Embryo and Fetal Pathology

COLOR ATLAS WITH ULTRASOUND CORRELATION

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The Human Embryo and Embryonic Growth Disorganization

STAGES OF EMBRYONIC DEVELOPMENT

Carnegie staging in the development of the human embryo categorizes 23 stages.

Fertilization and Implantation (Stages 1–3)

Embryonic development commences with fertilization between a sperm and a secondary oocyte (Tables 1.1 to 1.5). The fertilization process requires about 24 hours and results in the formation of a **zygote** – a diploid cell with 46 chromosomes containing genetic material from both parents. This takes place in the ampulla of the uterine tube.

The embryo's sex is determined at fertilization. An X chromosome-bearing sperm produces an XX zygote, which normally develops into a female, whereas fertilization by a Y chromosome-bearing sperm produces an XY zygote, which normally develops into a male.

The zygote passes down the uterine tube and undergoes rapid mitotic cell divisions, termed cleavage. These divisions result in smaller cells – the **blastomeres.** Three days later, after the developing embryo enters the uterine cavity, compaction occurs, resulting in a solid sphere of 12–16 cells to form the **morula**.

At 4 days, hollow spaces appear inside the compact morula and fluid soon passes into these cavities, allowing one large space to form and thus converting the morula into the **blastocyst** (blastocyst hatching). The blastocyst cavity

Table 1.1 Human embryonic development and growth

Period	Conception* (d)	Gestational age** (d)	CR length (mm)	External characterizations	Carnegie staging
Blastogenesis					
First 2 weeks	0–14	0–28	0–0.4	Unicellular to bilaminar plate	1–6b
Days 14–28	15–28	29–35	0.4–4.6	Trilaminar embryo to open neural groove	7–10
Organogenesis					
Second 4 weeks	22–35	36–49	4.6–8	Neural tube closure to limb buds	11–13
Days 32–56	36–60	50–75	8–30	Limb growth to fused eyelids	14–22
Fetal	61–266	75–280	35–350	Fetal maturation	

* Embryonic development is dated from fertilization.
** Prenatal growth evaluation by ultrasound is dated from day of last menstrual period. This is termed "gestational age."
Adaped from Wilson RD: Prenatal evaluation of growth by ultrasound, *Growth Genetics & Hormones*, v.9(1), 1993.

separates the cells into an outer cell layer, the trophoblast, which gives rise to the placenta, and a group of centrally located cells, the **inner cell mass**, which gives rise to both embryo and extraembryonic tissue.

The **zona pellucida** hatches on day 5 and the blastocyst attaches to the endometrial epithelium. The trophoblastic cells then start to invade the endometrium.

Implantation of the blastocyst usually takes place on day 7 in the midportion of the body of the uterus, slightly more frequently on the posterior than on the anterior wall.

Gastrulation

Changes occur in the developing embryo as the bilaminar embryonic disc is converted into a trilaminar embryonic disc composed of three germ layers.

Table 1.2 Measurements of gestation age by ultrasound

Mean gestational age (wk)*	Mean gestational sac diameter (mm)†	Embryo CR length (mm)	BPD (mm)	Femur length (mm)
5 + 0	2	–	–	–
6 + 0	10	6	–	–
7 + 0	18	10	–	–
8 + 0	26	17	–	–
9 + 0	–	25	–	–
10 + 0	–	33	–	–
11 + 0	–	43	–	6
12 + 0	–	55	17	9
13 + 0	–	68	20	12
14 + 0	–	85	25	15

* From 1st day of last menstrual period †Daya et al., 1991 ‡Jeanty, 1983
Adaped from Wilson RD: Prenatal evaluation of growth by ultrasound, *Growth Genetics & Hormones*, v.9(1), 1993.

The process of germ layer formation, called gastrulation, is the beginning of embryogenesis (formation of the embryo).

Gastrulation begins at the end of the 1st week with the appearance of the hypoblast; it continues during the 2nd week with the formation of the epiblast and is completed during the 3rd week with the formation of intraembryonic mesoderm by the primitive streak. The three primary germ layers are called ectoderm, mesoderm, and endoderm. As the embryo develops, these layers give rise to the tissues and organs of the embryo.

The blastocyst begins to become attached to the uterine lining (the endometrium).

Table 1.3 Number of somites correlated to approximate age in days

Approximate age (days)	No. of somites
20	1–4
21	4–7
22	7–10
23	10–13
24	13–17
25	17–20
26	20–23
27	23–26
28	26–29
30	34–35

Implantation

Implantation includes dissolution of the zona pellucida and adhesion between the blastocyst and the endometrium, trophoblastic penetration, and migration

Table 1.4 Summary of embryonic development highlights

CR length (mm)	Days after ovulation	Carnegie stage	Main external features
0.1	0–2	1	Fertilized oocyte
	4–6	3	Blastocyst
0.2–0.4	6–15	5	Trilaminar embryo with primitive streak
1.5–2.0	20–22	9	Heart tubes begin to fuse
2.0–3.0	22–24	10	Neural folds begin to fuse; heart begins to beat
3.0–4.0	24–26	11	Rostral neuropore closing
4.0–5.0	26–30	12	Upper limb buds appear
5.0–6.0	28–32	13	Four pairs of branchial arches
6.0–7.0	31–35	14	Lens pits and nasal pits visible
Highlights 35–56 days, organogenesis			
7.0–10.0	35–38	15	Hand plates formed; retinal pigment visible
10.0–12.0	37–42	16	Foot plates formed
12.0–14.0	42–44	17	Finger rays appear; auricular hillocks developed
14.0–17.0	44–48	18	Toe rays appear
16.0–20.0	48–51	19	Trunk elongating; midgut herniation to umbilical cord
20.0–22.0	51–53	20	Fingers distinct but webbed
22.0–24.0	53–54	21	Fingers free and longer
24.0–28.0	54–56	22	Toes free and longer
28.0–30.0	56–60	23	Head more rounded; fusing eyelids

Table 1.5 Major landmarks for early development

Retinal pigment 35–37 days
Separation of common aorticopulmonary trunk (A & PA separate) 42 days
Distinct elbow and/or developing eyelids 44 days
Scalp vascular plexus 49 days
Intestines into umbilical cord 7–10 weeks
Perforation of anal membrane 51 days
Lack of tail 56 days
Fingernails and a well-defined neck 10–12 weeks (a fetus not embryo)

of the blastocyst through the endometrium. Implantation occurs by the intrusion of trophoblastic extensions, which penetrate between apparently intact endometrial cells.

Second Week of Development (Stages 4 and 5)

During the 2nd week, a bilaminar **embryonic disc** forms, **amniotic and primary yolk sac** cavities develop, and there are two layers of trophoblast (Figure 1.1).

