Gametogenesis, Fertilization, and First Week

1

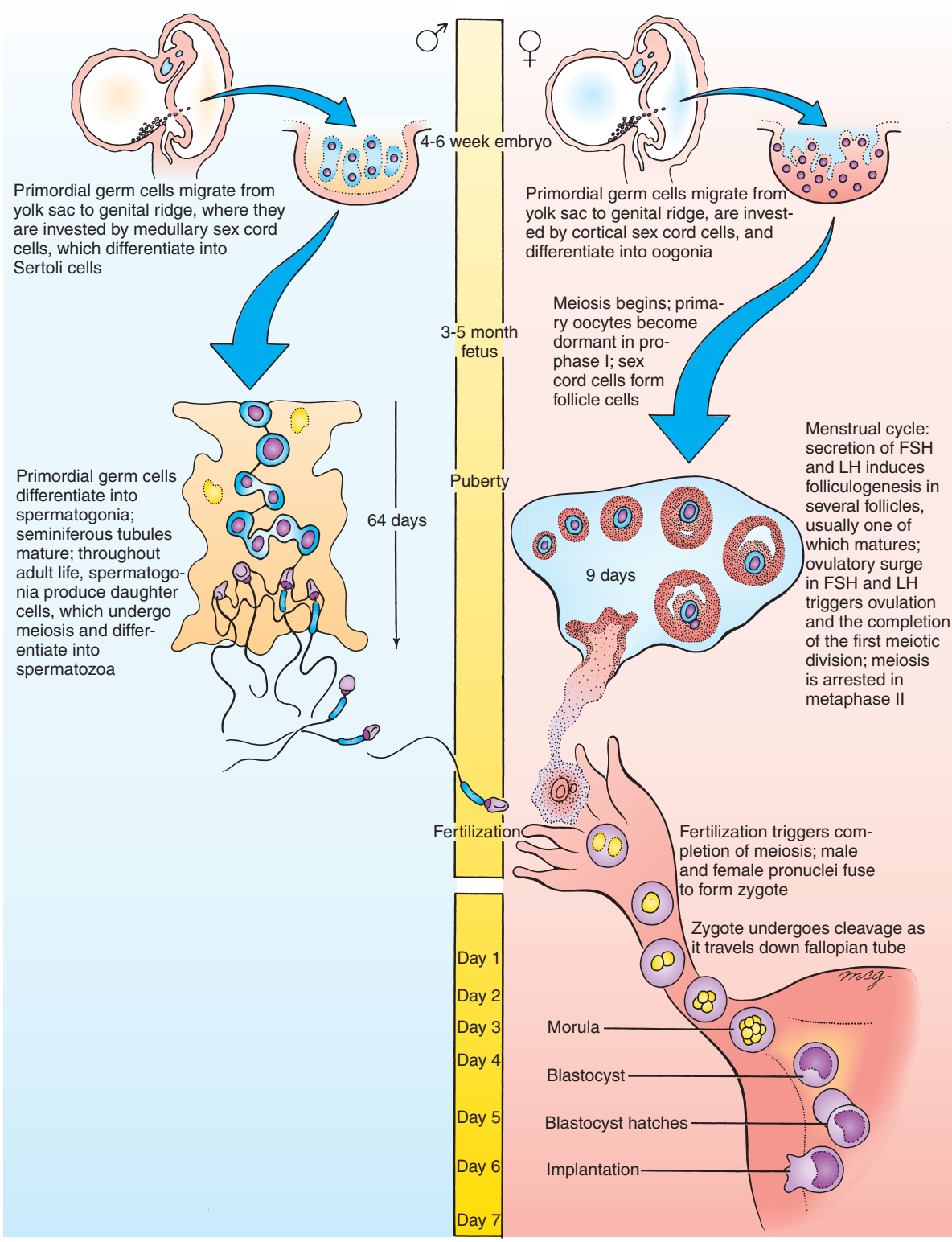
Summary

A textbook of human embryology could begin at any of several points in the human life cycle. This textbook starts with a discussion of the origin of specialized cells called **primordial germ cells (PGCs)**. PGCs can be first identified within the wall of the **yolk sac**, one of the extraembryonic membranes, during the 4th to 6th weeks of gestation. These PGCs will give rise to the **germ line**, a series of cells that form the sex cells, or **gametes** (i.e., the **egg** and **sperm)**. However, these gametes will not function to form the next generation for several decades (i.e., after the onset of **puberty**). Yet, remarkably, one of the first things that happens in the developing embryo is to set aside the germ line for the next generation. Similarly, the germ lines that gave rise to the developing embryo were established a generation earlier, when the embryo's father and mother were developing in utero (that is, when the embryo's maternal and paternal grandmothers were pregnant with the embryo's father and mother).

From the wall of the yolk sac, PGCs actively migrate between the 6th to 12th weeks of gestation to the dorsal body wall of the embryo, where they populate the developing gonads and differentiate into the gamete precursor cells called **spermatogonia** in the male and **oogonia** in the female. Like the normal somatic cells of the body, the spermatogonia and oogonia are **diploid;** that is, they each contain 23 pairs of chromosomes (for a total of 46 chromosomes each). When these cells eventually produce **gametes** by the process of **gametogenesis** (called **spermatogenesis** in the male and **oogenesis** in the female), they undergo **meiosis**, a sequence of two specialized cell divisions by which the number of chromosomes in the gametes is halved. The gametes thus contain 23 chromosomes (one of each pair) and are therefore **haploid**. The developing gametes also undergo cytoplasmic differentiation, resulting in the production of mature **spermatozoa** in the male and **definitive oocytes** in the female.

In the male, spermatogenesis takes place in the seminiferous tubules of the testes and does not occur until puberty. In contrast, in the female oogenesis is initiated during *fetal* life. Specifically, between the 3rd and 5th months of fetal life, oogonia initiate the first meiotic division, thereby becoming primary oocytes. However, the primary oocytes then quickly enter a state of meiotic arrest that persists until after puberty. After puberty, a few oocytes and their enclosing follicles resume development each month in response to the production of pituitary gonadotropic hormones. Usually, only one of these follicles matures fully and undergoes **ovulation** to release the enclosed oocyte, and the oocyte completes meiosis only if a spermatozoon fertilizes it. **Fertilization**, the uniting of the egg and sperm, takes place in the oviduct. After the oocyte finishes meiosis, the paternal and maternal chromosomes come together, resulting in the formation of a **zygote** containing a single diploid nucleus. Embryonic development is considered to begin at this point.

The newly formed embryo undergoes a series of cell divisions called **cleavage** as it travels down the oviduct toward the uterus. The cleavage divisions subdivide the zygote first into two cells, then into four, then into eight, and so on. These daughter cells do not grow between divisions, so the entire embryo remains the same size. Starting at the 8- to 16-cell stage, the cleaving embryo, or **morula**, differentiates into two groups of cells: a peripheral outer cell layer and a central **inner cell mass**. The outer cell layer, called the **trophoblast**, forms the fetal component of the placenta and associated extraembryonic membranes, whereas the inner cell mass, also called the **embryoblast**, gives rise to the embryo proper and associated extraembryonic membranes. By the 30-cell stage, the embryo begins to form a fluid-filled central cavity, the **blastocyst cavity**. By the 5th to 6th day of development, the embryo is a hollow ball of about 100 cells called a **blastocyst**. At this point, it enters the uterine cavity and begins to implant into the endometrial lining of the uterine wall.



f0010 **Time line.** Gametogenesis and first week of development.

Clinical Taster A couple, both in their late 30s, is having difficulty conceiving a child. Early in their marriage about, 10 years ago, they used birth control pills and condoms thereafter, but they stopped using all forms of birth control more than 2 years ago. Despite this and having intercourse three or four times a week, a pregnancy has not resulted. On routine physical examination, both the man and woman seem to be in excellent health. The woman is an avid runner and competes in occasional marathons, and she has had regular periods since her menarche at age 13. The man had a varicocele, which was corrected when he was 19; the urologist who performed the surgery assured him that there would be no subsequent adverse affect on his fertility.

Because no obvious cause of their fertility problem is noted, the couple is referred to a local fertility clinic for specialized treatment. At the clinic, the man has a semen analysis. This reveals that his sperm count (60 million sperm per ejaculate), sperm mobility (vigorous motility and forward progression [i.e., straight swimming movement]), sperm morphology (70% with an oval head and a tail 7 to 15 times longer than the head), and semen volume (3.5 mL with a normal fructose level) are within the normal ranges. Semen viscosity and sperm agglutination are also normal. As a next step, a postcoital test is planned. Using the woman's recent menstrual history to estimate the time of her midcycle, and daily basal body temperature measurements and urine LH (luteinizing hormone) tests to predict ovulation, intercourse is timed for the evening of the day on which ovulation is expected to occur. The next morning, the woman undergoes a cervical examination. It is noted that the cervical mucus contains clumped and immotile sperm, suggesting a sperm-cervical mucus incompatibility.

Based on the results of the postcoital test, the couple decides to undergo **artificial insemination**. After five attempts in which the man's sperm are collected, washed, and injected into the uterus through a sterile catheter passed through the cervix, a pregnancy still has not resulted. The couple is discouraged and decides to take some time off to consider their options.

After considering adoption, gestational surrogacy, and remaining childless, the couple returns three months later and requests **IVF (in vitro fertilization)**. On the second of two very regimented attempts, the couple is delighted to learn that a pregnancy has resulted. A few weeks later Doppler ultrasound examination detects two fetal heart beats. This is confirmed two months later by ultrasonography. Early in the 9th month of gestation two healthy babies are delivered, a 6-pound 2-ounce girl and a 5-pound 14-ounce boy.

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Primordial Germ Cells

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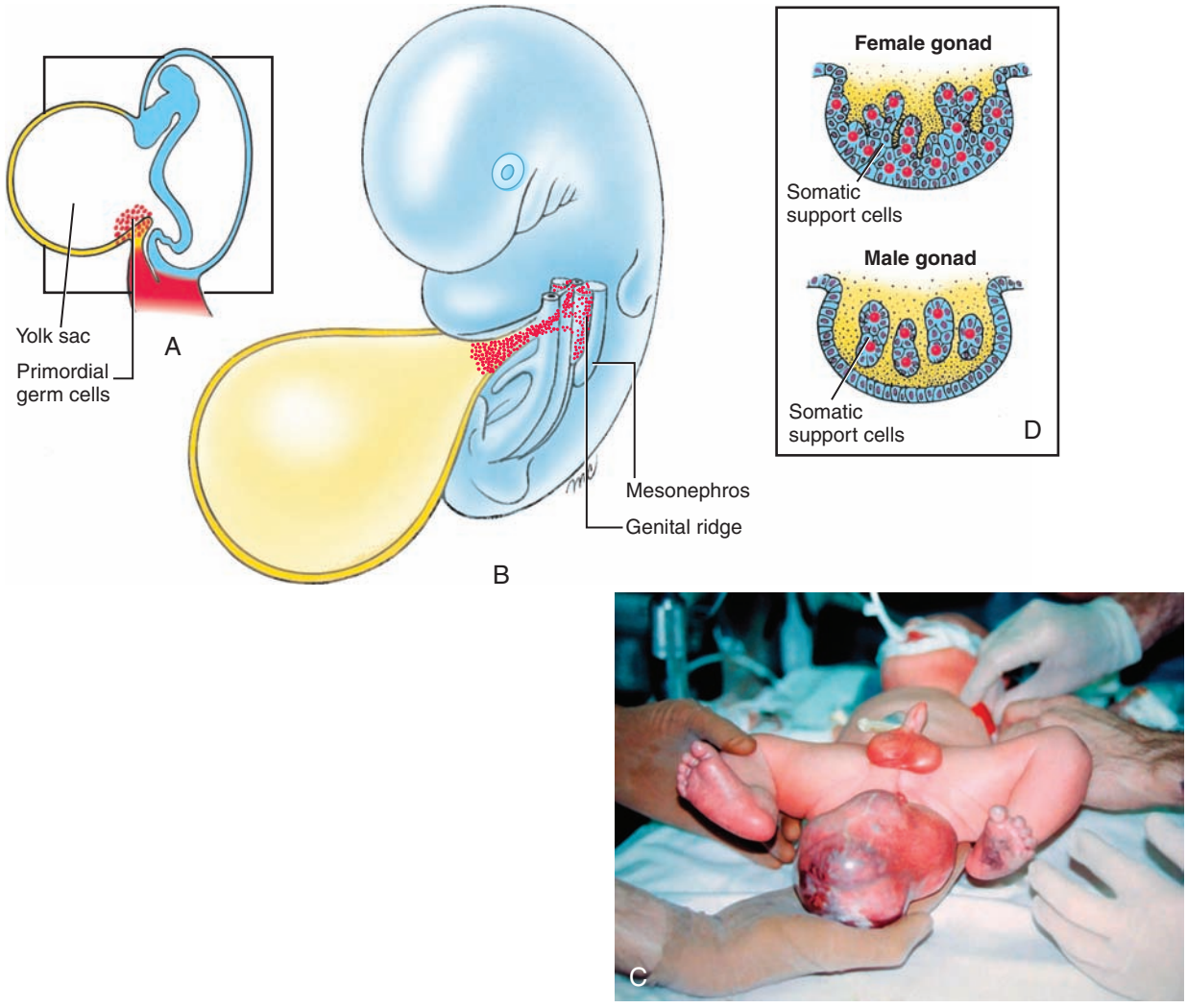
Primordial Germ Cells Reside in Yolk Sac

p0010 Cells that give rise to **gametes** in both males and females can be identified during the 4th week of gestation within an extraembryonic membrane called the **yolk sac** (Fig. 1-1*A*). Based on studies in animal models, it is believed that these cells arise earlier in gestation, during the phase of gastrulation (discussed in Ch. 3). These cells are called **primordial germ cells (PGCs)**, and their lineage constitutes the **germ line**. PGCs can be recognized within the yolk sac and during their subsequent migration (see next paragraph) because of their distinctive pale cytoplasm and ovoid shape and because they specifically stain intensely with reagents that localize the enzyme alkaline phosphatase.

