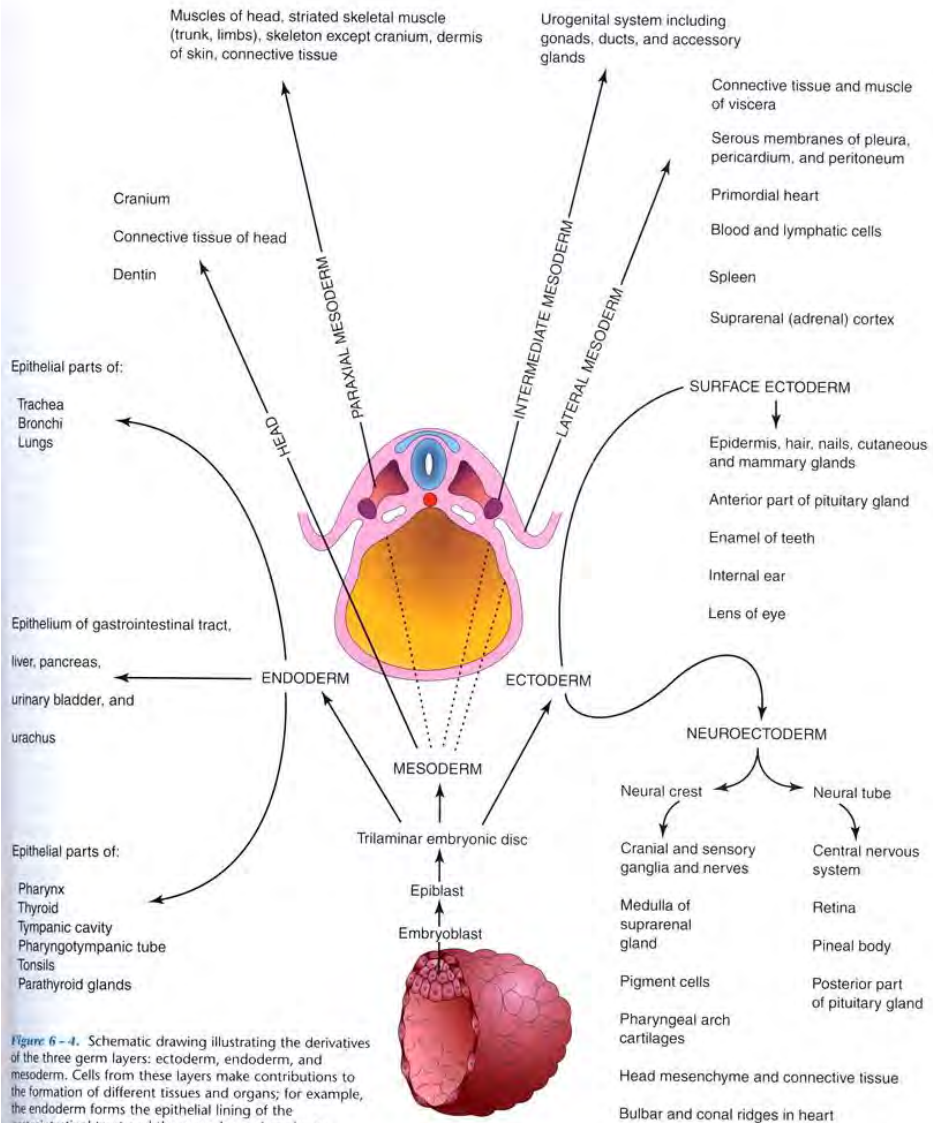


Figure 6–4. Schematic drawing illustrating the derivatives of the three germ layers: ectoderm, endoderm, and mesoderm. Cells from these layers make contributions to the formation of different tissues and organs; for example, the endoderm forms the epithelial lining of the gastrointestinal tract and the 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.

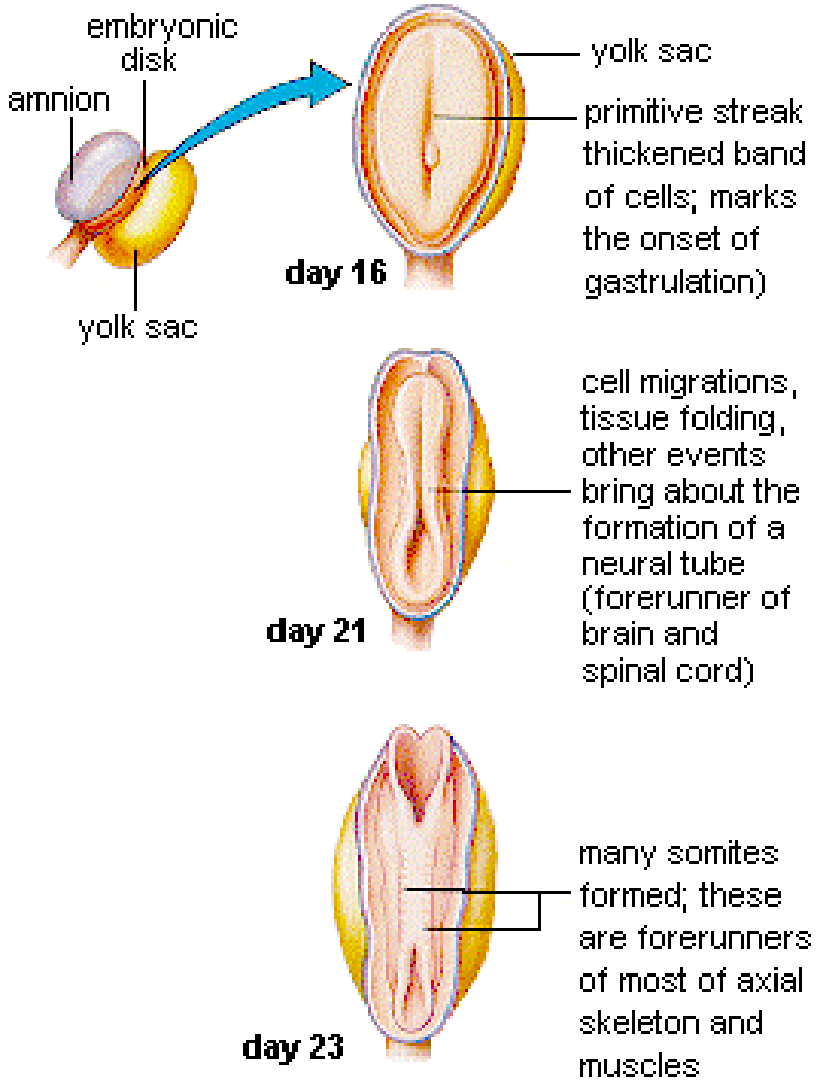
Primative groove develops in the streak

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



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 3rd week with angiogenesis

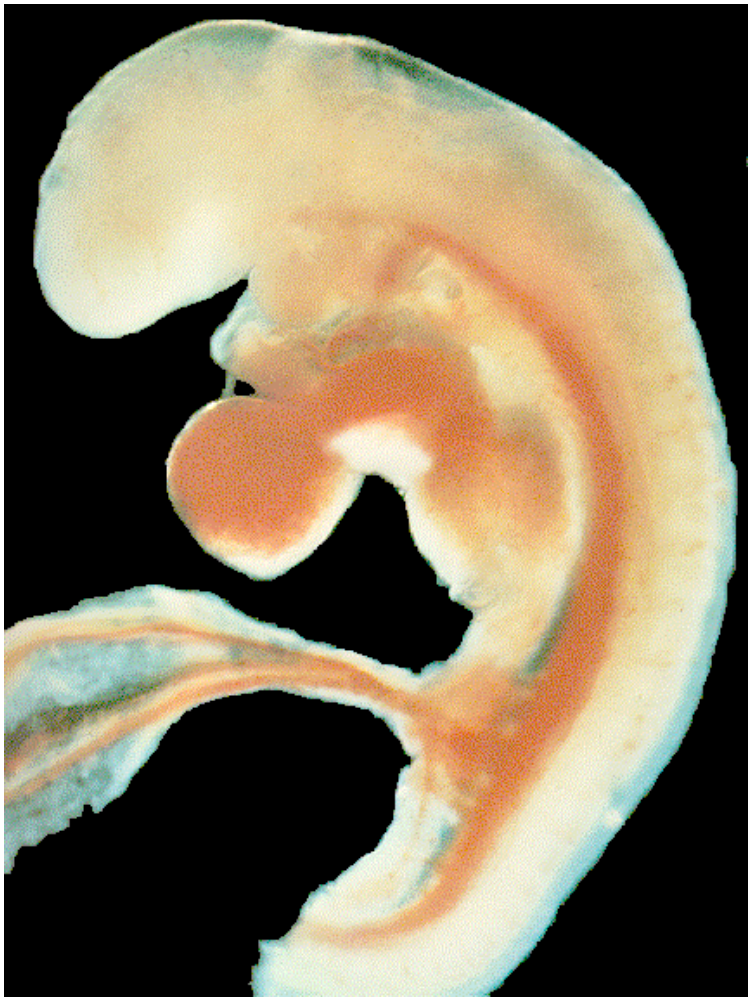
embryonic blood vessels form and differentiate into the muscular and connective tissue of the vessels

Paired cardiogenic heart tubes form and fuse to create the primordial heart tube. By the end of the 3rd week blood is circulating and the heart begins to beat on day 21-22.

Weeks 4-8 Organogenic Period

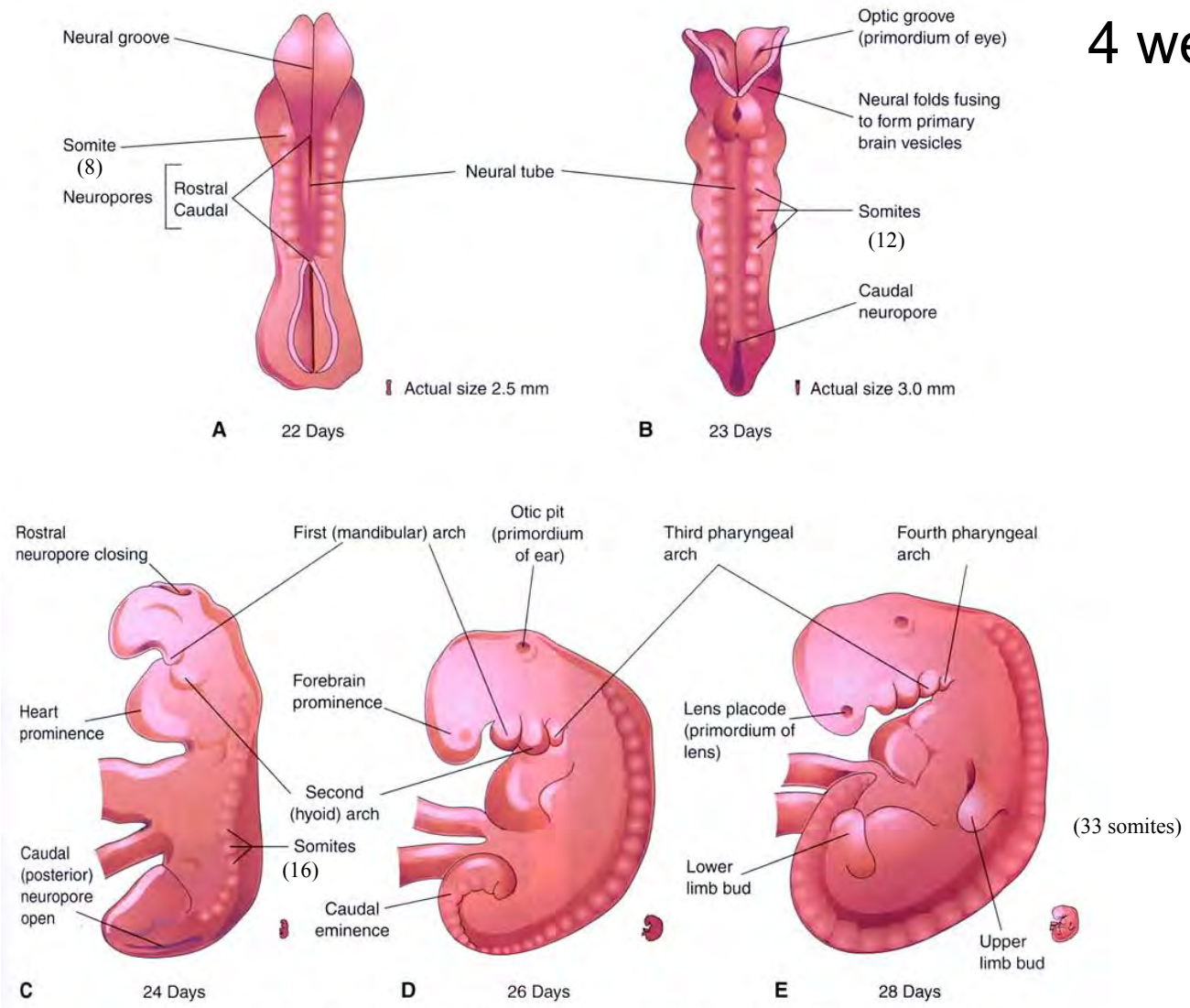
- Development of all major organ systems occur in the organogenic period
- Upper limb buds begin to show differentiation of elbows and large hand plates
- Digital rays-primordia of digits
- Embryo begins to show spontaneous movement
- External ear begins to develop
- Retinal Pigment forms- eye become 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

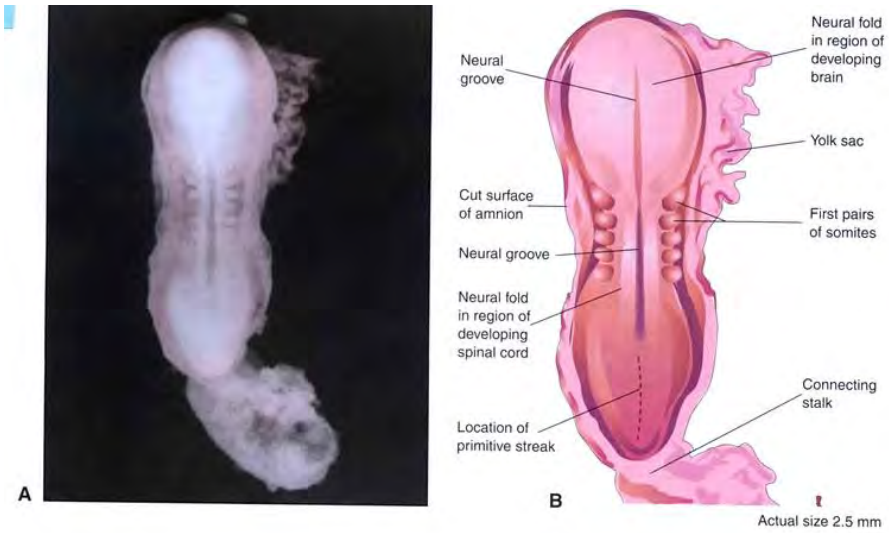
4 weeks



(33 somites)

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



23 days

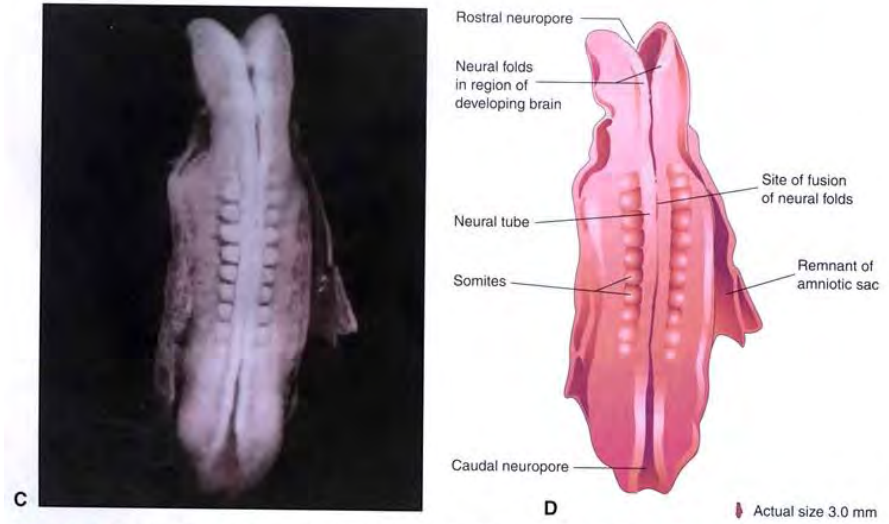


Figure 6 – 6. *A*, Dorsal view of a five-somite embryo at Carnegie stage 10, about 22 days. Observe the neural folds and neural groove. The neural folds in the cranial region have thickened to form the primordium of the brain. *B*, Drawing indicating the structures shown in *A*. Most of the amniotic 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