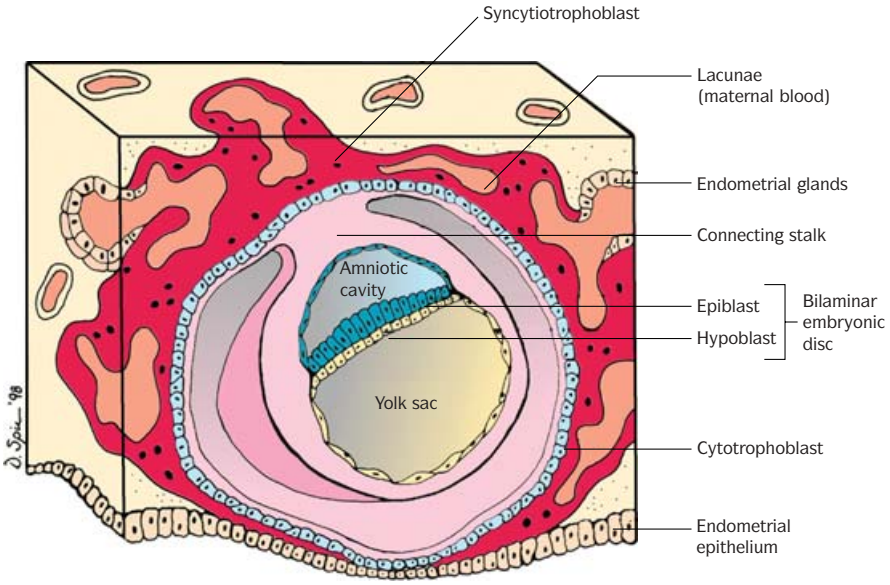
The two-layered disc separates the blastocyst cavity into two unequal parts (a smaller amniotic cavity and a larger primary yolk cavity). The thick layer of embryonic cells bordering the amniotic cavity is called the **epiblast** and a thin layer bordering the primary yolk cavity is called the **hypoblast**.

The trophoblast differentiates into two layers, an inner **cytotrophoblast** and an outer **syncytiotrophoblast**. The trophoblast continues to penetrate deeper into the endometrium. At the end of the 2nd week, the site of implantation is recognized as a small elevated area of endometrium having a central pore filled with a blood clot.

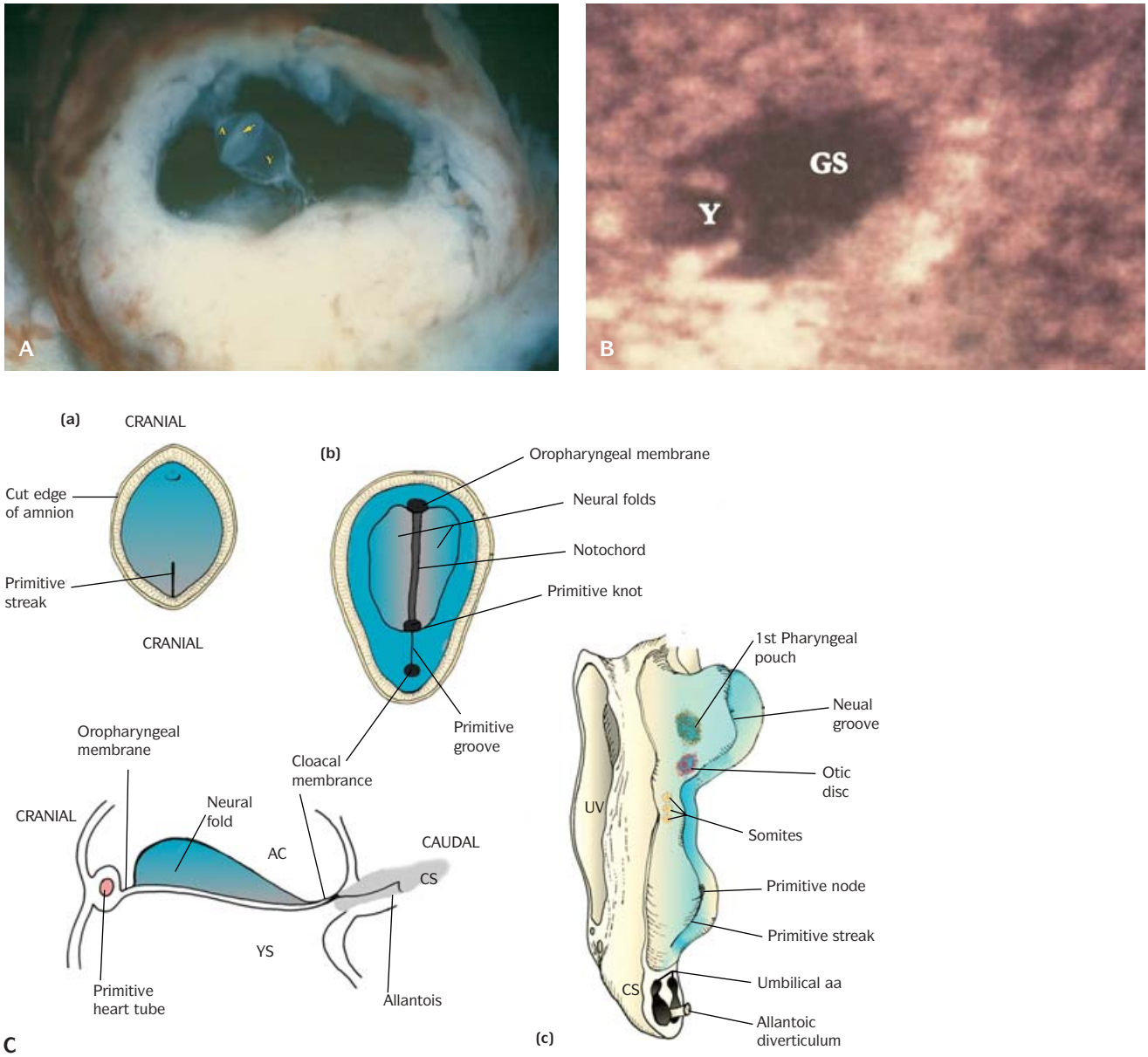
Third Week of Development (Stages 6–9)

Formation of the primitive streak and three germ layers (ectoderm, mesoderm, and endoderm) (Figure 1.2) occurs during the 3rd week.