s0030

Primordial Germ Cells Migrate into Dorsal Body Wall

p0020 Between four and six weeks, PGCs migrate by ameboid movement from the yolk sac to the wall of the gut tube, and from the gut tube via the mesentery of the gut to the dorsal body wall (Fig. 1-1*B*). In the dorsal body wall, these cells come to rest on either side of the midline in the loose mesenchymal tissue just deep to the membranous lining of the coelomic cavity. Most of the PGCs populate the region of the body wall at the level that will form the gonads (discussed in Ch. 15). PGCs continue to multiply by mitosis during their migration. Some PGCs may become stranded during their migration, coming to rest at extragonadal sites. Occasionally, stray germ cells of this type may give rise to a type of tumor called a **teratoma** (Fig. 1-1*C*).



f0010 **Figure 1-1.** *A*, Primordial germ cells (PGCs) reside in the endodermal layer of the caudal side of the yolk sac during 4 to 6 weeks of development and then migrate to the dorsal body wall. *B*, Between 6 and 12 weeks, PGCs stimulate formation of the genital ridges. *C*, Infant with a large sacrococcygeal teratoma. *D*, Somatic support cells differentiate and invest PGCs. In females, somatic support cells become ovarian follicle cells; in males, somatic support cells become Sertoli cells of the seminiferous tubules.

b0010 ## IN THE CLINIC

TERATOMA FORMATION

Teratomas, tumors composed of tissues derived from all three germ layers, can be extragonadal or gonadal and are derived from PGCs. Sacrococcygeal teratomas are the most common tumors in newborns and occur in 1 in 20,000 to 70,000 births (see Fig. 1-1*C*). They occur four times more frequently in female newborns than in male newborns, and they represent about 3% of all childhood malignancies. Gonadal tumors are usually diagnosed after the onset of puberty. Both ovarian and testicular teratomas can form. The **pluripotency** (ability to form many cell types, not to be confused with **totipotency**, the ability to form *all* cell types) of teratomas is exhibited by their ability to give rise to a variety of definitive anatomic structures, including hair, teeth, pituitary gland, and even a fully formed eye.

s0040 ## Primordial Germ Cells Stimulate Formation of Gonads

p0030 Differentiation of the gonads is described in detail in Chapter 15. When PGCs arrive in the presumptive gonad region, they stimulate cells of the adjacent coelomic epithelium to proliferate and form **somatic support cells** (Fig. 1-1*D*; see also Fig. 15-16). Proliferation of the somatic support cells create a swelling just medial to each mesonephros (embryonic kidney) on both the right and left sides of the gut mesentery. These swellings, the **genital ridges**, represent the primitive gonads. Somatic support cells invest PGCs and give rise to tissues that will nourish and regulate development of maturing sex cells—**ovarian follicles** in the female and **Sertoli cells** of the **germinal epithelium (seminiferous epithelium)** of the **seminiferous tubules** in the male. Somatic support cells are essential for germ cell development within the gonad: if germ cells are not invested by somatic support cells, they degenerate. Conversely, if PGCs fail to arrive in the presumptive gonadal region, gonadal development is disrupted.

b0020 ## IN THE RESEARCH LAB

ORIGIN OF PGCs

Although the exact time and place of origin of PGCs in humans is unknown, cell tracing and other experiments in the mouse demonstrate that PGCs arise from the epiblast (one of the layers of the bilaminar and trilaminar blastoderm stages; discussed in Chs. 2, 3). During gastrulation, these cells move through the caudal part of the primitive streak and into the extraembryonic area. From there, they migrate to the gut wall and through the gut mesentery to the gonadal ridges, as in humans.

MOLECULAR REGULATION OF PGC DEVELOPMENT

Development of the germ line involves the sequential activation of genes that direct the initial induction, proliferation, survival, migration, and differentiation of PGCs. Animal models have been very useful for understanding these events and have been used to show that the function of many genes controlling PGC development are conserved across diverse organisms. However, mechanisms underlying the initial events of PGC formation in mammals seem to be very different from those of lower organisms.

In some model organisms, such as the fruitfly, worm, and frog, **maternal effect genes** (discussed in Ch. 5) are required for initiation of germ cell formation. Activation of these maternal genes regulates the segregation of the **germ plasm** (cytoplasm containing determinants of the germ line) to a specific region of the zygote so that it becomes incorporated during cleavage into a unique group of cells that will form the germ cell precursors.

The Drosophila *Vasa* gene is segregated to germ cells in this fashion. *Vasa* transcripts are expressed ubiquitously in the oocyte cytoplasm, but *Vasa* protein becomes specifically localized in the germ plasm. *Vasa* is an RNA-binding protein of the DEAD box family and its possible role is to bind mRNAs involved in germ line determination, such as *Oskar* and *Nanos*, and to control the onset of their translation. Vertebrate orthologs of *Vasa* exist, and in some vertebrates *Vasa* protein is expressed in germ cell precursors as they are forming (however, in mice, *Vasa* is expressed in germ cells only much later, after they have differentiated and are about to colonize the gonads).

In contrast to lower organisms, where germ cells are usually specified by the inheritance of maternal gene products, in the mouse and probably also in humans the germ line is induced. All cells of the mammalian morula are seemingly capable of forming pluripotent germ cells, but their capacity to do so becomes rapidly restricted first to the inner cell mass and then to the epiblast. Therefore, in mammals, the **initiation of germ line development** requires activation of genes that maintain pluripotency within the precursors that will form the germ line. One such gene encodes a POU domain transcription factor (*Oct4*, also called *Pou5f1*; transcription factors are discussed in Ch. 5). Its activity is present initially in all cells of the morula, but then only in the inner cell mass. It is then restricted to the epiblast, and finally it is expressed only in the presumptive germ cells themselves.

Further development of the germ line requires an inductive signal from the trophoblast (induction is discussed in Ch. 5). One such signal is *Bone morphogenetic protein 4* (*Bmp4*). In chimeric mouse embryos (mouse injection chimeras are discussed in Ch. 5) lacking *Bmp4* specifically within the trophoblast, PGCs, as well as the allantois (an extraembryonic membrane), fail to form. *Bmp4* induces expression of two germ–line specific genes in mice: *Fragilis* and *Stella*; however, their exact roles in PGC development are currently unknown.

Proliferation and survival of PGCs is ensured by the expression of **trophic factors** (factors that promote cell growth and survival) within the PGCs or within associated cells. A trophic factor expressed by PGCs and required for their early survival and proliferation is the RNA-binding protein *Tiar*. Another is a mouse ortholog of the Drosophila *Nanos* gene (*Nanos3*). Many other trophic factors seem to

be required for the survival and proliferation of PGCs along their migratory pathway from the yolk sac to the gut and dorsal mesentery and then to the dorsal body wall. These include several factors expressed by tissues along the pathway, including the *c-Kit* ligand (*Stem cell factor* or *Steel factor*) and members of the *Interleukin/Lif* cytokine family (a cytokine is a regulatory protein released by cells of the immune system that acts as an intercellular mediator in the generation of an immune response). Study of *c-Kit* and *Steel* mutants has revealed that this signaling pathway suppresses **PGC apoptosis** (cell death) during migration. This finding provides an explanation for why PGCs that stray from their normal migratory path and come to rest in extragonadal sites usually (but not always; see above discussion of extragonadal teratomas) degenerate.

Other factors, including extracellular matrix proteins, must also be expressed by cells along the pathway to allow migration of PGCs and to direct their migration from the yolk sac to the gut and dorsal mesentery and then to the gonadal ridge (presumptive gonad) in the posterior body wall. *Tenascin C*, *β2 Integrin*, and *Laminin* all seem to be required for PGC migration. **Chemotropic signals** (i.e., attractive signals produced by the developing gonads) also seem to be involved to regulate PGC honing. One such factor is the chemokine (a type of cytokine) *Stromal cell-derived factor-1* (*Sdf1* or *Cxcl12*) and its receptor *Cxcr4*. PGC migration toward the gonad is disrupted in mouse or zebrafish embryos lacking the ligand or its receptor. In addition, *Sdf1* acts as a PGC survival factor.

Once PGCs arrive within the presumptive gonad, numerous genes must be expressed to **regulate the final differentiation of cells of the germ line**. Three new germ cell–specific genes are expressed shortly after PGCs enter the genital ridge (after which they are usually called **gonocytes**): *murine Vasa homolog* (*mVh*; the *Vasa* gene was discussed above), *Germ cell nuclear antigen 1* (*Gcna1*), and *Germ cell-less* (*Gcl1*). The last is expressed in the Drosophila germ line shortly after it is established, and it is named after the mutation in which the gene is inactivated and the germ line is lost.

s0050

Gametogenesis

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Timing of Gametogenesis Is Different in Males and Females

p0040 In both males and females, PGCs undergo further mitotic divisions within the gonads and then commence **gametogenesis**, the process that converts them into mature male and female gametes (**spermatozoa** and **definitive oocytes**, respectively). However, timing of these processes differs in the two sexes (see Timeline; Fig. 1-3). In males, PGCs (usually now called **gonocytes**) remain dormant from the 6th week of embryonic development until puberty. At puberty, **seminiferous tubules** mature and PGCs differentiate into **spermatogonia**. Successive waves of spermatogonia undergo **meiosis** (the process by which the number of chromosomes in the sex cells is halved; see following section) and mature into spermatozoa. Spermatozoa are produced continuously from puberty until death.

p0050 In contrast in females, PGCs (again, usually now called **gonocytes**) undergo a few more mitotic divisions after they are invested by the somatic support cells. They then differentiate into **oogonia**, and by the 5th month of fetal development all oogonia begin meiosis, after which they are called **primary oocytes**. However, during an early phase of meiosis all sex cells enter a state of dormancy, and they remain in meiotic arrest as primary oocytes until sexual maturity. Starting at puberty, each month a few ovarian follicles resume development in response to the monthly surge of pituitary gonadotropic hormones, but usually only one primary oocyte matures into a **secondary oocyte** and is ovulated. This oocyte enters a second phase of meiotic arrest and does not actually complete meiosis unless it is fertilized. These monthly cycles continue until the onset of menopause at approximately 50 years of age. The process of gametogenesis in the male and female (called **spermatogenesis** and **oogenesis**, respectively) is discussed in detail later in this chapter.

b0030

IN THE RESEARCH LAB

WHY IS TIMING OF GAMETOGENESIS DIFFERENT IN MALES AND FEMALES?

Experiments in mouse embryos provide insight into why the timing of gametogenesis differs in males and females. Shortly after PGCs enter the genital ridge, they stop their migration and undergo two or three further rounds of mitosis and then enter a premeiotic stage during which they upregulate meiotic genes. In the male genital ridge, germ cells then reverse this process and arrest, but in the female genital ridge they enter meiotic prophase as primary oocytes and progress through meiosis until the diplotene stage, at which time they arrest. If male (XY) PGCs are transplanted into female (XX) embryos, the male PGCs follow the course just described for normal female

PGCs in females. Moreover, PGCs in female or male embryos that fail to reach the gonad also progress through meiosis as oocytes, regardless of their genotype. These two results suggest that all germ cells, regardless of their chromosome constitution, are programmed to develop as oocytes and that the timing of meiotic entry seems to be a cell-autonomous property rather than being induced. In contrast in males, the genital ridge prevents prenatal entry into meiosis. Experiments suggest that there is a **male meiosis inhibitor** and that this inhibitor is a diffusible signaling factor produced by Sertoli cells. Possible candidates for this factor include the protein *Prostaglandin D2* and the protein encoded by the *Tdl* gene (a gene showing sequence homology to antimicrobial proteins called *beta-Defensins*; *Prostaglandins* are synthesized from fatty acids and modulate several physiological functions such as blood pressure, smooth muscle contraction, and inflammation).

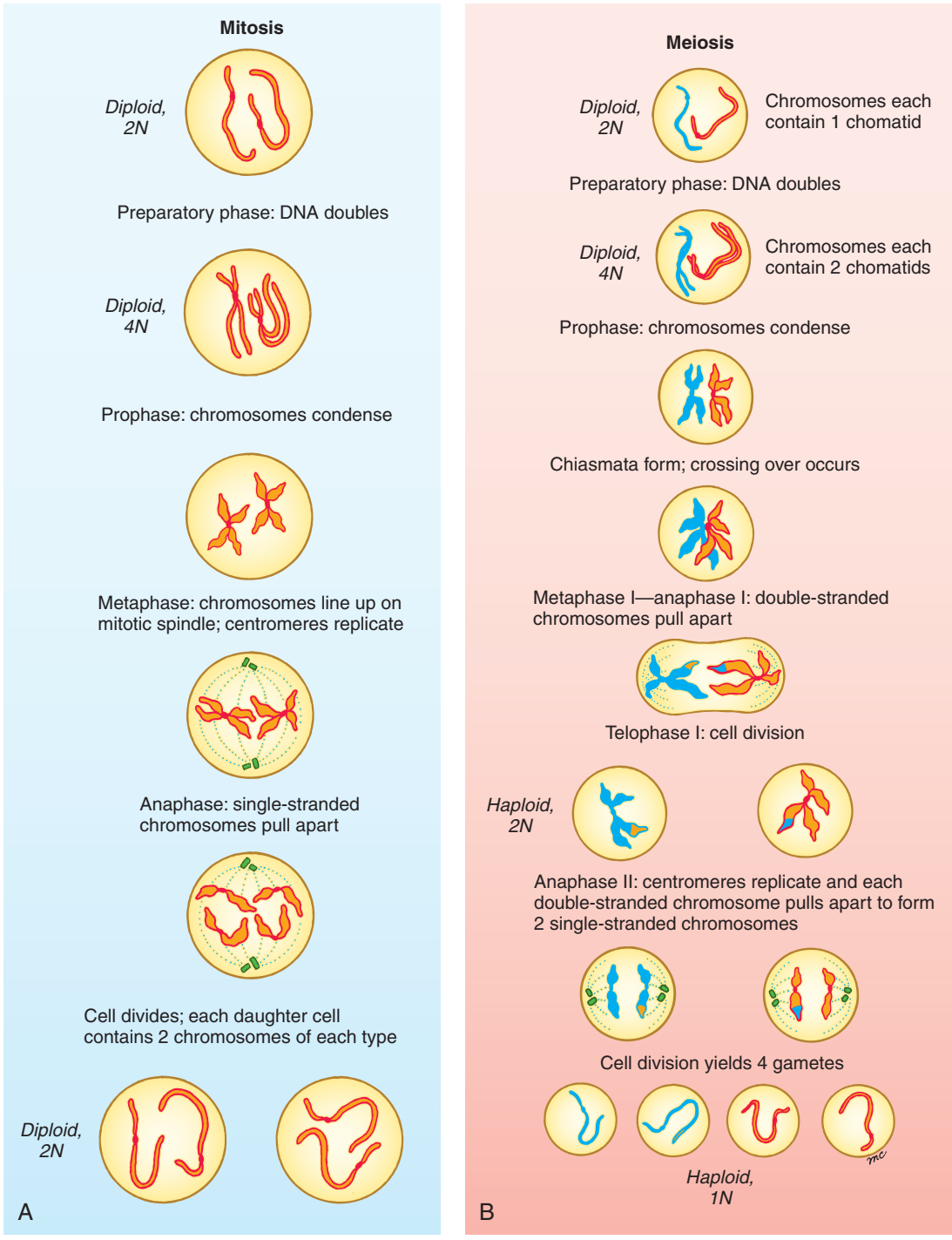
Meiosis Halves Number of Chromosomes and DNA Strands in Sex Cells

p0060 Although the timing of meiosis is very different in the male and female, the basic chromosomal events of the process are the same in the two sexes (Fig. 1-2). Like all normal somatic (nongerm) cells, PGCs contain 23 pairs of chromosomes, or a total of 46 chromosomes. One chromosome of each pair is obtained from the maternal gamete and the other from the paternal gamete. These chromosomes contain **deoxyribonucleic acid (DNA)**, which encodes information required for development and functioning of the organism. Of the total complement of 46 chromosomes, 22 pairs consist of matching, homologous chromosomes called **autosomes**. The remaining two chromosomes are called **sex chromosomes** because they determine the sex of the individual. There are two kinds of sex chromosome, X and Y. Individuals with one X chromosome and one Y chromosome (XY) are genetically male; individuals with two X chromosomes (XX) are genetically female. Nonetheless, one of the X chromosomes in the female genome is randomly inactivated, leaving only one active X chromosome in each cell (X-inactivation is discussed in Ch. 2). Mechanisms underlying sex determination are discussed further in Chapter 15.

