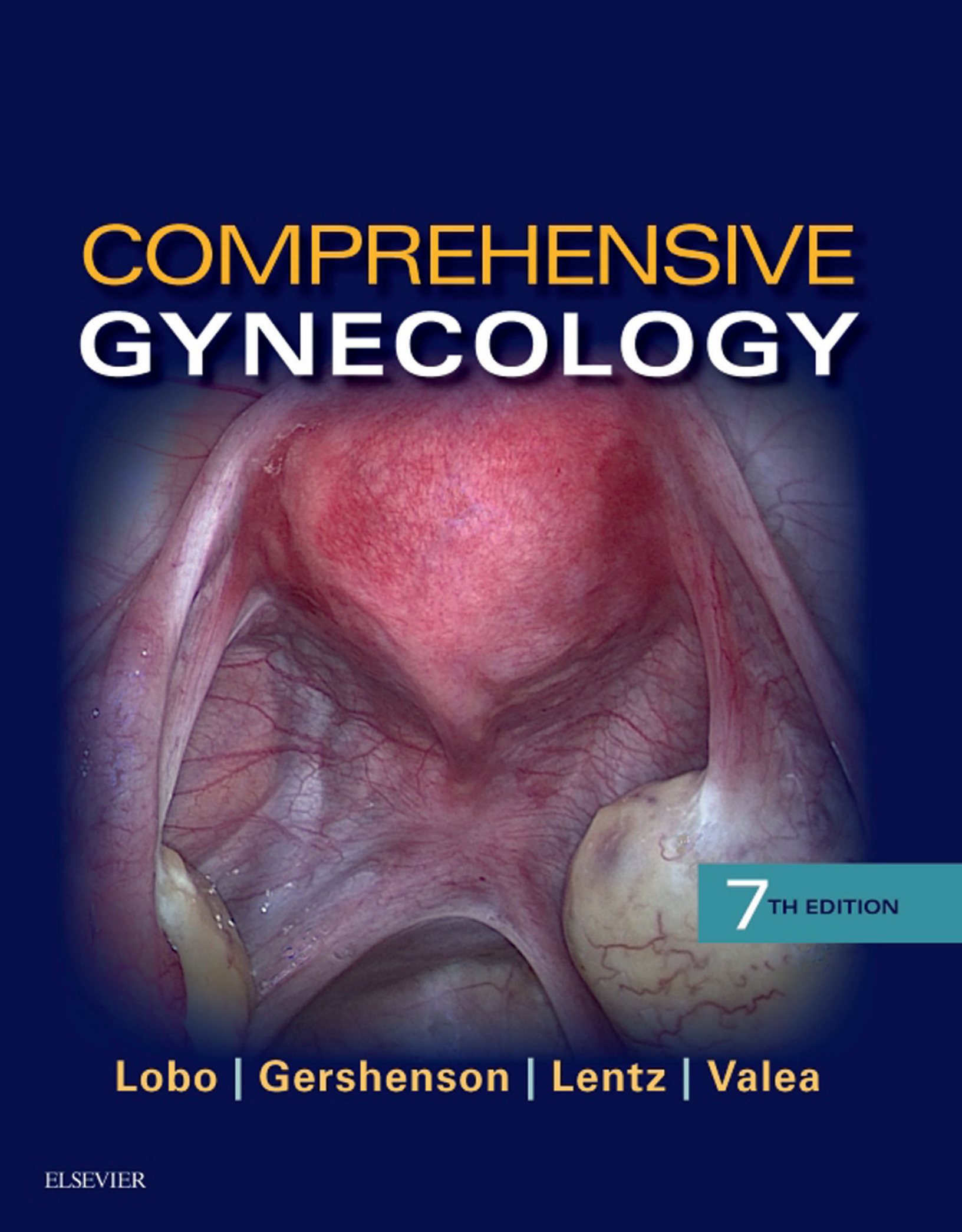
COMPREHENSIVE GYNECOLOGY



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7th Edition

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Preface

Try to learn something about everything and everything about something.

Thomas Huxley

Comprehensive Gynecology is now in its seventh edition, and it is humbling to note that it has almost been 30 years since the first edition was published in 1987.

The current editors are indebted to our mentors who pioneered the original work. The contributions of Drs. William Droegemueller, Authur L Herbst, Daniel R. Mishell, Jr., and Morton A. Stenchever were monumental and provided the impetus for our following in their footsteps. We are also saddened by the fact that in 2015, we lost Mort, who is now in a better place. Also, as we close out this edition, we have just learned that we have also lost our esteemed mentor, Dan Mishell, who passed away in May 2016. Both men have contributed so much to the field, and to us personally, and have done so much to improve the lives of women.

In line with the quote above from Huxley, the British biologist and philosopher, we continue to attempt to be *comprehensive.* We want the reader to be comfortable with all aspects of gynecology; some readers will wish to be more expert in certain subspecialty areas such as urogynecology, oncology, or reproductive endocrinology.

In this edition we are privileged to welcome Fidel Valea as one of the editors, taking over the duties from Vern L. Katz, who elected to retire. We would like to thank Vern once again for his contributions.

Rather than adding new chapters, we have split some in two to provide better focus on the subject areas, and also organized the chapters to provide better flow. We have added several co-authors, continuing the trend we established with the previous edition. This is a major departure from the early editions, where the four editors wrote all of the chapters. We feel adding additional talent to the authorship provides a more comprehensive and validated approach to the dissemination of knowledge in gynecology.

In this edition, we have provided the most important references in the body of the chapter, allowing the reader to have immediate access to the source, rather than having to search for the reference. However, we have maintained a full bibliography for many chapters, available online.

In this edition we have also provided video content to make this a more visual experience for the reader. New and better illustrations have also been added to assist in visual learning. The cover is also a departure from our previous editions and speaks to our wish to impart a visual experience and emphasize contemporary techniques of minimally invasive procedures for gynecological surgery.

Nearly every chapter has maintained key points of importance, which have been bundled together in an online synopsis of the entire book. This will allow rapid assessment of the content of each chapter for more in-depth reading of areas of greater interest as well as provide key learning facts in all areas of gynecology.

We hope readers will enjoy this edition and learn as much as they can from this ever-evolving field in order to provide better health care for women.

We would like to give a big thanks to our editors, Kate Dimock, and particularly Rae Robertson, who have stewarded us through this process.

We would also like to give a big thank you, with great appreciation and love, to our families, without whose support and encouragement this project could not have been accomplished.

Roger A. Lobo, MD
David M. Gershenson, MD
Gretchen M. Lentz, MD
Fidel A. Valea, MD

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Fertilization and Embryogenesis

Meiosis, Fertilization, Implantation, Embryonic Development, Sexual Differentiation

Thomas M. Price

Several areas of medical investigation have brought increased attention to the processes of fertilization and embryonic development, including teratology, stem cell research, immunogenetics, and assisted reproductive technology. The preimplantation, implantation, and embryonic stages of development in the human can now be studied because of the development of newer techniques and areas of research. This chapter considers the processes of oocyte meiosis, fertilization and early cleavage, implantation, development of the genitourinary system, and sex differentiation.

OOCYTE AND MEIOSIS

The oocyte is a unique and extremely specialized cell. The primordial germ cells in both males and females are large eosinophilic cells derived from endoderm in the wall of the yolk sac. These 700 to 1300 cells migrate to the germinal ridge by way of the dorsal mesentery of the hindgut by ameboid action by 5 to 6 weeks. Oogenesis begins with the replication of the diploid oogonia through mitosis to produce primary oocytes, reaching a peak number of 600,000 (95% prediction interval: 70,000-5,000,000) at 18 to 22 weeks of gestation. Through apoptosis the numbers decline to about 360,000 (95% prediction interval: 42,000 to 3,000,000) at menarche (Wallace, 2010). As can be seen, there is a large variance among individuals and a direct correlation between the number of fetal oocytes and the age of menopause. Accelerated apoptosis is seen in Turner syndrome resulting in few oocytes at birth (Modi, 2003).

The meiotic process actually begins at 10 to 12 weeks' gestation and is the mechanism by which diploid organisms reduce their gametes to a haploid state so that they can recombine again during fertilization to become diploid organisms. In humans, this process reduces 46 chromosomes to 23 chromosome structures in the gamete. The haploid gamete contains only one chromosome for each homologous pair of chromosomes, so it has either the maternal or paternal chromosome for each pair, but not both. Meiosis is also the mechanism by which genetic exchange is completed through chiasma formation and crossing over (recombination) between homologous chromosome pairs. Two meiotic cell divisions are required to produce haploid gametes. In the human female, oogonia enter meiosis in "waves" (Fig. 1.1)— that is, not all oogonia enter meiosis at the same time. Meiosis initiation is dependent on mesonephric-produced retinoic acid (Childs, 2011).

Oocytes in the first substage of prophase, leptotene, are found in the human fetal ovary as early as 10 weeks' gestation. With increasing gestational age, greater proportions of oocytes in later stages of meiosis may be observed, and by the end of the second trimester of pregnancy, the majority of oocytes in the fetal ovary have cytologic characteristics that are consistent with the diplotene/dictyotene substages of prophase I of meiosis I (the stage at which the oocytes are arrested until ovulation) (Fig. 1.2).

Meiosis is preceded by interphase I during which DNA replication occurs, thus transforming the diploid oogonia with a DNA content of 2*N* to an oocyte with a DNA content of 4*N*. Meiosis is defined in two stages. The first, known as the reduction division (division I, or meiosis I) initiates in the fetal ovary but is then arrested and completed at the time of ovulation.

Meiosis I starts with prophase I (prophase includes leptotene, zygotene, pachytene, and diplotene), which occurs exclusively during fetal life and sets the stage for genetic exchange that ensures genetic variation in our species (Fig. 1.3). Leptotene is

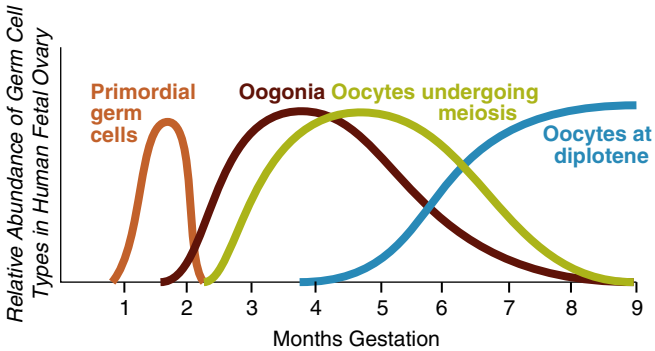

Figure 1.1 Diagrammatic representation of the different meiotic cell types and their proportions in the ovary during fetal life. (Courtesy of Edith Cheng, MD.)

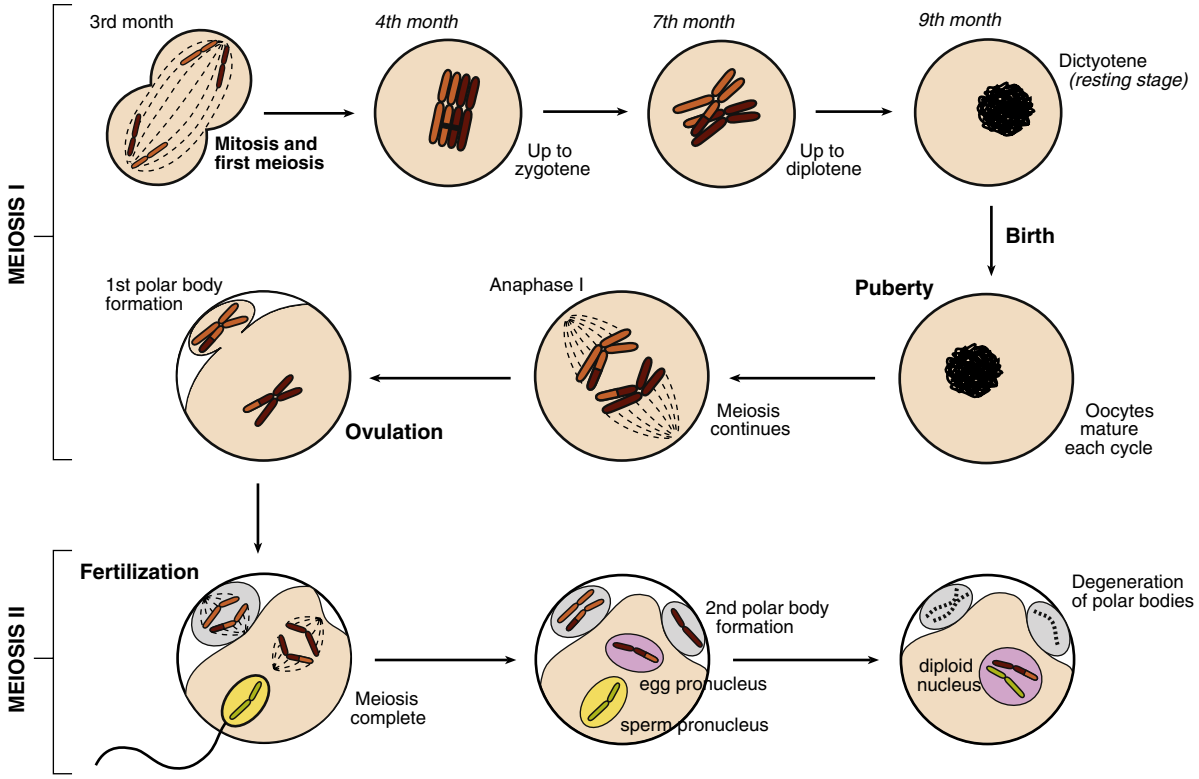

Figure 1.2 Diagram of oocyte meiosis. For simplicity, only one pair of chromosomes is depicted. Prophase stages of the first meiotic division occur in the female during fetal life. The meiotic process is arrested at the diplotene stage ("first meiotic arrest"), and the oocyte enters the dictyotene stages. Meiosis I resumes at puberty and is completed at the time of ovulation. The second meiotic division takes place over several hours in the oviduct only after sperm penetration. (Courtesy of Edith Cheng, MD.)

proportionately the most abundant of all the prophase I substages in the early gestations. Cells in this meiotic phase are characterized by a large nucleus with fine, diffuse, string-like chromatin evenly distributed within the nucleus (Fig. 1.3, *A*). Chromatin of homologous pairs occupies "domains" and does not occur as distinct linear strands of chromosomes. The zygotene substage is defined by the initiation of pairing, which is characterized by the striking appearance of the synaptonemal complex formation in some of the chromosomes (Fig. 1.3, *B*). There is cytologic evidence of chromosome condensation and linearization, and the chromatin is seen as a fine, string-like structure. The pachytene substage is the most easily recognizable period of the prophase and is characterized by clearly defined chromosomes that appear as continuous ribbons of thick beadlike chromatin (Fig. 1.3, *C*). By definition, this is the substage in which all homologues have paired. In this substage, the paired homologues are structurally composed of four closely opposed chromatids and are known as a *tetrad*. The frequency of oocytes in pachytene increases with gestational age and peaks in the mid-second trimester of pregnancy (at about 20 to 25 weeks' gestation). The diplotene substage is a stage of desynapsis that occurs as the synaptonemal complex dissolves and the two homologous chromosomes pull away from each other. However, these bivalents, which are composed of a maternally and a paternally derived chromosome, are held together at the centromere and at sites of chiasma formation

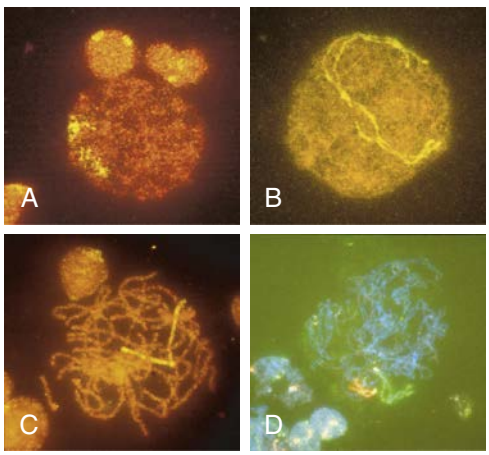

Figure 1.3 Fetal ovary with fluorescent in situ hybridization. The first three images are meiotic cells from a 21-week fetal ovary. **A,** Fluorescent in situ hybridization (FISH) with a whole chromosome probe for chromosome X was completed to visualize the pairing characteristics of the X chromosome during leptotene. **B,** Zygotene. **C,** Pachytene. **D,** Image of a meiotic cell from a 34-week fetal ovary that underwent dual FISH with probes for chromosomes 13 *(green signal)* and 21 *(red signal)* to illustrate the pairing characteristics of this substage of prophase in meiosis I. (Courtesy of Edith Cheng, MD.)

that represent sites where crossing over has occurred (Fig. 1.3, *D*). In general, chiasma formation occurs only between chromatids of homologous pairs and not between sister chromatids. Usually, one to three chiasmata occur for each chromosome arm. Oocytes at this stage of prophase I constitute the majority of third-trimester fetal and newborn ovaries. Diplotene merges with diakinesis, the last substage of meiosis I, and is a stage of transition to metaphase, lasting many years in humans (Speed, 1985).

With puberty, folliculogenesis occurs with progression of the follicle, consisting of the oocyte and granulosa cells from primordial to antral characterized by granulosa cell proliferation, development of gonadotropin receptors, and expression of enzymes for sex steroid production (Baerwald, 2012). It takes approximately 85 days for a follicle to mature to the point of ovulation. There is no change in the chromosome stage during folliculogenesis.

Meiosis I resumes with the surge of luteinizing hormone prior to ovulation completing metaphase, anaphase, and telophase. The result is two daughter cells, which are diploid (2*N*) in DNA content but contain 23 chromosome structures, each containing two closely held sister chromatids. One daughter cell, the oocyte, receives the majority of the cytoplasm, and the other becomes the first polar body. The polar body is located in the perivitelline space between the surface of the oocyte (oolemma) and the zona pellucida (ZP).

Meiosis II is rapid with the oocyte advancing immediately to metaphase II where the sister chromatids for each chromosome are aligned at the equatorial plate, held together by spindle fibers at the centromere. With sperm penetration, meiosis II is completed with extrusion of the second polar yielding a haploid oocyte (1*N*), entered by a haploid (1*N*) sperm (Fig. 1.4).

FERTILIZATION AND EARLY CLEAVAGE

In most mammals, including humans, the egg is released from the ovary in the metaphase II stage (Fig. 1.5). When the egg enters the fallopian tube, it is surrounded by a cumulus of granulosa cells (cumulus oophorus) and intimately surrounded by a clear zona pellucida (ZP). Within the zona pellucida are both the egg and the first polar body. Meanwhile, spermatozoa are transported through the cervical mucus and the uterus and into the fallopian tubes.