The **primitive streak** results from a proliferation of ectodermal cells at the caudal end of the embryonal disc. Cells at the primitive streak proliferate to form the embryonic endoderm and mesoderm. The cephalic end of



1.1. Bilaminar embryonic disc in the 2nd week of development (stage 5), with amniotic and primary yolk sac cavities.



1.2. (A) Ectopic pregnancy at day 17 showing an embryonic disc with opacity (arrow) representing the primitive streak. The amniotic cavity (A) and the primary yolk sac cavity (Y) are present. **(B)** Ultrasound of a human embryo at the same stage of development as A (GS, gestational sac; Y, yolk sac). **(C)** Diagram of development of the primitive streak (a), notochord (b), and neural folds (c) in a trilaminar embryo (stages 6–9).

the primitive streak is the primitive node, and this cord of cells is the **notochord**.

Thickening of ectodermal cells gives rise to the **neural plate**, the first appearance of the nervous system, which becomes depressed below the surface along the long axis of the embryo to form the neural groove. The **neural groove**

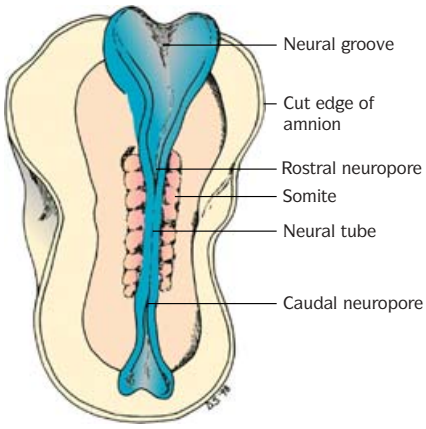
deepens and its margins elevate to form the **neural folds**. The fusion is completed during the 4th week of development. The neural tube ultimately will give rise to the central nervous system. The cephalic end will dilate to form the forebrain, midbrain, and hindbrain. The remainder of the neural tube will become the spinal cord.

The mesoderm on either side of the midline of the embryo (the paraxial mesoderm) undergoes segmentation, forming **somites**. The first pair of somites arises in the cervical region of the embryo at approximately day 20 of development. From there new somites appear in craniocaudal sequence, approximately three per day, until 42–44 pairs are present at the end of week 5. There are 4 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 8–10 coccygeal pairs. The first occipital and the last 5–7 coccygeal somites later disappear, while the remainder form the axial skeleton. During this period of development, the age of the embryo is expressed in the number of somites. Each somite differentiates into bones, cartilage, and ligaments of the vertebral column as well as into skeletal voluntary muscles, dermis, and subcutaneous tissue of the skin. The intermediate mesoderm and the lateral mesoderm give rise to portions of the urogenital system. The lateral plate mesoderm is involved in the development of pericardial, pleural, and peritoneal cavities as well as the muscle of the diaphragm.

Mesoderm also forms a primitive cardiovascular system during the 3rd week of development. Blood vessel formation begins in the extraembryonic mesoderm of the yolk sac, the connecting stalk, and the chorion. Embryonic vessels develop 2 days later. The linkage of the primitive heart tube with blood vessels takes place toward the end of week 3, after which blood circulation begins. The beating heart tube begins at 17–19 days.

The embryo changes shape from a disc to a tube with a cranial and a caudal end and the third germ layer, the endoderm, becomes incorporated into the interior of the embryo.

The formation of **chorionic villi** takes place in the 3rd week. The cytotrophoblast cells of the chorionic villi penetrate the layer of syncytiotrophoblast to form a cytotrophoblastic shell, which attaches the chorionic sac to the endometrial tissues.

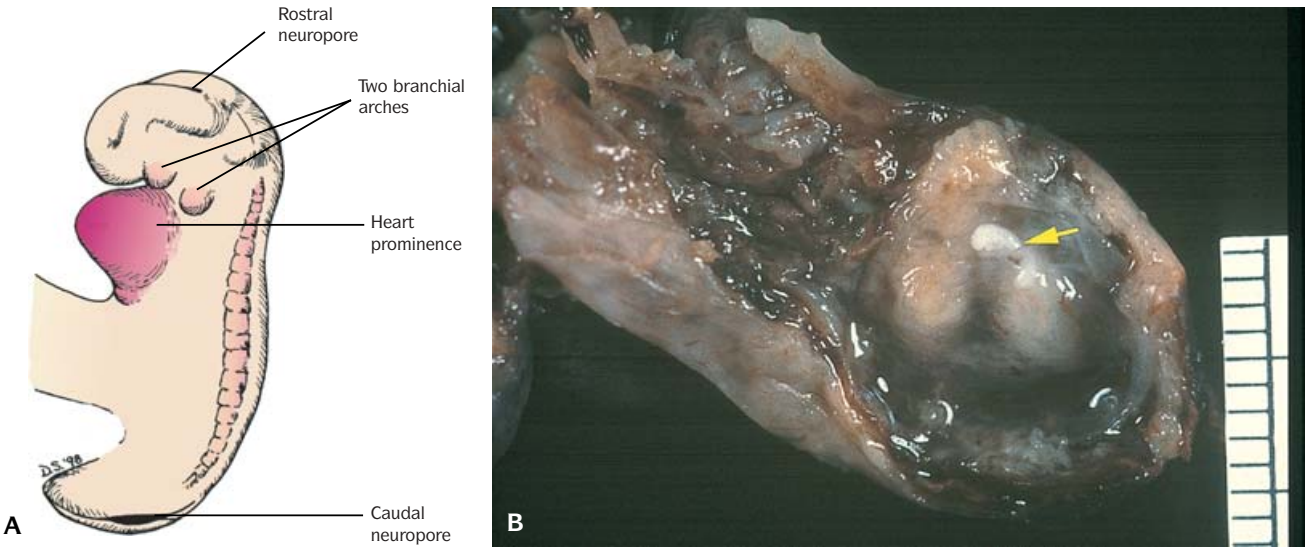


1.3. Diagram of human embryo at stage 10. Neural folds are partially fused with the neural tube open at the rostral and caudal neuropore.