p0070 Two designations that are often confused are the **ploidy** of a cell and its **N number**. *Ploidy* refers to the number of copies of each *chromosome* present in a cell nucleus, whereas the *N number* refers to the number of copies of each unique double-stranded *DNA molecule* in the nucleus. Each chromosome contains one or two molecules of DNA at different stages of the cell cycle (whether mitotic or meiotic), so the ploidy and N number of a cell do not always coincide. Somatic cells and PGCs have two copies of each kind of chromosome and hence are called **diploid**. Mature gametes, in contrast, have just one copy of each kind of chromosome and are called **haploid**. Haploid gametes with one DNA molecule per chromosome are said to be **1N**. In some stages of the cell cycle, diploid cells also have one DNA molecule per chromosome and hence are **2N**. However, during the earlier phases of meiosis or mitosis, each chromosome of a diploid cell has two molecules of DNA, and so the cell is **4N**.

p0080 **Meiosis** is a specialized process of cell division that occurs only in the germ line. Figure 1-2 compares mitosis (*A*) and meiosis (*B*). In **mitosis** (normal cell division), a diploid, 2N cell replicates its DNA (becoming diploid, 4N) and undergoes a single division to yield two diploid, 2N daughter cells. In meiosis, a diploid germ cell replicates its DNA (becoming diploid, 4N) and undergoes two successive, qualitatively different nuclear and cell divisions to yield four haploid, 1N offspring. In males, the cell divisions of meiosis are equal and yield four identical spermatozoa. However in females, the meiotic cell divisions are dramatically unequal and yield a single, massive, haploid definitive oocyte and three minute, nonfunctional, haploid **polar bodies**.

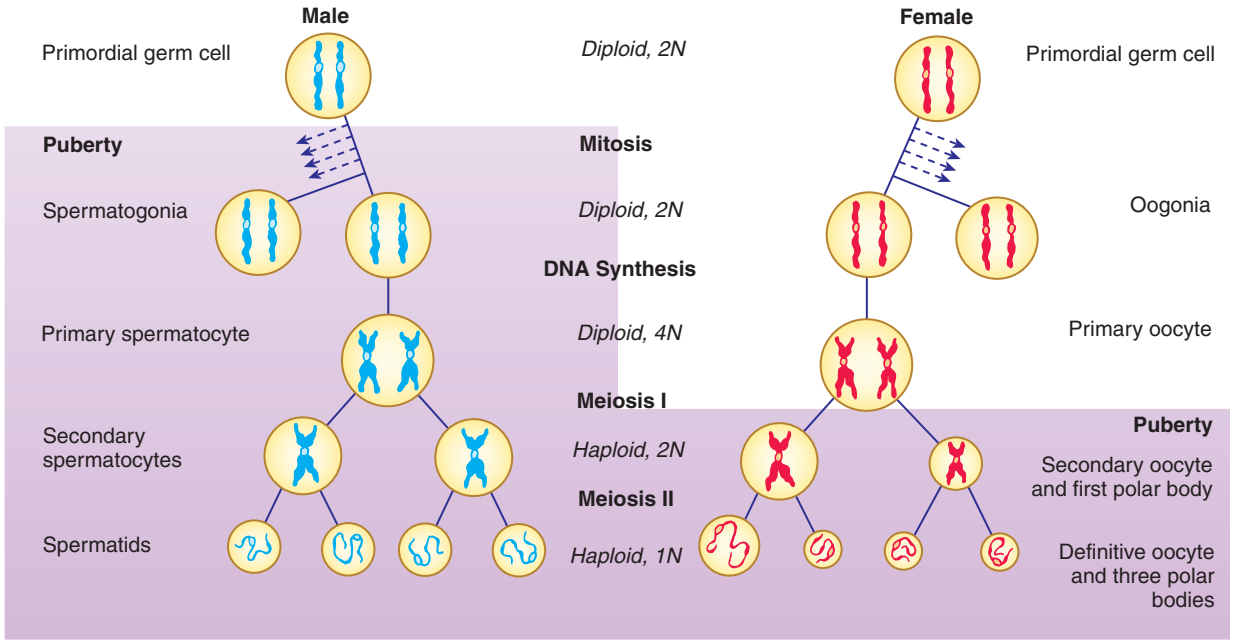
p0090 **First Meiotic Division: DNA Replication and Recombination, Yielding Four Haploid, 2N Daughter Cells**. The steps of meiosis are illustrated in Figure 1-2*B* and summarized in Table 1-1. The preliminary step in meiosis, as in mitosis, is the replication of each chromosomal DNA molecule; thus, the diploid cell is converted from 2N to 4N. This event marks the beginning of gametogenesis. In the female, the oogonium is now called a **primary oocyte**, and in the male, the spermatogonium is now called a **primary spermatocyte** (Fig. 1-3). Once the DNA replicates, each chromosome consists of two parallel strands or **chromatids** joined together at a structure called the **centromere**. Each chromatid contains a single DNA molecule (which is itself double stranded; don't confuse DNA double strands with the two chromatid strands composing each chromosome).



f0020 **Figure 1-2.** *A*, Mitosis. *B*, Meiosis. See Table 1-1 for a description of the stages.

t0010 **Table 1-1** Events during Mitotic and Meiotic Cell Divisions in the Germ Line

Stage	Events	Name of Cell	Condition of Genome
Resting interval between mitotic cell divisions	Normal cellular metabolism occurs	♀ Oogonium ♂ Spermatogonium	Diploid, 2N
Mitosis			
Preparatory phase	DNA replication yields double-stranded chromosomes	♀ Oogonium ♂ Spermatogonium	Diploid, 4N
Prophase	Double-stranded chromosomes condense		
Metaphase	Chromosomes align along the equator; centromeres replicate		
Anaphase and telophase	Each double-stranded chromosome splits into 2 single-stranded chromosomes, one of which is distributed to each daughter nucleus		
Cytokinesis	Cell divides	♀ Oogonium ♂ Spermatogonium	Diploid, 2N
Meiosis I			
Preparatory phase	DNA replication yields double-stranded chromosomes	♀ Primary oocyte ♂ Primary spermatocyte	Diploid, 4N
Prophase	Double-stranded chromosomes condense; 2 chromosomes of each homologous pair align at the centromeres to form a 4-limbed chiasma; recombination by crossing over occurs		
Metaphase	Chromosomes align along the equator; *centromeres do not replicate*		
Anaphase and telophase	1 double-stranded chromosome of each homologous pair is distributed to each daughter cell		
Cytokinesis	Cell divides	♀ one secondary oocyte and the first polar body ♂ two secondary spermatocytes	Haploid, 2N
Meiosis II			
Prophase	*No DNA replication takes place during the second meiotic division*; double-stranded chromosomes condense		
Metaphase	Chromosomes align along the equator; *centromeres replicate*		
Anaphase and telophase	Each chromosome splits into 2 single-stranded chromosomes, one of which is distributed to each daughter nucleus		
Cytokinesis	Cell divides	♀ one definitive oocyte and three polar bodies ♂ four spermatids	Haploid, 1N



f0030 **Figure 1-3.** Nuclear maturation of germ cells in meiosis in the male and female. In the male, primordial germ cells (PGCs) remain dormant until puberty, when they differentiate into spermatogonia and commence mitosis. Throughout adulthood, spermatogonia produce primary spermatocytes, which undergo meiosis and spermatogenesis. Each primary spermatocyte yields four spermatozoa. In the female, PGCs differentiate into oogonia, which undergo mitosis and then commence meiosis as primary oocytes during fetal life. The primary oocytes remain arrested in prophase I until stimulated to resume meiosis during a menstrual cycle. If fertilization occurs, each primary oocyte yields one definitive oocyte and three polar bodies.

p0100 In the next step, called **prophase**, the chromosomes condense into compact, double-stranded structures (i.e., two chromatids joined by one centromere). During the late stages of prophase, the double-stranded chromosomes of each homologous pair match up, centromere to centromere, to form a joint structure called a **chiasma** (composed of four chromatids, two centromeres, and two chromosomes). Chiasma formation makes it possible for the two homologous chromosomes to exchange large segments of DNA by a process called **crossing over**. The resulting **recombination** of the genetic material on homologous maternal and paternal chromosomes is largely random and, therefore, increases the genetic variability of the future gametes. As mentioned earlier, the primary oocyte enters a phase of meiotic arrest during the first meiotic prophase.

p0110 During **metaphase**, the four-stranded chiasma structures are organized on the equator of a spindle apparatus similar to the one that forms during mitosis, and during **anaphase**, one double-stranded chromosome of each homologous pair is distributed to each of the two daughter nuclei. During the first meiotic division, the centromeres of the chromosomes do not replicate, and, therefore, the two chromatids of each chromosome remain together. The resulting daughter nuclei thus are haploid but 2N: they contain the same amount of DNA as the parent germ cell but half as many chromosomes. After the daughter nuclei form, the cell itself divides (undergoes **cytokinesis**). The first meiotic cell division produces two **secondary spermatocytes** in the male and a **secondary oocyte** and a **first polar body** in the female (see Fig. 1-3).

p0120 **Second Meiotic Division: Double-Stranded Chromosomes Divide, Yielding Four Haploid, 1N Daughter Cells**. No DNA replication occurs during the second meiotic division. The 23 double-stranded chromosomes condense during the second meiotic prophase and line up during the second meiotic metaphase. The chromosomal centromeres then replicate, and during anaphase, the double-stranded chromosomes pull apart into two single-stranded chromosomes, one of which is distributed to each of the daughter nuclei. In males, the second meiotic cell

division produces two **definitive spermatocytes**, more commonly called **spermatids** (i.e., a total of four from each germ cell entering meiosis). In the female, the second meiotic cell division, like the first, is radically unequal, producing a large **definitive oocyte** and another diminutive polar body. The first polar body may simultaneously undergo a second meiotic division to produce a third polar body (see Fig. 1-3).

p0130 In the female, the oocyte enters a second phase of meiotic arrest during the second meiotic metaphase before replication of the centromeres. Meiosis does not resume unless the cell is fertilized.

s0080 Spermatogenesis

p0140 Now that meiosis has been described, it is possible to describe and compare the specific processes of spermatogenesis and oogenesis. At puberty, the testes begin to secrete greatly increased amounts of the steroid hormone **testosterone**. This hormone has a multitude of effects. In addition to stimulating development of many secondary sex characteristics, it triggers growth of the testes, maturation of seminiferous tubules, and commencement of spermatogenesis.

p0150 Under the influence of testosterone, Sertoli cells differentiate into a system of seminiferous tubules. The dormant PGCs resume development, divide several times by mitosis, and then differentiate into spermatogonia. These spermatogonia are located immediately under the basement membrane surrounding the seminiferous tubules, where they occupy pockets between Sertoli cells (Fig. 1-4*A*). Each spermatogonium is connected to the adjacent Sertoli cells by specialized membrane junctions (see next section). In addition, Sertoli cells are joined to each other by dense bands of intercellular membrane junctions that surround each Sertoli cell and thus isolate spermatogonia from the tubule lumen.

s0090 Male Germ Cells Are Translocated to Seminiferous Tubule Lumen during Spermatogenesis

p0160 Cells that will undergo spermatogenesis arise by mitosis from the spermatogonia. These cells are gradually translocated between the Sertoli cells from the basal to the luminal side of the seminiferous epithelium while spermatogenesis takes place (see Fig. 1-4*A*). During this migratory phase, primary spermatocytes pass without interruption through both meiotic divisions, producing first two secondary spermatocytes and then four spermatids. The spermatids undergo dramatic changes that convert them into mature sperm while they complete their migration to the lumen. This process of sperm cell differentiation is called **spermiogenesis.**