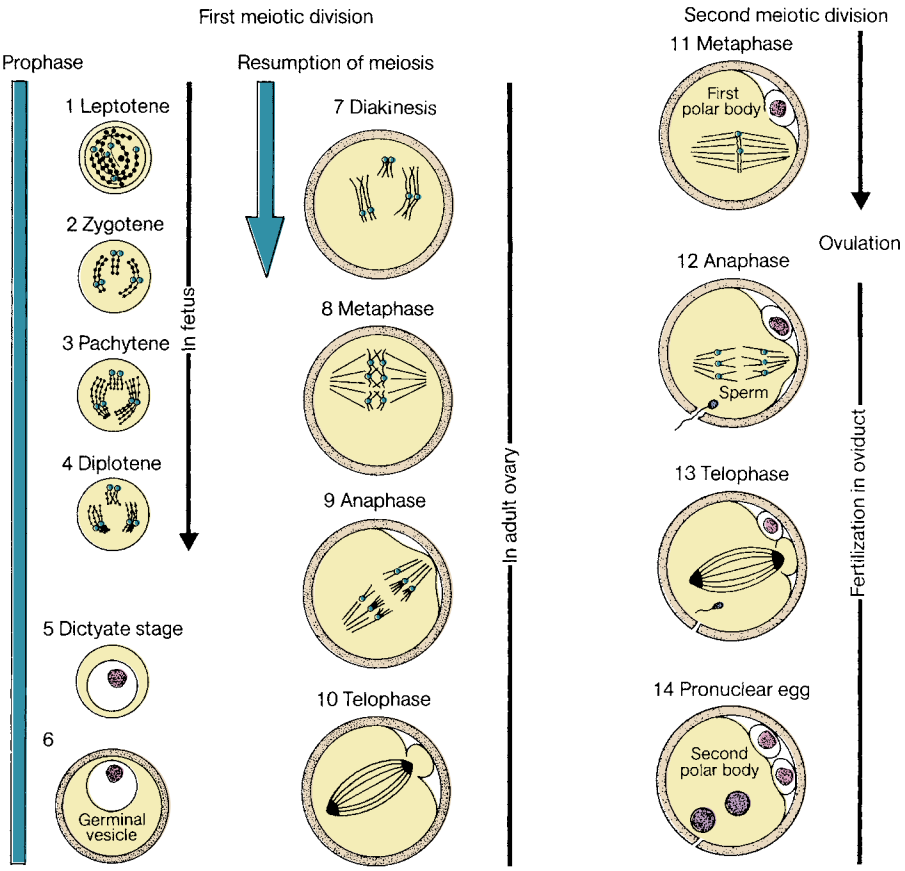

Figure 1.4 Diagram of oocyte meiosis. For simplicity, only three pairs of chromosomes are depicted (1 to 4). Prophase stages of the first meiotic division, which occur in most mammals during fetal life. The meiotic process is arrested at the diplotene stage ("first meiotic arrest"), and the oocyte enters the dictyate stages (5 to 6). When meiosis is resumed, the first maturation division is completed (7 to 11). Ovulation occurs usually at the metaphase II stage (11), and the second meiotic division (12 to 14) takes place in the oviduct only after sperm penetration. (From Tsafriri A. Oocyte maturation in mammals. In: Jones RE, ed. *The Vertebrate Ovary.* New York: Plenum; 1978.)

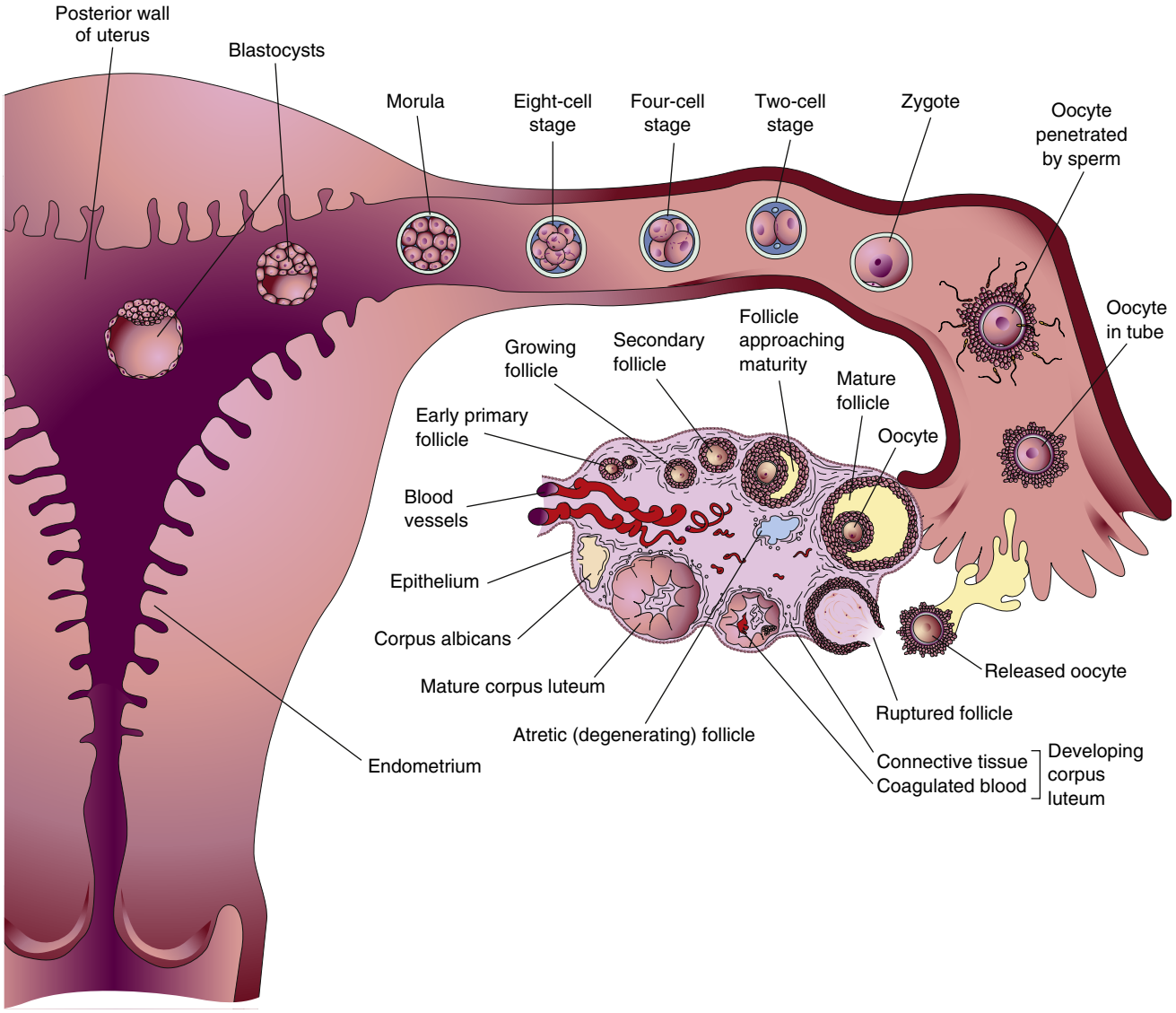

Figure 1.5 Summary of the ovarian cycle, fertilization, and human development during the first week. Stage 1 of development begins with fertilization in the uterine tube and ends when the zygote forms. Stage 2 (days 2 to 3) comprises the early stages of cleavage (from 2 to about 32 cells, the morula). Stage 3 (days 4 to 5) consists of the free (unattached) blastocyst. Stage 4 (days 5 to 6) is represented by the blastocyst attaching to the posterior wall of the uterus, the usual site of implantation. The blastocysts have been sectioned to show their internal structure. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

Although 20 million to 200 million sperm may enter the vagina during intercourse, only 1 in 25,000 will make it to the fallopian tubes (Williams, 1993). This journey involves processes of capacitation, chemotaxis, hyperactivated motility, and acrosome reaction (Fig. 1.6). Capacitation precedes all other changes and involves initial removal of cholesterol from the plasma membrane altering the permeability and fluidity. This allows the influx of calcium and bicarbonate with many downstream effects such as increased cyclic adenosine monophosphate (cAMP), protein tyrosine phosphorylation, and activation of protein kinases (Aitken, 2013). A function of capacitation is to allow localization of protein complexes in the head of the sperm, which will subsequently bind the ZP. Chemotaxis is shown by a greater number of sperm in the ampullary portion of the fallopian tube containing a cumulus-oocyte-complex (COC) compared with the side lacking a COC. In vitro, follicular fluid acts as a chemoattractant, possibly due to progesterone, but the exact responsible constituent(s) of the fluid continues to be debated (Eisenbach, 1999). Hyperactivated motility involves increased vigorous movement of the sperm in order to penetrate the cumulus (granulosa) cells surrounding the oocyte and is most likely due to progesterone. A major action of progesterone is to increase calcium influx into the sperm with multiple downstream effects. Likely, the

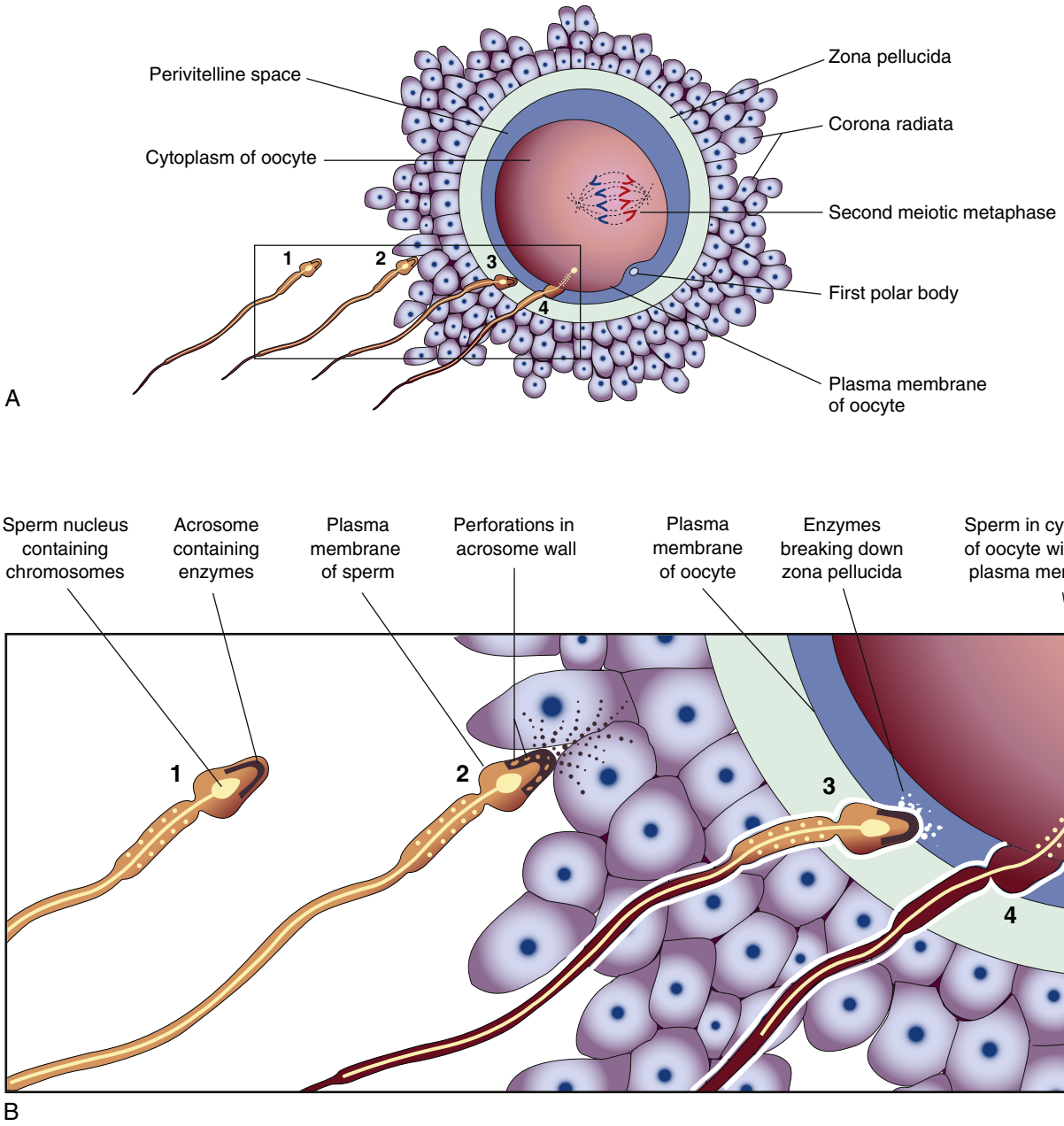

Figure 1.6 Acrosome reaction and a sperm penetrating an oocyte. The detail of the area outlined in **A** is given in **B**. *1*, Sperm during capacitation, a period of conditioning that occurs in the female reproductive tract.*2*, Sperm undergoing the acrosome reaction, during which perforations form 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, leaving the sperm's plasma membrane attached to the oocyte's plasma membrane. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

progesterone concentration increases as the sperm approaches the egg, resulting in more aggressive motility. When the egg is reached, receptor complexes on the outer most plasma membrane bind to specific ZP glycoprotein receptors (primarily ZP 3). These interactions are very species specific. Human sperm can only bind to the ZP of human, baboon, and gibbon oocytes. Binding results in fenestrations forming between the plasma membrane and the underlying acrosome membrane releasing enzymes including acrosin (a serine protease) to locally degrade the ZP (Chiu, 2014).

As many sperm may initially bind the ZP, a mechanism must be in place to prevent fertilization by more than one sperm

(polyspermia). With initial binding of the sperm membrane to the oolemma, a calcium-dependent release of cortical granules occurs. Cortical granules are vesicles containing protein made during oogenesis and located in the periphery of the cell. Contents are released into the perivitelline space and function to modify ZP proteins and enlarge the perivitelline space to prevent sperm entry (Talbot, 2003). With sperm entry, the oocyte completes its second meiotic division, casting off the second polar body into the perivitelline space.

The majority of a single sperm enters the oocyte, and this is indeed the case during intracytoplasmic sperm injection (ICSI) for infertility. Only the centrioles and the nucleus survive, whereas mitochondria in the midpiece and tail are destroyed. The sperm centrioles interact with α-tubulin from the oocyte to form a microtubule network for migration of pronuclei and subsequent separation of chromosomes during the first mitosis (Schatten, 2009). Thus mitochondria are of maternal origin, whereas centrioles are paternal.

Early cell division (cleavage) is not synchronous and varies in time (Fig. 1.7). Time intervals from two pronuclei to two-cell, two-cell to three-cell, three-cell to four-cell, and four-cell to five-cell are 26 hours, 12 hours, 0.8 hours, and 14 hours, respectively, as determined with time-lapse photography during in vitro fertilization (IVF) (Meseguer, 2011). A significant number of fertilized oocytes do not complete cleavage for a number of reasons, including failure of appropriate chromosome arrangement on the spindle, specific gene defects that prevent the formation of the spindle, and environmental factors. Importantly, teratogens acting at this point are usually either completely destructive or cause little or no effect. Twinning may occur by the separation of the two cells produced by cleavage, each of which has the potential to develop into a separate embryo (Hall, 2003). Twinning may occur at any stage until the formation of the blastocyst (blast), because each cell is totipotent. Both genetic and environmental factors are probably involved in the causation of twinning.

MORULA AND BLASTULA STAGE: EARLY DIFFERENTIATION

After fertilization, the *zygote* (the term for a fertilized egg) has a diameter of 83 to 105 μm and undergoes rapid mitotic division to reach the next stage of approximately 16 cells called a *morula*. After 4 to 5 days traversing the fallopian tube, the embryo arrives into the uterine cavity at the blast stage. The blast is characterized by a cavity (blastocoele) and differentiation of cells into the trophectoderm (TE), which will ultimately produce the fetal membranes and placenta and the inner cell mass (ICM), which will produce the fetus. During IVF, the blast forms 5 days after fertilization with a diameter of 155 to 265 μm consisting of about 40 TE cells and 20 ICM cells. In the human, implantation generally takes place 3 days after the embryo enters the uterus. The development of the blast with the separation of the ICM from the developing TE together make up the first stage of differentiation in the embryo. Differentiation within the ICM proceeds fairly rapidly, and if separation of cells and twinning occur at this point, the twins may be conjoined in some fashion.

Advances in assisted reproductive technology and genetics now provide practitioners assess to the early embryo for preimplantation genetic diagnosis (PGD) of single-gene disorders or preimplantation genetic screening (PGS) for chromosome abnormalities (Fig. 1.8). This technique involves removal of up to 20 TE cells from the day 5 blast for analysis. For PGD of single-gene disorders, DNA is extracted from the cells and the mutation analyzed by polymerase chain reaction (PCR) amplification or single nucleotide polymorphism (SNP) microarray. For PGS of chromosomal defects such as aneuploidy or structural rearrangements, analysis of DNA is performed with comparative genomic hybridization (CGH)-array (Fiorentino, 2014) or partial genomic sequencing (next-generation sequencing).

IMPLANTATION

Implantation consists of apposition, attachment, and invasion. This complex process has much redundancy and involves multiple factors including ovarian hormones, cytokines, transcription factors, growth factors, and extracellular matrix proteins (ECMs) (Table 1.1). Both the endometrium and the embryo produce these factors. Communication between the embryo and the endometrium is key. Implantation occurs 7 to 10 days after ovulation corresponding to cycle days 21 to 24 of an idyllic 28-day cycle with ovulation on day 14. During apposition the human embryo is oriented with the ICM and polar TE (TE next to the ICM) adjacent to the endometrium.

For attachment to the endometrium, the embryonic cells must first be expelled from the surrounding ZP in the process of "hatching." Hatching involves rupture of the ZP in one small area as opposed to a general dissolution of the entire ZP. This may involve hydrostatic pressure from inside the ZP and from zonalytic proteases produced by the TE and endometrium. These cysteine proteases, named *cathepsins*, are essential for hatching. Attachment of the embryonic cells to the endometrial cells involves cell adhesion proteins, integrins, and ECM proteins such as fibronectin, laminin, and collagen. Integrins are cell surface proteins, which bind extracellular matrix proteins and are expressed on both the luminal epithelium and TE.

Invasion of the TE cells next occurs by penetrating between the luminal epithelial cells, through the basement membrane and into the stroma of the endometrium. These initial TE cells form the extravillous trophoblasts (EVTs), which invade down to the inner third of the myometrium for anchoring and into the spiral arteries for remodeling. During spiral artery remodeling, endovascular EVT disorganize and partially replace the smooth muscle wall and the vascular endothelial cells. Proliferation of endovascular EVT leads to plugging and obstruction of the decidual spiral arteries resulting in a decrease in blood flow and oxygen tension. Low oxygen promotes the proliferation and transformation of cytotrophoblast to syncytiotrophoblast. Prior to 8 weeks' gestation, nutrition to the embryo is derived from endometrial gland secretion and plasma seeping through the obstructed spiral arteries into the intervillous space. With continued remodeling of the spiral arteries, patency is reestablished and maternal blood cells enter the intervillous space at around 9 weeks' gestation with a rise in oxygen tension. Lack of adequate EVT invasion and spiral artery remodeling is a key feature in preeclampsia, intrauterine growth restriction, and stillbirth (Brosens, 2002).