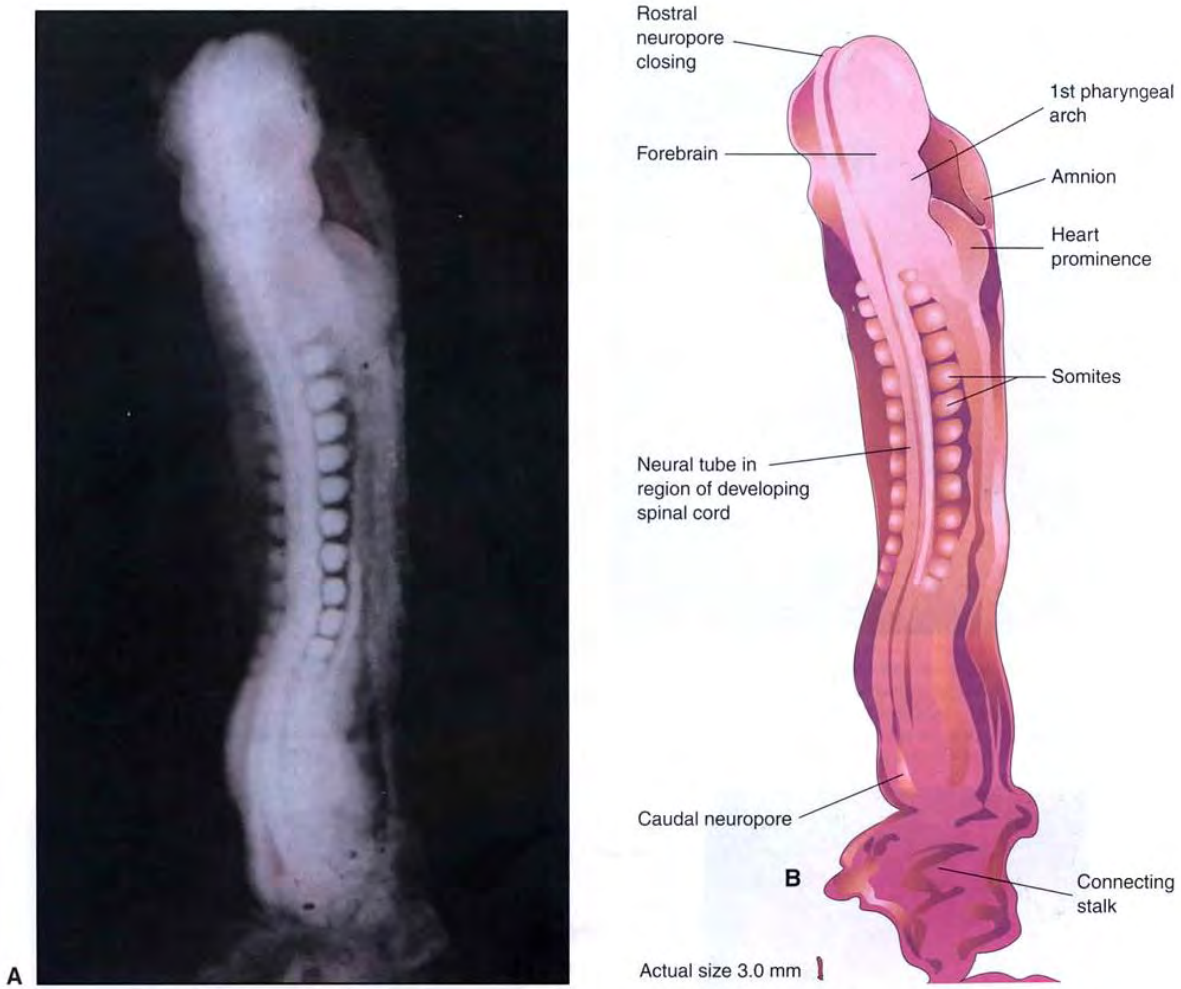


Figure 6 - 7. *A*, Dorsal view of a 13-somite embryo at Carnegie stage 11, about 24 days. The rostral neuropore is closing, but the caudal neuropore is wide open. *B*, Drawing indicating the structures shown in *A*. The embryo is curved because of folding at the cranial and caudal ends.

26 days

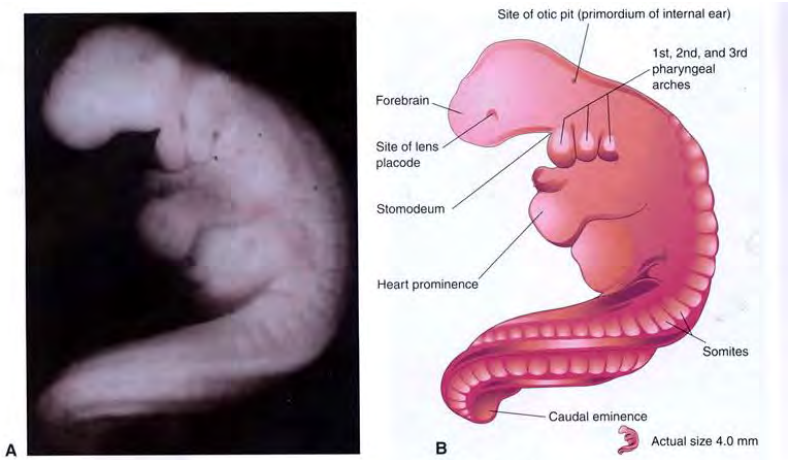


Figure 6 - 8. *A*, Lateral view of a 27-somite embryo at Carnegie stage 12, about 26 days. The embryo is very curved, especially its long tail-like caudal eminence. Observe the lens placode (primordium of the lens of the eye) and the otic pit, indicating early development of the internal ear. *B*, Drawing indicating the structures shown in *A*. The rostral neuropore is closed, and three pairs of pharyngeal arches are present. (*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.)

28 days

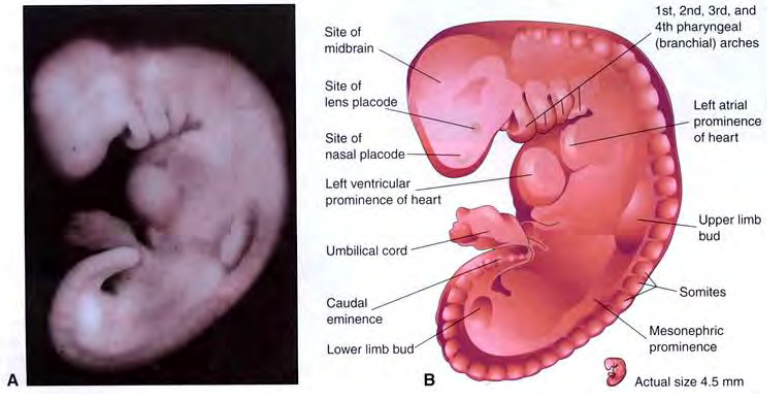
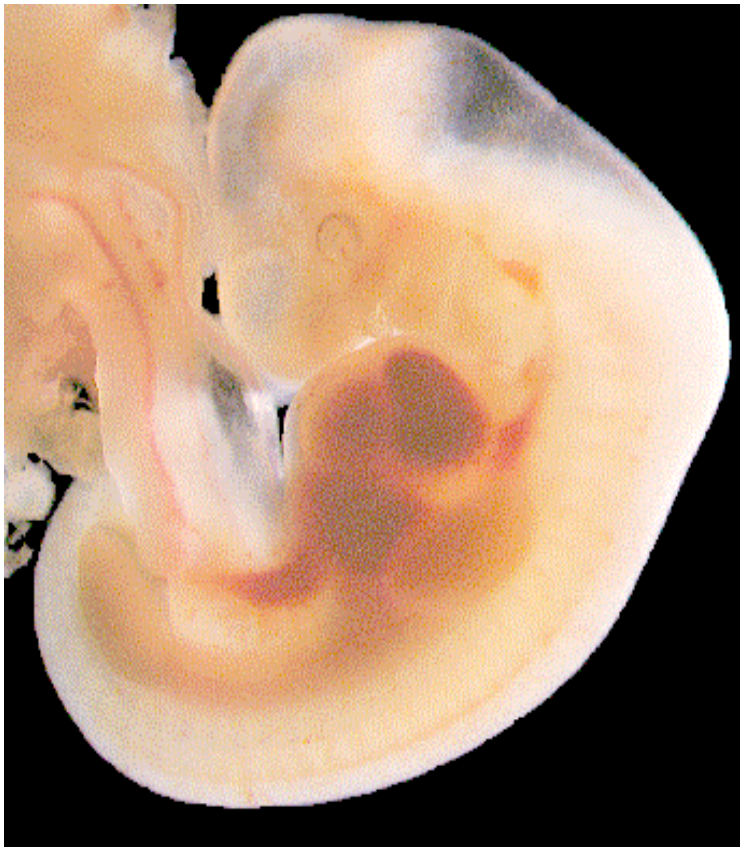


Figure 6 - 9. *A*, Lateral view of an embryo at Carnegie stage 13, about 28 days. The primordial heart is large, and its division into a primordial atrium and ventricle is visible. The rostral and caudal neuropores are closed. *B*, Drawing indicating the structures shown in *A*. The embryo has a characteristic C-shaped curvature, four pharyngeal arches, and upper and lower limb buds. (*A*, From Nishimura H, Semba H, Tanimura 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

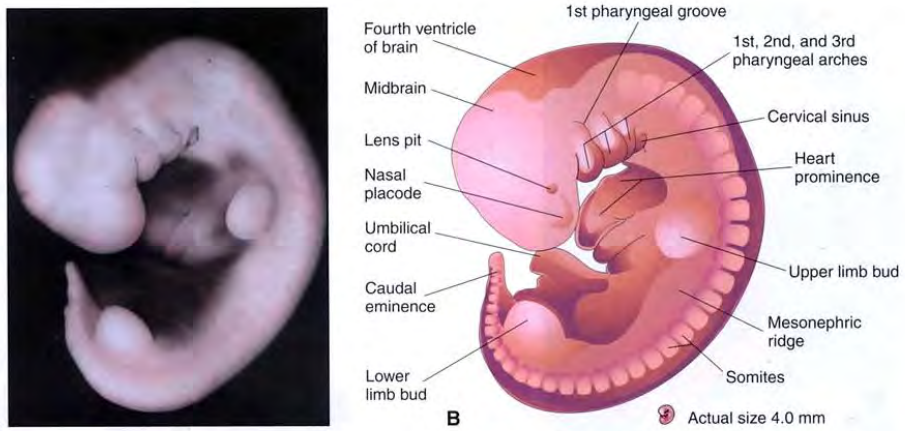
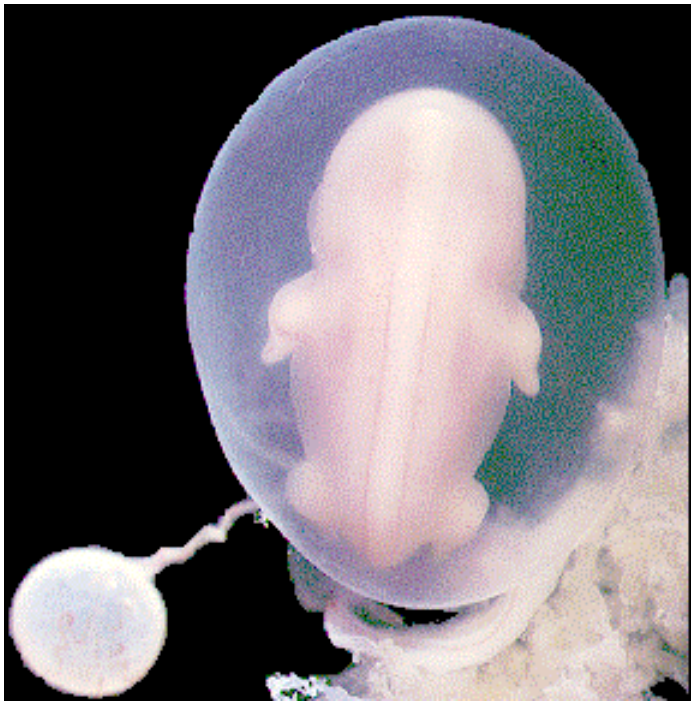


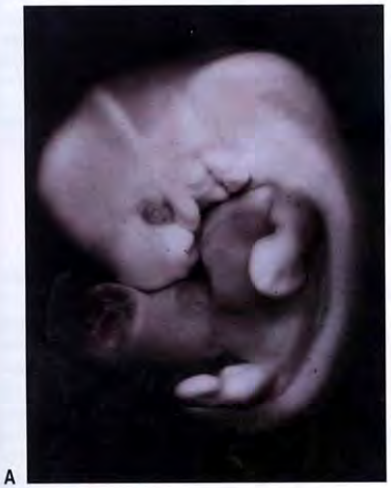
Figure 6 - 10. *A*, Lateral view of an embryo at Carnegie stage 14, about 32 days. The second pharyngeal arch has overgrown the third arch, forming a depression known as the cervical sinus. The mesonephric ridge indicates the site of the mesonephric kidney, an interim kidney (see Chapter 14). *B*, Drawing indicating the structures shown in *A*. The upper limb buds are paddle-shaped, whereas the lower limb buds are flipperlike. (*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.)

32 days
(5 weeks)

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



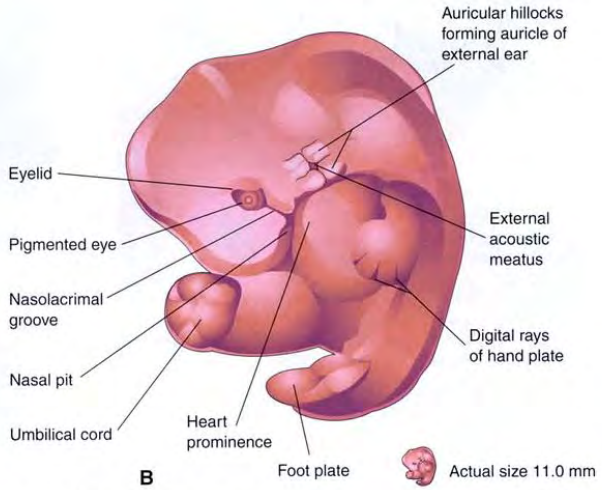


Figure 6 - 11. *A*, Lateral view of an embryo at Carnegie stage 17, about 42 days. Digital rays are visible in the hand plate, indicating the future site of the digits (fingers). *B*, Drawing indicating the structures shown in *A*. The eye, auricular hillocks, and external acoustic meatus are now clearly discernible.

42 days
(6 weeks)

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)

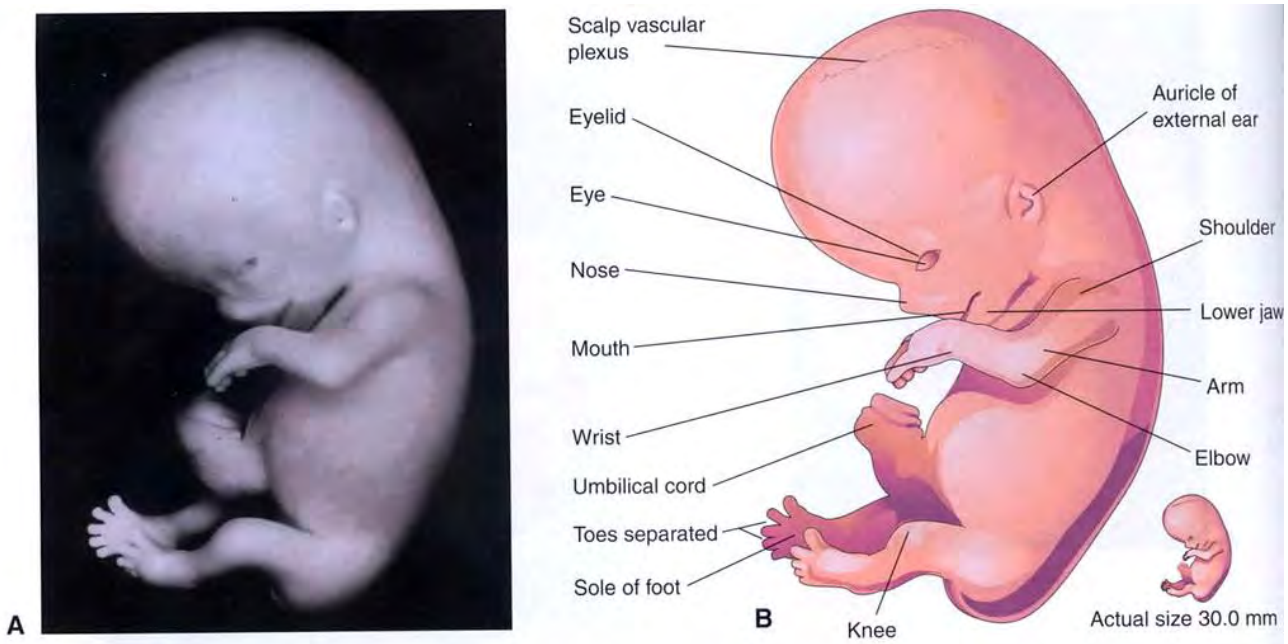


Figure 6 – 12. *A*, Lateral view of an embryo at Carnegie stage 23, about 56 days. The embryo now has a distinctly human appearance. *B*, Drawing indicating the structures shown in *A*. The scalp vascular plexus is reduced and the caudal eminence has disappeared. (*A*, From Nishimura H, Semba H, Tanimura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

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..)