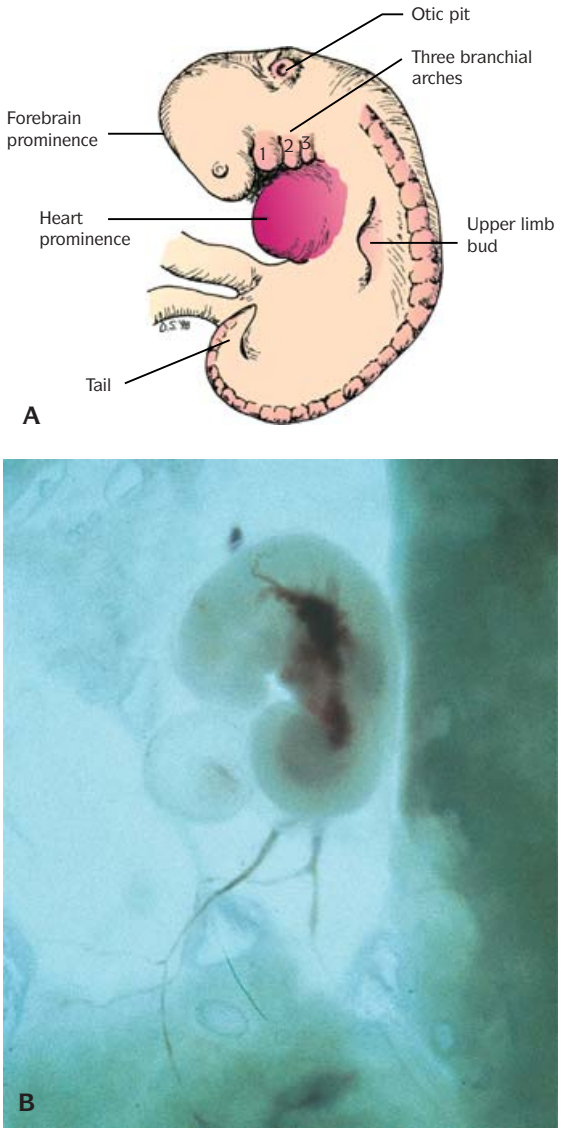
Fourth Week of Development (Stages 10–12: Up to Day 28, End of Blastogenesis)

At this stage, the embryo measures 2–5 mm (Figures 1.3 to 1.6). At *stage 10*, the embryo (at 22–24 days) is almost straight and has between 4 and 12 somites that produce conspicuous surface elevations. The neural tube is closed between the somites but is widely open at the rostral and caudal neuropore. The first and second pairs of branchial arches become visible.

During *stage 11*, a slight curve is produced by folding of the head and tail. The heart produces a large ventral prominence. The rostral neuropore continues to close and optic vesicles are formed.



1.4. (A) Diagram of a human embryo at stage 11. **(B)** A human embryo at stage 11 (arrow) showing a slight curve. The size should range from 2 to 5 mm. **(C)** Human embryo at stage 11 with a slight curve, two pairs of branchial arches, heart prominence (H), and optic vesicle (O). Rostral neuropore (arrow) continues to close.

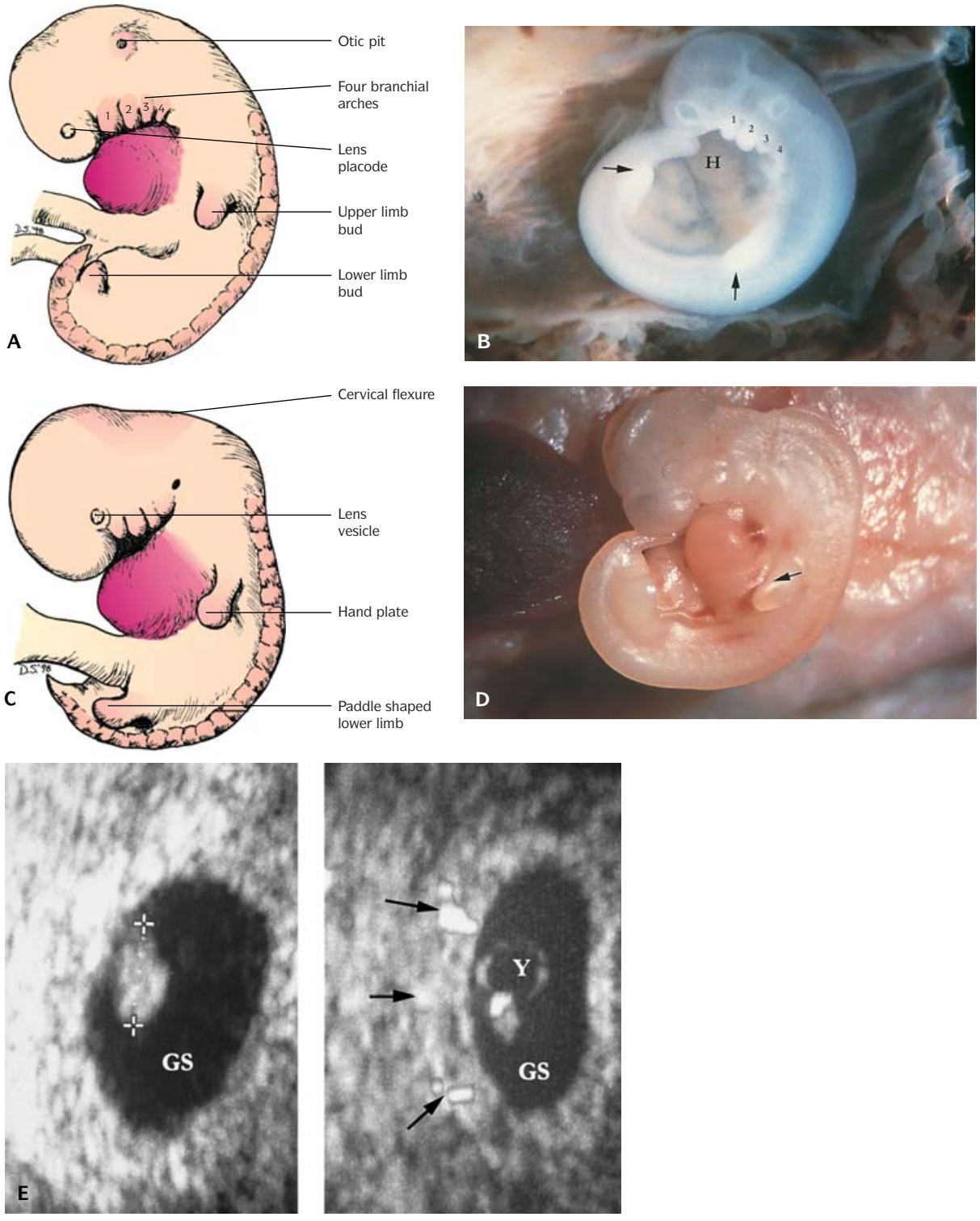


1.5. (A) Drawing of a human embryo at stage 12. **(B)** Embryo at stage 10–12 (4th week of development) with early vascular development.