Sertoli Cells Are Also Instrumental in Spermiogenesis s0100

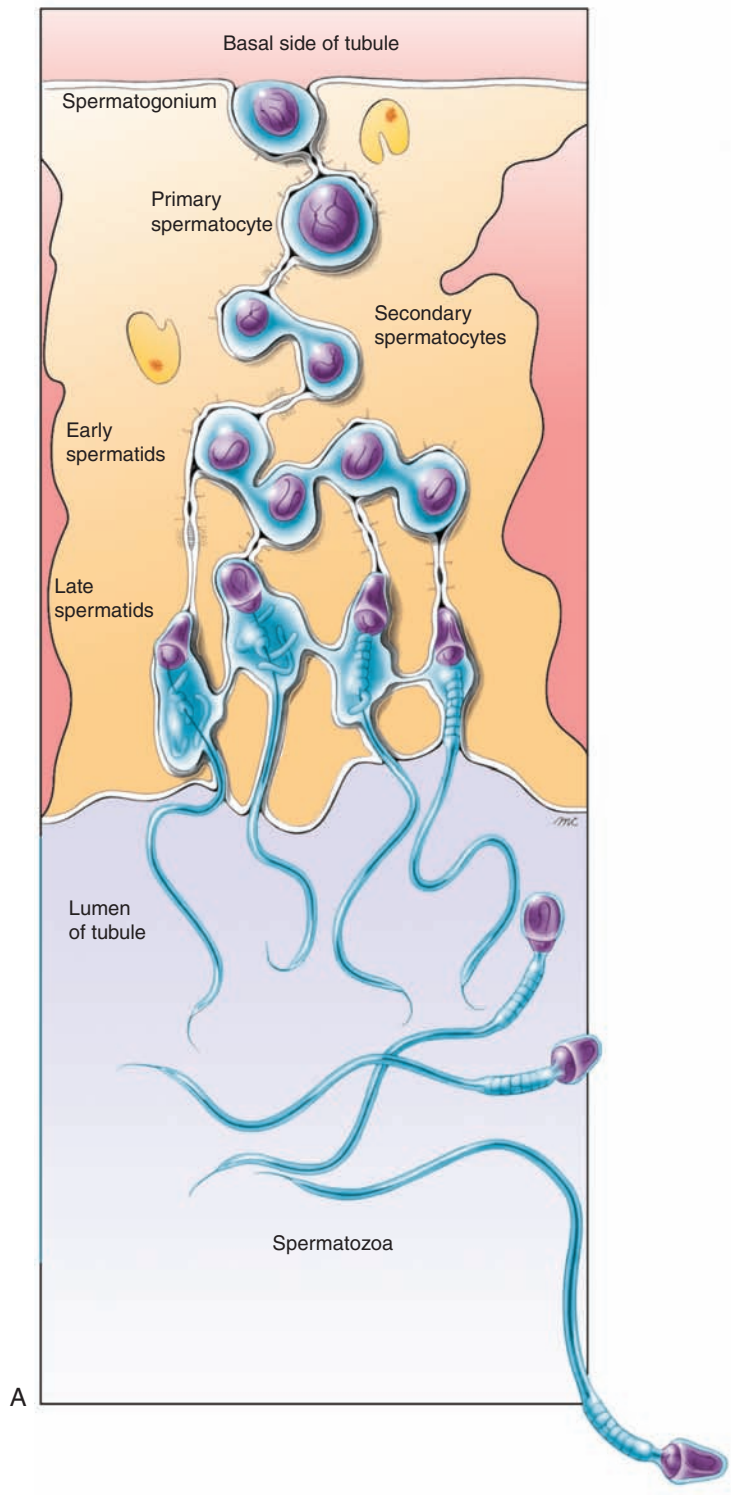
p0170 Sertoli cells participate intimately in the differentiation of the gametes. Maturing spermatocytes and spermatids are connected to surrounding Sertoli cells by intercellular junctions, typical of those found on epithelial cells, and unique cytoplasmic processes called **tubulobulbar complexes** that extend into the Sertoli cells. The cytoplasm of developing gametes shrinks dramatically during spermiogenesis; the tubulobulbar complexes are thought to provide a mechanism by which the excess cytoplasm is transferred to Sertoli cells. As cytoplasm is removed, spermatids undergo dramatic changes in shape and internal organization that transform them into spermatozoa. Finally, the last connections with Sertoli cells break, releasing the spermatozoa into the tubule lumen. This final step is called **spermiation**.

p0180 As shown in Figures 1-4*B*, *C*, a spermatozoon consists of a **head, midpiece**, and **tail**. The head contains the condensed nucleus and is capped by an apical vesicle filled with hydrolytic enzymes (e.g., acrosin, hyaluronidase, and neuraminidase). This vesicle, the **acrosome**, plays an essential role in fertilization. The midpiece contains large, helical mitochondria and generates energy for swimming. The long tail contains microtubules that form part of the propulsion system of the spermatozoon.

IN THE CLINIC b0040

SPERMATOZOA ABNORMALITIES

Errors in spermatogenesis or spermiogenesis are common. Examination of a sperm sample will reveal spermatozoa with abnormalities such as small, narrow, or piriform (pear-shaped) heads, double or triple heads, acrosomal defects, and double tails. If at least 50% of the spermatozoa in an ejaculate have a normal morphology, fertility is not expected to be impaired. Having a larger number of abnormal spermatozoa (called teratospermia if excessive) can be associated with infertility.



f0040 **Figure 1-4.** *A*, Schematic section through the wall of the seminiferous tubule. Spermatogonium just under the outer surface of the tubule wall (basal side) undergoes mitosis to produce daughter cells, which may either continue to divide by mitosis (thus renewing the spermatogonial stem cell population) or commence meiosis as primary spermatocytes. As spermatogenesis and spermiogenesis occur, the differentiating cell is translocated between adjacent Sertoli cells to the tubule lumen. Daughter spermatocytes and spermatids remain linked by cytoplasmic bridges. The entire clone of spermatogonia derived from each primordial germ cell is linked by cytoplasmic bridges.

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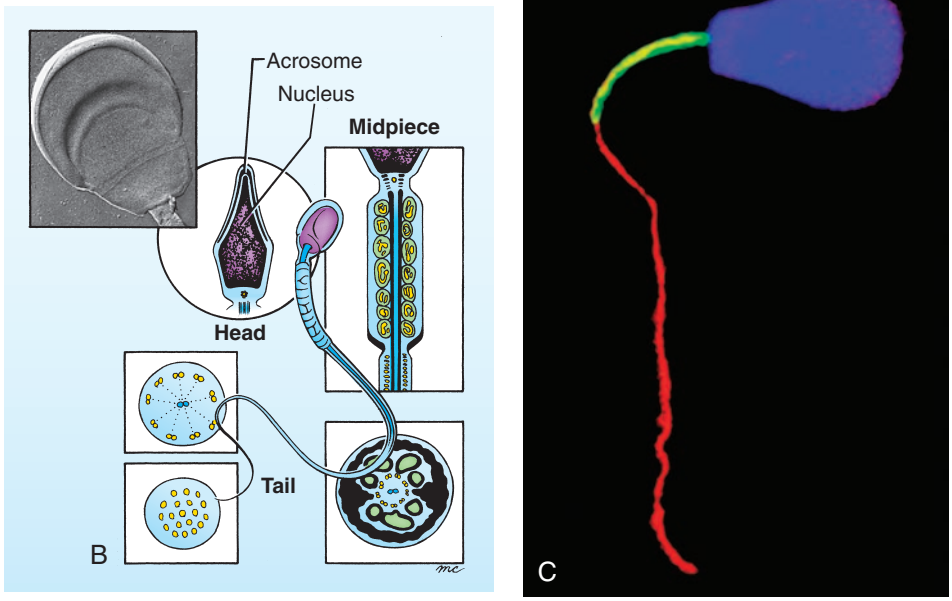


f0040 **Figure 1-4, cont'd.** *B*, Structure of the mature spermatozoon. The head contains the nucleus capped by the acrosome; the midpiece contains coiled mitochondria; the tail contains propulsive microtubules. The inset micrograph shows the head of a human sperm. *C*, Bull sperm labeled with fluorescent markers to reveal its nucleus (blue) in its head, mitochondria (green) in its midpiece, and microtubules (red) in its tail. The red labeling around the perimeter of the head is background labeling.

s0110 ## Continual Waves of Spermatogenesis Occur throughout Seminiferous Epithelium

p0190 Spermatogenesis takes place continuously from puberty to death. Gametes are produced in synchronous waves in each local area of the germinal epithelium, although the process is not synchronized throughout the seminiferous tubules. In many different mammals, the clone of spermatogonia, derived from each spermatogonial stem cell, populates a local area of the seminiferous tubules and displays synchronous spermatogenesis. That may be the case in humans as well. About four waves of synchronously differentiating cells can be observed in a given region of the human tubule epithelium at any time. Ultrastructural studies provide evidence that these waves of differentiating cells remain synchronized because of incomplete cytokinesis throughout the series of mitotic and meiotic divisions between the division of a spermatogonium and formation of spermatids. Instead of fully separating, daughter cells produced by these divisions remain connected by slender cytoplasmic bridges (see Fig. 1-4*A*) that could allow passage of small signaling molecules or metabolites.

p0200 In the human male, each cycle of spermatogenesis takes about 64 days. Spermatogonial mitosis occupies about 16 days, the first meiotic division takes about 8 days, the second meiotic division takes about 16 days, and spermiogenesis requires about 24 days.

s0120 ## Spermatozoa Undergo a Terminal Step of Functional Maturation Called Capacitation

p0210 During its journey from the seminiferous tubules to the ampulla of the oviduct, a sperm cell undergoes a process of functional maturation that prepares it to fertilize an oocyte. Sperm produced in the seminiferous tubules are stored in the lower part of the **epididymis**, a 40-foot long

highly coiled duct connected to the **vas deferens** near its origin in the testis. During ejaculation, sperm are propelled through the vas deferens and urethra and are mixed with nourishing secretions from the **seminal vesicles, prostate**, and **bulbourethral glands** (these structures are further discussed in Ch. 15). As many as 300 million spermatozoa may be deposited in the vagina by a single ejaculation, but only a few hundred succeed in navigating through the cervix, uterus, and oviduct and into the expanded ampullar region. In the **ampulla** of the oviduct, sperm survive and retain their capacity to fertilize an oocyte for 1 to 3 days.

p0220 **Capacitation**, the final step of sperm maturation, consists mainly of changes in the acrosome that prepare it to release the enzymes required to penetrate the zona pellucida, a shell of glycoprotein surrounding the oocyte. Capacitation takes place within the female genital tract and is thought to require contact with secretions of the oviduct. Spermatozoa used in in vitro fertilization (IVF) procedures are artificially capacitated. Spermatozoa with defective acrosomes may be injected directly into oocytes to assist reproduction in humans (assisted reproduction technology, or ART, is discussed later in the chapter under **"In the Clinic"**).

s0130 Oogenesis

s0140 Primary Oocytes Form in Ovaries by Five Months of Fetal Life

p0230 As mentioned earlier, female germ cells undergo a series of mitotic divisions after they are invested by somatic support cells and then differentiate into oogonia (see Fig. 1-3). By 12 weeks of development, oogonia in the genital ridges enter the first meiotic prophase and then almost immediately become dormant. The nucleus of each of these dormant **primary oocytes**, containing the partially condensed prophase chromosomes, becomes very large and watery and is referred to as a **germinal vesicle**. The swollen condition of the germinal vesicle is thought to protect the oocyte's DNA during the long period of meiotic arrest.

p0240 A single-layered, squamous capsule of epithelial follicle cells derived from the somatic support cells tightly encloses each primary oocyte. This capsule and its enclosed primary oocyte constitute a **primordial follicle** (see Fig. 1-6). By 5 months, the number of primordial follicles in the ovaries peaks at about 7 million. Most of these follicles subsequently degenerate. By birth only 700,000 to 2 million remain, and by puberty, only about 400,000.

Hormones of Female Cycle Control Folliculogenesis, Ovulation, and Condition of Uterus s0150

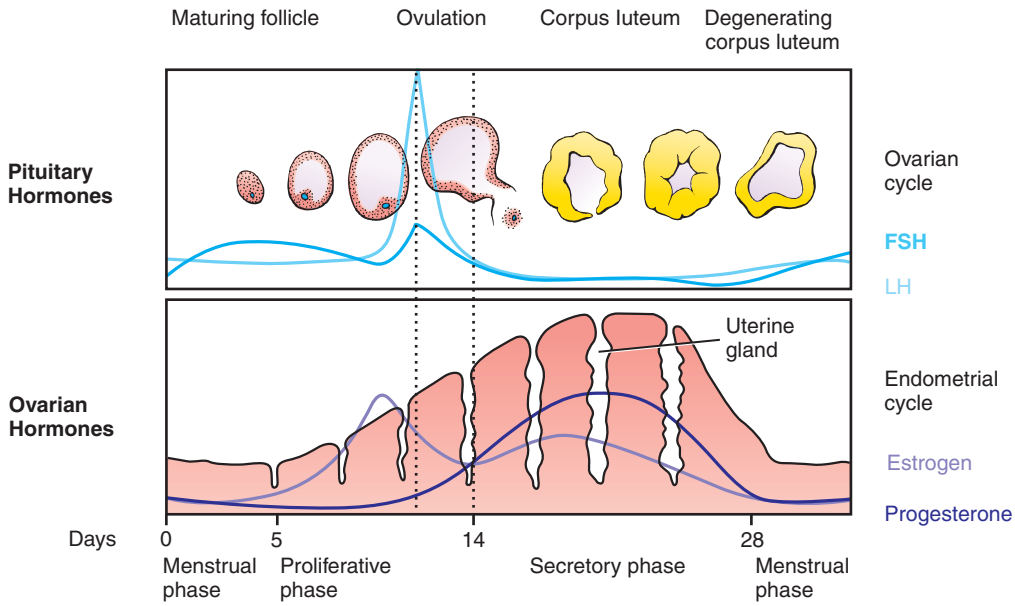
After reaching puberty, also called **menarche** in females, and until the woman enters **menopause** several decades later, monthly cycles in the secretion of hypothalamic, pituitary, and ovarian hormones control a **menstrual cycle**, which results each month in the production of a female gamete and a uterus primed to receive a fertilized embryo. Specifically, this 28-day cycle consists of: p0250

- The monthly maturation of (usually) a single oocyte and its enclosing follicle p0260
- The concurrent proliferation of the uterine endometrium p0270
- The process of ovulation by which the oocyte is released from the ovary p0280
- The continued development of the follicle into an endocrine corpus luteum p0290
- The sloughing of the uterine endometrium and involution of the corpus luteum (unless a fertilized ovum implants in the uterus and begins to develop) p0300

The menstrual cycle is considered to begin with menstruation (also called the menses), the shedding of the degenerated uterine endometrium from the previous cycle. On about the 5th day of the cycle (the 5th day after the beginning of menstruation), an increase in secretion by the hypothalamus of the brain of a small peptide hormone, gonadotropin-releasing hormone (GnRH), stimulates the pituitary gland to increase its secretion of two gonadotropic hormones (gonadotropins): follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Fig. 1-5). The rising levels of pituitary gonadotropins regulate later phases of folliculogenesis in the ovary and the proliferative phase in the uterine endometrium. p0310