Carnegie Stages

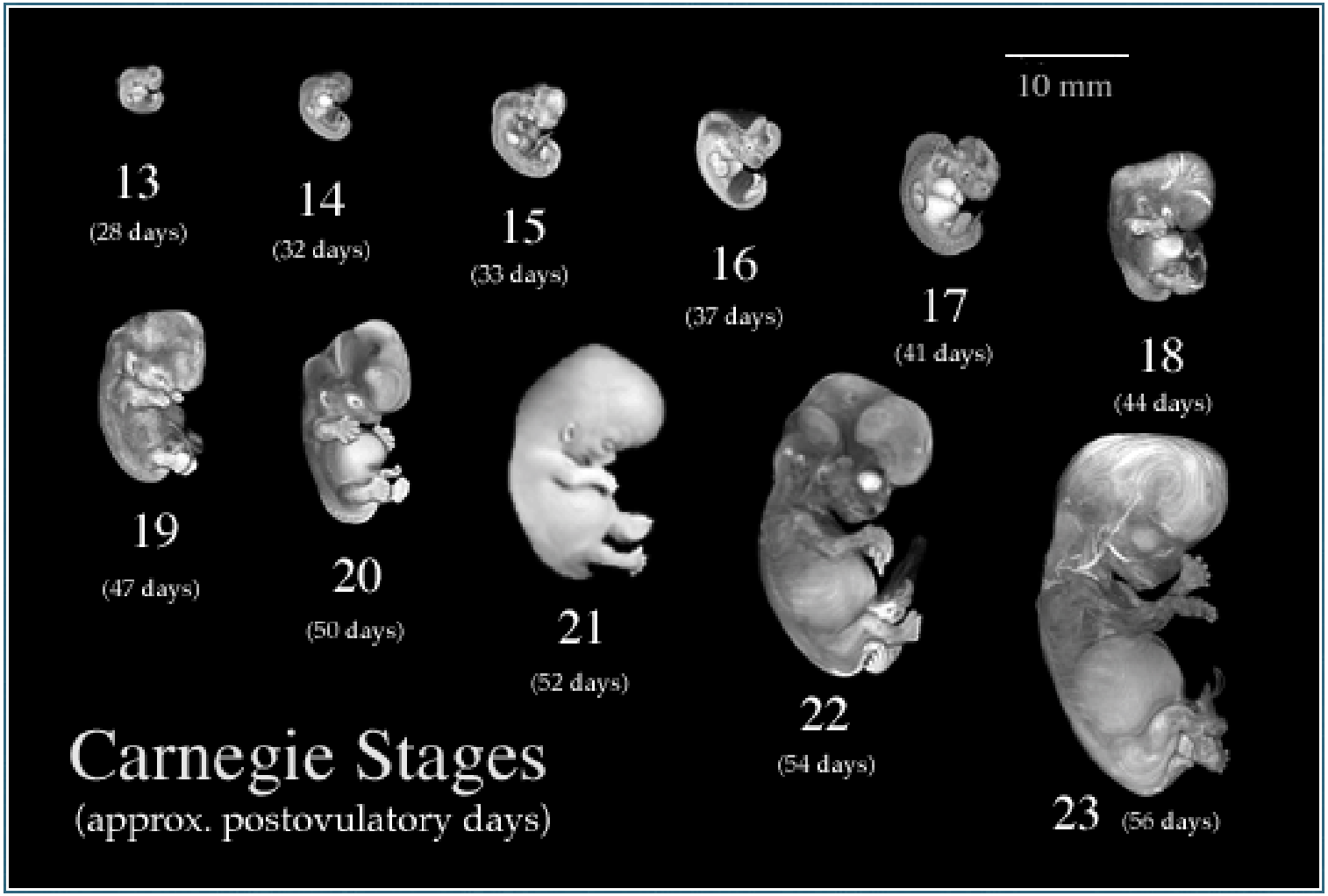


Table 6 – 1. Criteria for Estimating Developmental Stages in Human Embryos

Age (Days)	Figure Reference	Carnegie Stage	No. of Somites	Length (mm)*	Main External Characteristics†
20–21	6–1*A*, 6–2*A*	9	1–3	1.5–3.0	*Flat embryonic disc. Deep neural groove and prominent neural folds.* One to three pairs of somites present. Head fold evident.
22–23	6–5*A* 6–6*A*, *C*	10	4–12	2.0–3.5	*Embryo straight or slightly curved.* Neural tube forming or formed opposite somites, but widely open at rostral and caudal neuropores. First and second pairs of pharyngeal arches visible.
24–25	6–5*C* 6–7*A*	11	13–20	2.5–4.5	*Embryo curved owing to head and tail folds.* Rostral neuropore closing. Otic placodes present. Optic vesicles formed.
26–27	6–5*D* 6–8*A*	12	21–29	3.0–5.0	*Upper limb buds appear.* Rostral neuropore closed. Caudal neuropore closing. Three pairs of pharyngeal arches visible. Heart prominence distinct. Otic pits present.
28–30	6–5*E* 6–9*A*	13	30–35	4.0–6.0	*Embryo has* C*-shaped curve.* Caudal neuropore closed. Upper limb buds are flipperlike. Four pairs of pharyngeal arches visible. Lower limb buds appear. *Otic vesicles present.* Lens placodes distinct. Tail-like caudal eminence present.
31–32	6–10*A*	14	‡	5.0–7.0	*Upper limbs are paddle-shaped.* Lens pits and nasal pits visible. Optic cups present.
33–36		15		7.0–9.0	*Hand plates formed, digital rays present.* Lens vesicles present. Nasal pits prominent. *Lower limbs are paddle-shaped.* Cervical sinuses visible.
37–40		16		8.0–11.0	*Foot plates formed.* Pigment visible in retina. Auricular hillocks developing.
41–43	6–11*A*	17		11.0–14.0	*Digital rays clearly visible in hand plates.* Auricular hillocks outline future auricle of external ear. Trunk beginning to straighten. Cerebral vesicles prominent.
44–46		18		13.0–17.0	*Digital rays clearly evident in foot plates.* Elbow region visible. Eyelids forming. Notches between the digital rays in the hands. Nipples visible.
47–48		19		16.0–18.0	*Limbs extend ventrally.* Trunk elongating and straightening. Midgut herniation prominent.
49–51		20		18.0–22.0	*Upper limbs longer and bent at elbows. Fingers distinct but webbed.* Notches between the digital rays in the feet. Scalp vascular plexus appears.
52–53		21		22.0–24.0	*Hands and feet approach each other. Fingers are free and longer. Toes distinct but webbed.* Stubby tail present.
54–55		22		23.0–28.0	*Toes free and longer.* Eyelids and auricles of external ears more developed.
56	6–12	23		27.0–31.0	*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.*

*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.

† Based mainly on O'Rahilly R, Müller F: *Developmental Stages in Human Embryos.* Washington, Carnegie Institute of Washington, 1987.

‡At this and subsequent stages, the number of somites is difficult to determine and so is not a useful criterion. Refer to Moore KL, Persaud TVN, Shiota K: *Color Atlas of Clinical Embryology,* 2nd ed. Philadelphia, WB Saunders, 2000 for more color photographs of embryos.

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

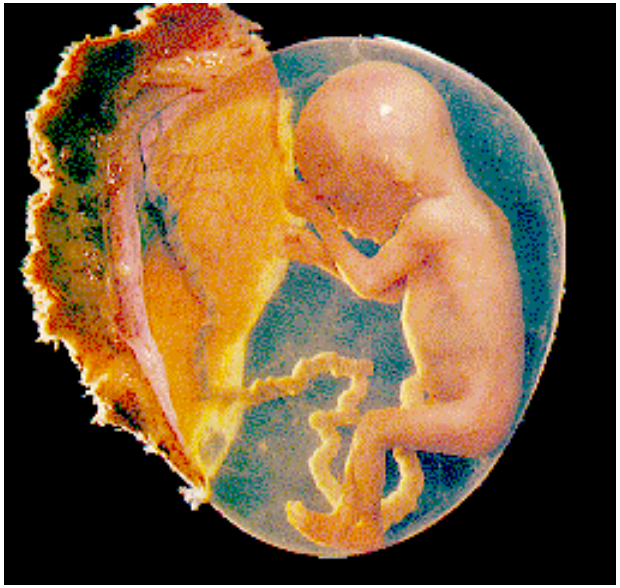
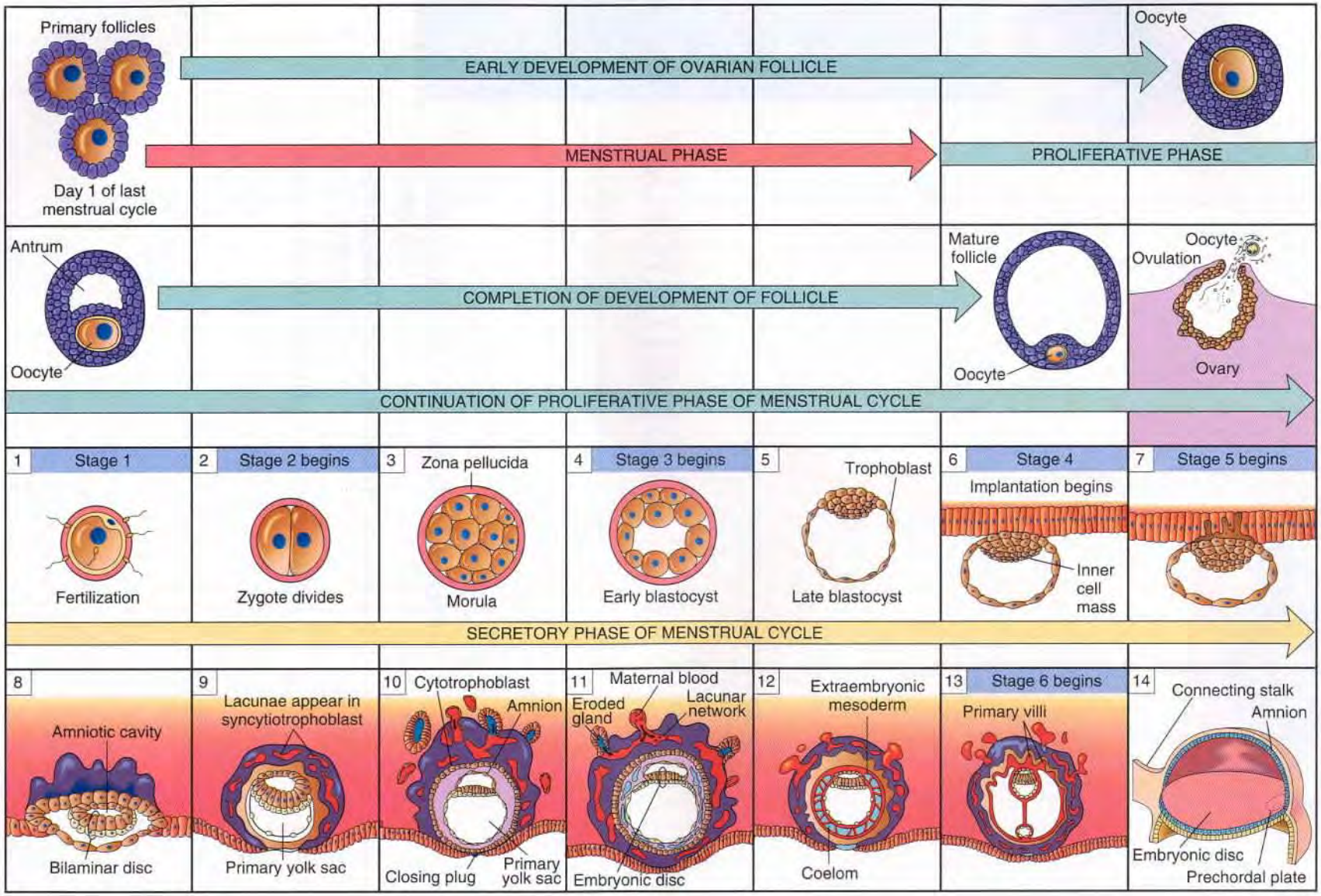


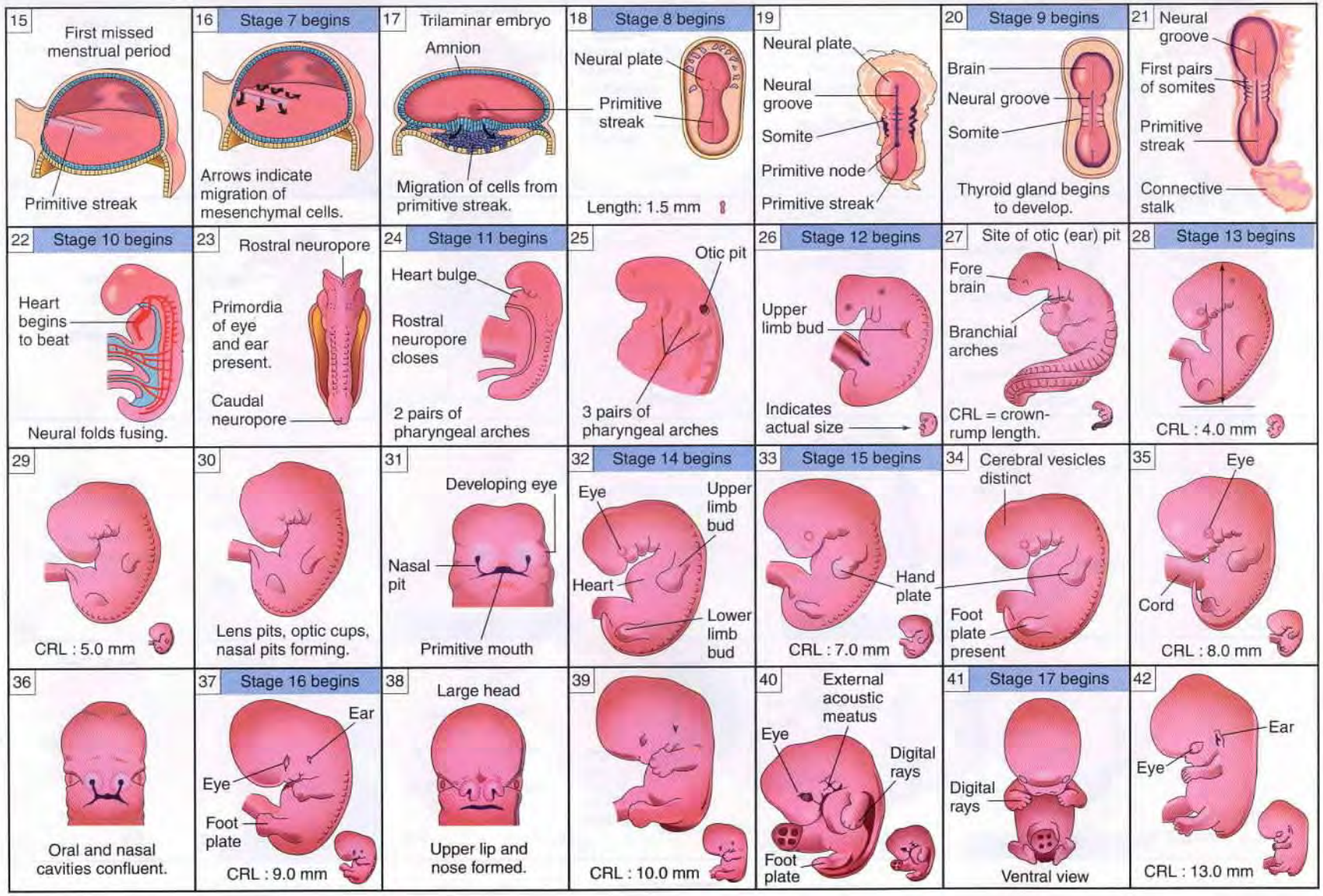
Table 7 - 1. Criteria for Estimating Fertilization Age During the Fetal Period

Age (weeks)	CR Length (mm)*	Foot Length (mm)*	Fetal Weight (gm)†	Main External Characteristics
Previable Fetuses				
9	50	7	8	*Eyelids closing or closed.* Head round. External genitalia still not distinguishable as male or female. Intestines in umbilical cord.
10	61	9	14	*Intestine in abdomen.* Early fingernail development.
12	87	14	45	*Sex distinguishable externally.* Well-defined neck.
14	120	20	110	*Head erect.* Lower limbs well developed. Early toenail development.
16	140	27	200	*Ears stand out from head.*
18	160	33	320	*Vernix caseosa covers skin.* Quickening (signs of life felt by mothers).
20	190	39	460	*Head and body hair (lanugo) visible.*
Viable Fetuses‡				
22	210	45	630	*Skin wrinkled* and red.
24	230	50	820	*Fingernails present.* Lean body.
26	250	55	1000	*Eyes partially open.* Eyelashes present.
28	270	59	1300	*Eyes open.* Good head of hair. Skin slightly wrinkled.
30	280	63	1700	*Toenails present.* Body filling out. Testes descending.
32	300	68	2100	*Fingernails extend to fingertips.* Skin smooth.
36	340	79	2900	*Body usually plump.* Lanugo almost absent. Toenails extend to toe tips. Flexed limbs; firm grasp.
38	360	83	3400	*Prominent chest*; breasts protrude. Testes in scrotum or palpable in inguinal canals. Fingernails extend beyond fingertips.