The idea of low oxygen tension during early embryo development has been explored with IVF. With a limited number of

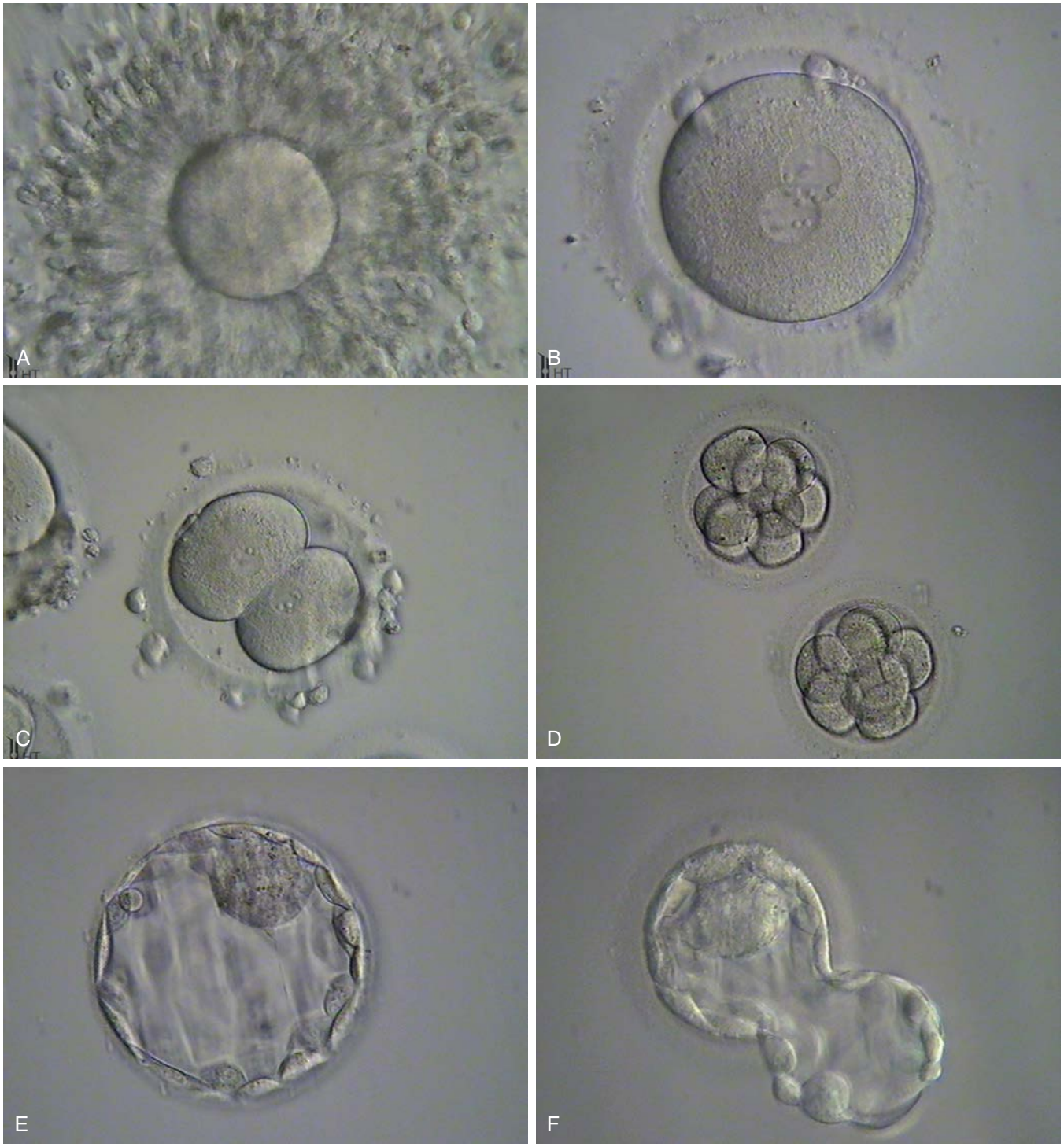

Figure 1.7 Six photomicrographs of fresh, unmounted human eggs and embryos. **A,** Recently retrieved human oocyte surrounded by cumulus cells. **B,** Fertilized oocyte demonstrating male and female pronuclei and both polar bodies at approximately 11 and 12 o'clock position. **C,** Two-cell zygote with scattered cumulus cells remaining attached to the zone pellucida. **D,** Eight-cell zygotes. **E,** Blastocyst with the inner cell mass seen at 12 o'clock. **F,** A hatching blastocyst in which a portion of the trophectoderm has extruded from the zona pellucida at the 4 o'clock position. (Courtesy of Douglas Raburn, PhD.)

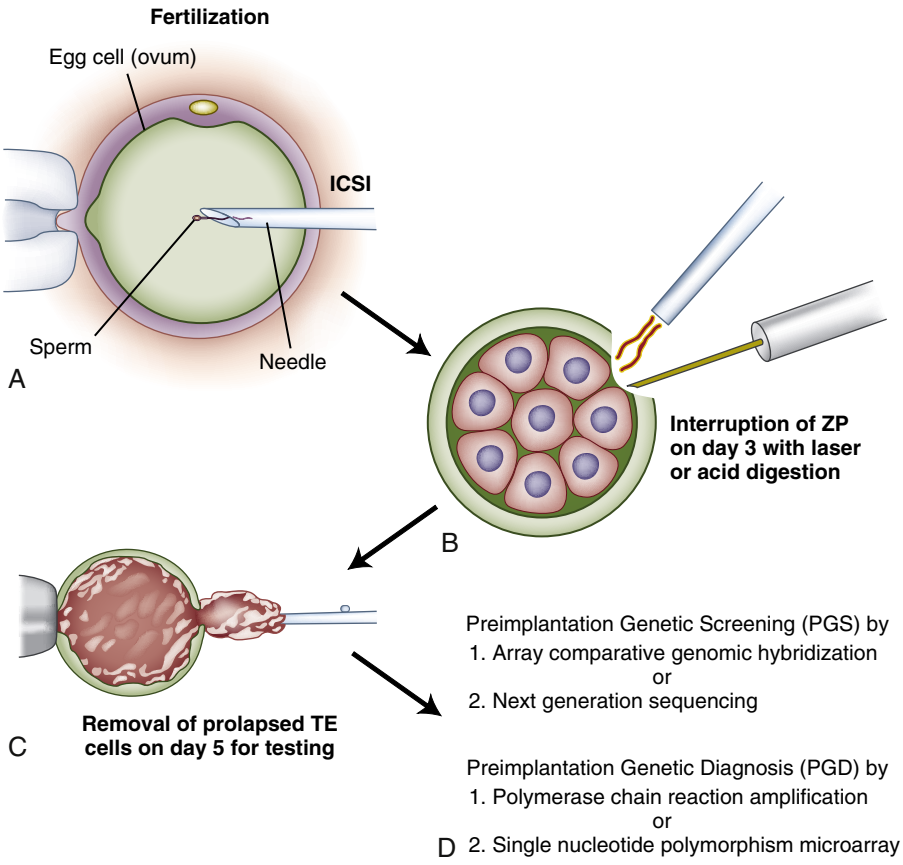

Figure 1.8 Schematic of preimplantation testing. **A,** Commonly the oocyte is fertilized with a single sperm using the technique of intracytoplasmic sperm injection (ICSI). This precludes the possibility of contamination from sperm remaining attached to the outside of the embryo during embryo biopsy. **B,** On day 3 of culture, when the embryo has cleaved to about eight cells, a small opening is made in the zona pellucida (ZP) with either a laser or brief exposure to an acid solution. **C,** By day 5 of culture, the embryo has progressed to the blastocyst stage and a portion of the trophectoderm (TE) cells have prolapsed out the opening in the zona pellucida. These cells are removed for subsequent DNA isolation. **D,** DNA from trophectoderm cells is used to determine chromosome number, insertions, and deletions for preimplantation genetic screening (PGS) using techniques of array comparative genomic hybridization (aCGH) or next-generation sequencing (NGS). DNA may also be used to detect single-gene abnormalities for different diseases using single nucleotide polymorphism (SNP) microarray or polymerase chain reaction (PCR) amplification in the process of preimplantation genetic diagnosis (PGD).

Table 1.1 Events of Implantation

Event	Days after Ovulation
Zona pellucida disappears	4-5
Blastocyst attaches to epithelial surface of endometrium	6
Trophoblast erodes into endometrial stroma	7
Trophoblast differentiates into cytotrophoblastic and syncytial trophoblastic layers	7-8
Lacunae appear around trophoblast	8-9
Blastocyst burrows beneath endometrial surface	9-10
Lacunar network forms	10-11
Trophoblast invades endometrial sinusoids, establishing a uteroplacental circulation	11-12
Endometrial epithelium completely covers blastocyst	12-13
Strong decidual reaction occurs in stroma	13-14

trials, culturing embryos in 5% oxygen as opposed to 20% oxygen results in a modest increase in the implantation rate (Bontekoe, 2012).

Villous trophoblast form finger-like projections extending into the intervillous space and thus surrounded by maternal blood. Syncytiotrophoblast form the outer layer with an underlying layer of precursor cytotrophoblast surrounding matrix containing capillaries, fibroblasts, and macrophages. Cytotrophoblasts become less numerous as pregnancy progresses.

Blood levels of the pregnancy hormone human chorionic gonadotropin (hCG) can be detected within 48 hours of implantation. Regular hCG is produced by the syncytiotrophoblast of placental villi. Blood levels peak at 56 to 68 days, reach a nadir at 18 weeks, and then remain fairly constant until delivery. Gonadotropin-releasing hormone (GnRH) produced in the cytotrophoblast and syncytiotrophoblast induces expression of hCG. In spontaneous pregnancies hCG can be detected 9 days following follicle rupture observed by ultrasound. In IVF pregnancies, the

hormone can be found 8 days after embryo transfer. hCG levels rise, exponentially up to 8 weeks from the last menstrual period, but the doubling time increases as the level increases. For example, in a conception cycle with ovulation on cycle day 14, the doubling time from cycle days 25 to 37 for hCG is 1.6 days and from days 38 to 44 it is 2.3 days (Zegers-Hochschild, 1994). The doubling time is independent of the number of gestations, although the absolute hCG level is higher for multiple pregnancies.

The classic action of regular hCG is maintenance of the corpus luteum (CL) by binding the luteinizing hormone (LH) receptor for continued estrogen and progesterone production. Yet other identified actions include promotion of angiogenesis in the uterus, myometrial relaxation, inhibition of immune interaction at the utero-placental interface, stimulation of fetal testosterone production, and mediation of hyperemesis through receptors in the brain.

Hyperglycosylated hCG (H-hCG) is produced by the EVT. H-hCG is key in promoting angiogenesis and cell invasion and correspondingly is found in the early first trimester. The protein does not activate the LH receptor and does not preserve CL function. Instead it appears to function via the transforming growth factor beta (TGF-β) receptor (Berndt, 2013). Low levels of H-hCG indicate poor EVT development and are associated with spontaneous abortion and early preeclampsia (Fournier, 2015).

DECIDUALIZATION

Progesterone is responsible for "decidualization" of the endometrium. This refers to morphologic and functional changes in stromal cells. In humans, stromal cells close to the spiral arteries undergo progesterone-induced decidual changes in the late secretory phase, and this process progresses throughout the stroma with implantation and hCG production. A pregnancy within the uterus is not required and decidualization is a common finding with ectopic pregnancies. Decidual cells show morphologic changes of increased size with increased glycogen and lipid accumulation (Maruyama, 2008). With pregnancy the endometrium is now referred to as the *decidua*, separated into areas of the decidua basalis or placentalis, which interacts with the TE (area of mature placenta), the decidua vera or parietalis (decidua distant from the implantation site), and the decidua capsularis (surrounding the embryo on the side opposite the placenta).

Another classic histologic change seen in early pregnancy is the Arias-Stella reaction (Fig. 1.9). This occurs in the glandular cells with a hallmark of nuclear enlargement. These cells may be misinterpreted as atypical or malignant. In the presence of hCG, the Arias-Stella reaction may be seen in extrauterine tissues such as endometriosis, vaginal adenosis, paraovarian cysts, and mucinous cystadenomas (Arias-Stella, 2002).

Morphologically luminal epithelial cells develop extensions of the plasma membrane called *pinopods* (also called *uterodomes*) during the window of receptivity. Pinopods function to release key proteins including leukemia inhibitory factor (LIF) through exocytosis and apocrine secretion (Kabir-Salmani, 2005).

Downstream effects of progesterone-dependent decidualization have not been completely elucidated, but loss-of-function studies show the necessity of transcription factors including CCAAT/enhancer binding protein Beta (C/EBPβ), Homeobox A10 (Hoxa10), Forkhead/winged helix protein (Fox01), and chicken ovalbumin upstream promoter (COUP-TFII).

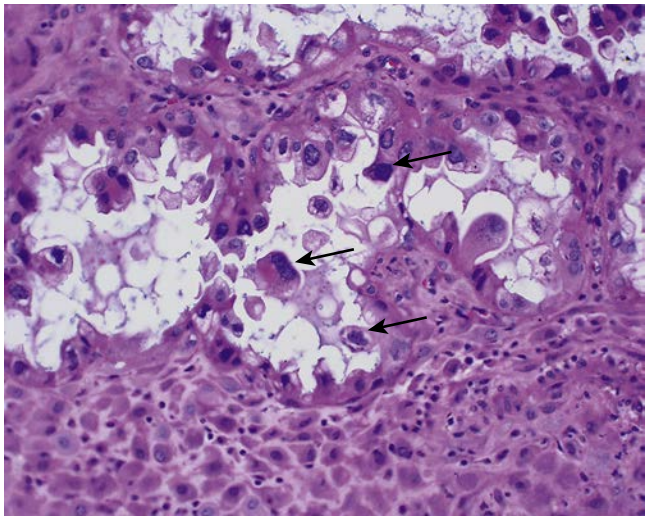

Figure 1.9 Photomicrograph of the Arias-Stella reaction. hCG action results in nuclear enlargement in endometrial glandular cells *(arrows)* resulting in visual characteristics of malignant cells. Magnification, ×200. (Courtesy of Rex Bentley, MD.)

A functional progesterone receptor requires interaction with "chaperone" proteins. In mice, one of these proteins, named *FK506 binding protein 52 (FKBP52)*, is expressed in the endometrium during the window of receptivity, and a loss of function mutation disrupts decidualization.

LIF is a cytokine produced by endometrial glandular cells around the time of implantation. LIF acts on EVT to increase the fibronectin production necessary for embryo attachment and invasion. Mice lacking expression of LIF (knockout) have both failure of decidualization and implantation (Chen, 2000).

Indian hedgehog (Ihh) protein is a morphogen produced by luminal epithelial cells under the control of progesterone. Morphogens are signaling proteins that diffuse throughout the decidua yielding a concentration gradient. Signaling is dependent on the concentration in a given area. *Ihh* knockout mice fail to decidualize or implant (Ramathal, 2010).

EMBRYO-ENDOMETRIAL COMMUNICATION

Implantation involves molecular interactions between the embryo and the adjacent endometrium. For example, the embryo produces heparin-binding epidermal growth factor-like growth factor (HB-EGF), which is both found on the cell membrane and is released from the cell (soluble). HB-EGF induces expression of itself in the adjacent endometrial cells (auto-induction loop). HB-EGF on the endometrial cells then acts to attach the embryo via EGF receptors expressed on the embryo (Lim, 2009). Additionally, the soluble HB-EGF from the embryo induces expression of cyclooxygenase to increase prostacyclin (PGI_2) in the endometrium resulting in enhanced endometrial vascular permeability to help with embryo invasion (Kim, 1999).

IMMUNOLOGY OF IMPLANTATION

The paternal contribution to the embryo results in the mother being exposed to allogenic cells. Although villous trophoblasts do not express major human leukocyte antigens (HLA), the EVTs express HLA-C, E, and G, which may be recognized by

the maternal immune system. Thus the maternal immune system must be locally suppressed to prevent rejection.

The majority of immune cells in the decidua are uterine natural killer (uNK) cells. These cells are present in the secretory endometrium, under the control of progesterone, and increase in number with pregnancy to form an infiltrate around the invading EVT. These cells start to dissipate in the second trimester. uNK cells are not cytotoxic to trophoblast cells and in fact appear to be supportive. A low number of uNK cells in the decidua of early pregnancy is associated with poor invasion of the EVT. Cytokines such as interferon gamma and angiogenic factors secreted by uNK cells are key to proper EVT development and function.

T-helper (Th) cells are also found in the decidua and are functionally classified as Th1 (cellular immunity), Th2 (humoral immunity), Th3 (production of transforming growth factor-beta for immunosuppression), and Tr1 (production of interleukin 10 for immunosuppression) (Saito, 2007). In early pregnancy, there is an increase in the percentage of decidual Th2 and Th3 cells.

T-regulatory cells (Tregs) function in antigen recognition for future immune tolerance. Mice lacking Treg cells experience abortion when mated with an allogenic male but not when mated with a syngenic male (Darasse-Jèze, 2006). These cells are key in developing tolerance to male antigens. Development of immunity to specific paternal antigens may explain observations including lower preeclampsia rates in women exposed to their partner's semen prior to pregnancy compared with women conceiving with donor insemination (Salha, 1999), and the lower preeclampsia rate in the second pregnancy with the same partner as opposed to a new partner.

EARLY ORGANOGENESIS IN THE EMBRYONIC PERIOD

During the third week after fertilization, the primitive streak forms in the caudal portion of the embryonic disk, and the embryonic disk begins to grow and change from a circular to a pear-shaped configuration. At that point the epithelium superiorly is considered ectoderm and will eventually give rise to the developing central nervous system, and the epithelium facing downward toward the yolk sac is endoderm. During this week the neuroplate develops with its associated notochordal process. By the sixteenth day after conception the third primitive germ layer, the intraembryonic mesoderm, begins to form between the ectoderm and endoderm. Early mesoderm migrates cranially, passing on either side of the notochordal process to meet in front in the formation of the cardiogenic area. The heart soon develops from this area. Later in the third week, extraembryonic mesoderm joins with the yolk sac and the developing amnion to contribute to the developing membranes.

An intraembryonic mesoderm develops on each side of the notochord and neural tube to form longitudinal columns, the paraxial mesoderm. Each paraxial column thins laterally into the lateral plate mesoderm, which is continuous with the extraembryonic mesoderm of the yolk sac and the amnion. The lateral plate mesoderm is separated from the paraxial mesoderm by a continuous tract of mesoderm called the *intermediate mesoderm*. By the twentieth day, paraxial mesoderm begins to divide into paired linear bodies known as *somites*. About 38 pairs of somites form during the next 10 days. Eventually a total of 42 to 44 pairs will develop, and these will give rise to body musculature (O'Rahilly, 1979).

Angiogenesis, or blood vessel formation, can be seen in the extraembryonic mesoderm of the yolk sac by day 15 or 16. Embryonic vessels can be seen about 2 days later and develop when mesenchymal cells known as *angioblasts* aggregate to form masses and cords called *blood islands*. Spaces then appear within these islands, and the angioblasts arrange themselves around these spaces to form primitive endothelium. Isolated vessels form channels and then grow into adjacent areas by endothelial budding. Primitive blood cells develop from endothelial cells as the vessels develop on the yolk sac and allantois. However, blood formation does not begin within the embryo until the second month of gestation, occurring first in the developing liver and later in the spleen, bone marrow, and lymph nodes. Separate mesenchymal cells surrounding the primitive endothelial vessels differentiate into muscular and connective tissue elements. The primitive heart forms in a similar manner from mesenchymal cells in the cardiogenic area. Paired endothelial channels, called *heart tubes*, develop by the end of the third week and fuse to form the primitive heart. By the twenty-first day, this primitive heart has linked up with blood vessels of the embryo, forming a primitive cardiovascular system. Blood circulation starts about this time, and the cardiovascular system becomes the first functioning organ system within the embryo (Clark, 1987). All the organ systems form between the fourth week and seventh week of gestation.