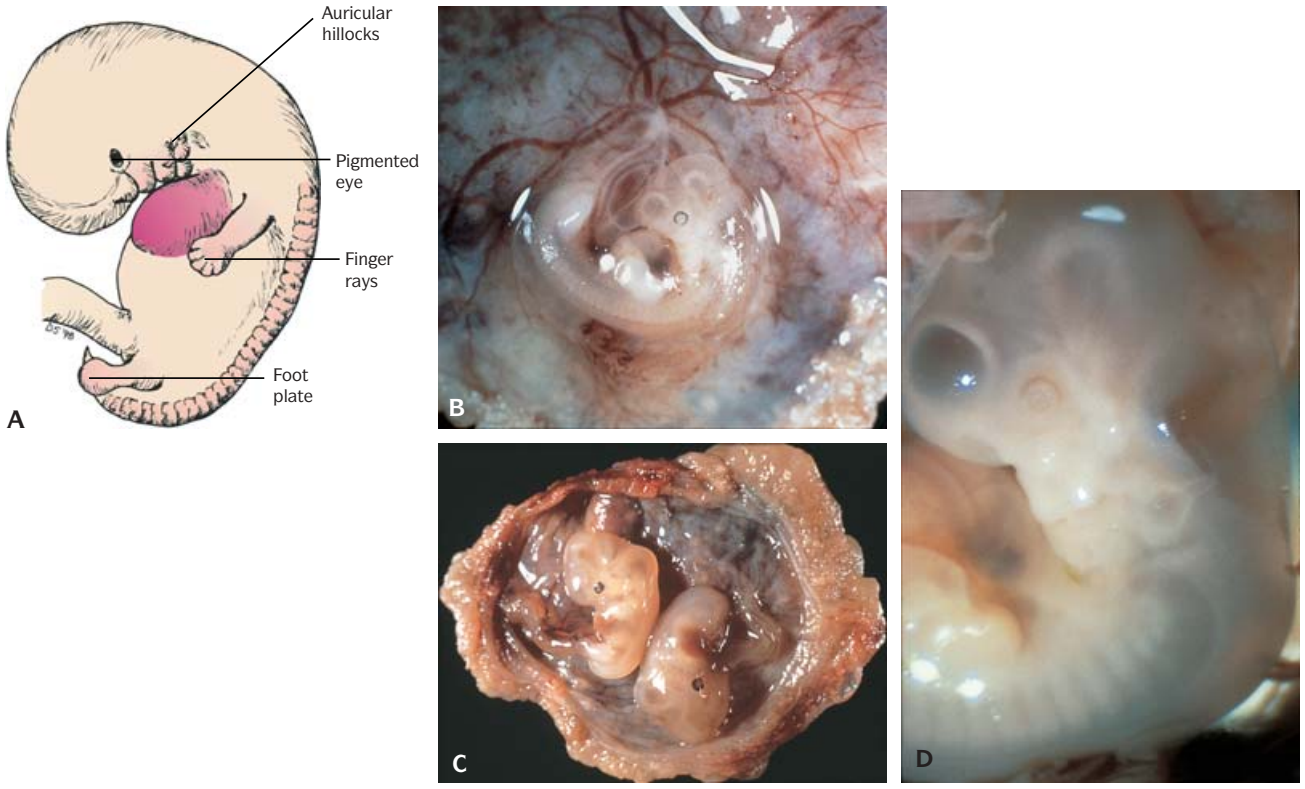
In *stage 12*, three pairs of **branchial arches** complete closure of the rostral hemisphere and recognizable upper-limb buds on the ventral lateral body wall appear. The **otic pits** and the primordia of the inner ears become visible. Growth of the forebrain produces an enlargement of the head, and further folding of the embryo in the longitudinal plane results in a C-shaped curvature. Narrowing of the connection between the embryo and the yolk sac produces a **body stalk** containing one umbilical vein and two umbilical arteries.

Fifth Week of Development (Stages 13–15)

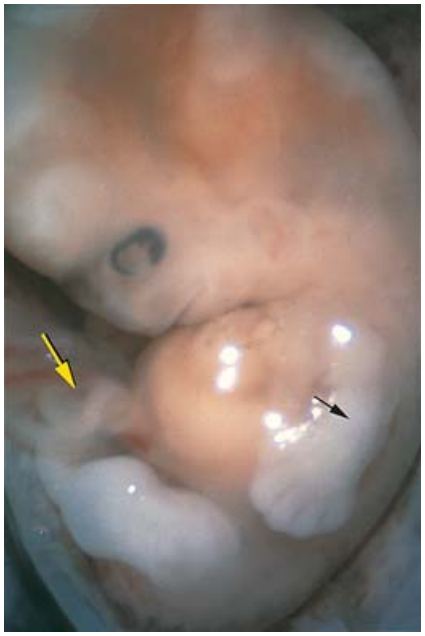
At this stage, the embryo measures 5–10 mm in length. Rapid head growth occurs, caused mainly by rapid development of the brain. The upper limbs



1.6. (A) Drawing of a human embryo at stage 13. **(B)** Human embryo at stage 13. Note body curvature, four pairs of branchial arches, heart prominence (H), and upper and lower limb buds (arrows). The lens placode and otic pit are identifiable and the neural tube is closed. **(C)** Drawing of a human embryo at stage 15. **(D)** Human embryo at stage 15 with well-defined lens vesicle and an area representing hand plate formation (arrow). The cervical flexure is prominent. **(E)** Ultrasound at stages 13–15: (Right) CR length of embryo in the gestational sac. (Left) Doppler imaging showing blood flow (arrows) surrounding the gestational sac (GS) and in the embryo (transverse plane at the level of the heart). Yolk sac (Y) is also indicated.



1.7. (A) Drawing of a human embryo at stage 17, lateral view. **(B)** Human embryo with early formation of retinal pigment, finger rays and foot plate. **(C)** Monochorionic monoamniotic twin embryos with well-developed retinal pigment. **(D)** Embryo at 12 weeks fertilization age showing auricular hillocks.



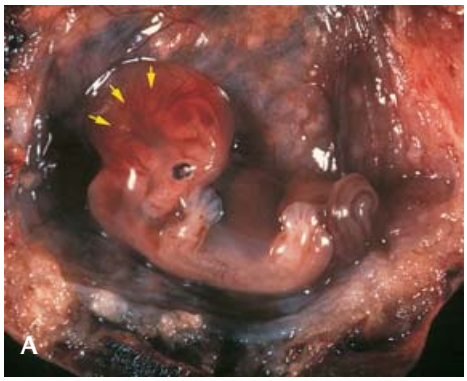
1.8. Human embryo at stage 18 and 19 showing elbow region (black arrow), toe rays, and herniation of intestinal loops into the umbilical cord (yellow arrow).

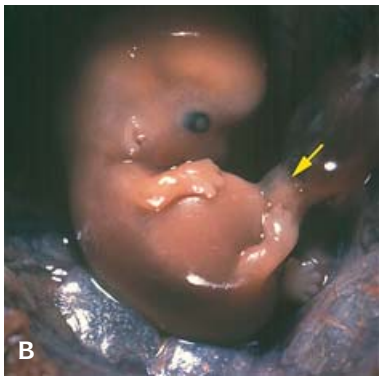
begin to show differentiation as the **hand plates** develop toward the end of this week. The fourth pair of **branchial arches** and the **lower-limb buds** are present by 28–32 days of development. Lens placodes of the eyes are visible on the sides of the head. The attenuated tail with its somites is a characteristic feature at the beginning of week 5.