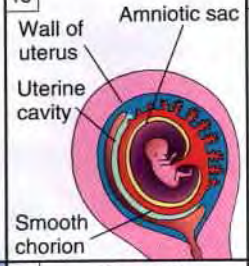
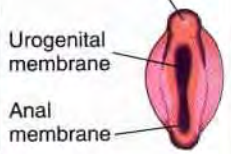
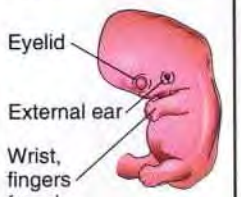
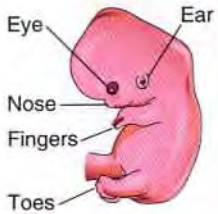
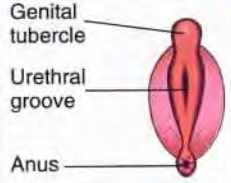
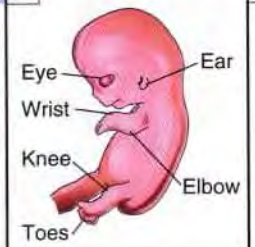
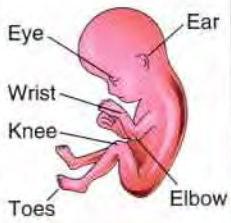
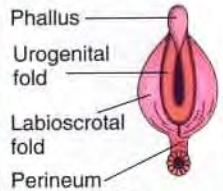
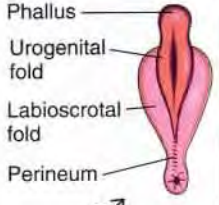
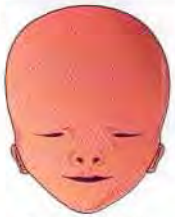
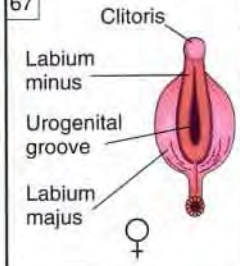
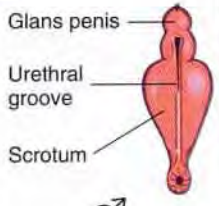
*These measurements are averages and so may not apply to specific cases; dimensional variations increase with age.
†These weights refer to fetuses that have been fixed for about 2 weeks in 10% formalin. Fresh specimens usually weigh about 5% less.
‡There is no sharp limit of development, age, or weight 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.

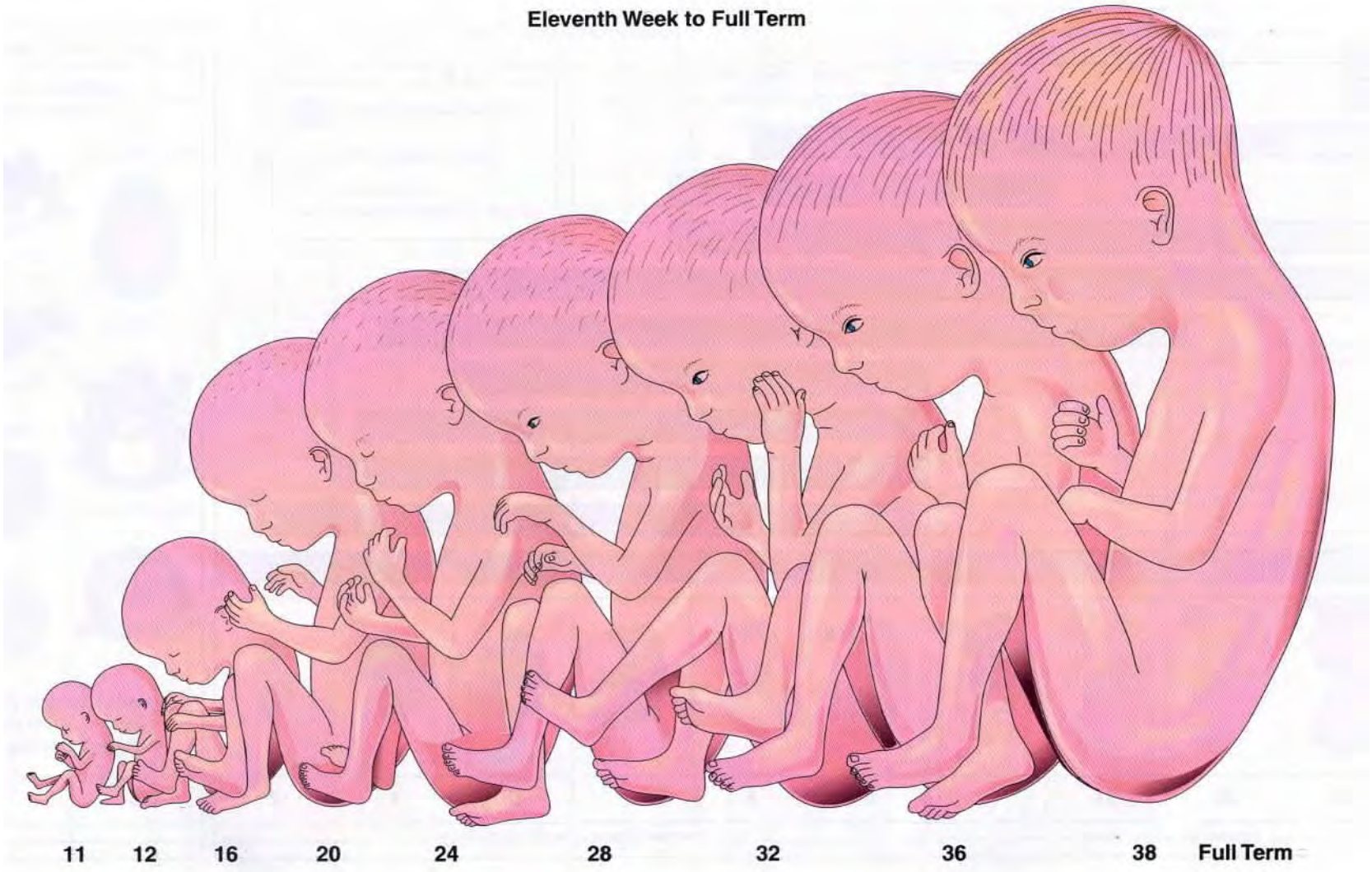
TIMETABLE OF HUMAN PRENATAL DEVELOPMENT
1 TO 6 WEEKS




3
4
5
6
15
First missed menstrual period
Primitive streak
16
Stage 7 begins
Arrows indicate migration of mesenchymal cells.
17
Trilaminar embryo
Amnion
Migration of cells from primitive streak.
18
Stage 8 begins
Neural plate
Primitive streak
Length: 1.5 mm
19
Neural plate
Neural groove
Somite
Primitive node
Primitive streak
20
Stage 9 begins
Brain
Neural groove
Somite
Thyroid gland begins to develop.
21
Neural groove
First pairs of somites
Primitive streak
Connective stalk
22
Stage 10 begins
Heart begins to beat
Neural folds fusing.
23
Rostral neuropore
Primordia of eye and ear present.
Caudal neuropore
24
Stage 11 begins
Heart bulge
Rostral neuropore closes
2 pairs of pharyngeal arches
25
Otic pit
3 pairs of pharyngeal arches
26
Stage 12 begins
Upper limb bud
Indicates actual size
27
Site of otic (ear) pit
Fore brain
Branchial arches
CRL = crown-rump length.
28
Stage 13 begins
CRL : 4.0 mm
29
CRL : 5.0 mm
30
Lens pits, optic cups, nasal pits forming.
31
Developing eye
Nasal pit
Primitive mouth
32
Stage 14 begins
Eye
Upper limb bud
Heart
Lower limb bud
33
Stage 15 begins
Hand plate
CRL : 7.0 mm
34
Cerebral vesicles distinct
Foot plate present
35
Eye
Cord
CRL : 8.0 mm
36
Oral and nasal cavities confluent.
37
Stage 16 begins
Ear
Eye
Foot plate
CRL : 9.0 mm
38
Large head
Upper lip and nose formed.
39
CRL : 10.0 mm
40
External acoustic meatus
Eye
Digital rays
Foot plate
41
Stage 17 begins
Digital rays
Ventral view
42
Ear
Eye
CRL : 13.0 mm

TIMETABLE OF HUMAN PRENATAL DEVELOPMENT
7 to 38 weeks

AGE (weeks)							
7	43 Actual size  CRL: 16 mm	44 Stage 18 begins 	45 Head large but chin poorly formed. Grooves between digital rays indicate fingers.	46 	47 Genital tubercle  ♀ or ♂	48 Stage 19 begins 	49 Actual size  CRL: 18 mm
8	50 Upper limbs longer and bent at elbows. Fingers distinct but webbed.	51 	52 Stage 21 begins  Large forehead	53 Stage 21 External genitalia still in sexless state but have begun to differentiate.	54 Stage 22 begins  ♀ or ♂	55 	56 Stage 23  CRL: 30 mm
9	57 Beginning of fetal period.	58 	59 Placenta 	60 Genitalia  ♀	61  CRL: 45 mm	62 Genitalia  ♂	63  CRL: 50 mm
10	64 Face has human profile. Note growth of chin compared to day 44.	65 	66  Ears still lower than normal.	67  ♀	68 Genitalia have ♀ or ♂ characteristics but still not fully formed.	69  ♂	70  CRL: 61 mm


Eleventh Week to Full Term
11
12
16
20
24
28
32
36
38
Full Term

Sexual Differentiation

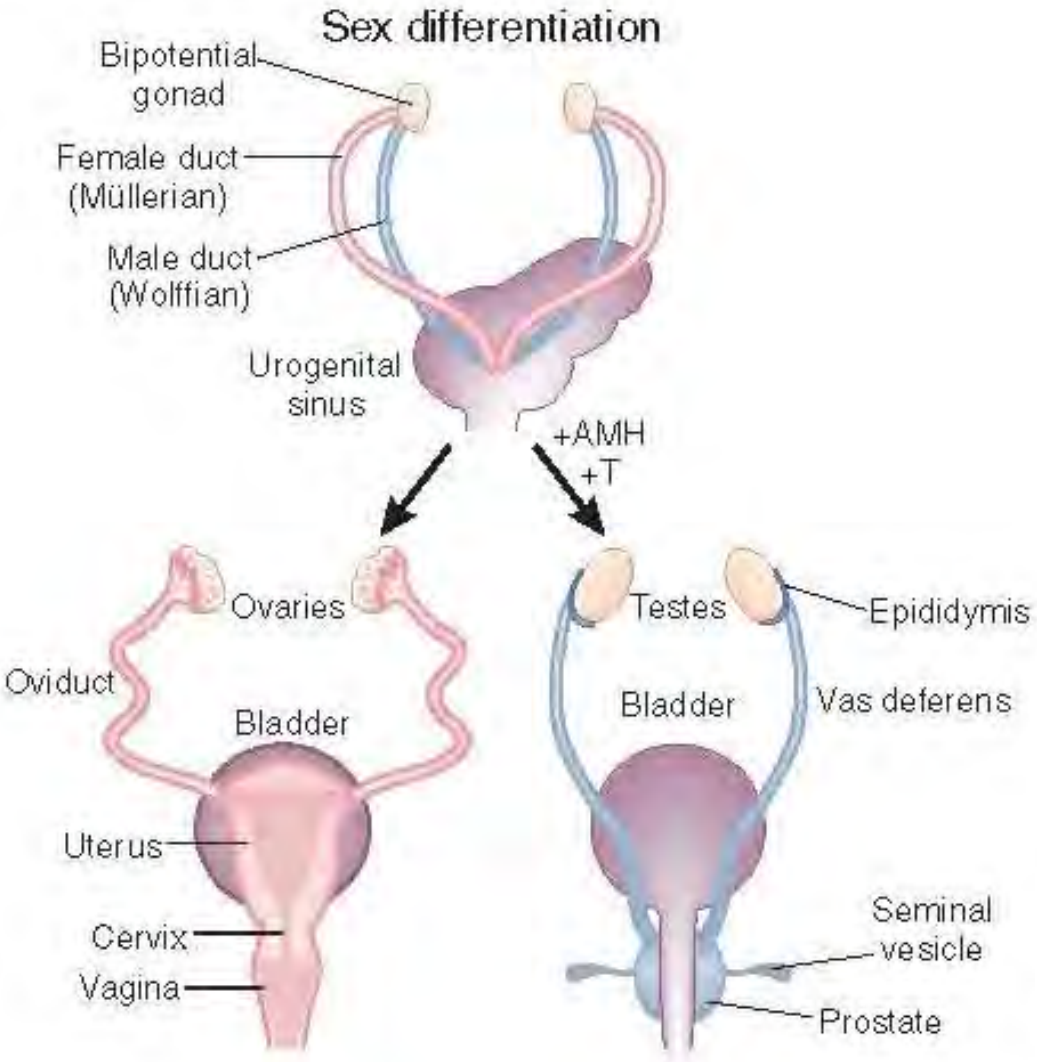
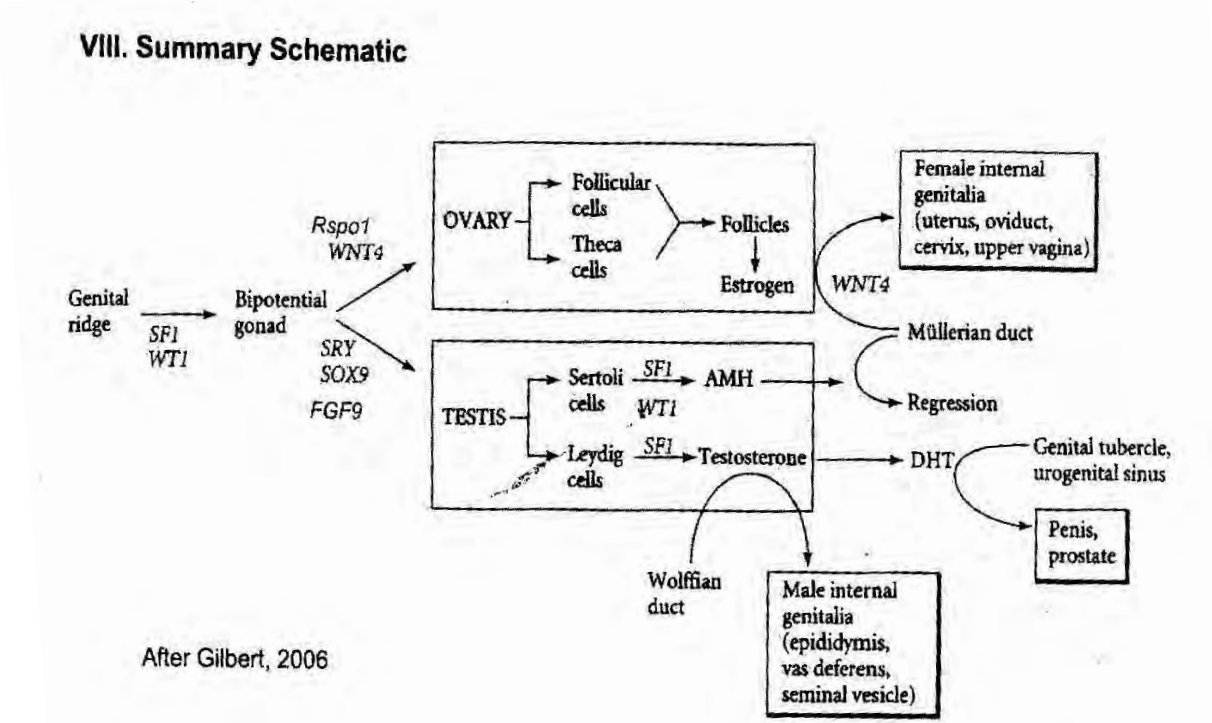