A teratogenic event that takes place during the embryonic period gives rise to a constellation of malformations related to the organ systems that are actively developing at that particular time. Thus cardiovascular malformations tend to occur because of teratogenic events early in the embryonic period, whereas genitourinary abnormalities tend to result from later events. Teratogenic effects before implantation often cause loss of the embryo but not malformations. The effects of a particular teratogen depend on the individual's genetic makeup, other environmental factors in play at the time, the embryonic developmental stage during which the teratogenic exposure occurred, and in some cases the dose of the teratogen and the duration of exposure. Some teratogens in and of themselves are actually harmless, but their metabolites cause the damage. Teratogens may be chemical substances and their by-products, or they may be physical phenomena, such as temperature elevation and irradiation. The embryo is most sensitive to teratogens during organogenesis of the embryonic period from 18 to 56 days postconception. Prior to day 18, exposure is most likely to result in either embryo death with miscarriage or no effect, as the majority of cells are pluripotent (Polifka, 2002). Teratogen exposure after the embryonic period of development may injure or kill the embryo or cause developmental and growth retardation but usually will not be responsible for specific malformations. The period of embryonic development is said to be complete at 56 days (8 weeks) from fertilization or 70 days (10 weeks) from the last menstrual period followed by the fetal stage.

DEVELOPMENT OF THE GENITOURINARY SYSTEM

The development of the genital organs is intimately involved with the development of the renal system.

RENAL DEVELOPMENT

Nephrogenic cords develop from the intermediate mesoderm as early as the 2-mm embryo stage, beginning in the more cephalad portions of the embryo. Three sets of excretory ducts and tubules

develop bilaterally (Little, 2010). The first, the pronephros, with its pronephric ducts, forms in the most cranial portion of the embryo at about the beginning of the fourth week after conception. The tubules associated with the duct probably have no excretory function in the human, but the caudal end will form the adrenal gland. Late in the fourth week, a second set of tubules, the mesonephric tubules, and their accompanying mesonephric ducts begin to develop. These are associated with tufts of capillaries, or glomeruli, and tubules for excretory purposes. Thus the mesonephros functions as a fetal kidney, producing urine for about 2 or 3 weeks. As new tubules develop, those derived from the more cephalad tubules degenerate. Usually about 40 mesonephric tubules function on either side of the embryo at any given time. The gonads arise from the central region of the mesonephros. The metanephros, or permanent kidney, begins its development early in the fifth week of gestation and starts to function late in the seventh or early in the eighth week. The metanephros develops both from the metanephrogenic mass of mesoderm, which is the most caudal portion of the nephrogenic cord, and from its duct system, which is derived from the metanephric diverticulum (ureteric bud). It is a cranially growing outpouching of the mesonephric duct close to where it enters the cloaca. The metanephric duct system gives rise to the ureter, the renal pelvis, the calyces, and the collecting tubules of the adult kidney. A critical process in the development of the kidney requires that the cranially growing metanephric diverticulum meets and fuses with the metanephrogenic mass of mesoderm so that formation of the kidney can take place. Originally the metanephric kidney is a pelvic organ, but by differential growth it becomes located in the lumbar region (Moritz, 1999).

The fetus produces urine starting at 8 weeks' gestation (Underwood, 2005). Starting in the second trimester, fetal urine is a major contributor to amniotic fluid volume. The fetus may swallow the amniotic fluid and recirculate it through the digestive system. Congenital abnormalities that impair normal development or function of the fetal kidneys generally result in little or no amniotic fluid (oligohydramnios or anhydramnios), whereas structural abnormalities of the gastrointestinal tract or neuromuscular conditions that prevent the fetus from swallowing can lead to excess amniotic fluid (polyhydramnios).

BLADDER AND URETHRA

The embryonic cloaca is divided by the urorectal septum into a dorsal rectum and a ventral urogenital sinus. The urogenital sinus, in turn, is divided into three parts: the cranial portion (the vesicourethral canal), which is continuous with the allantois; a middle pelvic portion; and a caudal urogenital sinus portion, which is covered externally by the urogenital membrane. The epithelium of the developing bladder is derived from the endoderm of the vesicourethral canal. The muscular layers and serosa of the bladder develop from adjacent splanchnic mesenchyme. As the bladder develops, the caudal portion of the mesonephric ducts is incorporated into its dorsal wall. The portion of the mesonephric duct distal to the points where the metanephric duct is taken up into the bladder becomes the trigone of the bladder. Although this portion is mesoderm in origin, it is probably epithelialized eventually by endodermal epithelium from the urogenital sinus. In this way the ureters, derived from the metanephric duct, come to open directly into the bladder.

In the male the mesonephric ducts open into the urethra as the ejaculatory ducts. Also in the male, mesenchymal tissue surrounding the developing urethra where it exits the bladder develops into the prostate gland, through which the ejaculatory ducts traverse. Figure 1.10 demonstrates graphically the development of the male and female urinary systems.

The epithelium of the female urethra is derived from endoderm of the vesicourethral canal. The urethral sphincter develops from a mesenchymal condensation around the urethra after the division of the cloaca in the 12- to 15-mm embryo. Following the opening of the anal membrane at the 20- to 30-mm stage, the puborectalis muscle appears. At 15 weeks' gestation, striated muscle can be seen, and a smooth muscle layer thickens at the level of the developing bladder neck, forming the inner part of the urethral musculature. Thus the urethral sphincter is composed of both central smooth muscle and peripheral striated muscle. The sphincter develops primarily in the anterior wall of the urethra in a horseshoe or omega shape (Matsuno, 1984).

MOLECULAR BASIS OF SEX DIFFERENTIATION

Genetic sex is determined at the time of conception. A Y chromosome is necessary for the development of the testes, and the testes are responsible for the organization of the sexual duct system into a male configuration and for the suppression of the paramesonephric (müllerian) system of the female. In the absence of a Y chromosome or in the absence of a gonad, development will be female in nature. Male differentiation is determined by expression of the *SRY* gene found on the short arm of the Y chromosome. SRY protein is a transcription factor and expression is unique to the Sertoli cell of the developing testis. SRY induces expression of another transcription factor, SOX9, which is also obligatory for male sex differentiation. A loss of function mutation of either SRY or SOX9 results in XY sex reversal, in which genetic males are phenotypic females. Several genes regulate SRY/SOX9 expression including Wilms' tumor suppressor 1 (Wt1) and steroidogenic factor 1 (Sf1). WT1 is a transcription factor expressed in both urinary tract and gonadal tissue. A loss of function mutation results in glomerulosclerosis and gonadal dysgenesis. Sf1 encodes a nuclear receptor necessary for steroidogenesis, gonadal differentiation, and adrenal formation. A loss of function mutation is associated with adrenal failure and XY sex reversal (Ozisik, 2003).

Although ovarian formation can only occur in the absence of *SRY/SOX9*, there are unique genes necessary for development. *FoxL2* encodes a transcription factor necessary for granulosa cell expansion. A loss of function mutation causes ovarian failure with other associated abnormalities found in blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (De Baere, 2001). *BMP15,* located on the X chromosome, and *GDF9* on chromosome 5 encode growth factors expressed in oocytes required for granulosa cell proliferation. A heterozygous loss of function mutation results in ovarian failure (Di Pasquale, 2004).

The understanding of the molecular basis of sex determination continues to expand with more than 25 genes so far identified in the process (Wilhelm, 2007).

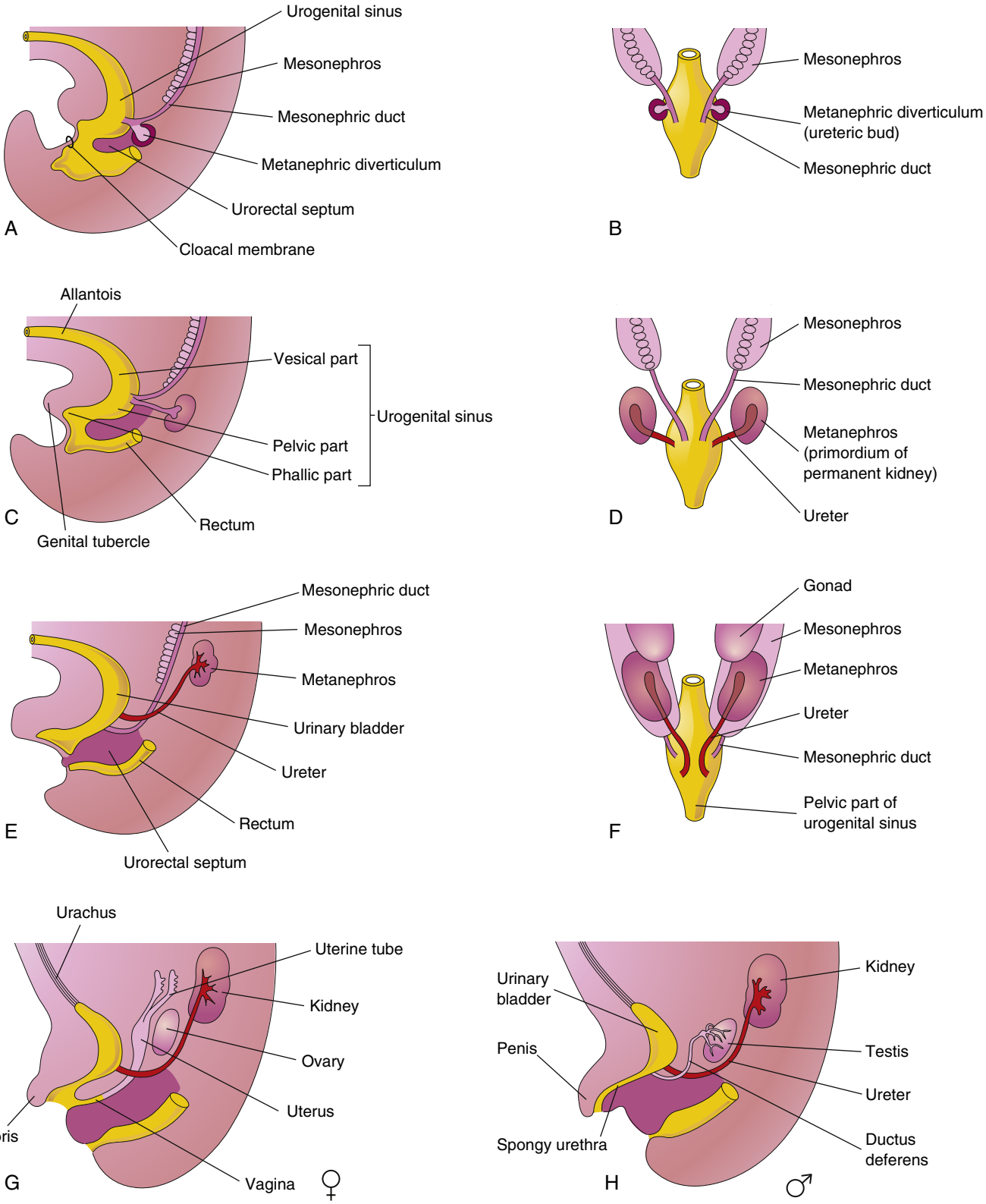

Figure 1.10 Diagrams showing division of the cloaca into the urogenital sinus and rectum; absorption of the mesonephric ducts; development of the urinary bladder, urethra, and urachus; and changes in the location of the ureters. **A,** Lateral view of the caudal half of a 5-week embryo. **B, D,** and **F,** Dorsal views. **C, E, G,** and **H,** Lateral views. The stages shown in **G** and **H** are reached by the twelfth week. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

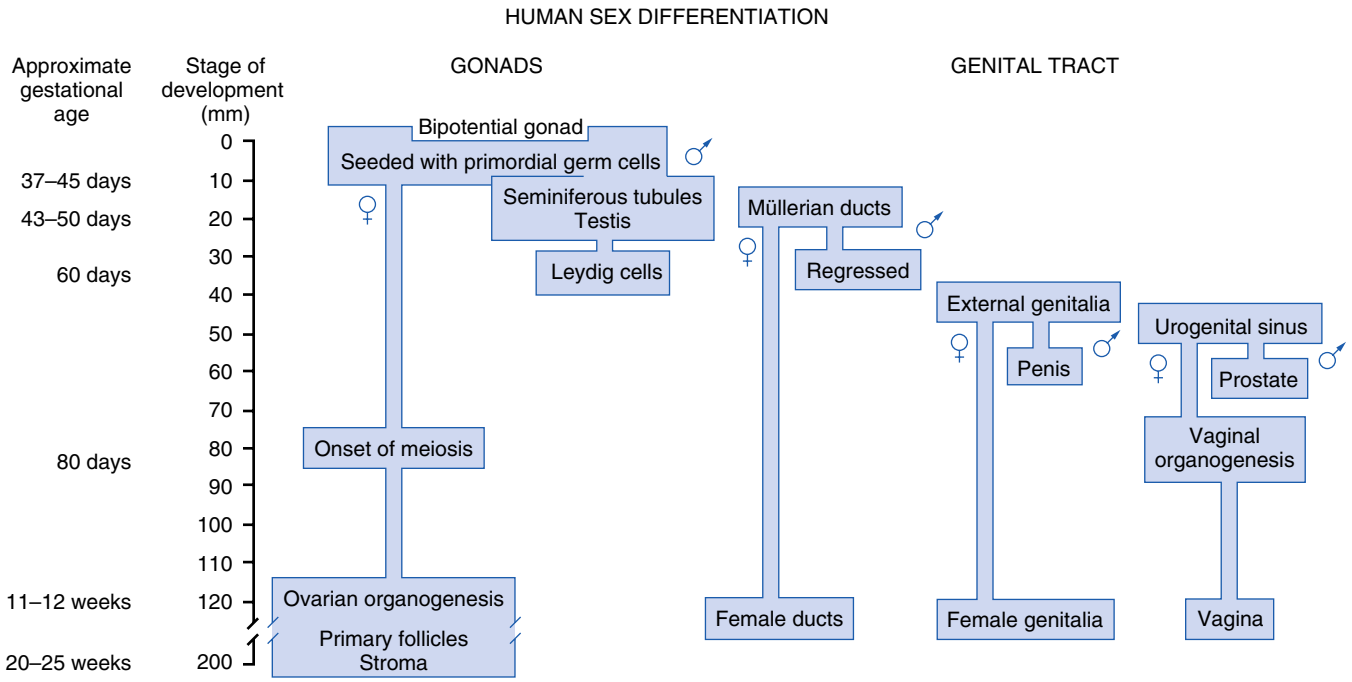

Figure 1.11 Development of sexual differentiation in the human. Note the lag from male to female development. (Modified from Grumbach MM, Hughes IA, Conte FA. Disorders of sex differentiation. In: Larsen PR, Kronenberg HM, Melmed S, et al, eds. *Williams Textbook of Endocrinology.* 10th ed. Philadelphia: WB Saunders: 2003:870.)

GENITAL DEVELOPMENT

Male gonadal development precedes female development (Fig. 1.11). During the fifth week after conception, coelomic epithelium, later known as *germinal epithelium*, thickens in the area of the medial aspect of the mesonephros. As germinal epithelial cells proliferate, they invade the underlying mesenchyme, producing a prominence known as the *gonadal ridge*. In the sixth week the primordial germ cells, which have formed at about week 4 in the wall of the yolk sac, migrate up the dorsal mesentery of the hindgut and enter the undifferentiated gonad. The somatic cells of the primitive gonadal ridge then differentiate into interstitial cells (Leydig cells) and Sertoli cells. As they do so, the primordial germ cells and Sertoli cells become enclosed within seminiferous tubules, and the interstitial cells remain outside these tubules. Sertoli cells are encased in the seminiferous tubules in the seventh and eighth weeks. In the eighth week, Leydig cells differentiate and begin to produce testosterone. At this point the mesonephric (wolffian) duct differentiates into the vas deferens, epididymis, and seminal vesicles, whereas the paramesonephric duct (müllerian duct) is suppressed because of the secretion and action of anti-müllerian hormone (AMH), also known as *müllerian inhibitory substance* (MIS), by Sertoli cells.

Primary sex cords, meanwhile, have condensed and extended to the medullary portion of the developing testes. They branch and join to form the rete testis. The testis therefore is primarily a medullary organ, and eventually the rete testis connects with the tubules of the mesonephric system and joins the developing epididymal duct.

Development of the ovary occurs at about the eleventh or twelfth week, although the primordial germ cells have migrated several weeks earlier to the germinal ridge (Fig. 1.12). Two functional X chromosomes are necessary for optimal development of the ovary. Deletion of either the short arm or the long arm of a single X chromosome precludes normal ovarian function, with the former being associated with Turner syndrome (Simpson, 1999). The processes of gonadal development are schematically summarized in Figure 1.13.

GENITAL DUCT SYSTEM

Early in embryonic life, two sets of paired genital ducts develop in each sex: the mesonephric (wolffian) ducts and the paramesonephric (müllerian) ducts. The mesonephric duct development precedes the paramesonephric duct development. The paramesonephric ducts develop on each side of the mesonephric ducts from the evaginations of the coelomic epithelium. The more cephalad ends of the ducts open directly into the peritoneal cavity, and the distal ends grow caudally, fusing in the lower midline to form the uterovaginal primordium. This tubular structure joins the dorsal wall of the urogenital sinus and produces an elevation, the müllerian tubercle. The mesonephric ducts enter the urogenital sinus on either side of the tubercle.

MALE GENITAL DUCTS

Seminiferous tubules are produced in the fetal testes during the seventh and eighth weeks after conception. During the eighth week, interstitial (Leydig) cells differentiate and begin to produce testosterone. Male internal genital development is mainly dependent on testosterone, whereas external genitalia are dependent on 5α-dihydrotestosterone (DHT). Testosterone produced

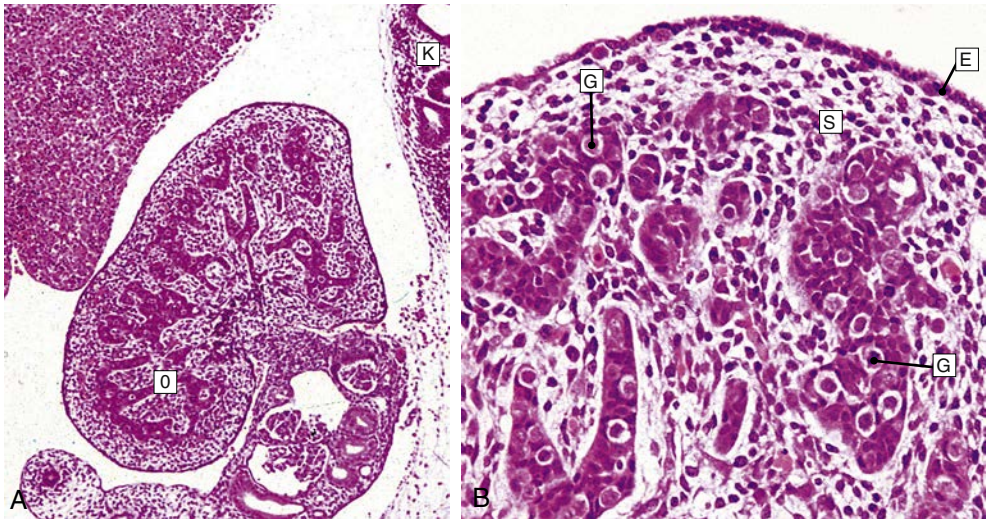

Figure 1.12 Ovary in embryo. **A,** The developing ovary *(O)* in a 9-week-old fetus is shown close to the developing kidney *(K)*. **B,** At this stage of development, the columns of primordial germ cells *(G)* are embedded in a mesenchymal stroma *(S)* covered by a layer of cuboidal surface cells *(E)*. (From Stevens A, Lowe J. *Human Histology.* 3rd ed. Philadelphia: Elsevier Mosby; 2005:357.)

by the Leydig cells stimulates growth and development of the wolffian duct structures of vas deferens, epididymis, and seminal vesicles. DHT formed in target tissues by the enzyme type 2 5α-reductase is responsible for formation of the prostate, scrotum, and penis (Thigpen, 1993).