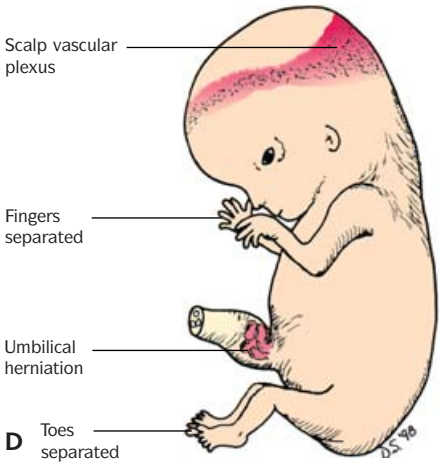
Sixth Week of Development (Stages 16 and 17)

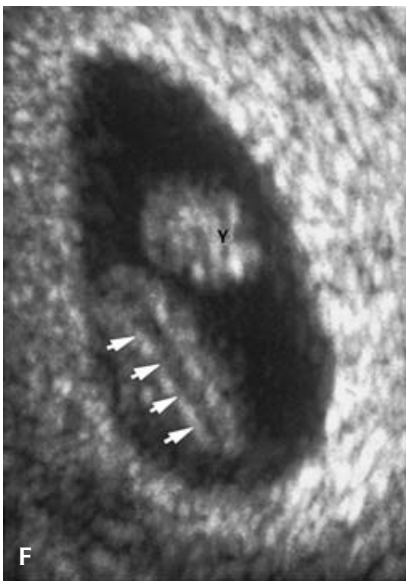
The crown–rump (CR) length of the embryo in this time period is 10–14 mm. At *stage 16*, **nasal pits** face ventrally, **retinal pigment** becomes visible, **auricular hillocks** appear, and the **foot plate** is formed. In *stage 17*, the C-shape of the embryo is still present. Development of **finger rays** and basic facial-structure formation advances (Figure 1.7). The **upper lip** appears when medial nasal prominences and maxillary prominences merge. The nostrils become clearly defined and the eyes are directed more anteriorly.

1.9. (A) Human embryo at stage 20 showing webbed fingers and notches between the toe rays. The vascular plexus becomes visible (arrows). **(B)** Human embryo at stage 21 and 22 with free fingers. The hands and feet approach each other. Note the intestine in the umbilical cord (arrow). **(C)** Human embryo at stage 23 with more typical human characteristics

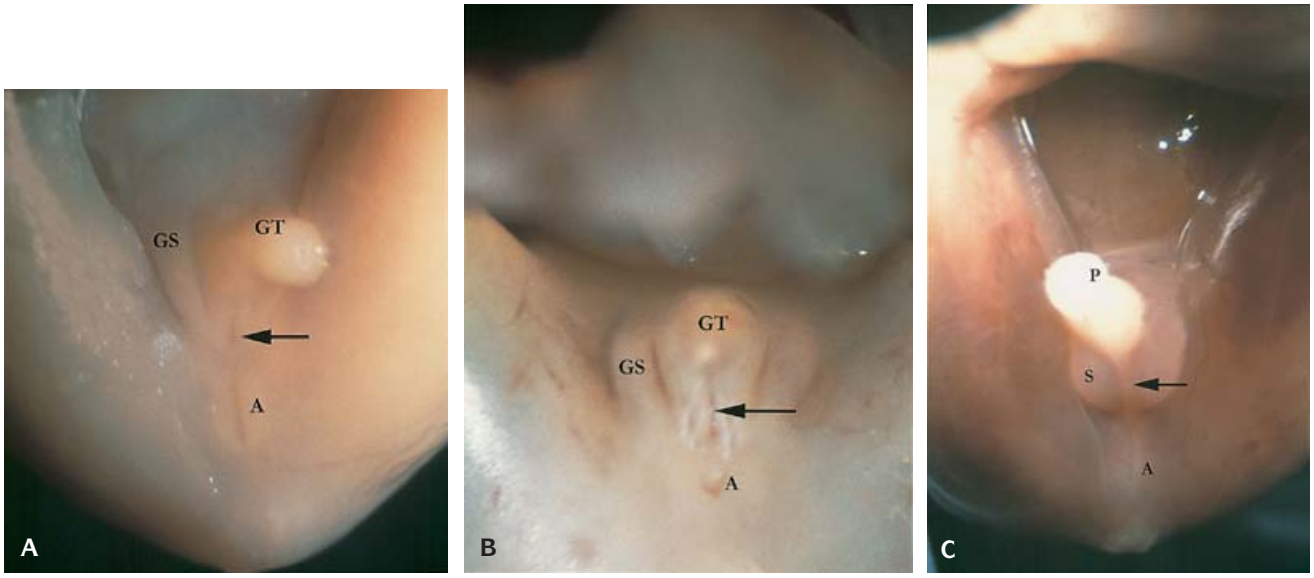








1.9. (*cont.*) such as a rounder head and completed development of the face, hands and feet. **(D)** Drawing of a human embryo at stage 23. **(E)** Posterior view of the embryo shown in **(C)** with an intact neural tube. **(F)** Ultrasound showing a posterior view of an embryo with the characteristic appearance of an intact neural tube (arrows) (Y, yolk sac). **(G)** Fetus at beginning of the fetal period (9 developmental weeks).



1.10. (A) Sexual differentiation of male and female cannot be determined until the 12th week of fertilization age. At 9 weeks the genitalia are ambiguous (GT, genital tubercle; urogenital groove, arrow; GS, genital swelling; A, anus). **(B)** Female at 12 weeks fertilization age (GT, genital tubercle; urogenital groove, arrow; GS, genital swelling; A, anus). **(C)** Male at 12 weeks fertilization age (P, penis; S, scrotum; A, anus; arrow, scrotal raphe).

Seventh Week of Development (Stages 18 and 19)

At the end of the 7th week, the embryo attains a CR length of 20 mm. The head continues to enlarge rapidly and the trunk straightens. **Elbow regions** can be recognized on upper limbs, toe rays appear on the lower limbs, and the nipples become visible. **Physiological herniation of the intestinal tract into the umbilical cord** occurs (Figures 1.8 to 1.10). The intestinal loops normally return to the abdomen by the end of the 10th week.

Eighth Week of Development (Stages 20–23)

At this stage, the **fingers are distinct but are still webbed**. There are **notches between the toe rays**, and a **scalp vascular plexus** appears. Toward the end of week 8, the fingers become free and longer and the development of hands and feet approach each other. The head becomes more rounded and shows typical human characteristics. The embryo has a CR length of 20 mm at the beginning of the 8th week and is 30 mm in CR length at the end of the 8th week. All major organ systems are formed by the end of the 8th week – the completion of blastogenesis, organogenesis, and embryonic development. Then the fetal period begins.