Figure 2 Sex differentiation in humans. The presence of a fetal testis that secretes both testosterone (T) and anti-Mullerian hormone (AMH) results in the induction of the Wolffian duct into the future vas deferens, epididymis, and seminal vesicles and the regression of the Müllerian duct, respectively[6]. 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 (oviducts), 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 *in vivo*, millions of spermatozoa leave the seminiferous tubules of the testis to mature in the epididymis before traveling through the vas deferens and urethra to enter the female, where they transverse the vagina, cervix and uterus before typically encountering a single oocyte in one of the fallopian tubes. Surgical contraception involves closing this pathway by removing segments of the two vas deferens (that is, vasectomy) in a man or both fallopian tubes (that is, tubal ligation) 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 Rspo1

Females

Rspo1 encodes a secreted protein to amplify Wnt4

Wnt4 up regulation represses SOX9 and FGF9

Loss of Wnt4 or Rspo1 causes female to male differentiation

Physiological Actions of Androgen

In utero:

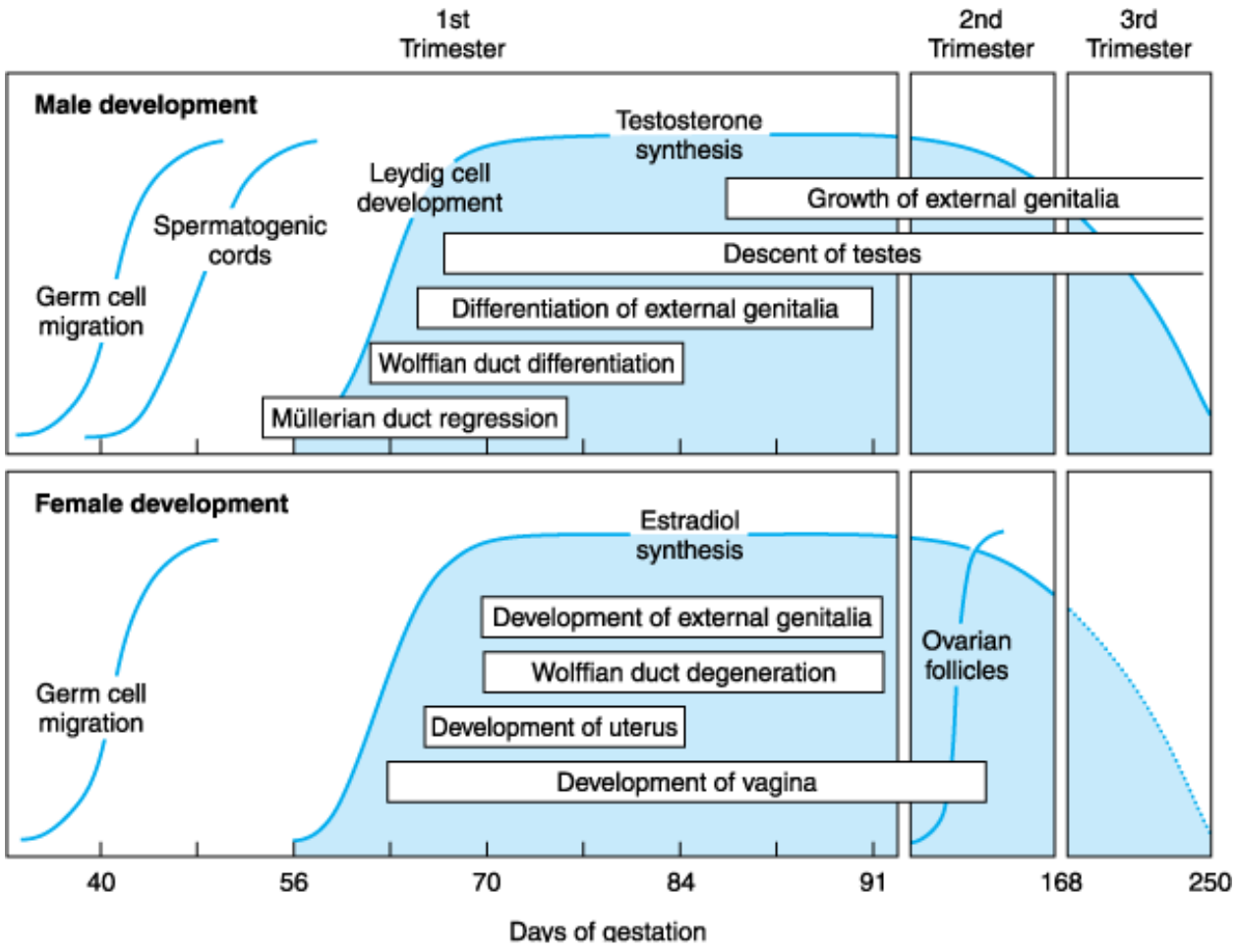
External genitalia development
Wolffian Duct development

Adult:

Hair growth, baldness
Phyche (sexual potency)
Bone loss prevention
Maintenance of spermatogenesis

Pubertal:

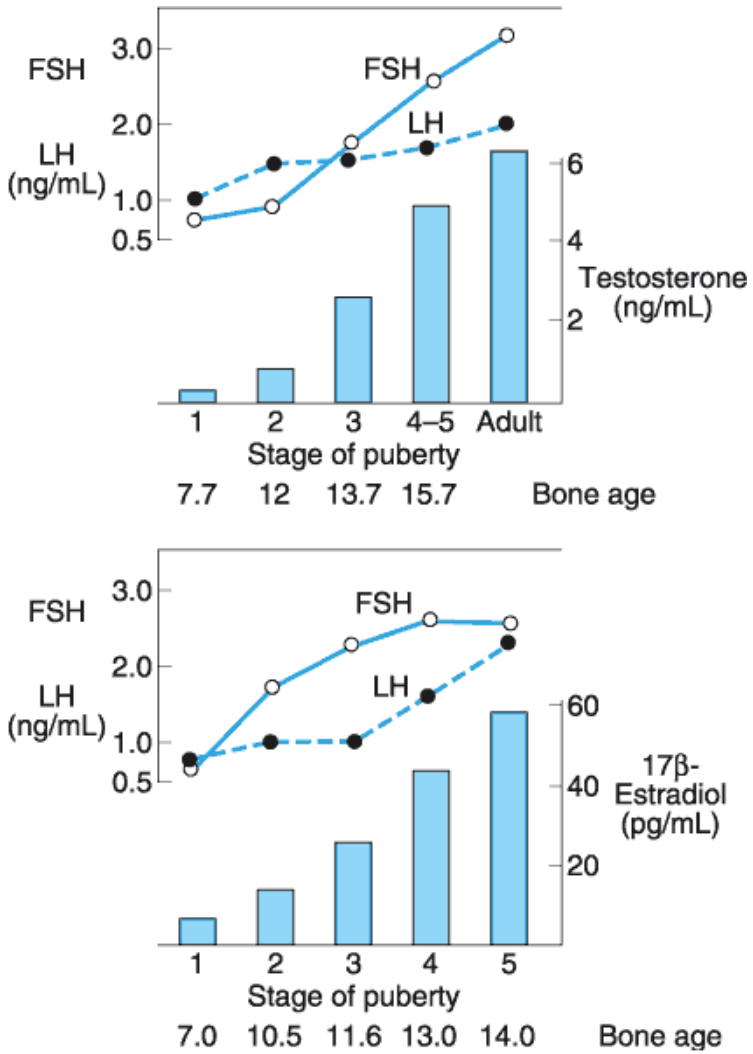
External genitalia
Hair growth
Linear growth
Accessory sex organs
Voice
Pyche (aggressive attitudes, sexual potency)
Muscle mass increase
Initiation of spermatogenesis



Source: McPhee SJ, Ganong WF: *Pathophysiology of Disease: An Introduction to Clinical Medicine*, 5th Edition: http://www.accessmedicine.com

Boys

Girls



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

Source: Ganong WF: *Review of Medical Physiology*, 22nd Edition: http://www.accessmedicine.com

Reproductive Hormone Signaling

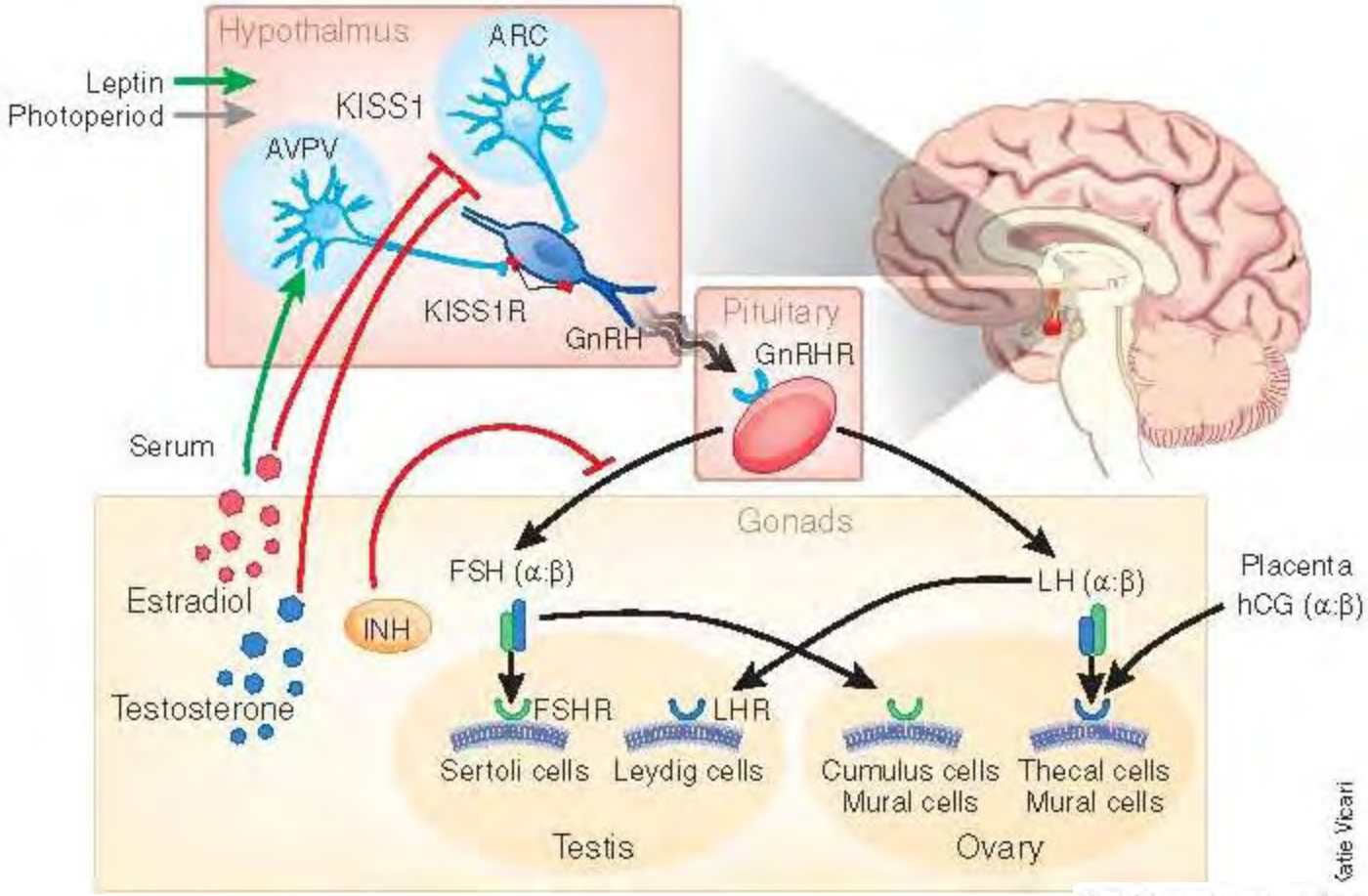


Figure 3 Neuroendocrine control of pituitary and gonadal function. The hypothalamus, which has a number of nuclei and pathways that affect reproductive behavior, secretes a key decapeptide, GnRH, that binds to its receptor, GnRHR, on the gonadotropes and is involved in induction of sexual maturity through 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 (KISSR1) 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

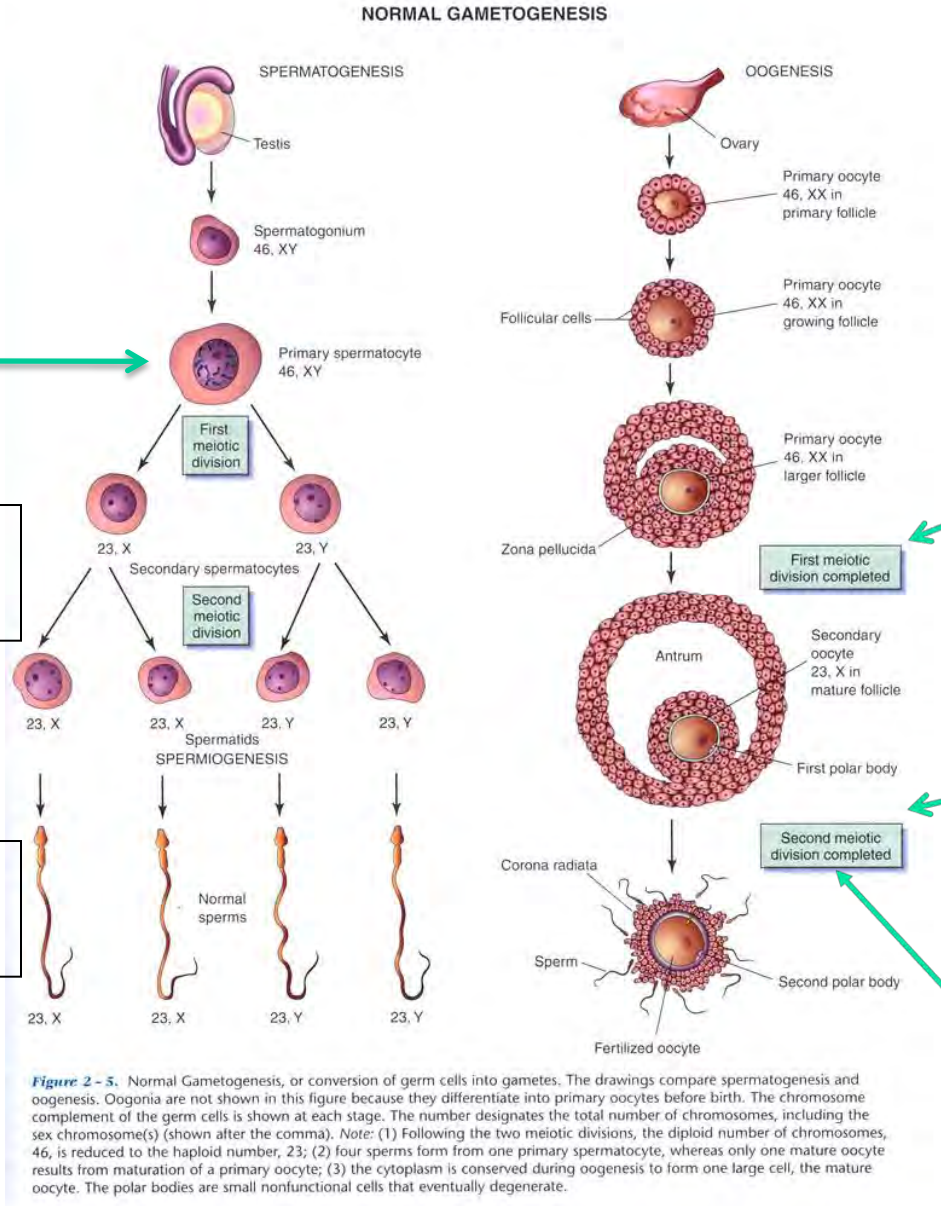


Figure 2 - 5. Normal Gametogenesis, or conversion of germ cells into gametes. The drawings compare spermatogenesis and oogenesis. Oogonia are not shown in this figure because they differentiate into primary oocytes before birth. The chromosome complement of the germ cells is shown at each stage. The 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.