About Five to Twelve Primary Follicles Resume Development Each Month s0160

Before a particular cycle, and independent of pituitary gonadotropins, the follicular epithelium of a small group of primordial follicles thickens, converting the single-layered follicular epithelium from a layer of p0320



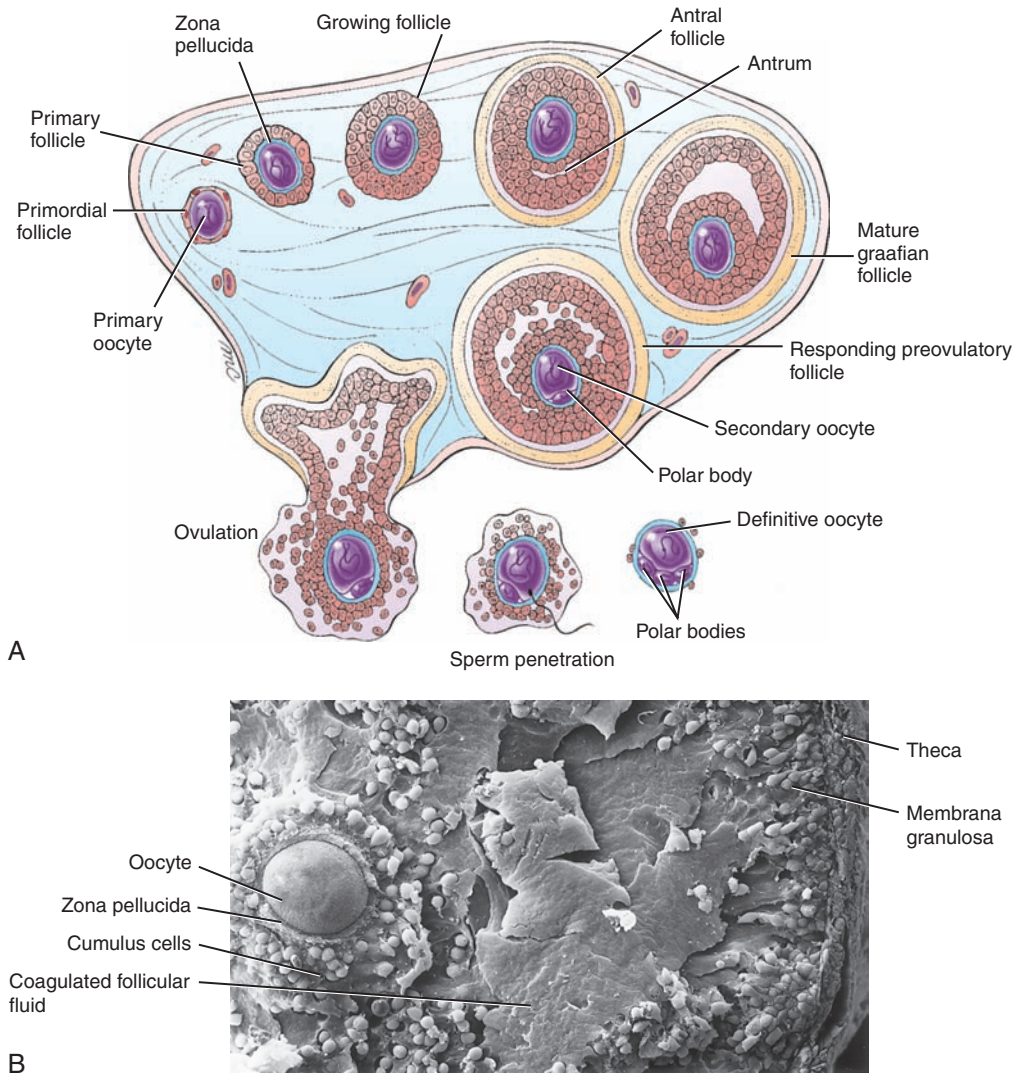
f0050 **Figure 1-5.** Ovarian, endometrial, and hormonal events of the menstrual cycle. Pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) directly control the ovarian cycle and also control production of estrogen and progesterone by responding follicles and corpus luteum of the ovary. These ovarian hormones in turn control the cycle of the uterine endometrium.

squamous cells to cuboidal cells (Fig. 1-6*A*). These follicles are now called **primary follicles**. The follicle cells and the oocyte jointly secrete a thin layer of acellular material, composed of only a few types of glycoprotein, onto the surface of the oocyte. Although this layer, the **zona pellucida**, appears to form a complete physical barrier between the follicle cells and oocyte (Figs. 1-6*B*, 1-7*A*), actually it is penetrated by thin extensions of follicle cells that are connected to the oocyte cell membrane by intercellular junctions (Fig. 1-7*B*). These extensions and their intercellular junctions remain intact until just before ovulation, and they probably convey both developmental signals and metabolic support to the oocyte. The follicular epithelium of five to twelve of these primary follicles then proliferates to form a multilayered capsule of follicle cells around the oocyte (see Fig. 1-6). The follicles are now called **growing follicles**. At this point, some of the growing follicles cease to develop and eventually degenerate, whereas a few continue to enlarge in response to rising levels of FSH, mainly by taking up fluid and developing a central fluid-filled cavity called the **antrum**. These follicles are called **antral** or **vesicular follicles**. At the same time, the connective tissue of the ovarian stroma surrounding each of these follicles differentiates into two layers, an inner layer called the **theca interna** and an outer layer called the **theca externa**. These two layers become vascularized, in contrast to the follicle cells, which do not.

Single Follicle Becomes Dominant and Remainder Degenerate

s0170

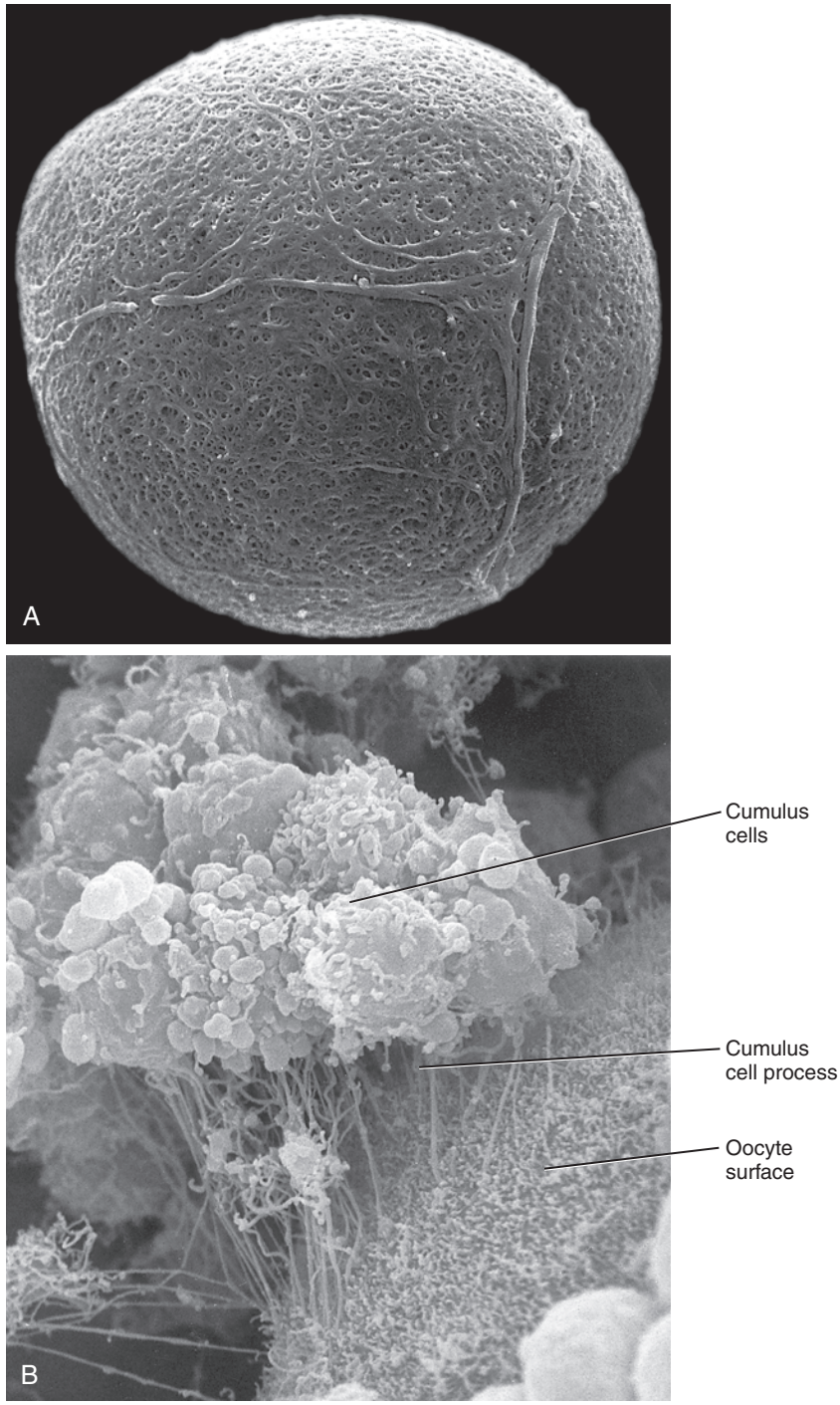
p0330 Eventually, one of the growing follicles gains primacy and continues to enlarge by absorbing fluid, whereas the remainder of the follicles recruited during the cycle degenerate (undergo **atresia**). The oocyte, surrounded by a small mass of follicle cells called the **cumulus oophorus**, increasingly projects into the expanding antrum but remains connected to the layer of follicle cells that lines the antral cavity and underlies the basement membrane of the follicle. This layer is called the **membrana granulosa**. The large, swollen follicle is now called a **mature vesicular follicle** or **mature graafian follicle** (see Fig. 1-6). At this point, the oocyte still has not resumed meiosis.



f0060 **Figure 1-6.** *A*, Schematic depiction of the ovary showing folliculogenesis and ovulation. Five to 12 primordial follicles initially respond to the rising levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), but only one matures. In response to the ovulatory surge in LH and FSH, the oocyte of this mature graafian follicle resumes meiosis and ovulation occurs. Final steps of meiosis take place only if the released oocyte is penetrated by a sperm. *B*, Scanning electron micrograph of a preovulatory follicle.

s0180 ## Why Is Folliculogenesis Selectively Stimulated in Only a Few Follicles Each Month?

p0340 The reason why only five to twelve primordial follicles commence folliculogenesis each month—and why, of this group, all but one eventually degenerate—is not known. One possibility is that follicles become progressively more sensitive to the stimulating effects of FSH as they advance in development. Follicles that are slightly more advanced simply on a random basis would, therefore, respond more acutely to FSH and would be favored. Another possibility is that the selection process is regulated by a complex system of feedback between the pituitary and ovarian hormones and growth factors.



f0070 **Figure 1-7.** *A*, Scanning electron micrograph of the zona pellucida after removal of the cumulus cells. The zona consists of protein and mucopolysaccharide and forms a barrier that the sperm penetrates by means of its acrosomal enzymes. *B*, Scanning electron micrograph of the oocyte surface and cumulus oophorus, with the zona pellucida digested away. The cumulus cells maintain contact with the oocyte via thin cell processes that penetrate the zona pellucida and form intercellular junctions with the oocyte cell membrane.

b0050

IN THE CLINIC

CHROMOSOMAL ABNORMALITIES RESULT IN SPONTANEOUS ABORTION OR ABNORMAL DEVELOPMENT

It is estimated that one third of all conceptions in normal, healthy women abort spontaneously; approximately one fourth of these occur before pregnancy is detected. Chromosomal anomalies seem to cause about 40% to 50% of spontaneous abortions in those cases in which the conceptus has been recovered and examined. However, many chromosomal anomalies allow the fetus to survive to term. The resulting infants display nonrandom patterns of developmental abnormalities; that is, **syndromes**. One of these syndromes, Down syndrome, is discussed in the detail in the following section; others are discussed in detail in subsequent chapters.

MANY CHROMOSOMAL ANOMALIES ARISE DURING GAMETOGENESIS AND CLEAVAGE

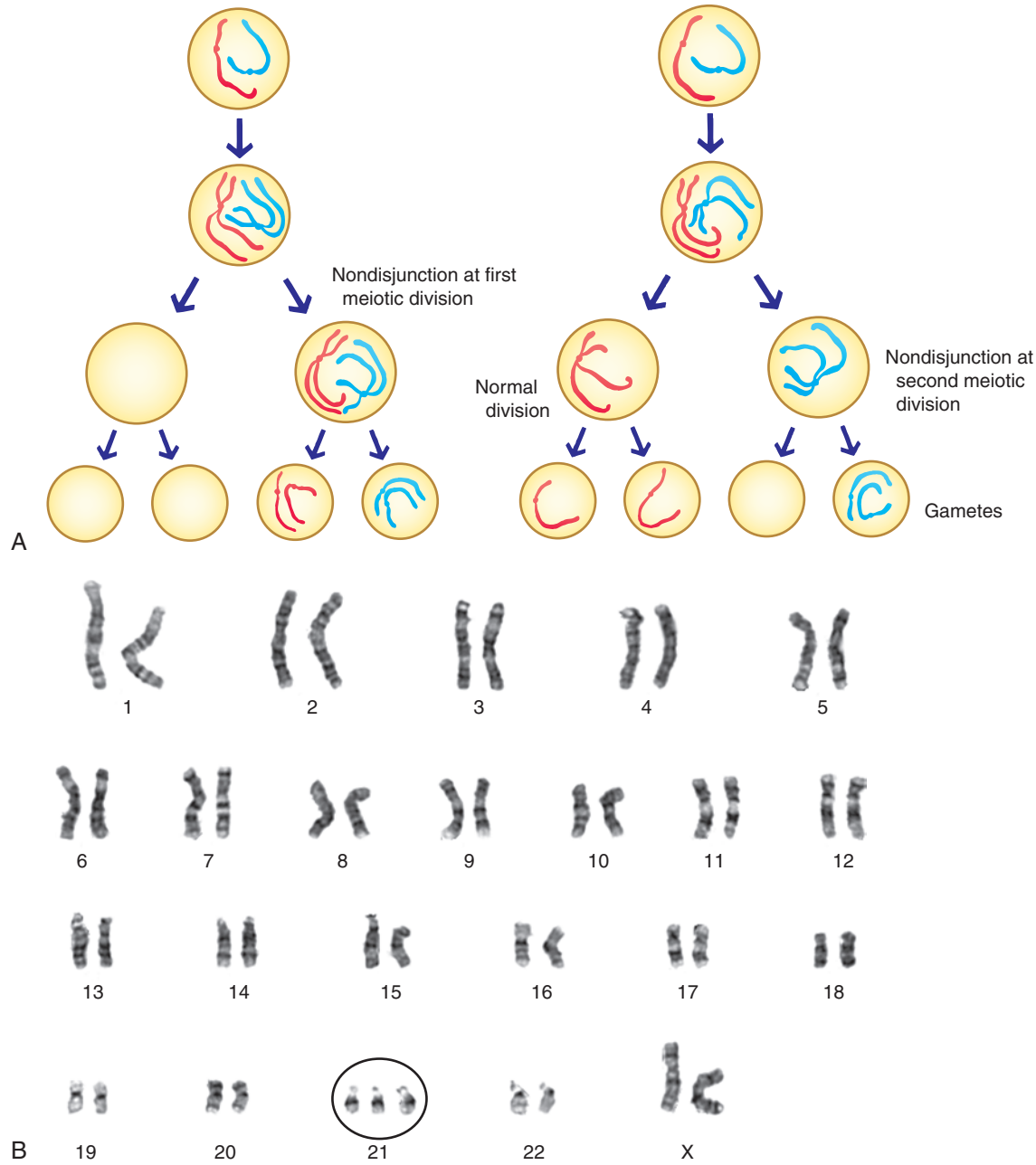
Abnormal chromosomes can be produced in the germ line of either parent through an error in meiosis or fertilization, or can arise in the early embryo through an error in mitosis. Gametes or blastomeres that result from these events contain missing or extra chromosomes, or chromosomes with duplicated, deleted, or rearranged segments. Absence of a specific chromosome in a gamete that combines with a normal gamete to form a zygote results in a condition known as **monosomy** (because the zygote contains only one copy of the chromosome rather than the normal two). Conversely, the presence of two of the same kind of chromosome in one of the gametes that forms a zygote results in **trisomy**.