Maternal hCG production may be key to male genital development. The maximum serum level of hCG at approximately 8 weeks postconception or 10 menstrual weeks correlates with the timing of male genital formation, and the highest fetal testosterone levels are seen at 11 to 17 weeks with a subsequent decline (Reyes, 1974). hCG acting via the LH receptor is responsible for stimulating Leydig cell testosterone production.

The bulbourethral glands, which are small structures that develop from outgrowths of endodermal tissue from the membranous portion of the urethra, incorporate stroma from the adjacent mesenchyme. The most distal portion of the paramesonephric duct remains, in the male, as the appendix of the testes. The most proximal end of the paramesonephric duct remains as a small outpouching within the body of the prostate gland, known as the prostatic utricle. Rarely, the prostatic utricle is developed to the point where it will excrete a small amount of blood and cause hematuria in adult life (Schuhrke, 1978).

FEMALE GENITAL DUCTS

In the absence of AMH, the mesonephric ducts regress, and the paramesonephric ducts develop into the female genital tract. This process begins at about 6 weeks and proceeds in a cephalad to caudal fashion. The more cephalad portions of the paramesonephric ducts, which open directly into the peritoneal cavity, form the fallopian tubes. The fused portion, or uterovaginal primordium, gives rise to the epithelium and glands of the uterus and cervix. Endometrial stroma and myometrium are derived from adjacent mesenchyme. Failure of development of the paramesonephric ducts leads to agenesis of the cervix and the uterus referred to as *müllerian agenesis* or Mayer-Rokitansky-Kuster-Hauser syndrome (Langman, 1982). Failure of fusion of the caudal portion of these ducts may lead to a variety of uterine anomalies, including complete duplication of the uterus and cervix or partial duplication of a variety of types, which are outlined in Chapter 11. Peritoneal reflections in the area adjacent to the fusion of the two paramesonephric ducts give rise to the formation of the broad ligaments. Mesenchymal tissue here develops into the parametrium.

Pietryga and Wózniak studied the development of uterine ligaments, documenting the development of the round ligament at the eighth week, the cardinal ligaments at the tenth week, and the broad ligament at week 19. From weeks 8 to 17, the round ligament is connected to the uterine tube (Pietryga, 1992). Beginning at week 18 it comes to arise from the edge of the uterus.

The vagina develops from paired solid outgrowths of endoderm of the urogenital sinus—the sinovaginal bulbs. These grow caudally as a solid core toward the end of the uterovaginal primordium. This core constitutes the fibromuscular portion of the vagina. The sinovaginal bulbs then canalize to form the vagina. Abnormalities in this process may lead to either transverse or horizontal vaginal septa. The junction of the sinovaginal bulbs with the urogenital sinus remains as the vaginal plate, which forms the hymen. This remains imperforate until late in embryonic life, although occasionally, perforation does not take place completely (imperforate hymen). Failure of the sinovaginal bulbs to form leads to agenesis of the vagina (Griffin, 1976).

Auxiliary genital glands in the female form from buds that grow out of the urethra. The buds derive contributions from the surrounding mesenchyme and form the urethral glands and the paraurethral glands (Skene glands). These glands correspond to the prostate gland in males. Similar outgrowths of the urogenital sinus form the vestibular glands (Bartholin glands), which are homologous to the bulbourethral glands in the male. The remnants of the mesonephric duct in the female include a small structure called the *appendix vesiculosa*, a few

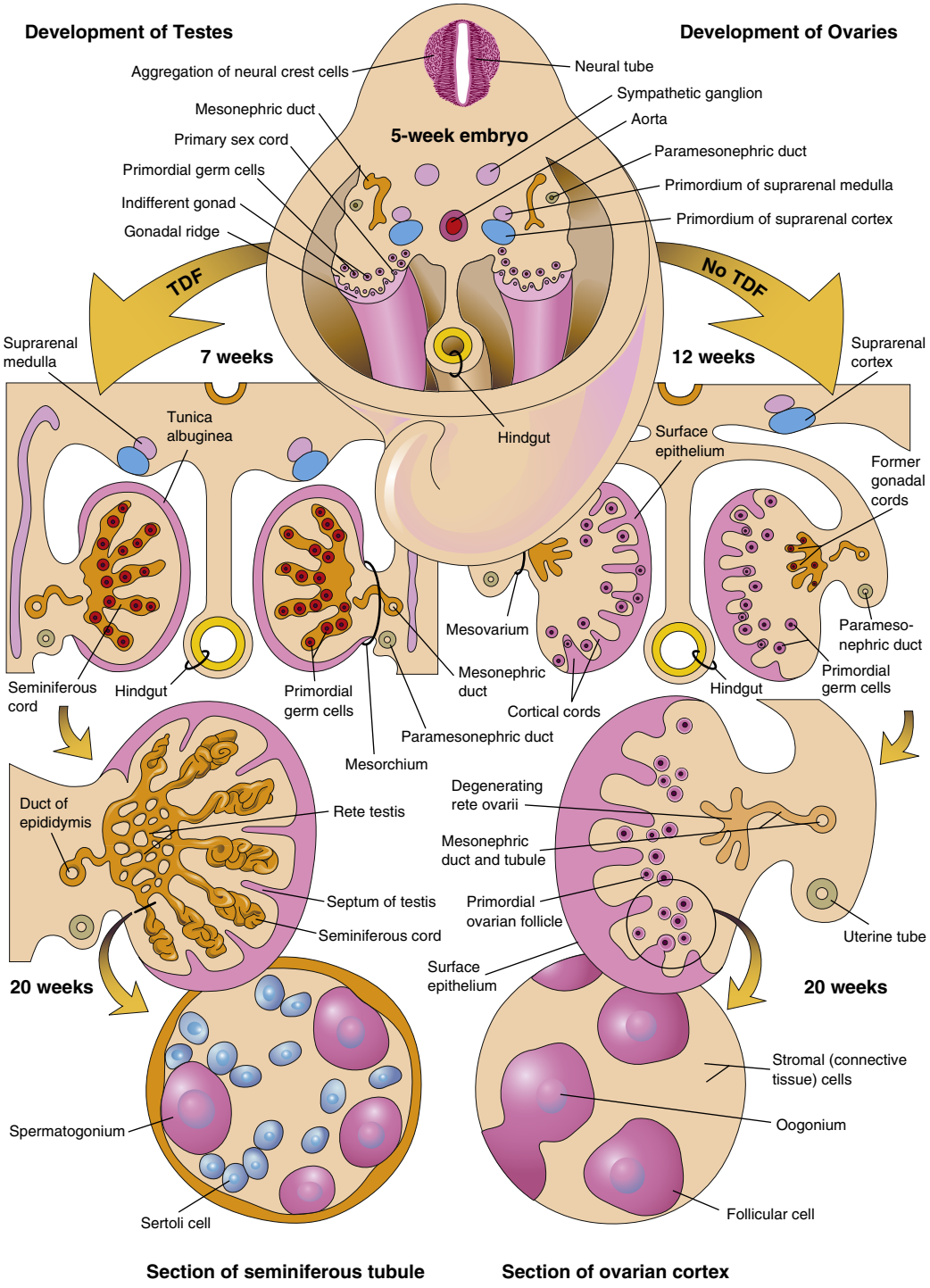

Figure 1.13 Schematic illustration showing differentiation of the indifferent gonads of a 5-week embryo *(top)* into ovaries or testes. Left side shows the development of testes resulting from the effects of the testis-determining factor (TDF), also called the *SRY gene*, located on the Y chromosome. Note that the gonadal cords become seminiferous cords, the primordium of the seminiferous tubules. The parts of the gonadal cords that enter the medulla of the testis form the rete testis. In the section of the testis at the bottom left, observe that there are two kinds of cells: spermatogonia derived from the primordial germ cells and sustentacular (Sertoli) cells derived from mesenchyme. The right side shows the development of ovaries in the absence of TDF. Cortical cords have extended from the surface epithelium of the gonad, and primordial cells have entered them. They are the primordia of the oogonia. Follicular cells are derived from the surface epithelium of the ovary. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

blind tubules in the broad ligaments (the epoophoron), and a few blind tubules adjacent to the uterus (collectively called the *paroöphoron*). Remnants of the mesonephric duct system are often present in the broad ligaments or may be present adjacent to the uterus or the vagina as Gartner duct cysts (Deppisch, 1975). The epoophoron or paroöphoron may develop into cysts. Cysts of the epoophoron are known as *paraovarian cysts* (Chapter 18). Remnants of the paramesonephric duct in the female may be seen as a small, blind cystic structure attached by a pedicle to the distal end of the fallopian tube—the hydatid of Morgagni. Table 1.2 categorizes the adult derivatives and residual remnants of the urogenital structures in both the male and the female. Figure 1.14 outlines schematically the development of the internal sexual organs in both sexes.

EXTERNAL GENITALIA

In the fourth week after fertilization, the genital tubercle develops at the ventral tip of the cloacal membrane. Two sets of lateral bodies—the labioscrotal swellings and urogenital folds—develop soon after on either side of the cloacal membrane. The genital tubercle then elongates to form a phallus in both males and females. By the end of the sixth week, the cloacal membrane is joined by the urorectal septum. The septum separates the cloaca into the urogenital sinus ventrally and the anal canal and rectum dorsally (Hynes, 2004). The point on the cloacal membrane where the urorectal septum fuses becomes the location of the perineal body in later development. The cloacal membrane is then divided into the ventral urogenital membrane and the dorsal anal membrane. These membranes then open, yielding the vulva and the anal canal. Failure of the anal membrane to open gives rise to an imperforate anus. With the opening of the urogenital membrane, a urethral groove forms on the undersurface of the phallus, completing the undifferentiated portion of external genital development. Differences between male and female embryos can be noted as early as the ninth week, but the distinct final forms are not noted until 12 weeks' gestation (Fig. 1.15).

The phallus grows in length to form a penis, and the urogenital folds are pulled forward to form the lateral walls of the urethral groove on the undersurface of the penis. These folds then fuse to form the penile urethra. Defects in fusion of various amounts give rise to various degrees of hypospadias. The skin at the distal margin of the penis grows over the glans to form the prepuce (foreskin). The vascular portion of the penis (corpora cavernosa penis and corpus cavernosum urethrae) arises from the mesenchymal tissue of the phallus. Finally, the labioscrotal swellings grow toward each other and fuse in the midline to form the scrotum. Later in embryonic life, usually at about the twenty-eighth week, the testes descend through the inguinal canal guided by the gubernaculum (Frey, 1984).

Feminization of the undifferentiated external genitalia occurs in the absence of androgen stimulation. The embryonic phallus does not demonstrate rapid growth and becomes the clitoris. Urogenital folds do not fuse except in front of the anus. The unfused urogenital folds form the labia minora. The labioscrotal folds fuse posteriorly in the area of the perineal body but laterally remain as the labia majora. Beyond 12 weeks' gestation, the labioscrotal folds will not fuse if the fetus is exposed to androgens, though masculinization may occur in other organs of the external genitalia such as growth of the clitoris. The labioscrotal

Table 1.2 Male and Female Derivatives of Embryonic Urogenital Structures

	Derivatives	
Embryonic Structure	**Male**	**Female**
Labioscrotal swellings	Scrotum	Labia majora
Urogenital folds	Ventral portion of penis	Labia minora
Phallus	Penis	Clitoris
	Glans, corpora cavernosa penis, and corpus spongiosum	Glans, corpora cavernosa, bulb of the vestibule
Urogenital sinus	Urinary bladder	Urinary bladder
	Prostate gland	Urethral and paraurethral glands
	Prostatic utricle	Vagina
	Bulbourethral glands	Greater vestibular glands
	Seminal colliculus	Hymen
Paramesonephric duct	Appendix of testes	Hydatid of Morgagni
		Uterus and cervix
		Fallopian tubes
Mesonephric duct	Appendix of epididymis	Appendix vesiculosis
	Ductus of epididymis	Duct of epoophoron
	Ductus deferens	Gartner duct
	Ejaculatory duct and seminal vesicle	—
Metanephric duct	Ureters, renal pelvis, calyces, and collecting system	Ureter, renal pelvis, calyces, and collecting system
Mesonephric tubules	Ductuli efferentes	Epoophoron
	Paradidymis	Paroöphoron
Undifferentiated gonad	Testis	Ovary
Cortex	Seminiferous tubules	Ovarian follicles
Medulla	—	Medulla
	Rete testis	Rete ovarii
Gubernaculum	Gubernaculum testis	Round ligament of uterus

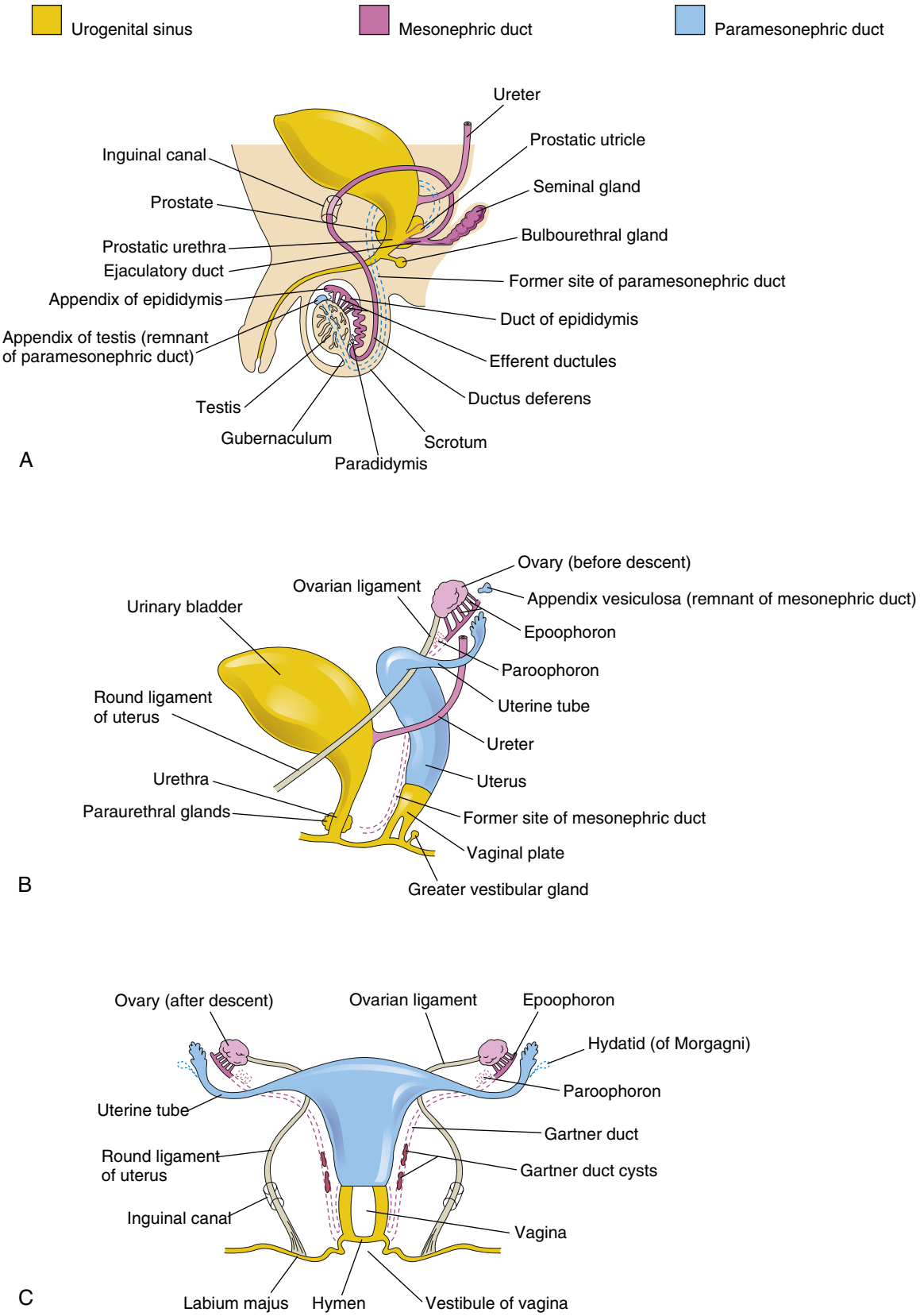

Figure 1.14 Schematic drawings illustrating development of the male and female reproductive systems from the genital ducts and urogenital sinus. Vestigial structures are also shown. **A,** Reproductive system in a newborn male. **B,** Female reproductive system in a 12-week fetus. **C,** Reproductive system in a newborn female. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

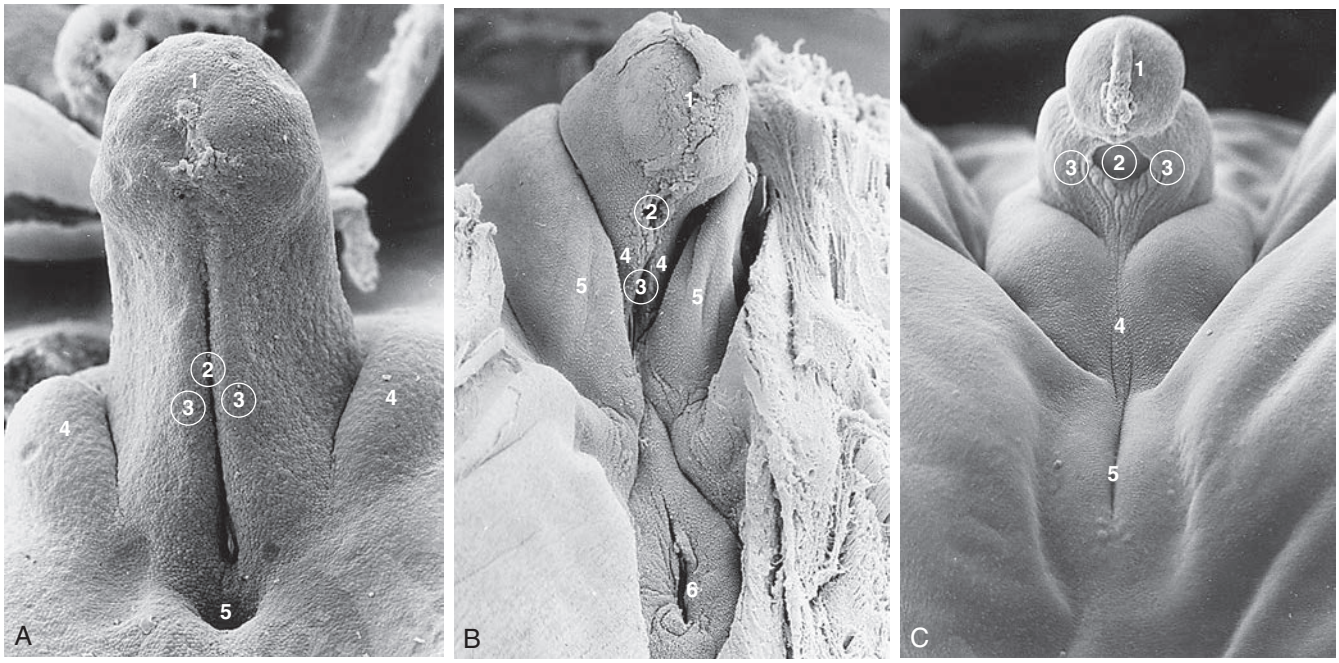

Figure 1.15 Scanning electron micrographs (SEMs) of the developing male external genitalia. **A,** SEM of the perineum during the indifferent state of a 17-mm, 7-week embryo (×100). *1*, Developing glans of penis with the ectodermal cord. *2*, Urethral groove continuous with the urogenital sinus. *3*, Urogenital folds. *4*, Labioscrotal swellings. *5*, Anus. **B,** External genitalia of a 7.2-cm, 10-week female fetus (×45). *1*, Glans of clitoris. *2*, External urethral orifice. *3*, Opening into urogenital sinus. *4*, Urogenital folds (labia minora). *5*, Labioscrotal swelling (labia majora). *6*, Anus. **C,** SEM of the external genitalia of a 5.5-cm, 10-week male fetus (×40). *1*, Glans of penis with ectodermal cord. *2*, Remains of urethral groove. *3*, Urogenital folds in the process of closing. *4*, Labioscrotal swelling fusing to form the raphe of the scrotum. *5*, Anus. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

folds fuse anteriorly to form the mons pubis. A portion of the urogenital sinus between the level of the hymen and the labia develops into the vestibule of the vagina, into which the urethra, the vagina, and the ducts of Bartholin glands enter. Female external genitalia are intensely estrogen receptor–positive compared with the genitalia of the male. These receptors may be seen primarily in the stroma of the labia minora and in the periphery of the glans and interprepuce (Kalloo, 1993). The presence of such receptors suggests that there may be a direct role of maternal estrogens in the development of female external genitalia.