Puberty

4 haploid sperm are formed from 1 primary spermatocyte

Sperm cell content is highly refined and specialized for movement

Prophase of the 1st meiotic division until 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

Cell Divisions During Human Spermatogenesis

12-14 years

PUBERTY

16 days

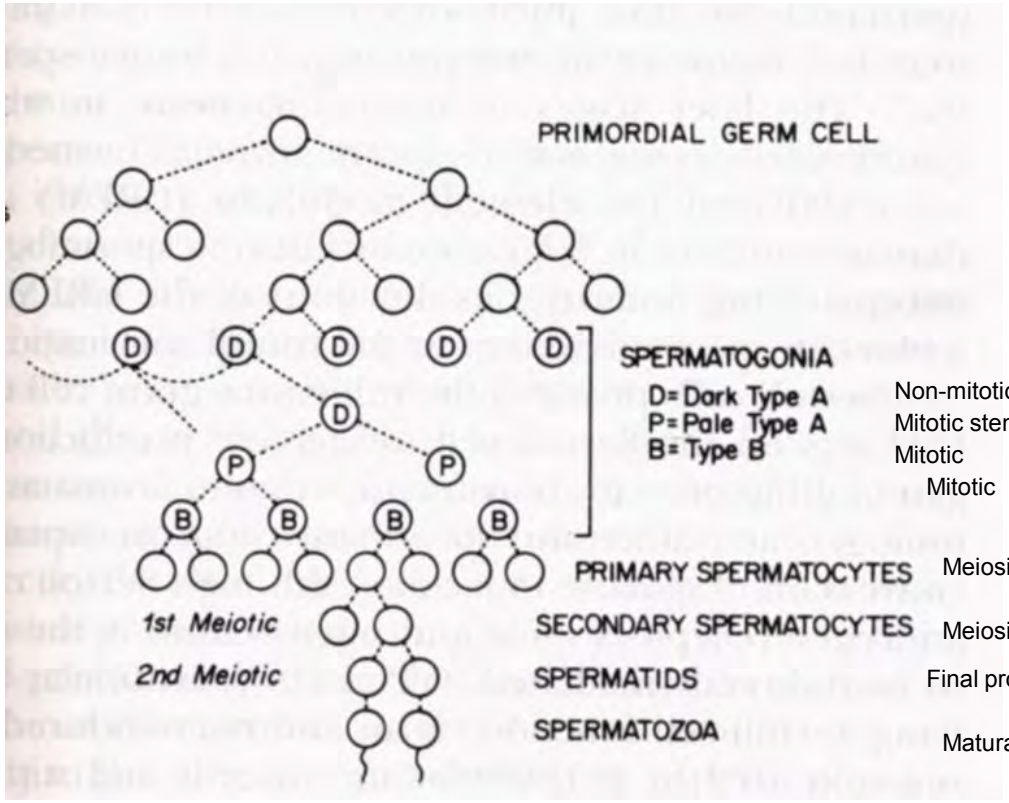
16 days

9 days

9 days

19 days

21 days

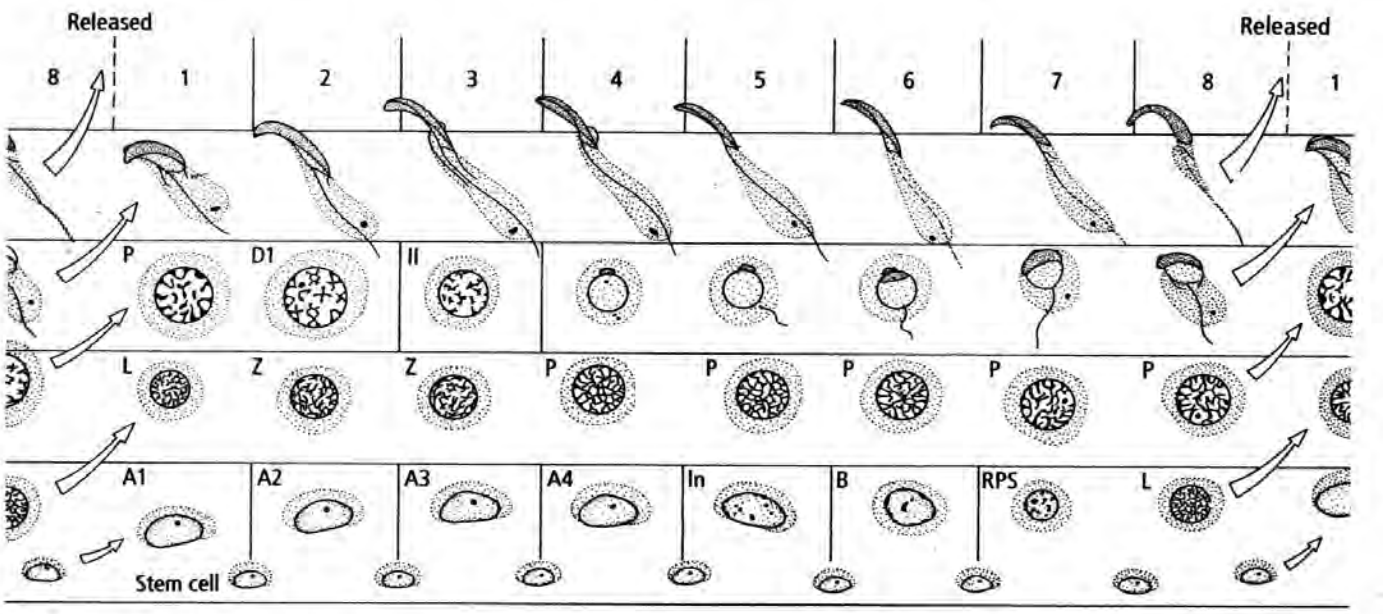


Spermatogenesis: Production of round haploid spermatids from diploid cells

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

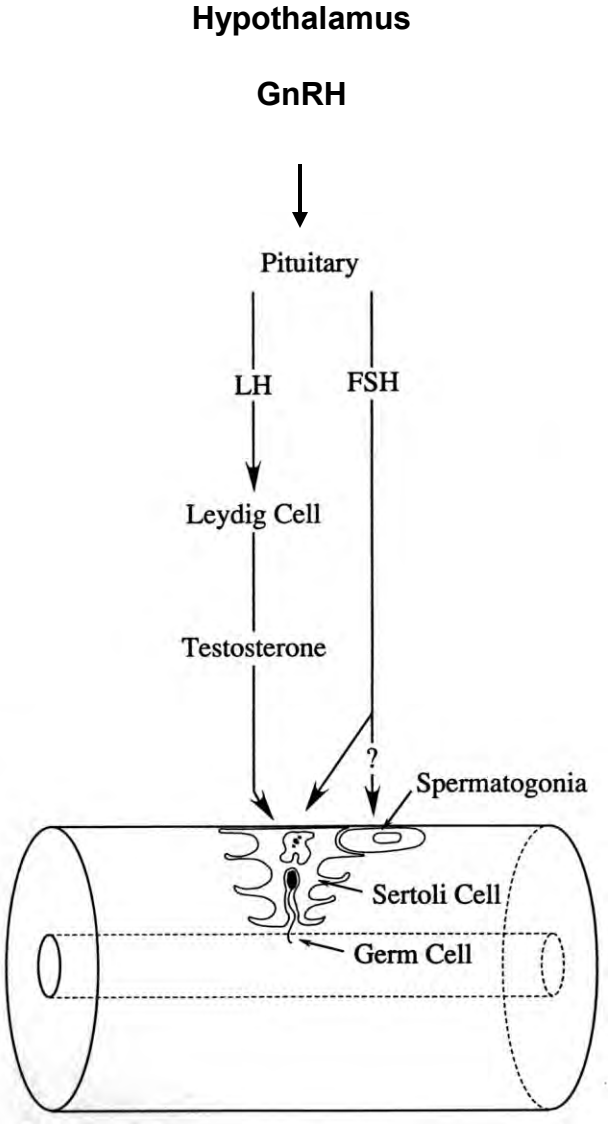
Spermiation: Release of spermatozoan into lumen of seminiferous tubule

Kinetics of Spermatogenesis



Species	Time to complete spermatogenesis	Duration of cycle
Man	70 day	16 days
Rat	48 days	12 days

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 Critera by WHO

>20 million sperm/mL
75% viability
50% forward mobility
At least 30% normal shape and form

Is Fertility Declining?

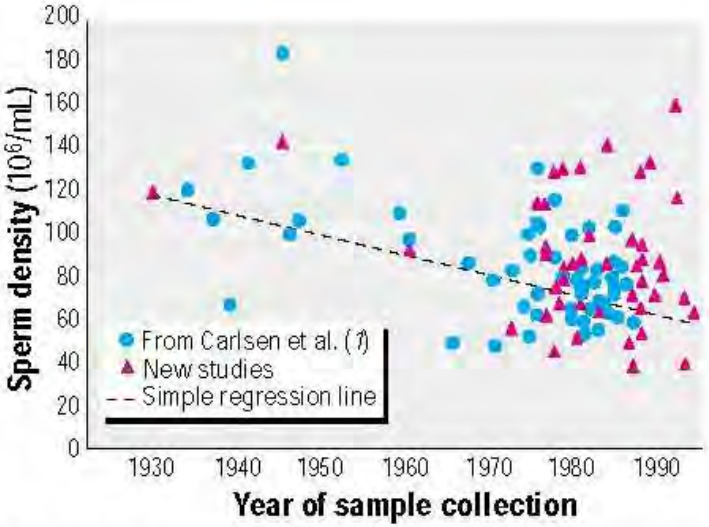


Figure 1. Mean sperm density in 101 studies published 1934–1996 and simple regression line.

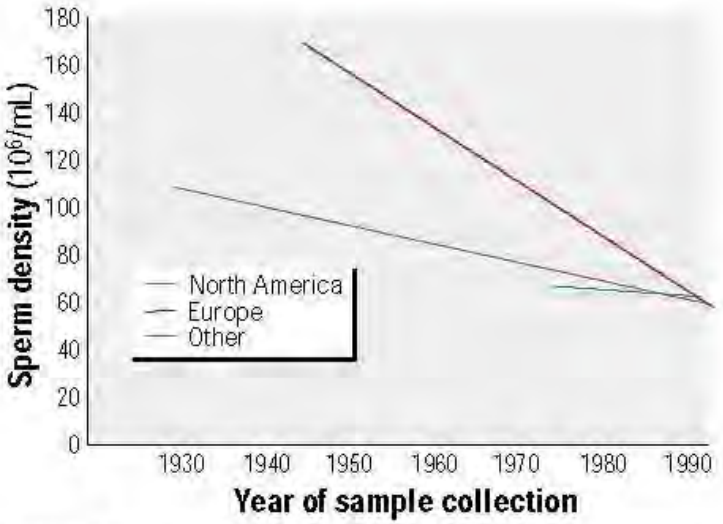


Figure 2. Interactive regression model for mean sperm density by year and geographic region, after controlling for proven fertility, abstinence time, age, specimen collection method, method of counting sperm, whether the study was included by Carlsen et al. (1), and interaction of region and study year.

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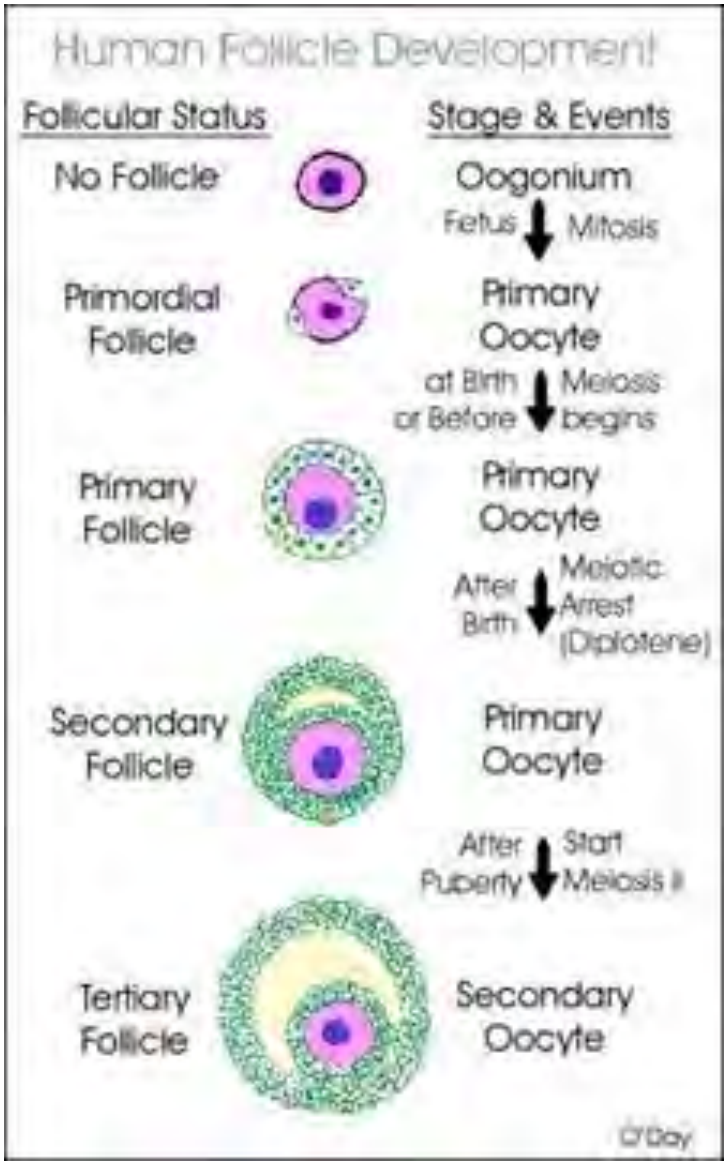
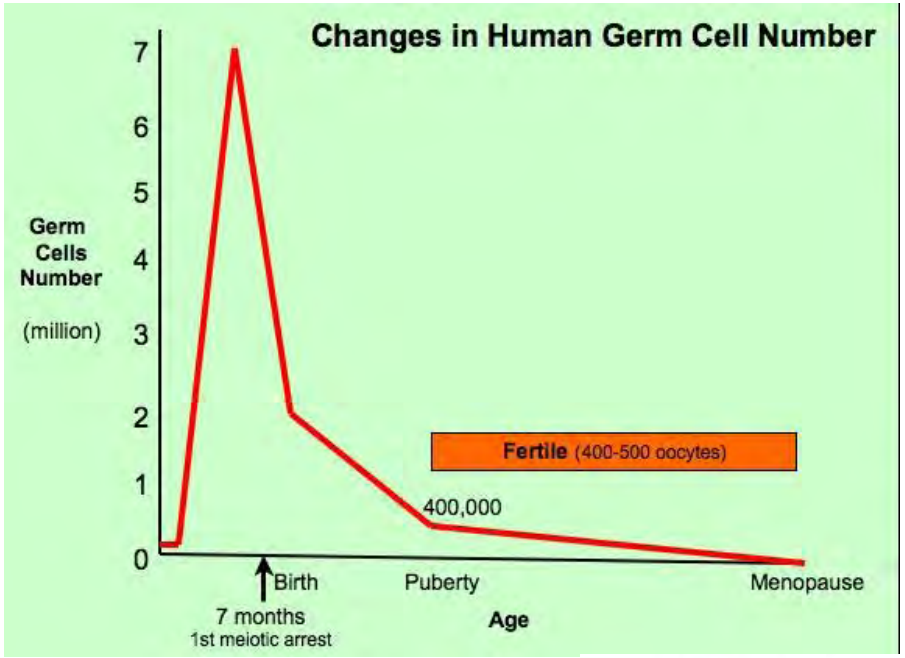
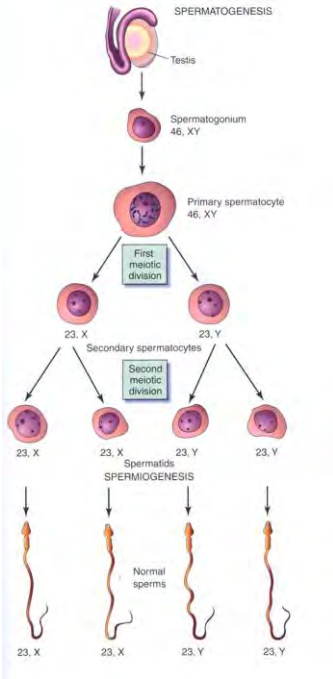
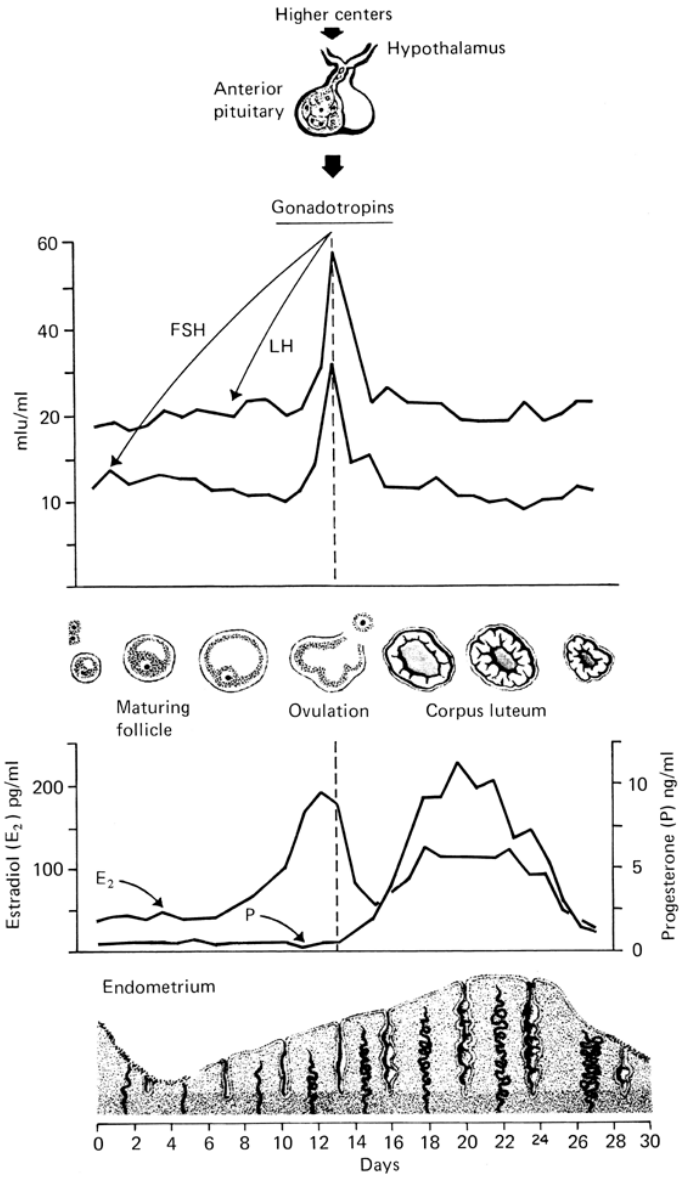
Swan, 2000