Prenatal Evaluation of Growth by Ultrasound

Prenatal evaluation is usually possible 3 weeks after fertilization.

Embryonic development and growth start with fertilization and progress through 4 weeks, blastogenesis (postconception days 0–28), and organogenesis (days 29–56). In humans, fusion of the eyelids (days 56–60) is regarded as an arbitrary end of the embryonic period.

Evaluation by ultrasound is dated from the first day of the last menstrual period, which is termed "gestational age" (2 weeks longer than embryonic age).

A gestational sac can usually be identified at 5 weeks and is an early indication of an intrauterine pregnancy. Ultrasound evaluation of the embryo reveals the following:

1. At 6 weeks, gestational age, embryonic structures and heart activity are almost always visible.
2. At 7 weeks, the embryo is 10 mm at a minimum and fetal heart activity should be visible in 100% of viable pregnancies.
3. At 8 weeks, fetal structures are visible and the yolk sac is identified as a circular structure measuring 5 mm in diameter. The detection of a yolk sac excludes the diagnosis of a blighted ovum because a viable embryo is necessary for yolk sac development.
4. An empty gestational sac with a mean diameter greater than 30 mm with no visible embryonic structures means that a nonviable pregnancy (blighted ovum) exists.
5. At 9–11 weeks, progressive ossification occurs with major centers in the calvaria and ilium.

The CR length is measured from the outer edge of the cephalic pole to the outer edge of the fetal rump. This measurement predicts the gestational age with an error of ± 3 days (90% confidence limits) after 7–10 weeks. The error increases to ± 5 days between 10 and 14 weeks of gestation. Fetal flexion may decrease maximal CR length by 5%.

The cephalic index is the ratio of the biparietal diameter (BPD) divided by the occipital frontal diameter. A normal ratio is 0.75 to 0.85. After 20 weeks of gestation, the BPD is less reliable for gestational dating because of changes in shape, growth disturbances, and individual variation.

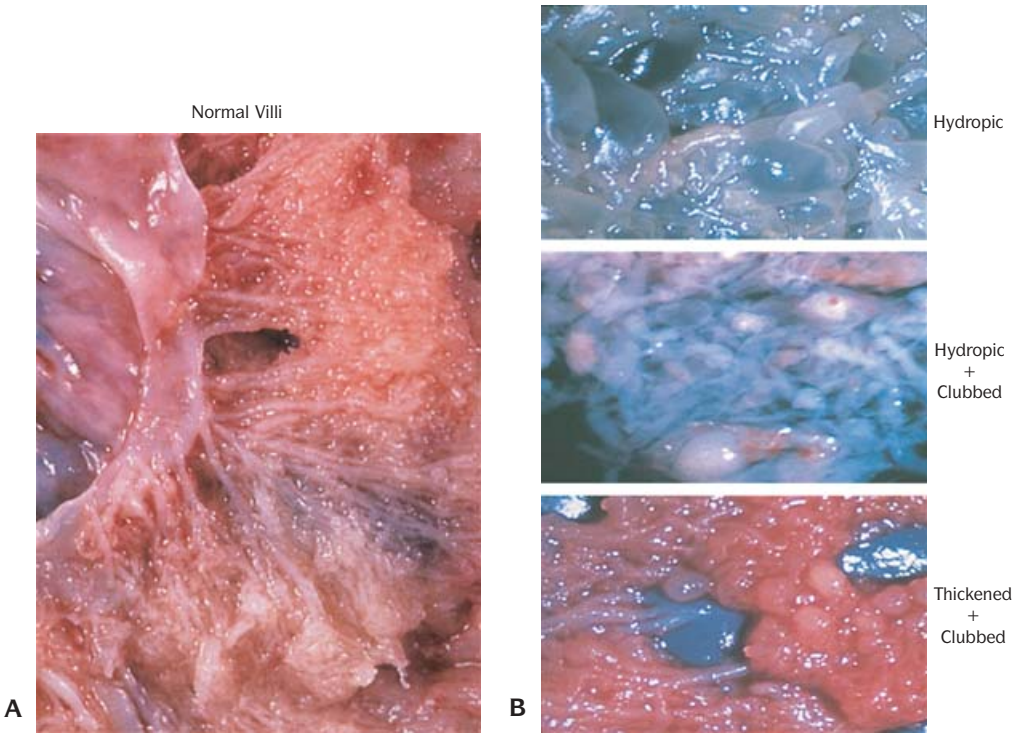
The femur can be measured as early as 10 weeks gestational age.

Fetal BPD and femur length for gestational age dating have a confidence interval of ± 1 week from 12 to 22 weeks, ± 2 weeks from 22 to 32 weeks, and ± 3 weeks from 32 to 41 weeks.

Small for gestational age (SGA) is defined as a birth weight less than the 10th centile. Therefore, the SGA group includes most normal but small infants. Large for gestational age (LGA) is defined as birth weight greater than the 90th centile. This LGA group also includes normal but large infants.