Virilization, masculinization, of a female (karyotype XX) fetus may occur from exposure to androgens, either from the mother or through fetal androgens as a result of genetic deficiencies in the steroid biosynthetic pathway such as occurs in congenital adrenal hyperplasia.

The ovaries do not descend into the labioscrotal folds. A structure similar to the gubernaculum develops in the inguinal canal, giving rise to the round ligaments, which suspend the uterus in the adult. Figure 1.16 summarizes the development of the external genitalia in each sex.

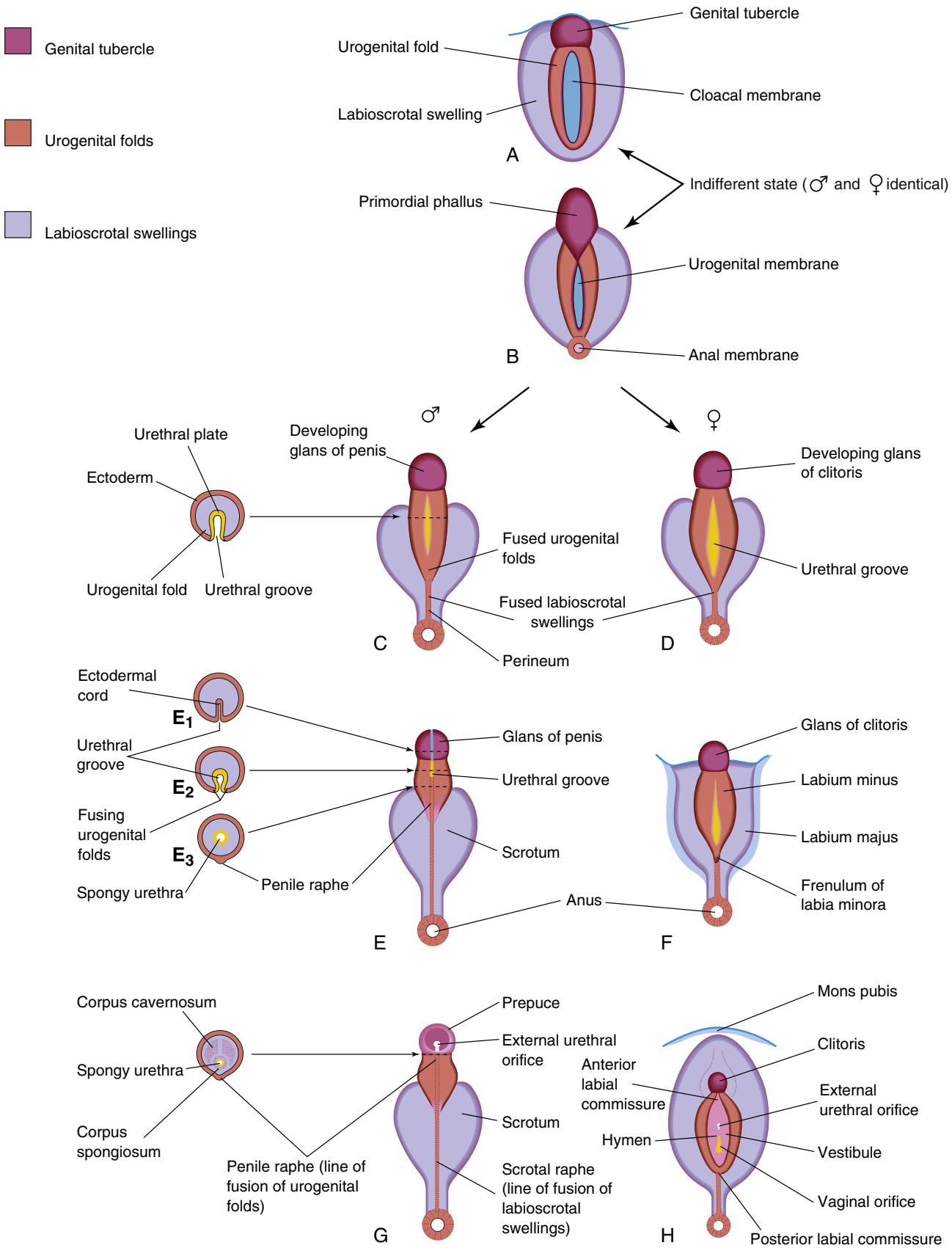

Figure 1.16 Development of the external genitalia. **A** and **B,** Diagrams illustrating the appearance of the genitalia during the indifferent state (fourth to seventh weeks). **C, E,** and **G,** Stages in the development of male external genitalia at 9, 11, and 12 weeks, respectively. To the left are schematic transverse sections of the developing penis, illustrating formation of the spongy urethra. **D, F,** and **H,** Stages in the development of female genitalia at 9, 11, and 12 weeks, respectively. (From Moore KL, Persaud TVN. *The Developing Human: Clinically Oriented Embryology.* 7th ed. Philadelphia: WB Saunders; 2003.)

KEY POINTS

- Oocyte meiosis is arrested at the prophase I from the fetal period until the time of ovulation.
- Fertilization occurs in the ampulla of the fallopian tube before the second polar body is cast off.
- After fertilization, first cell division leading to the two-cell embryo takes about 26 hours.
- The human embryo enters the uterus somewhere between 4 and 5 days after conception at the blastocyst stages of development.
- Implantation occurs when trophoblastic cells contact endometrium and burrow beneath the surface. This generally takes place 3 days after the embryo enters the uterus.
- Twinning due to embryo splitting may occur at any time until the formation of the blastocyst, after which time each cell is no longer pluripotent.
- The earliest fetal epithelium to develop is the ectoderm, the second is the endoderm, and the third is the mesoderm.
- hCG is secreted by the syncytiotrophoblast at about the time of implantation. It doubles in quantity every 1.2 to 2 days until 7 to 9 weeks' gestation.
- Angiogenesis is seen by day 15 or 16. Embryonic heart function begins in the third week of gestation.
- Organogenesis is complete by postconception day 56.
- The mesonephric duct system gives rise in the male to the epididymis, vas deferens, and seminal vesicles. Remnants of the mesonephric duct system in the female remain as parovarian cysts and the Gartner duct.
- The paramesonephric duct system develops in the female to give rise to the fallopian tube, uterus, and cervix. Remnants give rise to the hydatid of Morgagni at the end of the fallopian tubes. Remnants in the male remain as the appendix of the testes and prostatic utricle. This duct system is suppressed in the male by the action of AMH.
- The vagina develops from the sinovaginal bulbs, which are outgrowths of the urogenital sinus. Failure of these bulbs to form leads to agenesis of the vagina.
- The adult kidney develops from the metanephros, and its collecting system (ureter and calyceal system) develops from the metanephric (ureteric) bud from the mesonephric duct.
- The urinary bladder develops from the urogenital sinus.
- The *SRY* gene on the Y chromosome is responsible for the development of testes. Without the presence of this gene, the gonadal development is ovarian. With the absence of Sertoli cells, AMH is not produced, the paramesonephric duct system develops into a phenotypic female configuration, and the mesonephric duct system is suppressed.
- The genital tubercle elongates to form the penis in the male and the clitoris in the female.
- Two functional X chromosomes are necessary for optimal development of the ovary.

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2
Reproductive Genetics

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GENETIC BASIS OF DISEASE

Medical research and medical care have been profoundly influenced by the advancement of science that succeeded in sequencing the human genome, allowing scientists to concentrate on translating this genomic text to meaningful prose. As the code is deciphered with increasing resolution, it is apparent that virtually all human diseases have an underlying genetic component, although the conversion from *genotype* to ultimate clinical *phenotype* is not always easily understood.

The overarching goals of medical care have not changed: diagnose, treat, and focus on disease prevention. The new promise of medicine in the postgenomic era is to individualize these goals, such that lifestyle interventions, screening modalities, and pharmaceuticals can be tailored to each person based on his or her unique genomic sequence. These goals have begun to materialize, through examples such as detailed breast cancer screening for women in families with known *BRCA1* or *BRCA2* mutations, or tailored chemotherapeutic regimens based on molecular testing of an individual tumor. Furthermore, there is unprecedented public accessibility of the technology for genomic screening or application of genetic information to medical treatment. Since completion of the Human Genome Project in April 2003, technology has advanced at an extraordinary pace to allow *high-throughput* data generation at increasingly reasonable cost. High-throughput methods involve automation of experiments or assays to allow for simultaneous large-scale repetition. Over the first post-genomic decade, the time to prepare and sequence a complete human genome plummeted from 13 years to a matter of 3 to 4 days, and the cost dropped from just under $30 million to around $1000 (Topol, 2014).

As a result, genetics is a field that all health care professionals need a basic level of familiarity with, not just the subspecialists. Genetics, genomics, and the technology to interpret the information are now an integral part of mainstream medicine (Table 2.1). The obstetrician/gynecologist is often the first-line provider in helping patients navigate this complicated landscape. This chapter focuses on developing a basic understanding of genetic makeup, heritability, and the most commonly used tools for detecting genetic disorders in patients or their offspring.

BUILDING BLOCKS OF GENETICS

MOLECULAR BUILDING BLOCKS

Genetic information is encoded in *deoxyribonucleic acid (DNA)* in the nucleus of each cell of the body. DNA molecules are made up of two complementary linear sequences of nucleotides intertwined together as a double helix. The backbone of the linear DNA molecule is composed of a phosphate and a pentose sugar (deoxyribose) to which is attached a nitrogen base. Four such bases are found in a DNA molecule: two purines (adenine [A] and guanine [G]) and two pyrimidines (thymine [T] and cytosine [C]). Purine and pyrimidine occur in equal amounts; A is always paired with T in the two strands of the double helix, and G is always paired with C. The order of bases along the molecule is the genetic *sequence*, and the complete sequence of all 6 billion bases in an individual cell nucleus (3 billion paired bases, arranged in linear antisense strands) makes up the *human genome*.

The Central Dogma published by Francis Crick in 1970 remains at the heart of molecular biology (Crick, 1970). The DNA is transcribed to a complementary *ribonucleic acid (RNA)* molecule (messenger RNA), which may be modified by regulatory sequences or three-dimensional (3D) structure. Three-base *codons* are read and translated to amino acids that are linked to form a protein with some function within the cell or organism (Fig. 2.1). The traditional concept of a *gene* refers to a unit of DNA sequence that codes for production of a protein. Surprisingly, with completion of the Human Genome Project, this gene-centric view of biology turned out to be only the tip of the iceberg in understanding the complex manner in which the genetic sequence translates to human life. Of the 3 billion base pairs that make up the genome, only about 1.5% of the assembled sequence codes for proteins. This coding portion, or *exome* contains about 20,000 to 25,000 genes, which is only a fraction of previous estimates that were predicated on gene number correlating with complexity of the species (Gerstein, 2007). There is now significant interest in the remaining 98.5% of the genome, in how it carries out the blueprint of life. There is a growing field of discovery in the regulatory function of specialized noncoding RNA molecules, called *microRNA (miRNA)*, which appear to be the gatekeepers of many biologic processes (Pritchard, 2012).

Table 2.1 Publicly Available Online Resources for Human Genomic Information

General Reference		
National Human Genome Research Institute (NHGRI)		www.genome.gov
Sequence Databases		
GenBank: collection of all publicly available DNA sequences	National Institutes of Health (NIH)	www.ncbi.nlm.nih.gov/genbank
SNPedia: wiki investigating human genetics	River Road Bio, LLC (Cariaso, 2012)	www.SNPedia.com
HapMap: multi-ethnic project to catalog SNP haplotypes	Scientists and funding agencies from Japan, the United Kingdom, Canada, China, Nigeria, and the United States	hapmap.ncbi.nlm.nih.gov
ENCyclopedia Of DNA Elements (ENCODE)	International consortium to annotation functional elements in the genome (Birney, 2007)	www.encodeproject.org
Database of Genomic Variants	The Centre for Applied Genomics, Toronto, Canada (MacDonald, 2014)	dgv.tdag.ca
Genotype/Phenotype Correlation		
Online Mendelian Inheritance in Man (OMIM)	McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine (Amberger, 2015)	omim.org
Genome-Wide Association Study (GWAS) Central	(Beck, 2014)	www.gwascentral.org
Genome Browsers		
Ensembl	Wellcome Trust Sanger Institute/European Bioinformatics Institute	www.ensembl.org
University of California Santa Cruz (UCSC) Genome Bioinformatics	University of California at Santa Cruz	genome.ucsc.edu
NCBI	National Center for Biotechnology Information	www.ncbi.nlm.nih.gov/genome

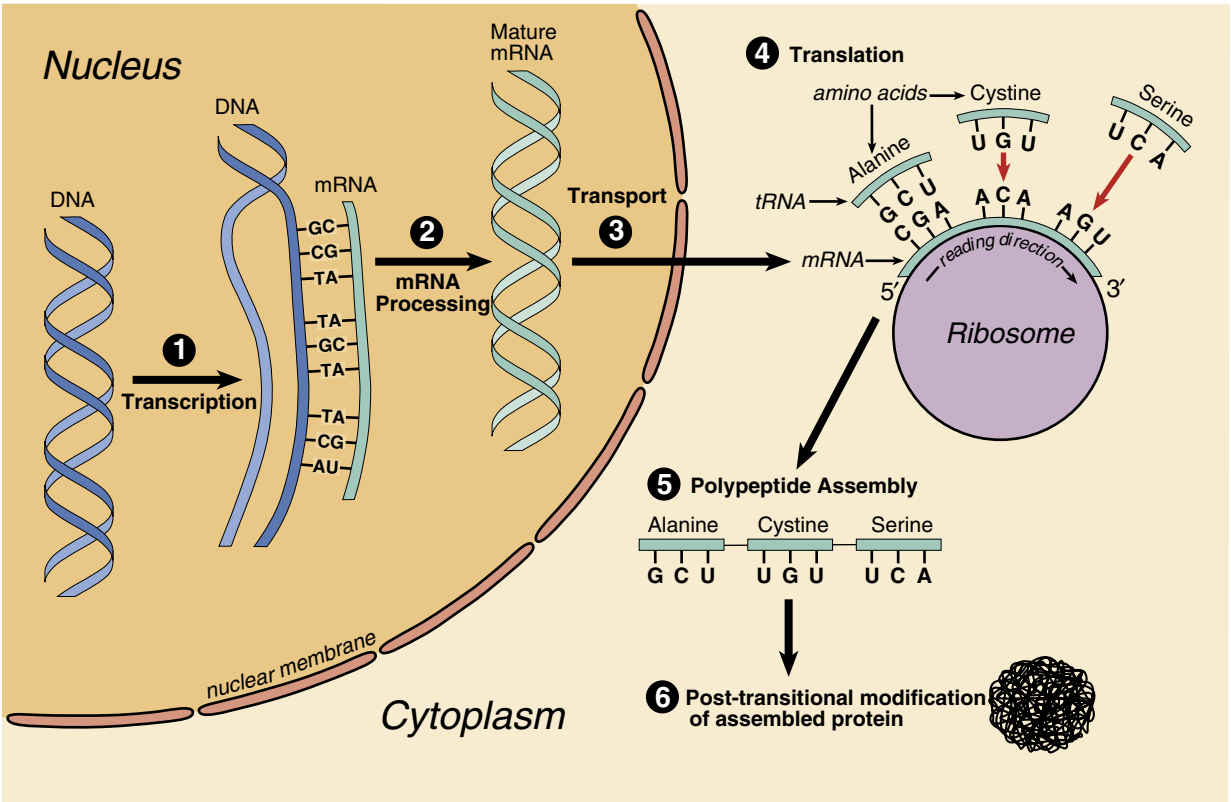

Figure 2.1 Schematic representation of polypeptide production from genetic message to final product. (Courtesy of Edith Cheng, MD.)

MITOSIS/MEIOSIS

The full genome consists of two copies of the total DNA sequence, packaged into two homologous sets of 23 separate *chromosomes* (22 autosome pairs and 1 allosome, or sex chromosome pair). During cell division, an exact replica of this biologic blueprint is passed to each daughter cell through the process of mitosis. The formation of gametes requires even distribution of the chromosomes to the progeny through the process of meiosis, as described in Chapter 1. Upon fertilization, the zygote regains a full diploid complement of genetic material, equally derived from each parent. This process of replicating, packaging, and passing on genetic material from generation to generation forms the basis of heredity. Furthermore, errors in these processes can cause sequence changes or rearrangement of larger portions of DNA, introducing genetic variation or pathology, depending on the location of the change.

GENOMIC VARIATION

Genetic Variation

On April 14, 2003, the Human Genome Project was declared complete, with successful sequencing of the full human genome. Initial interpretation of the sequence result claimed 99.9% similarity between healthy individuals at the DNA sequence level, leaving only 0.1% of the genome sequence to account for individual differences in phenotype (Lee, 2007). Each alternative form of genetic code at any given *locus* is referred to as an *allele*. An individual inherits two alleles of every genetic locus, one from each parent. If both inherited alleles are made up of the same sequence, the individual is *homozygous* for the given locus. If the alleles are different, the individual is *heterozygous*. The allelic options at any given genetic locus derive from single nucleotide substitutions within the DNA sequence. Population sampling has demonstrated that among healthy individuals, the genetic sequence differs at around 10 million points (out of 3.2 billion DNA base pairs). These naturally occurring differences are called *single nucleotide polymorphisms*, or SNPs. To be classified as an SNP, two or more versions of nucleotide sequence must be present in at least 1% of the general population. The term *SNP* is used to describe genetic variation of healthy individuals, as no disease-causing nucleotide change is this common. An example of an SNP known to mediate susceptibility to disease is the delta 32 allele of the beta-chemokine receptor 5, or CCR5. Individuals carrying one copy of the delta 32 allele are somewhat resistant to infection by HIV, the virus that causes AIDS, and individuals with two copies (delta 32 homozygotes, ~1% of whites) are almost completely immune to infection by HIV (Huang, 1996). Thus the genetic variant is not the cause of disease (HIV) but is importantly associated with the manifestation of disease in humans.

In addition to individual sequence variation, comparative genome studies between individual sequences have revealed a far more pervasive form of genetic variation, termed *copy number variants (CNV)* (Iafrate, 2004; Sebat, 2004). These are structural variants, made up of relatively large DNA segments (ranging in size from 1000 bp to 500,000 bp or more) that appear in a variable number at a given genetic locus, and cumulatively affect 360 million nucleotides, or about 12% of the human genome (Redon, 2006). A CNV can be either benign or pathogenic, and a large proportion of identified CNV have as yet unknown significance.