Human Follicle Development
Follicular Status
Stage & Events
No Follicle
Oogonium
Fetus
Mitosis
Primordial Follicle
Primary Oocyte
at Birth or Before
Meiosis begins
Primary Follicle
Primary Oocyte
After Birth
Meiotic Arrest (Diplotene)
Secondary Follicle
Primary Oocyte
After Puberty
Start Meiosis I
Tertiary Follicle
Secondary Oocyte
©Bay


Changes in Human Germ Cell Number
Germ Cells Number (million)
7
6
5
4
3
2
1
0
Fertile (400-500 oocytes)
400,000
Birth
7 months
1st meiotic arrest
Puberty
Menopause
Age


SPERMATOGENESIS
Testis
Spermatogonium
46, XY
Primary spermatocyte
46, XY
First meiotic division
23, X
23, Y
Secondary spermatocytes
Second meiotic division
23, X
23, X
23, Y
23, Y
Spermatids
SPERMIOGENESIS
Normal sperms
23, X
23, X
23, Y
23, Y

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 in utero 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 pathophysiological) 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 sys
- 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

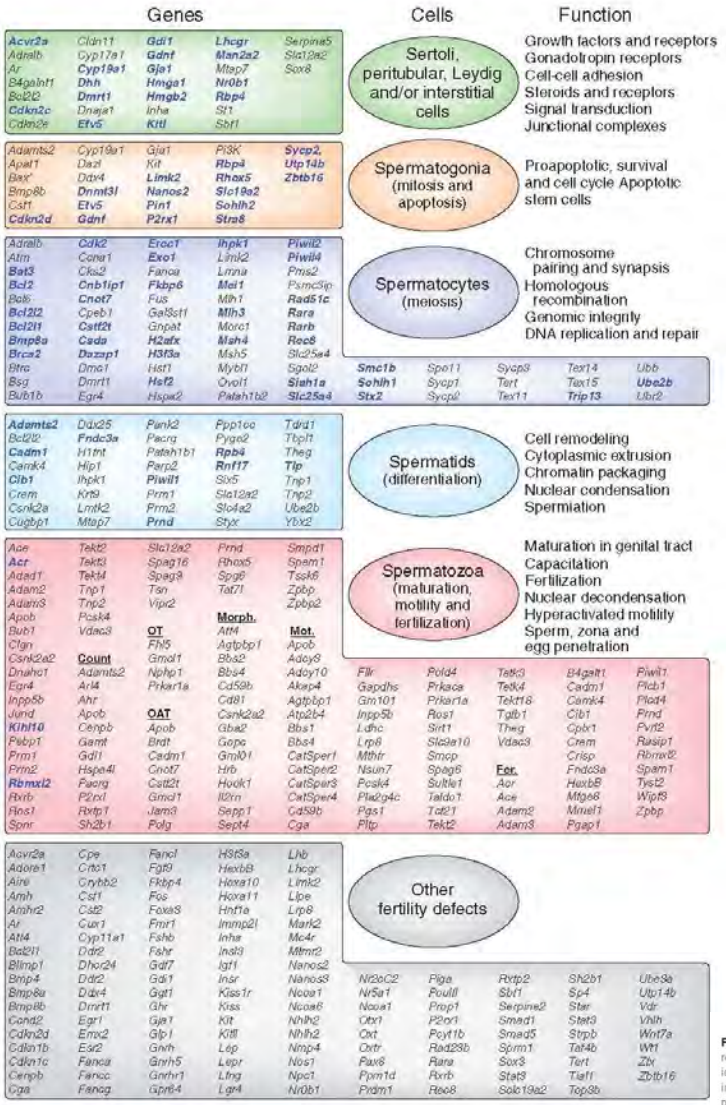


Figure 5 Mouse models of male reproductive defects provide new insights into the causes of male infertility. This figure, updated from Matzuk and Lamb[1], reveals the genes known today that influence testicular and sperm function in the mouse. The genes highlighted in ref. 1 are in black, with new genes identified since then and others not shown previously in blue. Communication between each cellular compartment within the testis (seminiferous tubules, interstitial cells and blood vessels, as well as between individual cell types (germ cells, Sertoli cells, peritubular myoid cells, Leydig cells and macrophages)) play essential parts in mitosis, meiosis and differentiated 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; oligoteratozoospermia; OAT, oligoasthenoteratozoospermia; Morph., morphology defects; Mot., motility defects.

Potential Genetic Targets for Female Fertility

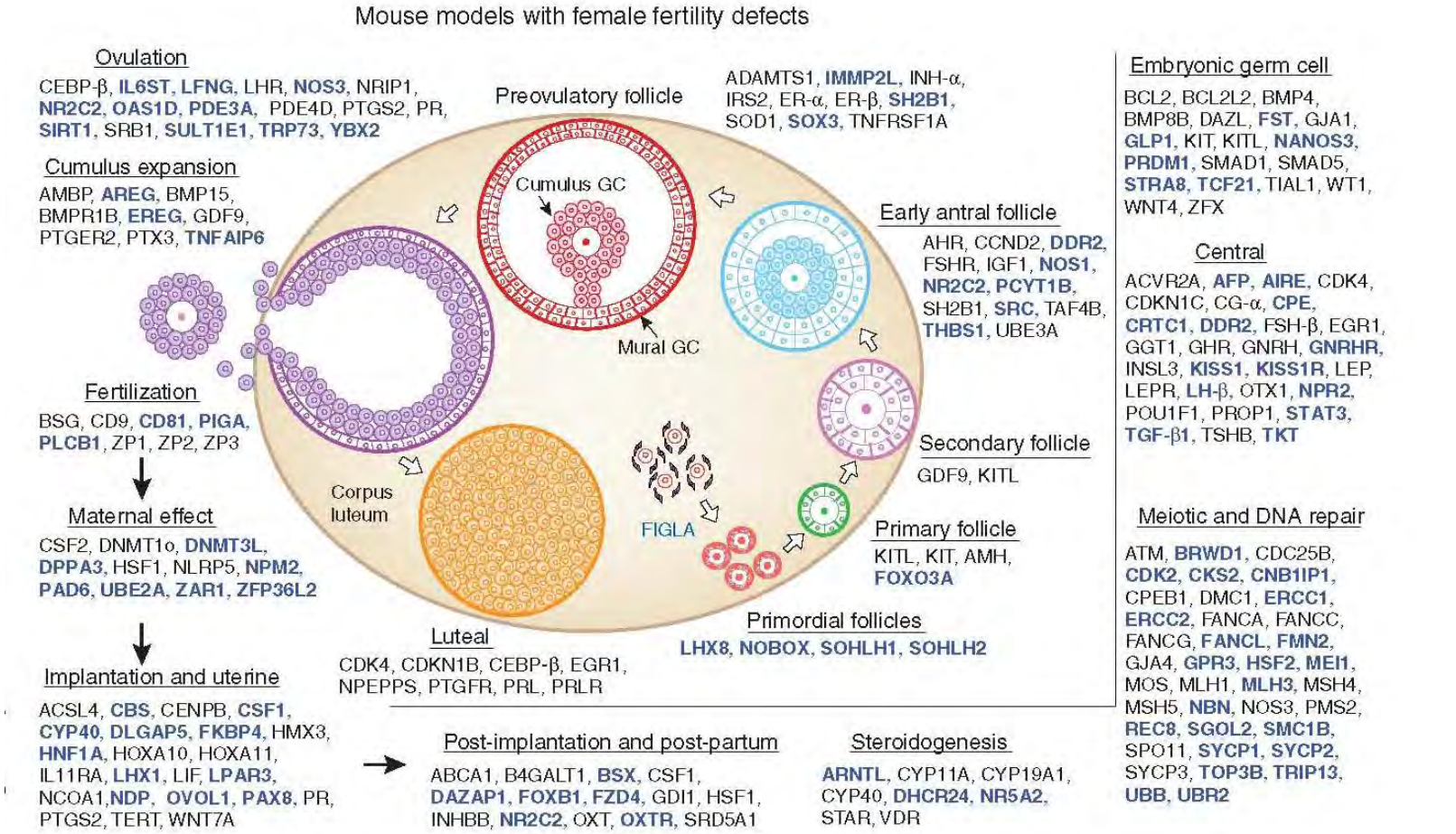


Figure 4 Genetic dissection of female fertility pathways in mice. An update of the figure from our review six years ago[1] shows a marked increase in the gene