Down syndrome is a disorder most frequently caused by an error during meiosis. If the two copies of chromosome 21 fail to separate during the first or second meiotic anaphase of gametogenesis in either parent (a phenomenon called **nondisjunction**), half the resulting gametes will lack chromosome 21 altogether and the other half will have two copies (Fig. 1-8*A*). Embryos formed by fusion of a gamete-lacking chromosome 21 with a normal gamete are called **monosomy 21** embryos. These embryos die rapidly; monosomies of autosomal chromosomes are invariably fatal during early embryonic development. If, on the other hand, a gamete with two copies of chromosome 21 fuses with a normal gamete, the resulting **trisomy 21** embryo may survive (Fig. 1-8*B*). Trisomy 21 infants display the pattern of abnormalities described as **Down syndrome**. In addition to recognizable facial characteristics, mental retardation, and short stature, individuals with Down syndrome may exhibit congenital heart defects (atrioventricular septal defect is most common, that is, a failure to form both the atrial and ventricular septae; discussed in Ch. 12), hearing loss, duodenal obstruction, a propensity to develop leukemia, and immune system defects. Trisomy in most Down syndrome individuals is the result of nondisjunction in the mother, usually during the first meiotic division (75% to 80% of the cases). Identification of the extra chromosome as maternal or paternal in origin was originally based on karyotype analysis that compared banding patterns of the extra chromosome 21 with chromosome 21 of the mother and father. These early studies concluded that about 70% to 75% of Down syndrome cases occurred as a consequence of nondisjunction in the mother. However, by the late 1980s, more sensitive karyotype analysis increased this frequency to 80%, and by the early 1990s, an even more sensitive molecular technique (Southern blot analysis of DNA polymorphisms) provided evidence that as many as 90% to 95% of Down syndrome cases arise through nondisjunction in the maternal germ line. Consequently, it is now accepted that only about 5% of the cases of Down syndrome result from an error in spermatogenesis.

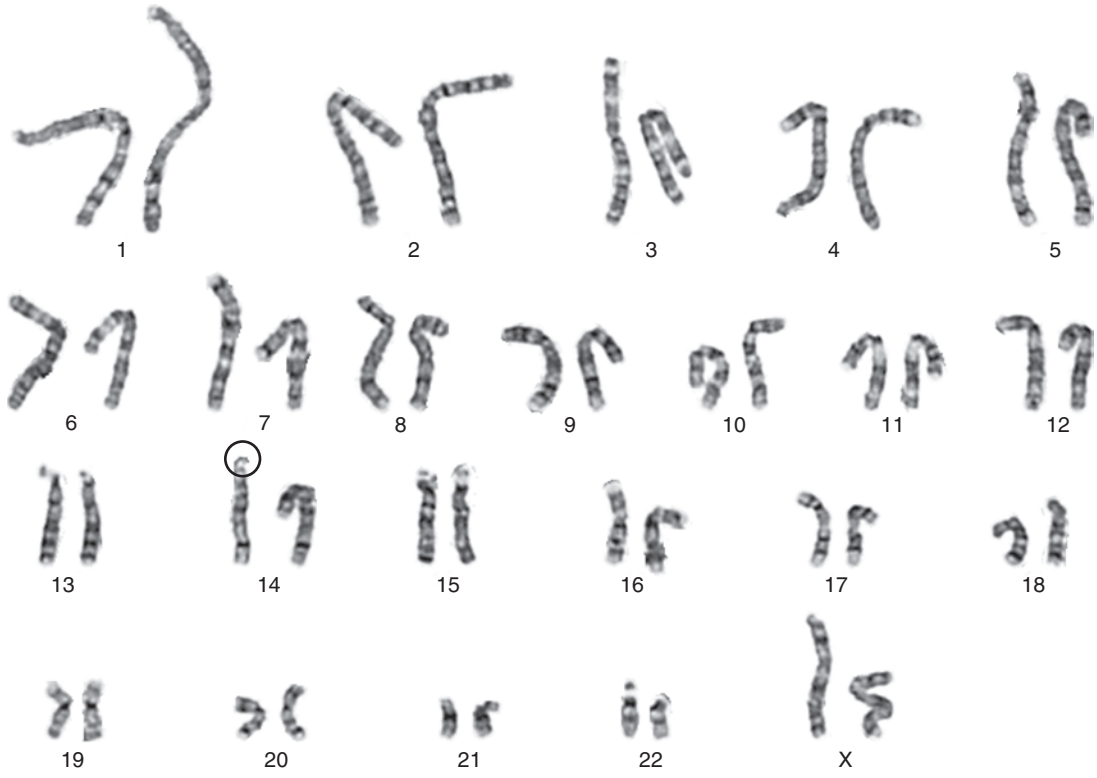
Occasionally, the extra chromosome 21 is lost from a subset of cells during cleavage. The resulting embryo develops as a **mosaic** of normal and trisomy 21 cells. Two percent to 5 percent of all individuals with Down syndrome are mosaics. These individuals may show a range of Down syndrome features depending on the abundance and location of abnormal cells. If nondisjunction occurs in the germ line, a seemingly normal individual could produce several Down syndrome offspring. Meiosis of a trisomic germ cell yields gametes with a normal single copy of the chromosome, as well as abnormal gametes with two copies, so normal offspring also can be produced.

Down syndrome does not always result from simple nondisjunction. Sometimes, a copy of chromosome 21 in a developing gamete becomes attached to the end of another chromosome, such as chromosome 14, during the first or second division of meiosis. This event is called a **translocation**. The zygote produced by fusion of such a gamete with a normal partner will have two normal copies of chromosome 21 plus an abnormal chromosome 14 carrying a third copy of chromosome 21 (Fig. 1-9). Two percent to five percent of all individuals with Down syndrome harbor such translocations.

Cases in which only a part of chromosome 21 is translocated have provided insight into which regions of chromosome 21 must be triplicated to produce specific aspects of Down syndrome, such as mental retardation, characteristic facial features, and cardiovascular defects. By determining which specific phenotypes occur in patients with Down syndrome having particular



f0080 **Figure 1-8.** *A*, Mechanism of chromosomal nondisjunction in meiosis. Failure of homologous double-stranded chromosomes to separate before cytokinesis during the first meiotic division (left-hand panel) results in their distribution to only one of the secondary gonocytes (or first polar body). Failure of the two strands of a double-stranded chromosome to separate before cytokinesis during the second meiotic division (right-hand panel) results in their distribution to only one of the definitive gonocytes (or second polar body). *B*, Karyotype of a female with trisomy 21 (circled), causing Down syndrome.



f0090 **Figure 1-9.** Karyotype of a female with Down syndrome caused by translocation of chromosome 21 onto chromosome 14 (circled).

translocated regions of chromosome 21, **Down syndrome candidate regions** on chromosome 21 have been identified. The completion of the sequencing of chromosome 21 (in May 2000) and the generation of transgenic mice (transgenic mice are discussed in Ch. 5) trisomic for these candidate regions is leading to the identification of those genes responsible for specific Down syndrome phenotypes in humans.

The incidence of Down syndrome increases significantly with the age of the mother but not with the age of the father. The risk of giving birth to a liveborn with Down syndrome at maternal age 30 is 1 in 900. The risk increases to 9 in 1000 by maternal age 40. However, it is not clear whether older women actually produce more oocytes with nondisjunction of chromosome 21 or whether the efficiency of spontaneously aborting trisomy 21 embryos decreases with age.

Trisomies of other autosomes (such as chromosomes 8, 9, 13, and 18) also produce recognizable syndromes of abnormal development, but these trisomies are present much less frequently in live births than is trisomy 21. Similarly, trisomies and monosomies of sex chromosomes occur (for example, **Klinefelter** and **Turner** syndromes, two syndromes in which there are extra or decreased numbers of sex chromosomes, respectively; discussed in Ch. 15). **Triploid** or **tetraploid** embryos, in which multiple copies of the entire genome are present, can arise by errors in fertilization (discussed in Ch. 2).

Several other types of chromosome anomalies are produced at meiosis. In some cases, errors in meiosis result in deletion of just part of a chromosome or duplication of a small chromosome segment. The resulting anomalies are called **partial monosomy** and **partial trisomy**, respectively. Other errors that can occur during meiosis are **inversions** of chromosome segments and the formation of **ring chromosomes.**

CHROMOSOME ANALYSIS CAN DETERMINE PARENTAL SOURCE OF DEFECTIVE CHROMOSOME AND PROVIDES BASIS FOR DIAGNOSIS AND POSSIBLE TREATMENT

Genetic analysis of congenital defects is a very recent development. The normal human **karyotype** was not fully characterized until the late 1950s. Improved staining and culture conditions now allow high-resolution chromosome

banding, increasing our ability to detect small deletions or duplications. Advances in molecular genetic techniques have led to a much finer analysis of DNA structure. As a result, it is possible to identify even smaller defects not evident with high-resolution banding. These techniques are used for both diagnosis and genetic counseling. Blood cells of a prospective parent can be checked for heritable chromosome anomalies, and embryonic cells obtained either from the amniotic fluid (**amniocentesis**) or from the chorionic villi (**chorionic villous sampling**) can be used to detect many disorders early in pregnancy (discussed in Ch. 6).

Three molecular approaches are used routinely for chromosomal analysis (Figs. 1-10, 1-11): fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and chromosome painting (whole chromosome painting and spectral karyotyping, or SKY). In all of these techniques, DNA probes linked to fluorescent dyes (fluorochromes, each of which emits a unique spectrum of light and is assigned a unique color by a computer) are used to probe specific loci on chromosomes. This is particularly useful for detecting changes in chromosome copy number (aneuploidy) or for characterizing chromosomal material involved in translocations.

s0190

Ovulation

s0200

Resumption of Meiosis and Ovulation Are Stimulated by an Ovulatory Surge in FSH and LH

p0350 On about day 13 or 14 of the menstrual cycle (at the end of the proliferative phase of the uterine endometrium), levels of FSH and LH suddenly rise very sharply (see Fig. 1-5). This **ovulatory surge** in pituitary gonadotropins stimulates the primary oocyte of the remaining mature graafian follicle to resume meiosis. This response can be observed visually about 15 hours after the beginning of the ovulatory surge, when the membrane of the swollen germinal vesicle (nucleus) of the oocyte breaks down (Fig 1-12*A*). By 20 hours, the chromosomes are lined up in metaphase. Cell division to form the secondary oocyte and first polar body rapidly ensues (Fig. 1-12*B*). The secondary oocyte promptly begins the second meiotic division but, about 3 hours before ovulation, is arrested at the second meiotic metaphase.

s0210

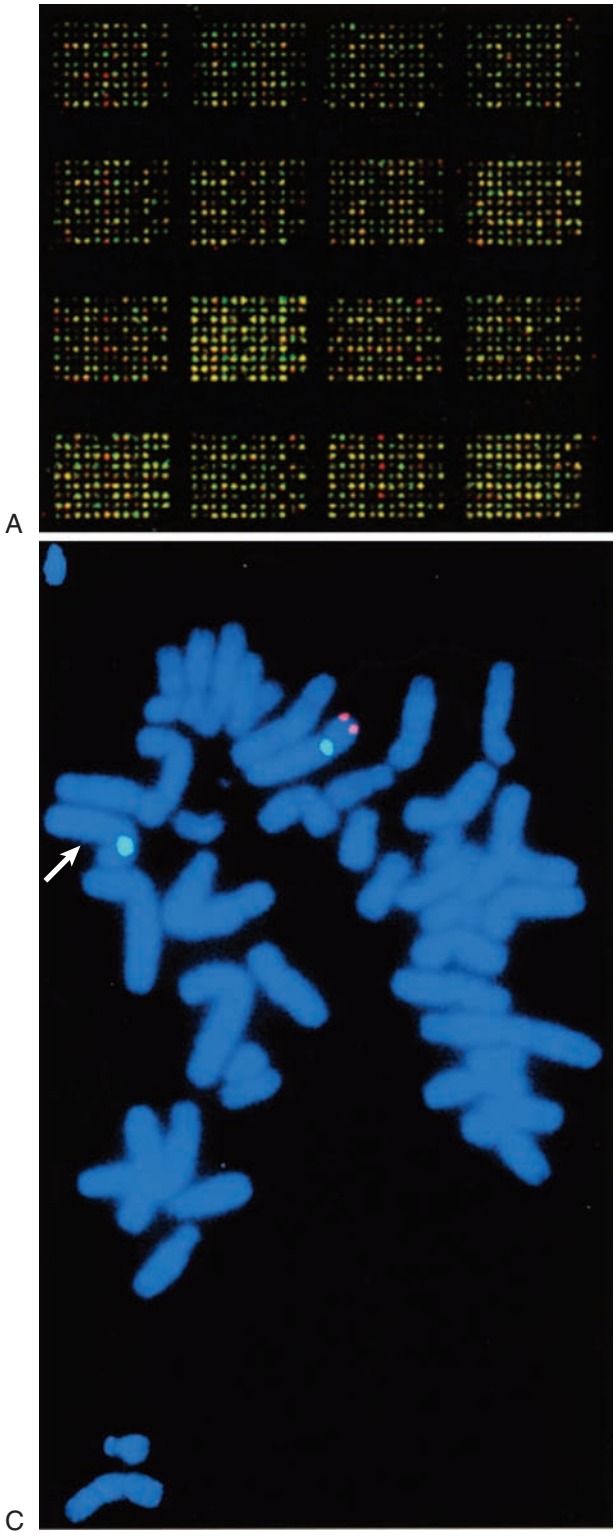
Cumulus Oophorus Expands in Response to Ovulatory Surge

p0360 As the germinal vesicle breaks down, the cumulus cells surrounding the oocyte lose their cell-to-cell connections and disaggregate. As a result, the oocyte and a mass of loose cumulus cells detach into the antral cavity. Over the next few hours, the cumulus cells secrete an abundant extracellular matrix, consisting mainly of hyaluronic acid, which causes the cumulus cell mass to expand severalfold. This process of **cumulus expansion** may play a role in several processes, including the regulation of meiotic progress and ovulation. In addition, the mass of matrix and entrapped cumulus cells that accompanies the ovulated oocyte may play roles in the transport of the oocyte in the oviduct, in fertilization, and in the early development of the zygote.