Thus, although SNPs introduce genetic variation at the level of individual base substitutions, CNVs represent variation in the "dose" of a relatively large DNA segment. The collection of genetic sequence variants (SNPs) or CNVs within an individual forms a sort of biologic landscape that will influence how that person experiences or responds to external influences such as challenge from an invading pathogen or ultraviolet ray exposure from the sun. Thus understanding genetic variation in the form of SNPs and CNVs and their biologic influence can reveal a predisposition toward disease, variable susceptibility to infections, or diverse responses to pharmaceuticals as well as side effects from the same compounds. In other words, genetic variation is at the core of our collective goal of "individualized medicine," in which preventive strategies or "designer drugs" can be tailored to an individual based on one's genomic information.

Epigenetic Variation

There are forms of genetic variation that do not involve a change in nucleotide sequence. Instead, persistent alterations in three-dimensional DNA structure can change the expression pattern of a gene. Covalent modification of histones to alter *chromatin* structure and the covalent addition of methyl groups to cytosine residues in the DNA are the most commonly seen three-dimensional DNA alterations. These patterns of *epigenetic* modification of genes are replicated through successive cell divisions despite unchanged DNA sequence and have the potential to be heritable (Portela, 2010).

Two well-studied mechanisms of epigenetic modification influencing disease phenotype include genomic imprinting and CpG island methylation patterns. Genomic imprinting is a process by which hypermethylation of a specific parental allele causes that allele of the gene to be silenced, and disease may arise if the remaining allele is abnormal. Variable methylation of CpG islands in cancer cells can promote tumorigenesis through loss of proliferative supervision of the cell. Epigenetics is a growing field for understanding complex genotype-phenotype interactions. Epigenetic mechanisms such as methylation have been shown to play a role in multiple other human disease types beyond cancer, including neurodevelopmental disorders, neurodegenerative and neurologic diseases, and autoimmune diseases (Portela, 2010).

GENETIC PATHOLOGY

The term *mutation* is generally reserved for new changes in the genetic code that lead to altered function and clinical consequences. A gene mutation occurs when there has been a change in the genetic code. The mutation may involve changing a single base, known as a *point mutation*, or a larger segment, in which bases are removed, duplicated, or inserted. Mutations occur as a result of environmental damage to DNA, through errors during DNA replication or repair, and through uneven crossing over and genetic exchange during meiosis. The loss or gain of bases in a protein-coding region may disrupt the reading frame of the triplet codons. Alternatively, a change in base sequence in a noncoding region of DNA may alter the ability of regulatory proteins or RNA molecules to bind to the DNA. Point mutations within the gene could result in an amino acid substitution, leading to different products with

altered functions. Figure 2.2 demonstrates such an occurrence for sickle cell anemia caused by the substitution of a single base at a single point. In contrast to sickle cell anemia where there is only one mutation, the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene is an example of a gene for which more than 1000 mutations or alleles have been described to date. Some genes also have regions that are more prone to mutational events (hot spots).

SINGLE GENE DISORDERS

Disease-causing genetic alterations are categorized by patterns of familial segregation. Any evaluation of the segregation pattern of a trait or disease in a family requires the development of a three-generation *pedigree* (Fig. 2.3). This graphic representation of family history data assists in determining the transmission pattern of the gene, as well as predicting the risk of recurrence. In some conditions, the pattern of transmission and the constellation of clinical characteristics of affected individuals in the pedigree provide the diagnosis, which otherwise would not be evident if only one individual were evaluated.

Hemoglobin-Binding Protein	DNA Triplet Codons		Amino Acid
HgbA *(normal)*	CTT	CTC	→ Glutamic acid
HgbS	CAT	CAC	→ Valine
HgbC	TTT	TTC	→ Lysine

Figure 2.2 A single base pair substitution in the same DNA triplet codon for glutamic acid at amino acid position 6 for normal hemoglobin results in hemoglobin S (valine in sickle cell disease) or hemoglobin C (lysine in HgbC disease). (Courtesy of Edith Cheng, MD.)

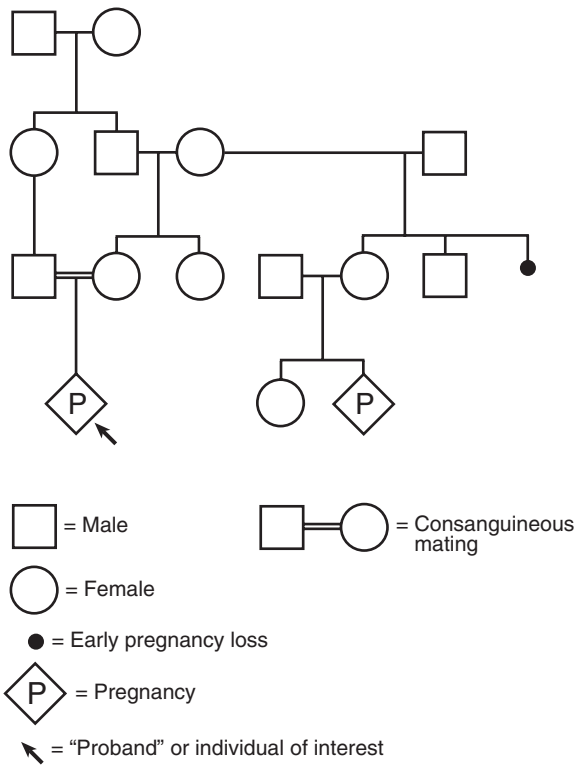

Figure 2.3 Standard figures and nomenclature for a pedigree. (Courtesy of Edith Cheng, MD.)

Mendelian Inheritance Patterns

Autosomal Dominant

In an autosomal dominant mode of inheritance, only one copy of the mutated gene is required for expression of the trait, and the individual is said to be heterozygous for the trait. There are more than 4000 known autosomal dominant conditions, and most occur in the heterozygous form in affected individuals. With a few exceptions, autosomal dominant conditions occurring in the homozygous form (two copies of the affected gene) are rare, the phenotype is more severe, and they are often lethal. An example is achondroplasia, in which two copies of the mutated gene result in a lethal condition.

The general characteristics of autosomal dominant inheritance are illustrated in Figure 2.4 and summarized as follows:

1. Every affected individual has an affected parent (unless this is a new mutation—to be discussed later). The inheritance pattern is vertical.
2. If reproductively fit, the affected person has a 50% risk of transmitting the gene with each pregnancy.
3. The sexes are affected equally.
4. There is father-to-son transmission.
5. An individual who does not carry the mutation will have no risk of transmission to his or her offspring.

Three additional properties associated with, but not exclusive to, autosomal dominant traits are variable *expressivity*, *penetrance*, and new mutations. Variable expressivity describes the severity of the phenotype in individuals who have the mutation. Some autosomal dominant conditions have a clear clinical demarcation between affected and unaffected individuals. However, some conditions express the clinical consequences of the mutation in varying degrees among members of the same family and between different families. These differences in expression are modified by age, sex of the affected individual, the individual's genetic background, and the environment. Variable expression of a condition can lead to difficulties in diagnosis and interpretation of inheritance pattern. Penetrance refers to the probability that a gene will have any clinical manifestation at all in a person

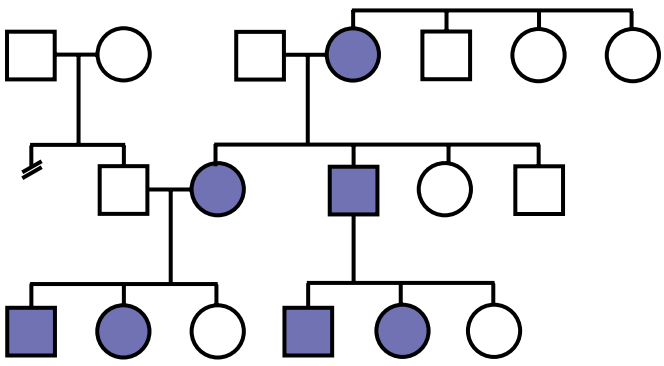

Figure 2.4 Example of autosomal dominant inheritance. (Courtesy of Edith Cheng, MD.)

known to have the mutation. A condition is 100% penetrant if all individuals with the mutation have any clinical feature of the disease (no matter how minor). A number of autosomal dominant conditions are the result of new mutations. For example, about 70% of achondroplasia cases occur as new mutations. Because this condition has 100% penetrance, the recurrence risk in subsequent pregnancies in the normal parents of an affected child is extremely low, but the risk to the offspring of the affected is 50%. If an autosomal dominant condition is associated with poor reproductive fitness, then the likelihood that the cases occurred because of new mutation is greater.

Autosomal Recessive

Autosomal recessive conditions are rare and require the affected individual to have two copies of the mutant allele (homozygous) in order to manifest the condition. In the heterozygote carrier, the product of the normal allele is generally able to compensate for the mutant allele and prevent occurrence of the disease. Figure 2.5 is a typical pedigree illustrating autosomal recessive inheritance. The following general statements can be made about an autosomal recessive trait:

1. The characteristic will occur equally in both sexes.
2. For an offspring to be at risk, both parents must have at least one copy of the mutation.
3. If both parents are heterozygous (carriers) for the condition, on average 25% of the offspring will be homozygous for the mutation and manifest the condition, and 50% will be carriers and unaffected. The remaining 25% will not have inherited the mutation at all, will be unaffected, and will not be at risk of transmitting the mutation to any offspring.
4. Consanguinity is often present in families demonstrating rare autosomal recessive conditions.

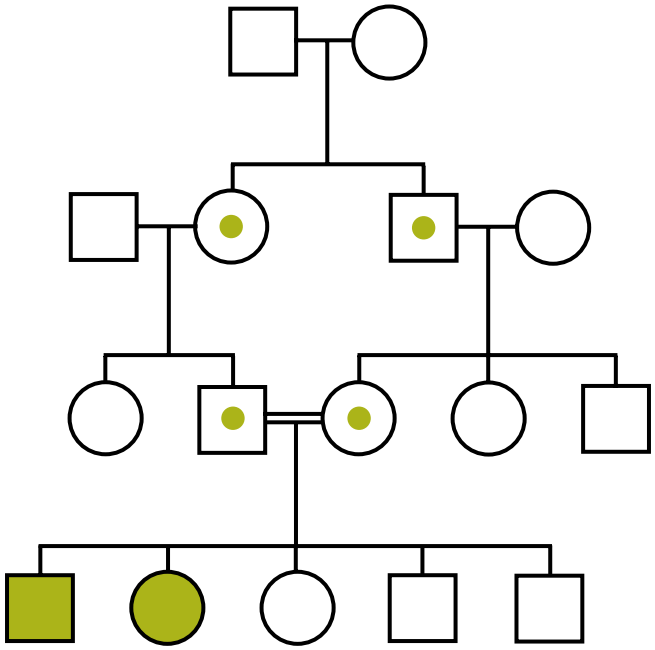

Figure 2.5 Pedigree illustrating autosomal recessive inheritance. Here the parents of the affected children are first cousins, as denoted by the double line connecting them. (Courtesy of Edith Cheng, MD.)

5. If the disease is relatively rare, it will be clustered among the siblings and will not be seen among other family members such as ancestors, cousins, aunts, and uncles.

Because autosomal recessive conditions require two copies of the mutant allele, and because most matings are not consanguineous, counseling couples about the risk for an autosomal recessive condition requires knowledge of the carrier frequency of the condition in the general population. Cystic fibrosis exemplifies the importance of knowing the population in which screening/counseling is being provided (Table 2.2). Depending on the ethnic group of the mother and father, the risk for a child having cystic fibrosis could be as high as 1 in 1936 (1/22 × 1/22 × 1/4) if they are Northern European white or considerably less so if they are of Asian descent.

X-linked Trait

The human X chromosome is quite large, containing about 160 million base pairs, or about 5% of the nuclear DNA. Of the 500 genes that have been mapped to the X chromosome, 70% are known to be associated with disease phenotypes. Diseases caused by genes on the X chromosome are said to be X linked, and most are recessive. In contrast, the Y chromosome is quite small, about 70 million base pairs, and contains only a few genes.

The expression of genes located on the X chromosome demonstrate a unique characteristic known as dosage compensation, a concept which was described by Mary Lyon in the 1960s to explain the equalization of X-linked gene products in males and females (Lyon, 1961). Achievement of dosage compensation is through the principles of X inactivation, also known as the *Lyon hypothesis*. The tenets of the Lyon hypothesis are as follows:

1. One X chromosome in each cell is randomly inactivated in the early female embryo (soon after fertilization).
2. The inactivation process is random: either the paternally or maternally derived X chromosome is chosen. The female is thus a mosaic for genes located on the X chromosome.
3. All descendants of the cell will have the same inactive X chromosome.

The Lyon hypothesis is supported by clinical evidence derived from animal and human observations of traits located on the X chromosome, such as the calico cat pattern of red and black patches of fur on female cats but not on male cats. In humans, males and females have equal quantities of the enzyme glucose-6-phosphate dehydrogenase (G6PD), which is encoded by a gene on the X chromosome. The mechanism for X inactivation

Table 2.2 Carrier Frequencies for Cystic Fibrosis in Different Populations

Ethnicity	Chance of Being Carrier	Chance Both Carriers*
European descent	1 in 29	1 in 841
Hispanic American	1 in 46	1 in 2116
African American	1 in 65	1 in 4225
Asian American	1 in 90	1 in 8100

*The chance for an affected child being born to these couples is the chance that both are carriers times ¼.

is unknown at this time but clearly requires the presence of the X inactivation center, which has been mapped to the proximal end of the long arm of the X chromosome (Xq). This center contains an unusual gene called the *X-inactive specific transcript (XIST)*, which seems to control X inactivation, a process that cannot occur in its absence.

The principles of the Lyon hypothesis remain true for the majority of genes located on the X chromosome. The silencing of these genes appears to occur as a function of DNA methylation at the promoter regions of these genes. However, several regions remain genetically active on both chromosomes. They include the pseudoautosomal regions located at the tips of the long and short arms, which are the regions that contain the genes for steroid sulfatase, the Xg blood group, and Kallman syndrome (hypogonadism and anosmia). The pseudoautosomal region on the short arm shares extensive homology with the Y chromosome and is the region involved in the pairing of the X and Y chromosome at meiosis.

Another exception to the Lyon hypothesis is that one X chromosome is nonrandomly, preferentially inactivated. This is observed for most cases of *translocations* between an X chromosome and an autosome. If the translocation is balanced, the structurally normal X chromosome is preferentially inactivated. If the translocation is unbalanced, then the structurally normal X chromosome is always active. These nonrandom patterns of inactivation are an attempt to minimize the clinical consequences of the chromosomal rearrangement. Studies can be done to look at patterns of inactivation, as in the case of prenatal diagnosis, to predict the clinical consequences of a de novo X/autosome translocation in the fetus.

Random inactivation confers a mosaic state for the carrier female. The normal allele is able to compensate for the abnormal allele (as in autosomal recessive traits), and carrier females of X-linked recessive conditions usually do not have clinical manifestations of the disease. Occasionally, however, there is skewed or less than 50-50 chance of inactivation such that the X chromosome carrying the normal allele is inactivated more frequently. In such cases, carrier females display some features of the condition and are referred to as *manifesting heterozygotes*. Manifesting heterozygotes have been described for hemophilia A, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency, and X-linked color blindness. Genetic counseling of recurrence risks for an X-linked recessive condition depends on the sex of the affected parent and of the offspring. Figure 2.6 is a pedigree illustrating X-linked recessive inheritance, the characteristics of which are the following:

1. Affected individuals are usually males unless X-chromosome activation is skewed in the carrier female or the female is homozygous for the trait.
2. The affected males in a kindred are related through females.
3. The gene is not transmitted from father to son.
4. All daughters of affected males will be carriers.
5. Daughters of carrier females have a 50% chance of being carriers; sons of carrier females have a 50% chance of being affected.

X-linked Dominant Inheritance

The major feature of X-linked dominant inheritance is that all heterozygotes, both male and female, manifest the condition. Although the pedigree may resemble autosomal dominant inheritance, the distinguishing feature is that affected males never have affected sons, and all daughters of affected males are affected. There are usually more affected females than males, and the majority of the females are heterozygotes. Examples of diseases with this mode of inheritance are hypophosphatemic rickets and Rett syndrome.

Non-Mendelian Inheritance Patterns (Complex Traits)

Trinucleotide-Repeat Disorders: Unstable Mutations

In the early 1990s a new class of genetic conditions was recognized as being due to unstable dynamic mutations in a gene. In classic genetic inheritance, the diseases and their inheritance patterns are due to mutations that are passed on from generation to generation in a stable form. That is, all affected members in a family have the identical inherited mutation. In 1991, however, a number of reports began to describe a new class of genetic condition in which the gene mutation was dynamic and would change with different affected individuals within a family. The most common group of disorders is known as *triplet*, or *trinucleotide repeat, disorders*. More than a dozen diseases are now known to be associated with unstable trinucleotide repeats (Table 2.3) (Cummings, 2000).

These conditions are characterized by an expansion of variable size, within the affected gene, of a segment of DNA that contains a repeat of three nucleotides such as CAGCAGCAG (CAG)*n*, or CCGCCG (CCG)*n*. These triplet repeats are unstable in that they tend to expand as the gene is passed on from generation to generation. The molecular mechanism is most likely misalignment at the time of meiosis. The result of increasing triplet expansion is progressively earlier onset or more severe manifestation of disease with each successive generation. This phenomenon is known as *anticipation*.

The commonality of this group of genetic conditions stops at the shared molecular mechanism. Each disease, otherwise, has its own features. Some, such as myotonic dystrophy, are inherited in an autosomal dominant pattern, but others, such as Friedrich ataxia, are autosomal recessive conditions. The susceptibility of the triplet repeat to expand also may depend on the parent of origin: paternal in Huntington disease and exclusively maternal in fragile X syndrome.

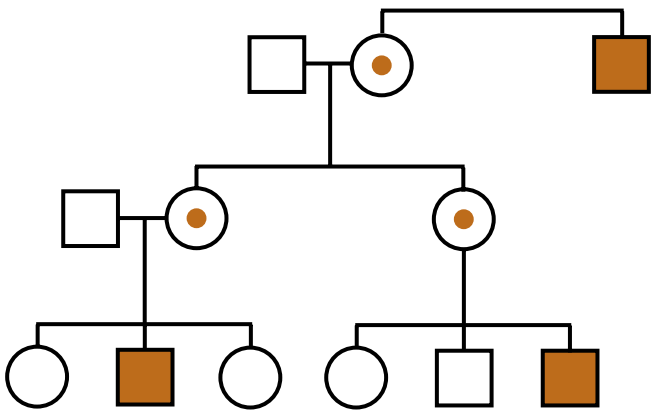

Figure 2.6 Pedigree illustrating X-linked recessive condition. (Courtesy of Edith Cheng, MD.)

Table 2.3 Some Commonly Known Disorders Associated with Unstable Triplet Repeats

Disease	Inheritance Pattern	Triplet Repeat	Location of Expansion	Repeat Number		
				Normal	Unstable	Affected
Huntington disease	Autosomal dominant	CAG	Exon coding region	<36	29-35	>35
Fragile X	X-linked	CGG	5′ untranslated region	<55	56-200	>200
Myotonic dystrophy	Autosomal dominant	GTG	3′ untranslated region	<35	50-100	>100
Spinal cerebellar ataxias*	Autosomal dominant	CAG	Exon	<40	Different for each subtype	>40
Friedrich ataxia	Autosomal recessive	GAA	Intron of gene	<33	34-65	>65

*Spinal cerebellar ataxias are a heterogeneous group of conditions, all of which appear to be associated with a CAG repeat. Each subtype has its own specific range of normal, unstable, and affected repeat sizes.