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

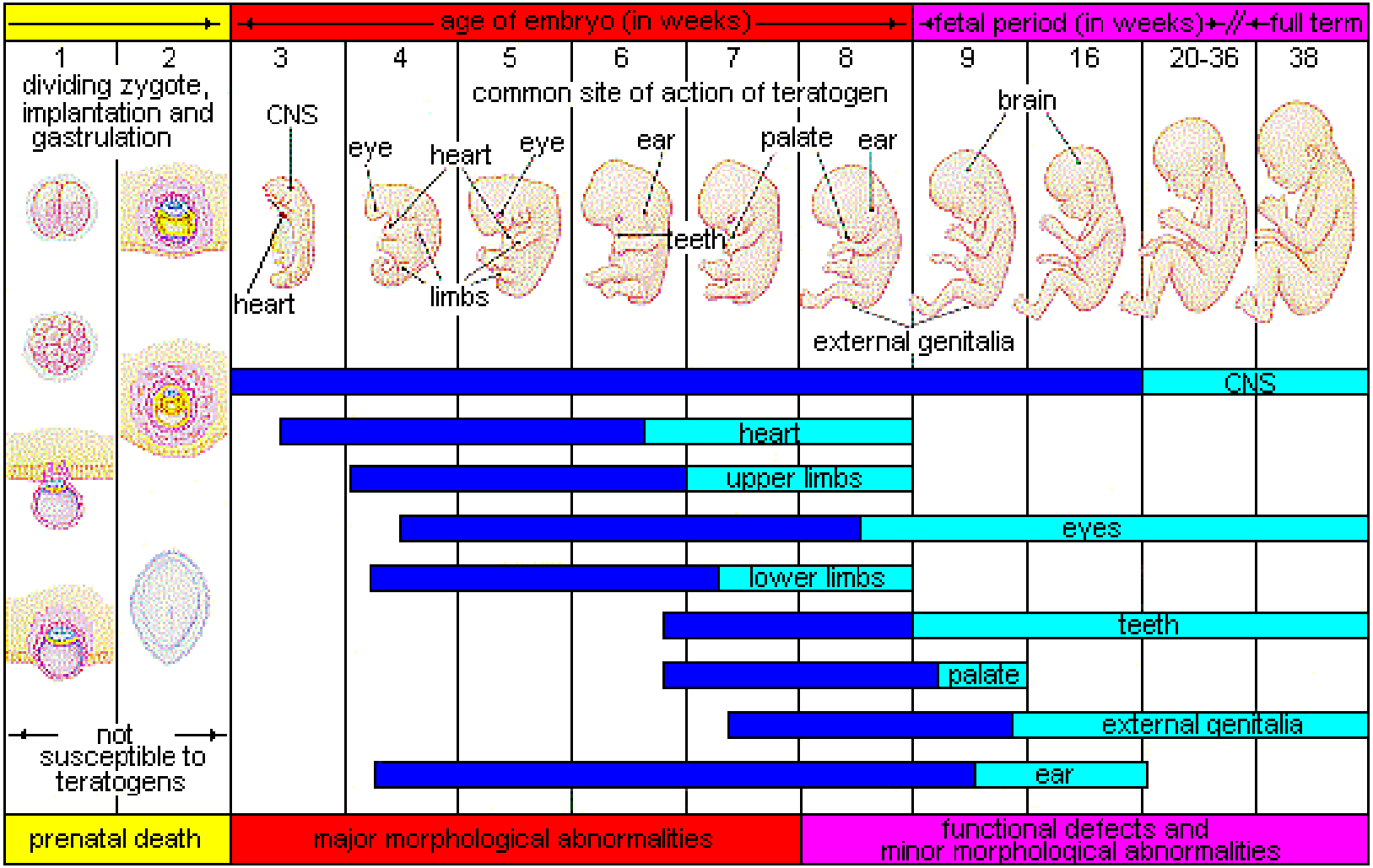
Mechanisms Developmental Toxicology

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

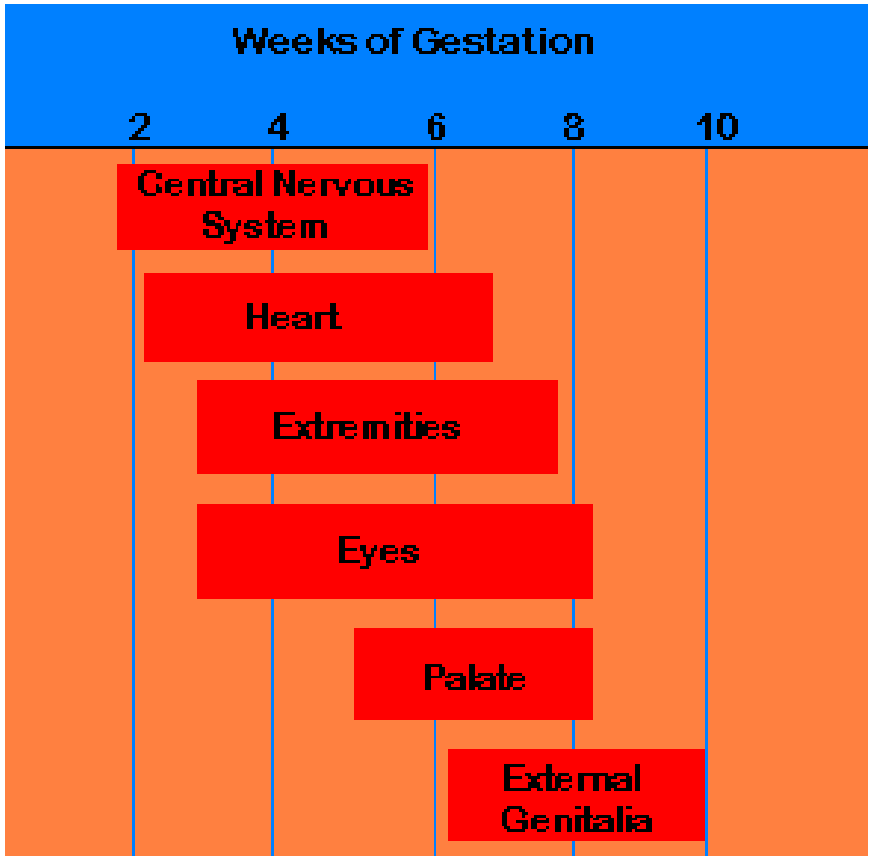
Outcomes of these Mechanisms

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



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

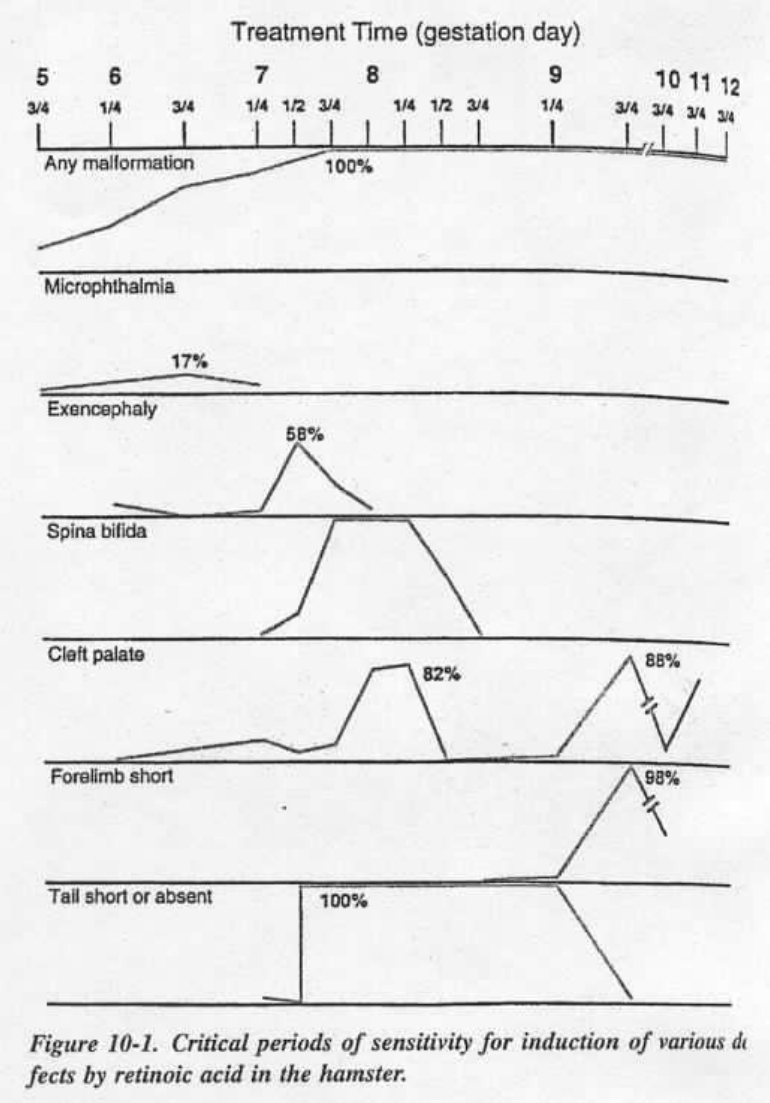


Figure 10-1. Critical periods of sensitivity for induction of various de fects by retinoic acid in the hamster.

Dose used was maternal LD_{50}

Total incidence of malformations lower prior to organogenesis but increases to 100% by gestational day $7_{3/4}$.

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

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

[a]Total frequency 2.2%.
[b]Parentheses indicate therapeutic abortions.
Reprinted with permission from Nelson K, Holmes L. Malformation due to presumed spontaneous mutations in newborn infants. N Engl J Med *1989;320:19.*

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

Drugs
Aminopterin/methotrexate (Amethopterin)
Androgenic hormones
Angiotensin-converting enzyme inhibitors
Busulfan
Carbamazepine
Chlorobiphenyls
Cocaine
Cyclophosphamide
Diethylstilbestrol
Etretinate
Heroin/methadone
Iodide
Isotretinoin (13-*cis*-retinoic acid)
Lithium
Phenobarbital
Phenytoin
Propylthiouracil
Prostaglandin
Tetracycline
Thalidomide
Trimethadione/paramethadione
Valproic acid
Warfarin
Heavy metals
Lead
Mercury
Radiation
Cancer therapy

Maternal conditions
Alcohol use
Insulin-dependent diabetes mellitus
Iodide deficiency
Maternal phenylketonuria
Myasthenia gravis
Smoking cigarettes or marijuana
Systemic lupus erythematosus
Vitamin A deficiency
Intrauterine infections
Cytomegalovirus
Herpes simplex
Parvovirus
Rubella
Syphilis
Toxoplasmosis
Varicella
Venezuelan equine encephalitis virus
Other exposures
Chorionic villus sampling
Dilation and curettage
Gasoline fumes (excessive)
Heat
Hypoxia
Methyl isocyanate
Methylene blue
Toluene (excessive)
Trauma, blunt

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 (insulinlike 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, *s*yphilis, *t*oxoplasmosis, *r*ubella, *c*ytomegalovirus, *h*erpesvirus.

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

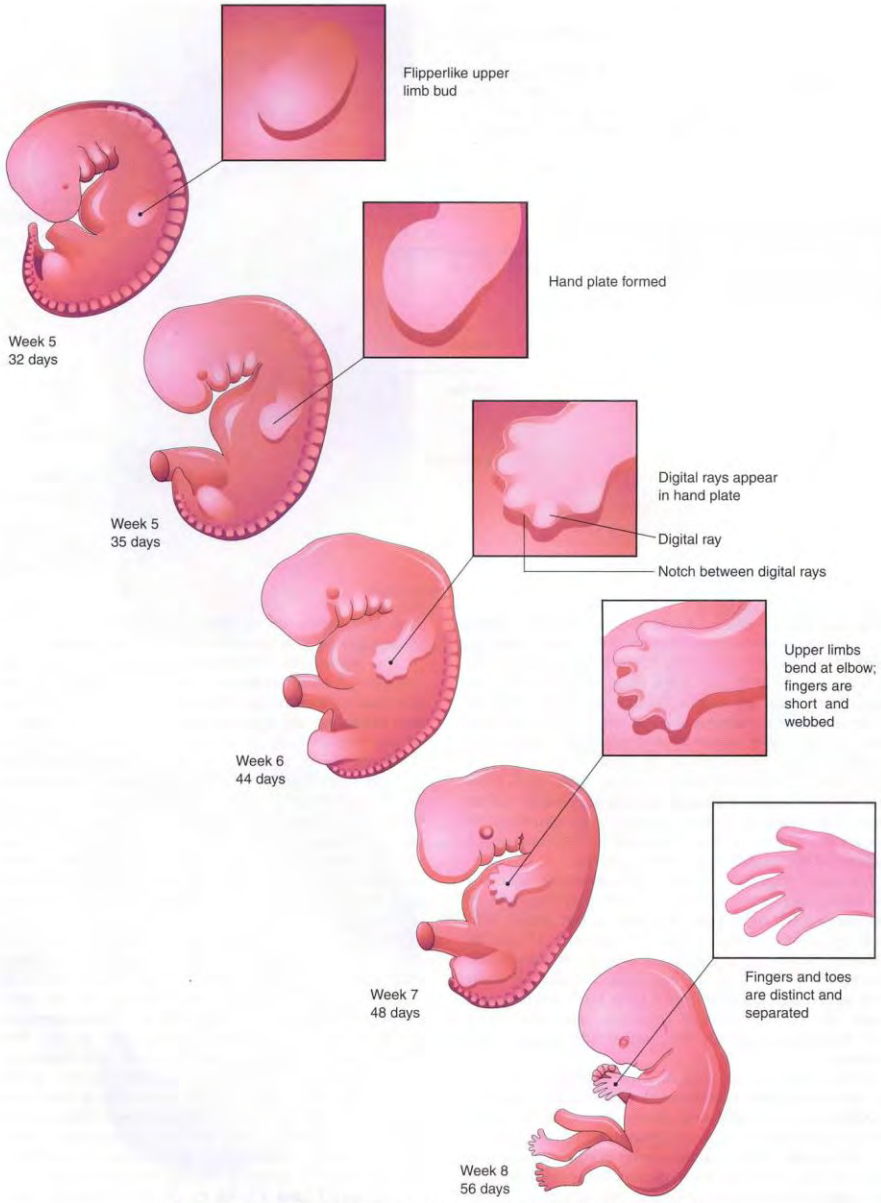
1. Passive diffusion
2. Facilitated transport
3. Active transport

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

1940-1959	No cases of reduced long bones of limbs
1959	1 case
1960	30 cases
1961	154 cases

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

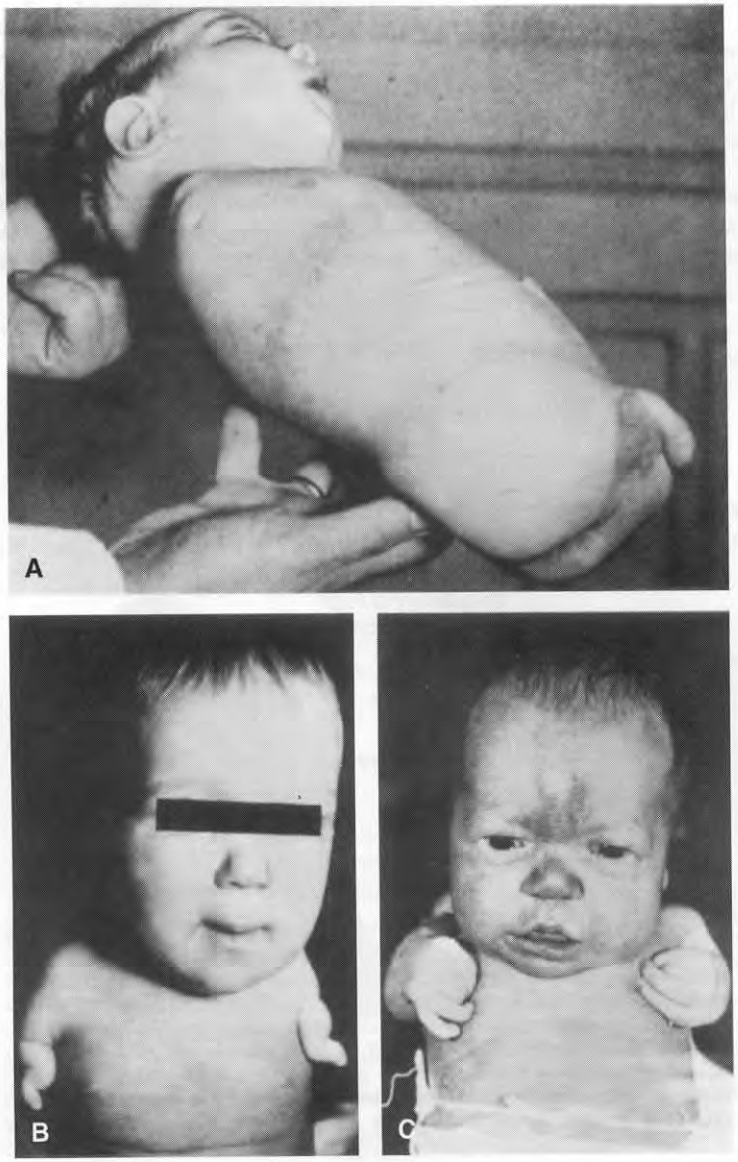
Animal Testing Followed:

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


A
B
C

Maternal Factors Affecting Development

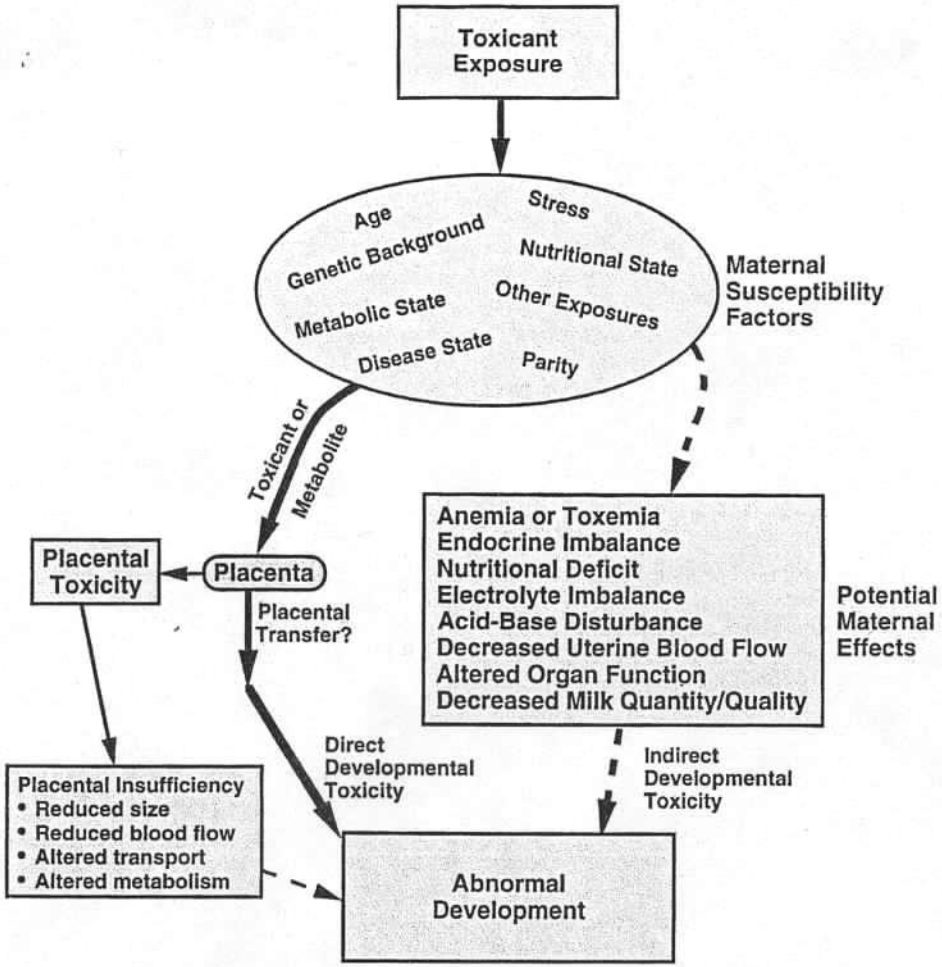


Figure 10- 7. Interrelationships between maternal susceptibility factors, metabolism, induction of maternal physiologic or functional alterations, placental transfer and toxicity, and developmental toxicity.

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.

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

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 congential 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