s0220

Ovulation Depends on Breakdown of the Follicle Wall

p0370 The process of **ovulation** (the expulsion of the secondary oocyte from the follicle) has been likened to an inflammatory response. The cascade of events that culminates in ovulation is thought to be initiated by the secretion of histamine and prostaglandins, well-known inflammatory mediators. Within a few hours after the ovulatory surge of FSH and LH, the follicle becomes more vascularized and is visibly pink and edematous in comparison with nonresponding follicles. The follicle is displaced to the surface of the ovary, where it forms a bulge (see Fig. 1-6*A*). As ovulation approaches, the projecting wall of the follicle begins to thin, resulting in formation of a small, nipple-shaped protrusion called the **stigma**. Finally, a combination of tension produced by smooth muscle cells in the follicle wall plus the release of collagen-degrading enzymes and other factors by fibroblasts in the region causes the follicle to rupture. Rupture of the follicle is not explosive: the oocyte, accompanied by a large number of investing cumulus cells bound in hyaluronic acid matrix, is slowly extruded onto the surface of the ovary. Ovulation occurs about 38 hours after the beginning of the ovulatory surge of FSH and LH.



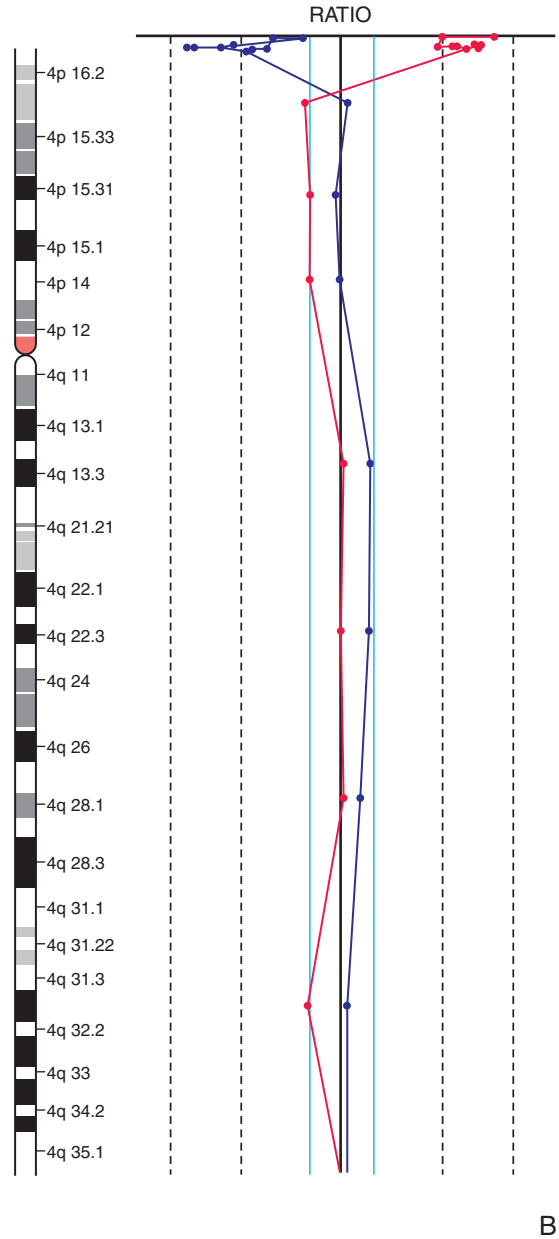


Figure 1–10. *For legend, see next page.*

f0100 **Figure 1-10.** Chromosomal deletions or duplications not apparent on high-resolution chromosome banding (karyotyping) can be detected using comparative genomic hybridization (CGH). This technology uses chip microarray (*A*) to compare fluorescently labeled DNA from a patient and a control. Two assays are run, swapping fluorescent dyes between the patient and control. The two assays are shown in *B* by the blue and red dots interconnected by lines. Each blue or red dot represents a different DNA probe, with multiple probes placed along the entire length of chromosome 4 (chromosome bands labeled at left). The patient has a deletion restricted to 4p16.2 (top). This is indicated by a deviation of the blue line (and multiple dots representing several telomeric probes) to the left, with reciprocal deviation of the red line to the right. The dashed lines indicate that there is a two-fold difference in copy number at this locus (center solid line represents a 1:1 ratio). *C*, The 4p16.2 deletion was verified using fluorescent in situ hybridization (FISH) on a metaphase chromosome spread. Green probe marks centromeres of the homologous chromosome 4 pair. Red probe marks the two sister chromatids of one 4p16.2; this region is deleted on the other chromosome 4 (arrow).

p0380 The sticky mass formed by the oocyte and cumulus is actively scraped off the surface of the ovary by the fimbriated mouth of the oviduct (Fig. 1-13). The cumulus-oocyte complex is then moved into the ampulla of the oviduct by the synchronized beating of cilia on the oviduct wall. Within the ampulla, the oocyte may remain viable for as long as 24 hours before it loses its capacity to be fertilized.

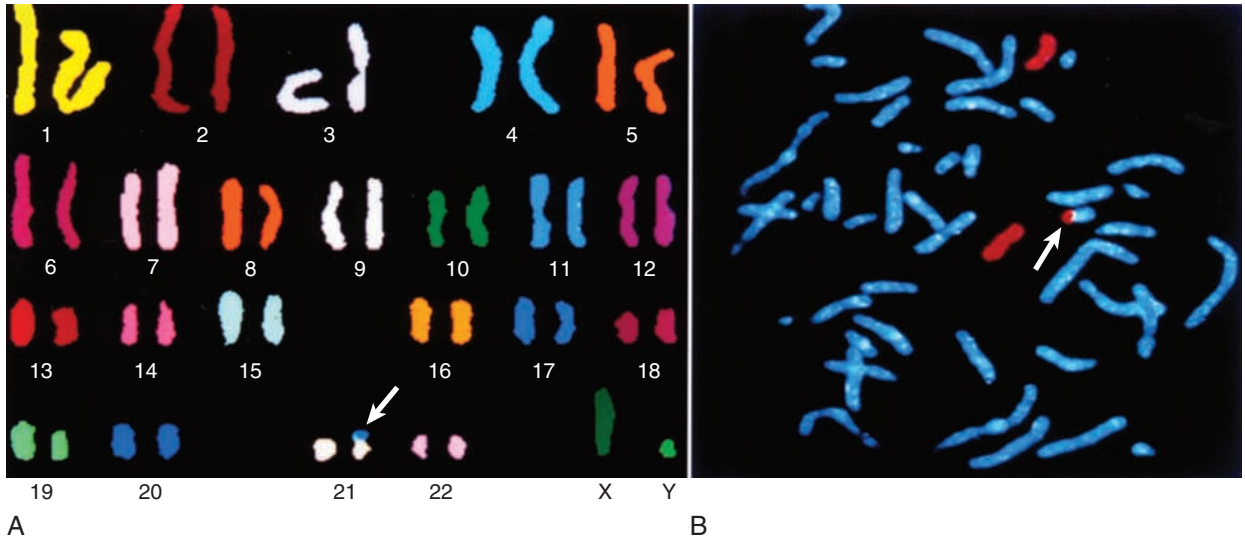
s0230 ### Ruptured Follicle Forms the Endocrine Corpus Luteum

p0390 After ovulation, membrane granulosa cells of the ruptured follicular wall begin to proliferate and give rise to the **luteal cells** of the **corpus luteum** (see Figs. 1-6 and 1-13). As described later, the corpus luteum is an endocrine structure that secretes steroid hormones to maintain the uterine endometrium in a condition ready to receive an embryo. If an embryo does not implant in the uterus, the corpus luteum degenerates after about 14 days and is converted to a scarlike structure called the **corpus albicans.**

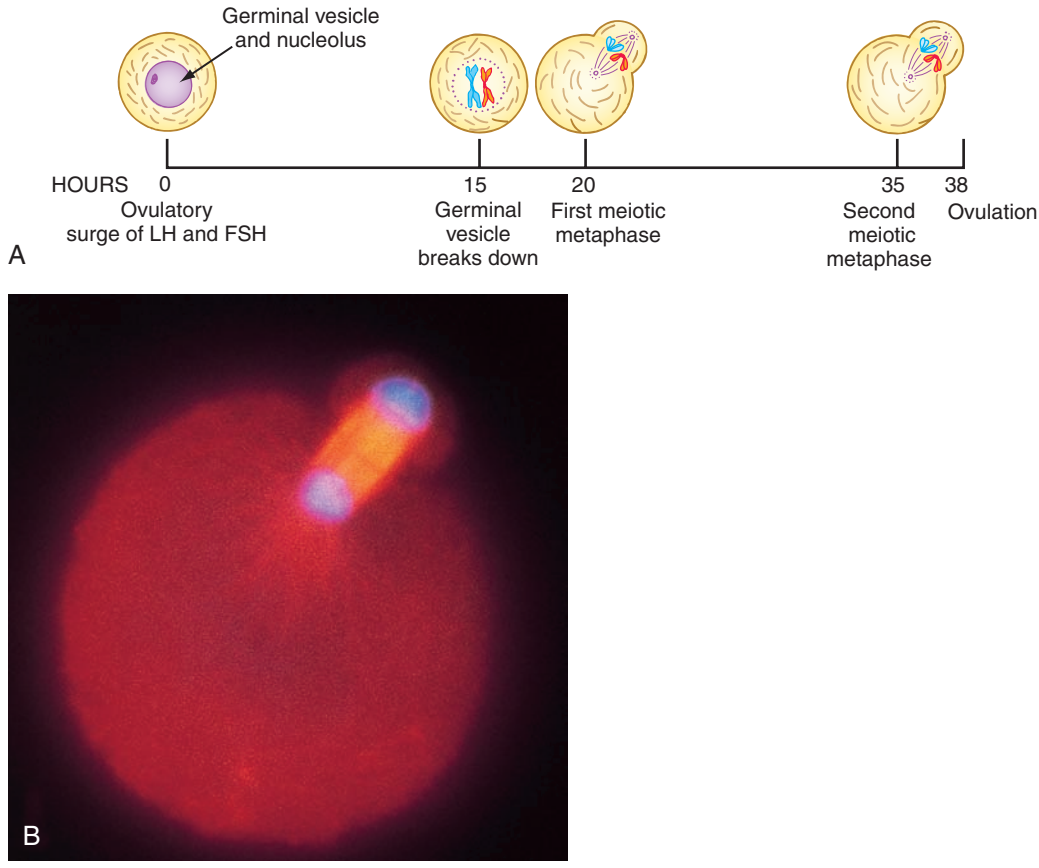
Menstrual Cycle

s0240

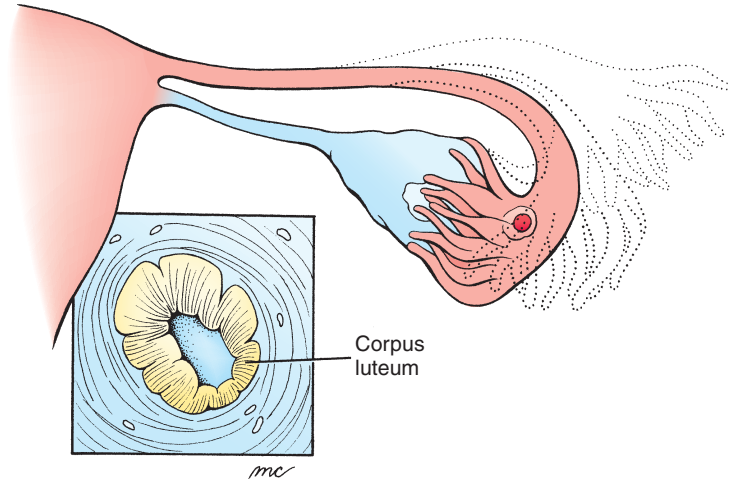
p0400 Beginning on about day 5 of the menstrual cycle, the thecal and follicle cells of responding follicles secrete



f0110 **Figure 1-11.** High-resolution chromosome banding occasionally detects complex chromosomal abnormalities where the origin of the deleted or duplicated chromosomal material is unknown. To identify such material, spectral karyotyping (SKY) can be used in which 24 combinatorially labeled chromosome painting probes are simultaneously hybridized (one probe for each of 22 chromosomes and probes for the X and Y chromosomes). *A*, Material attached to chromosome 21 was spectrally identified to be of chromosome 17 origin (arrow). *B*, This was verified using whole chromosome paint for chromosome 17 (red). The translocated chromosome 17 material is seen attached to chromosome 21 (arrow).



f0120 **Figure 1-12.** *A*, Timing of meiotic events during the ovarian cycle. *B*, Micrograph of preovulatory oocyte at the first meiotic metaphase. The cell is stained with fluorescent antibodies specific for the spindle proteins and shows the eccentric spindle apparatus and incipient first polar body.



f0130 **Figure 1-13.** The ovulated oocyte clings to the surface of the ovary by the gelatinous cumulus oophorus and is actively scraped off by the fimbriated oviduct mouth. After ovulation, the membrana granulosa layer of the ruptured follicle proliferates to form the endocrine corpus luteum.

steroids called **estrogens**. These hormones in turn cause the endometrial lining of the uterus to proliferate and undergo remodeling. This **proliferative phase** begins at about day 5 of the cycle and is complete by day 14 (see Fig. 1-5).

p0410 After ovulation occurs, thecal cells in the wall of the corpus luteum continue to secrete estrogens, and **luteal cells** that differentiate from remaining follicle cells also begin to secrete high levels of a related steroid hormone, **progesterone**. Luteal progesterone stimulates the uterine endometrial layer to thicken further and to form convoluted glands and increased vasculature. Unless an embryo implants in the uterine lining, this **secretory phase** of endometrial differentiation lasts about 13 days (see Fig. 1-5). At that point (near the end of the menstrual cycle), the corpus luteum shrinks and levels of progesterone fall. The thickened endometrium, which is dependent on progesterone, degenerates and begins to slough. The 4- to 5-day **menstrual phase**, during which the endometrium is sloughed (along with about 35 mL of blood and the unfertilized oocyte), is by convention considered the start of the next cycle.