Fragile X Syndrome

Fragile X syndrome, a disease within the unstable triplet group, is the most common heritable form of moderate mental retardation and is second to Down syndrome among the causes of mental retardation in males. In women, a mild carrier state may present as premature menopause. The gene is located on the X chromosome at Xq27.3 and causes a pattern of abnormalities, including mental retardation and characteristic facial features. Disease frequency is approximately 1 in 4000 male births. The condition is due to an expansion of the triplet repeat CGG located in the untranslated region of the first exon of the gene called *FMR1* (fragile X mental retardation 1). The triplet expansion blocks normal function of the *FMR1* gene, thus causing the syndrome.

Normal individuals have about 8 to 50 copies of the CGG triplet, whereas affected individuals have from 200 to more than 1000 copies. Individuals with an intermediate number of copies (52 to 200) are known as *premutation carriers*; this level of "expansion" renders the triplet-repeat segment unstable. These carriers are generally unaffected but are at risk for having affected children or descendants if the premutation expands in successive generations. The premutation, however, can be passed on without expanding.

Long-term follow-up of premutation carriers has revealed that these individuals are not necessarily "unaffected." Premature ovarian failure has been associated with female premutation carriers, and in men, a syndrome of atypical adult-onset ataxia (FXTAS) has now been described (Hagerman, 2004).

Although the unstable triplet is transmitted in an X-linked pattern, the probabilities of the different phenotypes are far from traditional X-linked inheritance. Understanding of this feature of the fragile X syndrome is crucial to genetic counseling and assessing recurrence risks. The possible outcomes of the offspring of a premutation carrier female are the following:

1. Male offspring—three possibilities:
   a. Unaffected by not having inherited the X chromosome with the premutation.
   b. Unaffected by inheriting the X chromosome with the premutation, which did *not* expand (about 20% of the time); this male, however, is at risk for passing the premutation to his daughters, who in turn will be at risk for having affected children. Therefore, for this male, his grandchildren will be at risk for the fragile X syndrome.
   c. Affected by having inherited the abnormal X chromosome, in which the premutation also expanded to a full mutation.
2. Female offspring—four possibilities:
   a. Unaffected by not having inherited the X chromosome with the premutation.
   b. Unaffected by inheriting the X chromosome with the premutation that did *not* expand.
   c. Unaffected, but inherited the X chromosome with an expansion—about 50% of females with the expansion appear to be clinically unaffected.
   d. Affected by inheriting the X chromosome with an expansion.

Genomic Imprinting and Uniparental Disomy

Genomic imprinting and uniparental disomy refers to the differential activation or expression of genes depending on the parent of origin. In contrast to Mendel's hypothesis that the phenotype of a gene is no different if inherited from the mother or the father, we now understand that there is a group of diseases in which the parent of origin of a gene or chromosome plays a role in the phenotype of the affected individual. The best-studied example of this mechanism is Prader-Willi syndrome (PWS) and Angelman syndrome (AS). Both diseases arise from loss of function of the same gene on chromosome 15, but two different disease phenotypes arise depending on which parental allele is affected. PWS is characterized by obesity, hyperphagia, small hands and feet, hypogonadism, and mental retardation (Jones, 2006). In about 70% of cases, cytogenetic deletion of the proximal arm of the paternally inherited chromosome 15 is observable (15q11-q13). In contrast, the same deletion of the maternally inherited chromosome 15 results in the Angelman phenotype of severe mental retardation, short stature, spasticity, and seizures (Jones, 2006). Interestingly, 30% of subjects with PWS do not have a cytogenetic deletion but rather inherit two intact chromosomes 15 from the mother. No genetic information on chromosome 15 is inherited from the father. This is referred to as *maternal uniparental disomy*. Individuals with Angelman syndrome without a cytogenetic deletion have two copies of the paternally derived chromosome 15 and no chromosome 15 from the mother, a condition termed *paternal uniparental disomy.* These findings indicate that for the region of 15q11-q13, the expression of the PWS phenotype is brought on by the absence of a paternal contribution of the genes in this region. Likewise, the expression of Angelman syndrome is due to the absence of the maternal contribution of genes located at 15q11-q13. The genes in this region are said to be "imprinted" because their parent of origin has been "marked."

Many regions of the human genome have now demonstrated evidence of imprinting. Knowledge of diseases that occur as a

result of imprinting has implications in prenatal diagnosis, especially when *mosaicism* is encountered.

Germline Mosaicism

Mosaicism is defined as the presence of two or more genetically different cell lines in the same individual or tissue derived from a single zygote. All females, because of X inactivation, are mosaics for genes on the X chromosome. Mosaicism, however, is not necessarily evenly or randomly distributed throughout the body. In other words, using the entire body as the whole organism, an individual is mosaic either because different organs or tissues have genetically different cells, but each organ or tissue has the same cell line, or because the genetically different cell lines are dispersed throughout many tissues in the body. The distinction between these two types of mosaicism is particularly important in making a prenatal diagnosis in cases in which mosaicism is identified in amniotic fluid cells. For instance, one cannot be confident that a fetus identified as being mosaic trisomy 21 would necessarily have a less severe mental retardation phenotype because of mosaicism. The brain cells could be all full trisomy 21, but the cells of the skin could all be normal diploid. In germline mosaicism, the implication is that the mutation is present in only one parent and arose during embryogenesis in all or some of the germ line cells but few or none of the somatic cells of the embryo. This concept was developed to explain recurrence of a genetic condition in a sibship (usually autosomal dominant) in which incorrect diagnosis, autosomal recessive inheritance, reduced penetrance, or variable expression could not be the reason for the recurrence. The best example of germline mosaicism is osteogenesis imperfecta type II (lethal form). At the molecular level, the mutation causing the condition is dominant—that is, only one copy of the abnormal gene is necessary to cause this perinatal lethal condition. Yet there are families in which multiple affected pregnancies are seen in the same couple or one parent has recurrences with different partners. If the spontaneous mutation rate for an autosomal dominant mutation is 1 chance in 10^5, then the probability of two independent spontaneous mutations for the same lethal autosomal dominant condition is $(1/10^5)^2$, a highly unlikely event. Germline mosaicism is now well documented for about 6% of cases of osteogenesis imperfecta type II (Zlotogora, 1998). Unfortunately, the exact recurrence risk is difficult to assess because the proportion of gametes containing the mutation is unknowable.

Mitochondrial Inheritance: Maternal Inheritance

Most inherited conditions occur as a result of mutations in the DNA of the nucleus (nuclear genome). However, mitochondria have their own DNA molecules, which contain a small fraction of genes whose product are vital to the function of the cell. Mitochondrial DNA (mtDNA), which was completely sequenced in 1981, is small, about 16.5 kilobase pairs (kbp), and is packaged as a circular chromosome located in the mitochondria. A growing number of conditions resulting from abnormalities of the mitochondria have now been identified. Because the mitochondrial apparatus and its function are under the control of both nuclear and mitochondrial genes, many diseases affecting the mitochondria do not follow the typical Mendelian pattern of inheritance. Each human cell contains a population of several hundred or more mitochondria in its cytoplasm. Most of the subunits that make up the mitochondrial apparatus are encoded by the nuclear genome.

Because the primary function of the mitochondria is to provide energy in the form of adenosine triphosphate (ATP) for the cell, mutations that affect the genes that code for oxidative phosphorylation will likely result in cell dysfunction and death. The organs most affected would be those that depend heavily on mitochondria. The diseases that result are generally neuromuscular in nature, such as encephalopathies, myopathies, ataxias, and retinal degeneration, but the mutations have *pleiotropic* effects, meaning multiple different clinical traits are caused by a single gene defect (Johns, 1995).

The most significant characteristic of mitochondrial diseases caused by mutations in mtDNA is that they are all maternally inherited. This is because the cytoplasm of the ovum is abundant with mitochondria, but the sperm contain very few mitochondria. Therefore an individual's mitochondria (and mtDNA) are essentially all inherited from the mother. If the mother has an mtDNA mutation, then all of her children will inherit that mutation. When a mutation arises in the DNA of a mitochondrion in the cytoplasm of the ovum, it is at first one mutation in one mitochondrion. However, as replication and division of this mutated mitochondrion occur, they become randomly distributed among the normal mitochondria and between the daughter cells. One daughter cell by chance may contain a large population of mitochondria with the mutation, but the other has none or very little. Fertilization of the egg with a large proportion of mitochondria containing the mutation would result in an offspring that is at risk for manifesting a mitochondrial disease. Leber hereditary optic neuropathy (LHON) is a well-known mitochondrial disease in which rapid, bilateral loss of central vision occurs as a result of a mutation in mitochondrial DNA. Males and females are affected equally, and all affected individuals are related through maternal lineage.

A second feature of mitochondrial diseases is that of variable expression. Within each cell and tissue, there is a threshold for energy production below which the cells will degenerate and die. Organ systems with large energy requirements will be most susceptible to mitochondrial abnormalities. Thus, if there is an mtDNA mutation, the severity of the mitochondrial disease will depend on the proportion of mitochondria with the mutation that the individual inherited from his or her mother and the susceptibility of different tissues to altered ATP metabolism.

In contrast, abnormalities of mitochondrial function caused by mutations of genes encoded in the nuclear genome will exhibit traditional Mendelian inheritance patterns; autosomal dominant, autosomal recessive and X-linked patterns of mitochondrial disorders have been observed. A few mitochondrial diseases occur as sporadic somatic mutations and have little or no recurrence risk. Table 2.4 lists some of the currently known diseases of mitochondrial function and their inheritance patterns.

Multifactorial Inheritance

Multifactorial inheritance is defined as traits or characteristics produced by the action of several genes, with or without the interplay of environmental factors. A number of structural abnormalities occurring as isolated defects and not part of a syndrome, such as cleft lip with or without cleft palate, open neural tube defect (including anencephaly and spina bifida), and

Table 2.4 Features of Some Disorders of Mitochondrial Function

Disease	Features	Genetics	Inheritance Pattern
Barth syndrome	Dilated cardiomyopathy, cyclic neutropenia, skeletal myopathy, growth deficiency, abnormal mitochondria	Nuclear DNA encoding mitochondrial protein tafazzin (*TAZ* gene)	X linked
Friedreich ataxia	Limb movement abnormalities, dysarthria, absent tendon reflexes	Nuclear DNA encoding mitochondrial protein frataxin (*FXN* gene, triplet repeat)	Autosomal recessive
Leber hereditary optic neuropathy (LHON)	Blindness, rapid optic nerve death in young adulthood	Mitochondrial DNA	Maternal
Leigh disease (subacute necrotizing encephalomyelopathy)	Infant onset progressive psychomotor regression following viral illness, hypotonia, peripheral neuropathy, lactic acidosis	Many genes: 20%-25% mitochondrial DNA (includes neuropathy, ataxia, and retinitis pigmentosa [NARP]) 75%-80% nuclear DNA	Mitochondrial DNA: maternal Nuclear DNA: autosomal recessive or X linked
MERRF	Myotonic epilepsy, ragged red fibers in muscle, ataxia, sensorineural deafness	Mitochondrial DNA	Maternal
MELAS	Mitochondrial encephalopathy, lactic acidosis, strokelike episodes, sensorineural deafness	Mitochondrial DNA	Maternal
MIDD	Maternally inherited diabetes and deafness	Mitochondrial DNA	Maternal
MNGIE	Mitochondrial neurogastrointestinal encephalopathy, childhood-onset gastrointestinal dysmotility, peripheral neuropathy	Nuclear DNA (*TYMP* gene) causes destabilization of mitochondrial DNA	Autosomal recessive

cardiac defects, are examples of such conditions. When both parents are normal and an affected child is produced, the chance of recurrence is generally between 2% and 5% for any given pregnancy. Because the underlying mechanisms by which the genes and the environment interact to cause these conditions are largely unknown, genetic counseling of recurrence risks must measure the observed recurrence risks in collections of families to generate a population-based empiric risk. These risk rates, however, are modified by many factors including ethnicity, the sex of the carrier parent, the sex of the affected parent and at-risk offspring, the presence of the defect in one or both parents, the number of affected family members, and consanguineous parentage (Kuller, 1996).

CHROMOSOMAL ABNORMALITIES

In general, genetic replication machinery of the cell is astonishingly accurate, and there are many repair mechanisms the cell uses to maintain fidelity of DNA copies. Thus the incidence of any given single gene disorder, even in high prevalence populations, is relatively low. In contrast, the distribution of genetic material during cell division by mitosis or meiosis is far more prone to mistakes, so that on a population level the risk of chromosome level genetic rearrangements occurs at least 100 times more frequently than single gene disorders. Consider the rate of 1 in 2500 for white babies affected by cystic fibrosis (the most common inherited disease in this population), compared with the estimation that an abnormal chromosome complement occurs in up to 4% of clinically recognized pregnancies (Creasy, 2014).

A variety of chromosome abnormalities may occur during meiosis or mitosis (see Chapter 1) leading to an abnormal *karyotype*, or chromosome complement visible under light microscopy. Chromosome abnormalities fall into several general categories, and many clinical conditions are associated with each type.

Numerical Chromosomal Abnormalities

Two terms are used in the description of numerical chromosomal abnormalities: *aneuploidy* refers to an extra or missing chromosome, such as in trisomy 21 (Down syndrome) or monosomy X (Turner syndrome), respectively; *polyploidy* refers to numerical chromosome abnormalities in which there is an addition of an entire complement of haploid chromosomes, such as in triploidy, in which three haploid sets occur (69, XXX or XXY or XYY). Numerical or aneuploid chromosome abnormalities involve either autosomes or sex chromosomes. Most occur as the result of *nondisjunction* during meiosis or mitosis in which homologous chromosome pairs fail to disjoin. The result in meiosis is that one daughter cell receives two copies of the homologues and the other receives none. Fertilization with a gamete containing a normal chromosome complement will result in a zygote that is either trisomic or monosomic (Fig. 2.7).

The majority of trisomic conceptions are nonviable, and autosomal trisomies have been seen in abortus material in all but chromosomes 1 and 17. However, trisomies 21, 18, 13, and 22 can result in live births and are associated with advanced maternal age (Fig. 2.8). Trisomy 13 (Patau syndrome) occurs in approximately 1/10,000 live births. The syndrome is characterized by prenatal growth restriction and multiple severe structural defects involving the midline (holoprosencephaly, cleft lip/palate,

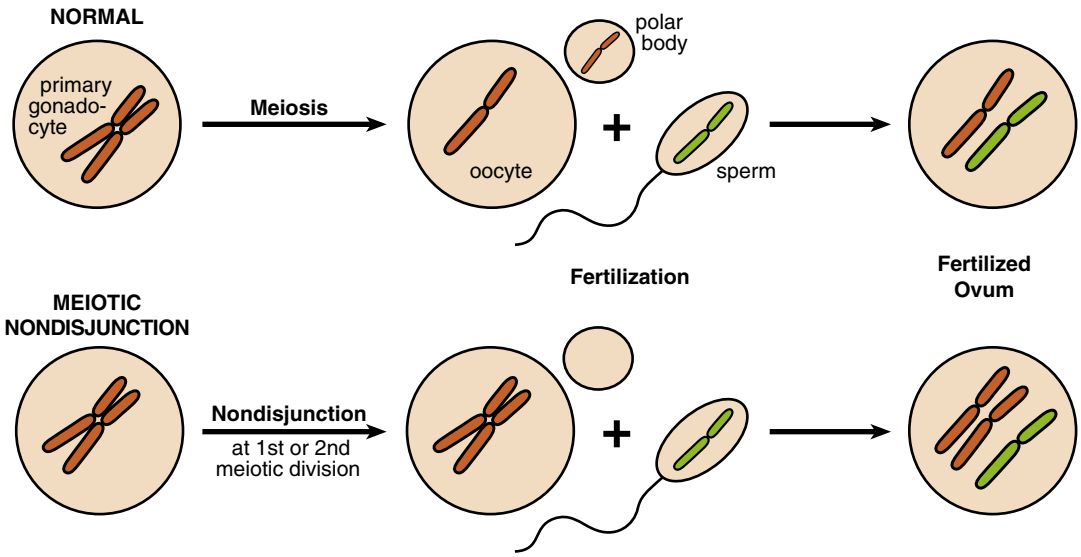

Figure 2.7 Graphic representation of meiotic nondisjunction. (Courtesy of Edith Cheng, MD.)

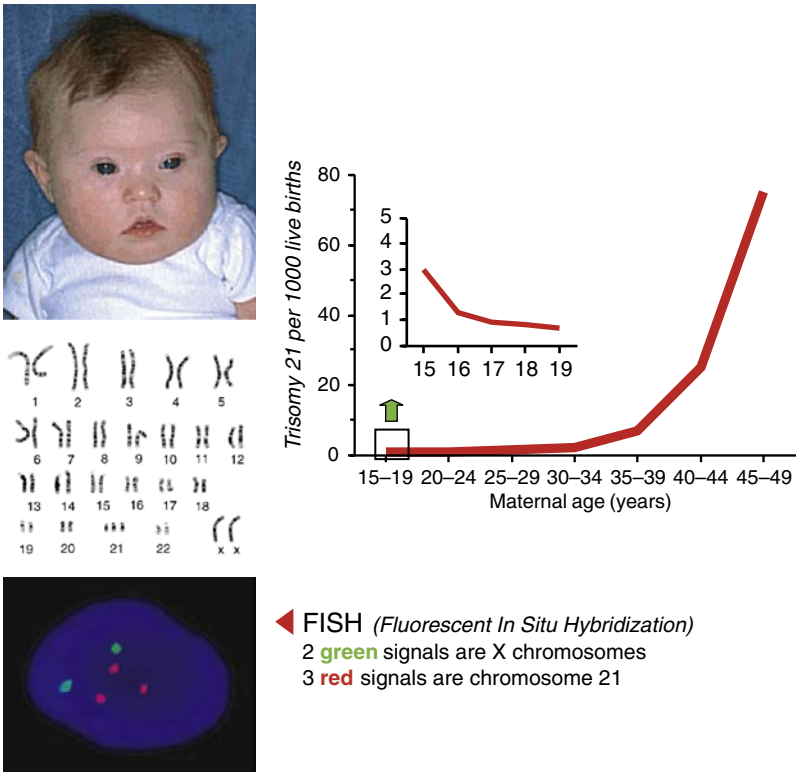

Figure 2.8 Trisomy 21 infant with karyotype demonstrating three separate chromosomes 21. Interphase fluorescent in situ hybridization (FISH) illustration of screening for trisomy 21. Graph illustrating the maternal age association and increasing risk for aneuploidy. Note that there is an increased risk at the peripubertal ages as well. (Courtesy of Edith Cheng, MD.)

cardiac defects), and postaxial polydactyly. Trisomy 18 (Edwards syndrome) is found in 1/6000 live births and is associated with prenatal growth restriction, rocker bottom feet, and cardiac and renal defects. Trisomy 21 is the most common viable autosomal trisomy and has an incidence of 1/800 live births. The majority (95%) of individuals with Down syndrome have complete trisomy 21—that is, three separate copies of chromosome 21 because of maternal nondisjunction. However, about 2% to 3% of individuals with clinical Down syndrome have a structural rearrangement (Robertsonian translocation—to be discussed in the next section), and another 1% to 3% are mosaic for trisomy 21 (Jones, 2006). Trisomy 22 has been seen in a few live-born