Examination of Products of Conception

Fetal weight can be estimated by ultrasound with established charts comparing

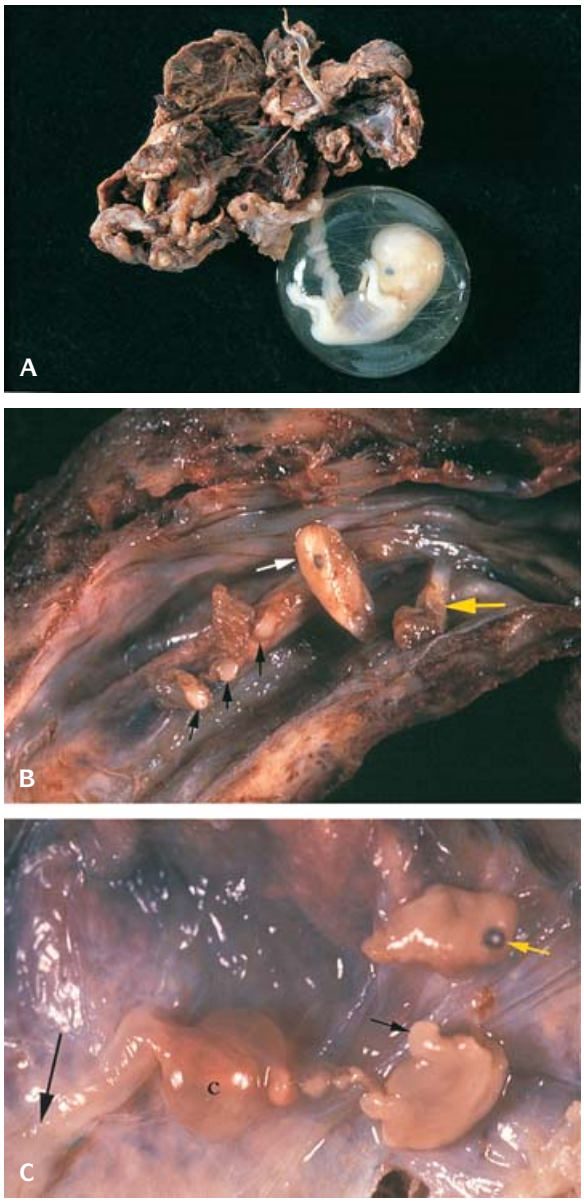


1.11. Illustration of **(A)** normal and **(B)** abnormal villi.

the BPD and abdominal circumference (AC). The BPD exceeds the AC until 38 weeks of gestation, when they become equal. The AC then exceeds the BPD.

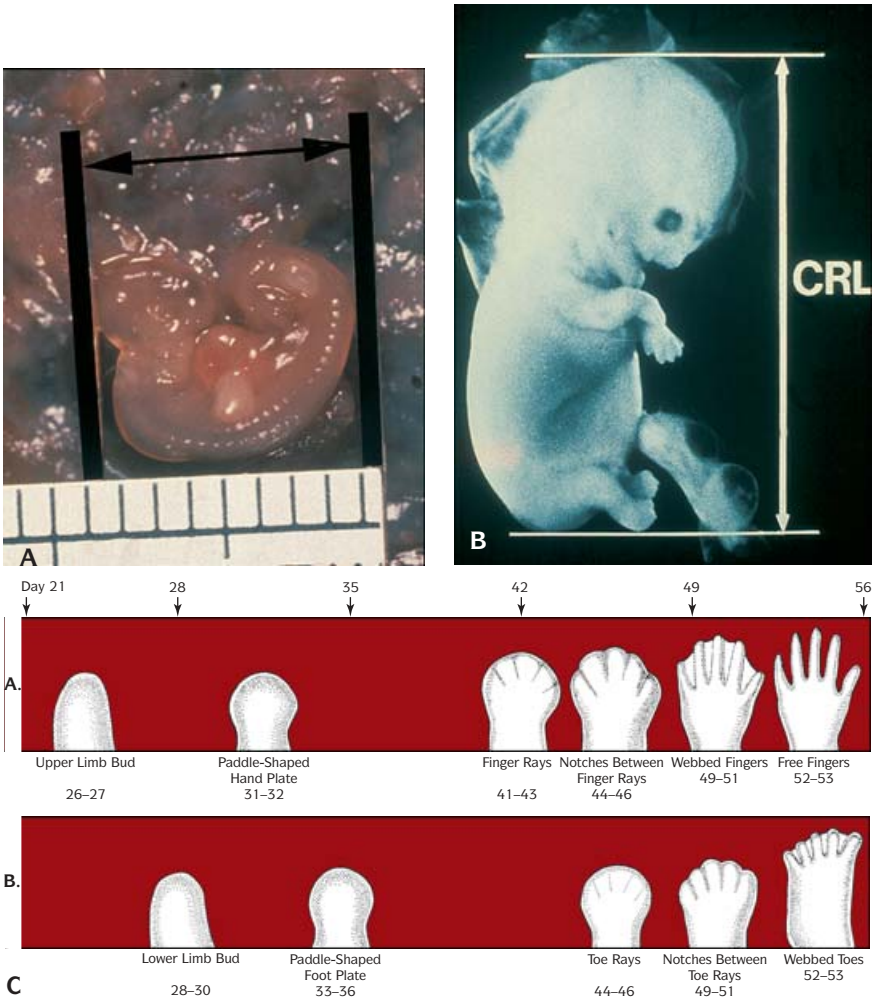
Adequate examination and evaluation of products of conception can yield important information that may benefit future pregnancies. The fate of the fertilized ova can be quite grim within the embryonic period; about 16% of those exposed to sperm fail to divide either because they are not penetrated by sperm or because the meiotic mechanism is not functioning. Another 15% fail to implant. Grossly abnormal embryos (27%) are spontaneously aborted at previllous stages with another 20% estimated to die after the first missed period for a total loss rate of 70–80%. Abortion is defined as the premature expulsion or removal of the conceptus from the uterus before it is able to sustain life on its own. Clinically, the term takes on many definitions, such as threatened abortion, incomplete abortion, missed abortion, recurrent abortion, and induced/therapeutic abortion. Early spontaneous abortion occurs in the embryonic period up to the end of the 8th developmental week.The embryonic period is from conception to the end of the 8th week, the fetal period from the 9th week to birth. Late spontaneous abortion occurs between the 9th week to the 20th week of development.

Initial examination of the products of conception should begin with assessing the villous component of the gestational sac (Figures 1.11 to 1.13). Normal



1.12. (A) Example of a complete specimen. The chorionic sac has been ruptured but the amniotic sac and embryo are still intact. **(B)** Cross-section of a complete specimen showing artifactual separation of the embryo from the umbilical cord (yellow arrow) secondary to maceration. The embryo is also fragmented (black arrows = limbs, white arrow = malformed head with retinal pigment). This embryo is a GD IV. **(C)** A complete specimen showing a macerated embryo (yellow arrow = head with retinal pigment, small black arrows = upper limb). Note the constricted, twisted, cystic (C) umbilical cord. (Large arrow at cord insertion.)

villi are very lush, fine and hairlike, covering the entire surface of the sac. Abnormal villi can appear hydropic, clubbed, and/or thickened and are usually sparse. A complete specimen consists of an intact chorionic sac that may be empty or contain various embryonic or extraembryonic tissues. The diagnosis



1.13. CR measurement of embryos. **(A)** Early embryo (32 days developmental age). **(B)** Embryo at about 7 weeks developmental age. **(C)** Illustrations demonstrating development of the hand and foot.

of a blighted ovum is an intact but empty sac without a trace of an embryo. Incomplete specimens consist of an opened or ruptured chorionic sac without an identifiable embryo. Curetted specimens are most often incomplete. When an embryo is identified, it should be measured and assessed for all developmental features, such as limb development, eyes, branchial arches, etc. (Tables 1.6 to 1.8). The embryo is measured in its natural position, from the curvature of the head to the curvature of the rump in younger embryos and from the crown to the rump in older embryos as they begin to straighten. Examination of the hand and foot also determines embryonic age. Under normal circumstances developmental age can be based primarily on length of hands and foot length.

Growth Disorganization (GD)

Four types of growth disorganization (GD) have been established by Poland