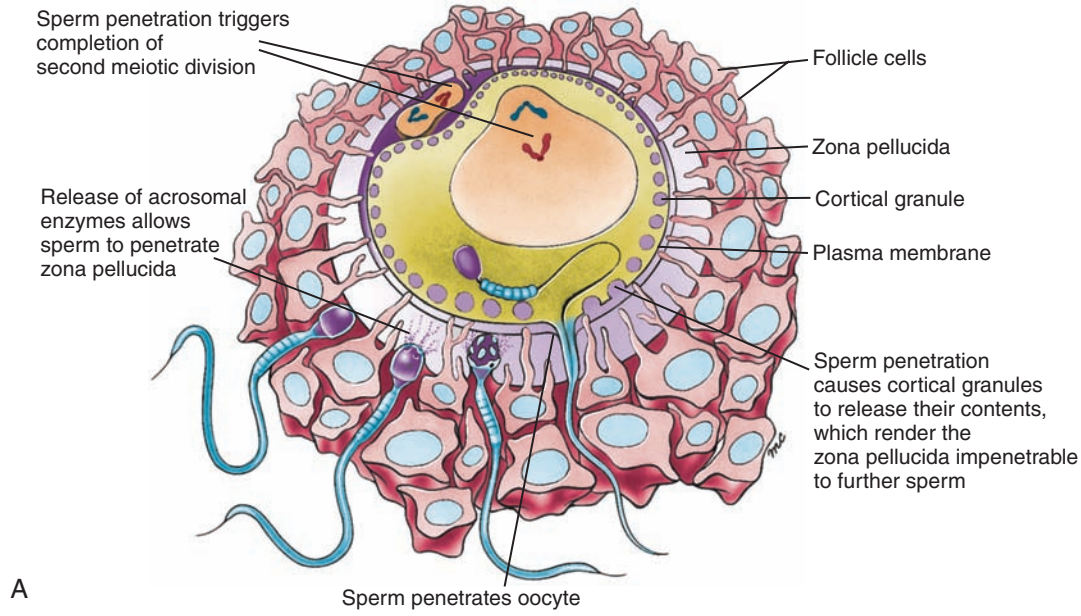
s0250 Fertilization

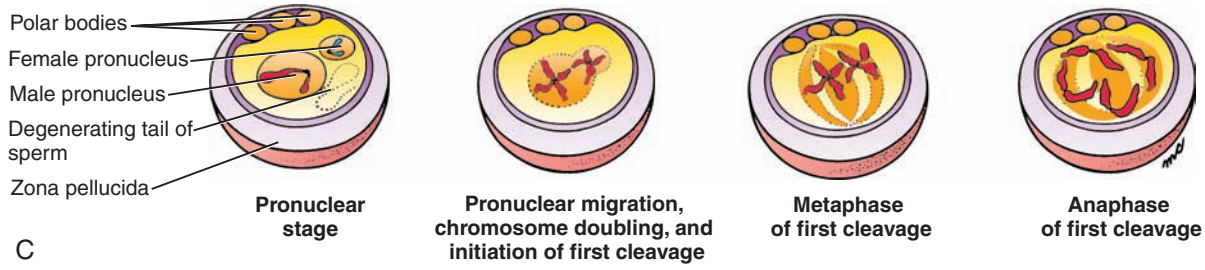
p0420 If viable spermatozoa encounter an ovulated oocyte in the ampulla of the oviduct, they surround it and begin forcing their way through the cumulus mass (Fig. 1-14*A*). In vitro evidence suggests that the ovulated follicle contains a currently unknown **sperm chemotropic factor** and that only capacitated sperm are able to respond to this factor by directed swimming toward the egg. Based on this, it might be said that the human sperm finds the human egg to be "attractive" (pun intended).

p0430 When a spermatozoon reaches the tough zona pellucida surrounding the oocyte, it binds in a species- (that is, human-) specific interaction with a glycoprotein sperm receptor molecule in the zona (ZP3, one of three glycoproteins composing the zona pellucida). Binding to ZP3 is mediated by a sperm surface protein called SED1. As a result of this binding, the acrosome is induced to release degradative enzymes that allow the sperm to penetrate the zona pellucida. When a spermatozoon successfully penetrates the zona pellucida and reaches the oocyte, the cell membranes of the two cells fuse {(Fig. 1-14*B*; see Fig. 1-14A)}. The egg tetraspanin (a 4-pass transmembrane protein), CD9, is required for this event, as is a sperm-specific protein named IZUMO after the Japanese shrine to marriage. (IZUMO is a member of the immunoglobulin superfamily and as such is likely to be an adhesion molecule.) Other factors implicated in fusion are members of the ADAM superfamily (all 30 or so family members contain a disintegrin and a metalloprotease domain). FERTILINβ, also known as ADAM2, is present on the surface of mammalian sperm and interacts with an integrin (integrins are discussed in Ch. 5) on the egg surface. Membrane fusion immediately causes two events to occur: formation of a calcium wave that radiates over the surface of the egg from the point of sperm contact; and release of the contents of thousands of small **cortical granules**, located just beneath the oocyte cell membrane, into the **perivitelline space** between the oocyte and the zona pellucida. These two events alter the sperm receptor molecules, causing the zona to become impenetrable by additional spermatozoa. Therefore, these changes prevent **polyspermy** or the fertilization of the oocyte by more than one spermatozoon. Because a few hundred spermatozoa reach the vicinity of the egg, the need to block polyspermy is extremely important.

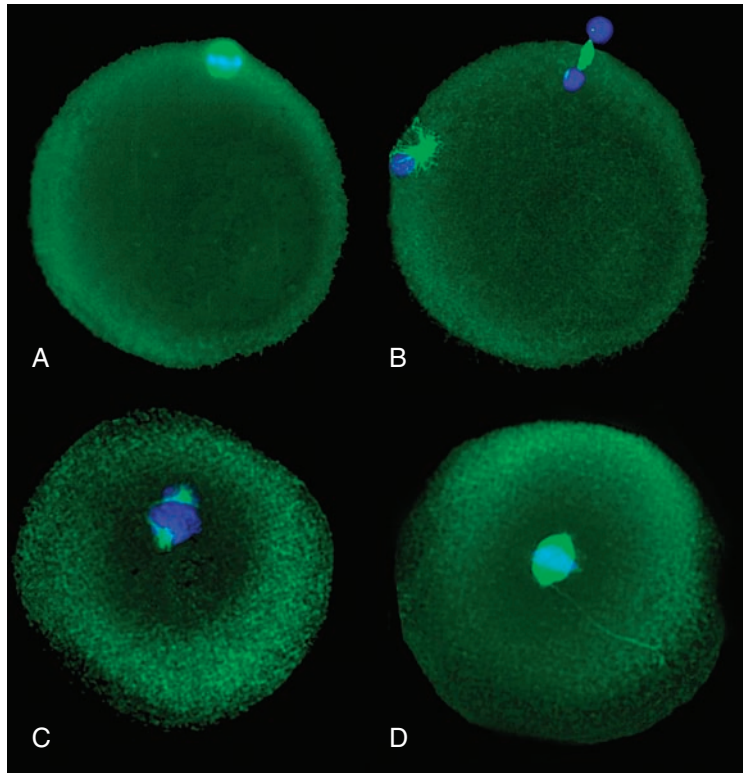
p0440 The fusion of the spermatozoon cell membrane with the oocyte membrane also causes the oocyte to resume meiosis. The oocyte completes the second meiotic metaphase and rapidly proceeds through anaphase, telophase, and cytokinesis, producing another polar body. Disregarding the presence of the sperm, the oocyte is now considered to be a **definitive oocyte** (considering only the oocyte's genome, it contains a haploid complement of chromosomes and a 1N quantity of DNA after completion of the second meiotic division). However, because the sperm has now penetrated the oocyte, the fertilized oocyte can also be called a **zygote** (from Greek *zugotos*, yoked**).** Although a single nucleus (surrounded by a nuclear membrane) containing both the oocyte's and sperm's chromosomes does not form in the zygote (see next paragraph and Figs. 1-14*C*, 1-15), taking into account both the oocyte's and sperm's genomes, the zygote contains a diploid complement of chromosomes and a 2N quantity of DNA.

p0450 After penetration of the oocyte by the sperm, the nuclei of the oocyte and sperm swell within the zygote and are called the **female** and **male pronuclei**, respectively (see Figs. 1-14*C*, 1-15). Their nuclear membranes quickly disappear as both maternal and





f0140 **Figure 1-14.** Fertilization. *A*, Spermatozoa wriggle through the cumulus mass and release their acrosomal enzymes on contact with the zona pellucida. Acrosomal enzymes dissolve the zona pellucida and allow sperm to reach the oocyte. Simultaneous with fusion of the membranes of the fertilizing sperm and oocyte, cortical granules of the oocyte release their contents, which causes the zona pellucida to become impenetrable to other sperm. Entry of the sperm nucleus into the cytoplasm stimulates the oocyte to complete the second meiotic division. *B*, Scanning electron micrograph showing a human sperm fusing with a hamster oocyte that has been enzymatically denuded of the zona pellucida. The ability of a man's sperm to penetrate a denuded hamster oocyte is often used as a clinical test of sperm activity. *C*, Early events in zygote development. After the oocyte completes meiosis, the female pronucleus and the larger male pronucleus approach each other as DNA is doubled in maternal and paternal chromosomes to initiate the first mitotic division. Pronuclear membranes then break down and maternal and paternal chromosomes assemble on the metaphase plate. Centromeres then replicate, and homologous chromosomes are distributed to the first two cells of the embryo.



f0150 **Figure 1-15.** Fertilization of human eggs in vitro. *A*, The first meiotic division has occurred, forming the first polar body and secondary oocyte. *B*, The second meiotic division is completed after the sperm has entered the oocyte. This results in the formation of the second polar body and female pronucleus. The male pronucleus and microtubules condensing around it are located at the 9 o'clock position. *C*, The sperm centriole has split into two centrioles, which are organizing a spindle in association with the merged chromosomes from the male and female pronuclei. *D*, The sperm and egg chromosomes are aligned on the metaphase plate.

paternal chromosomes are replicated in preparation for the first cleavage (see next section).

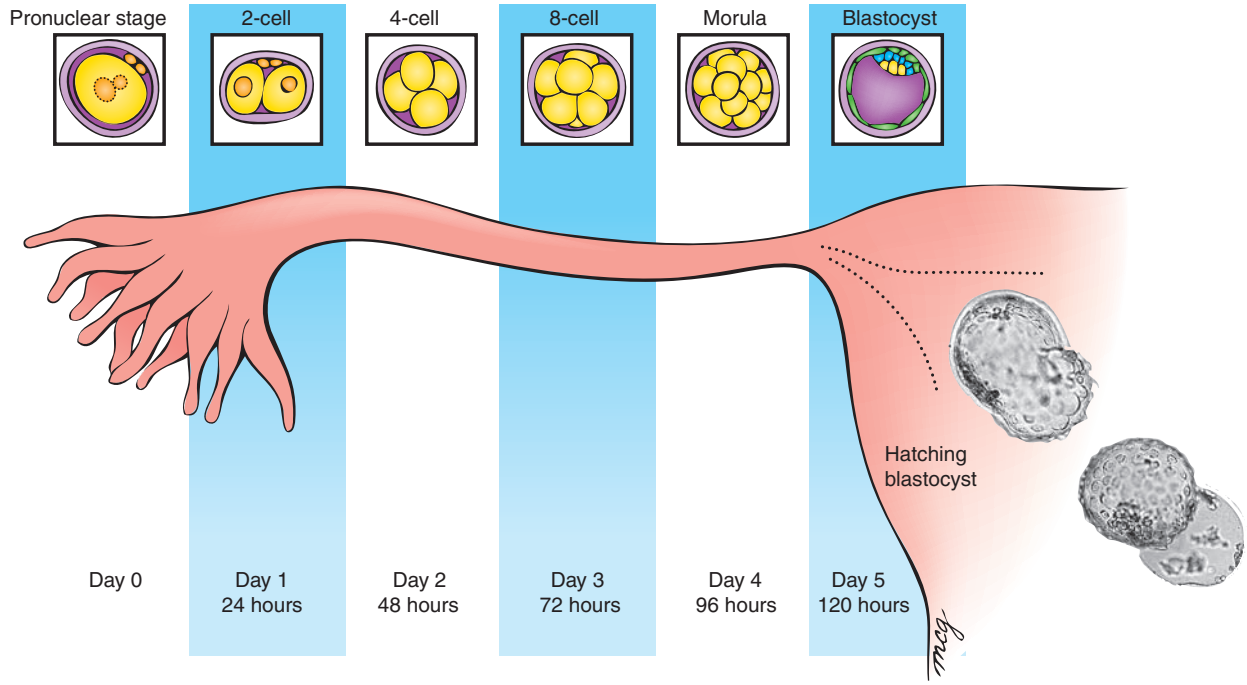
s0260 # Cleavage

s0270 ## Cleavage Subdivides Zygote without Increasing Its Size

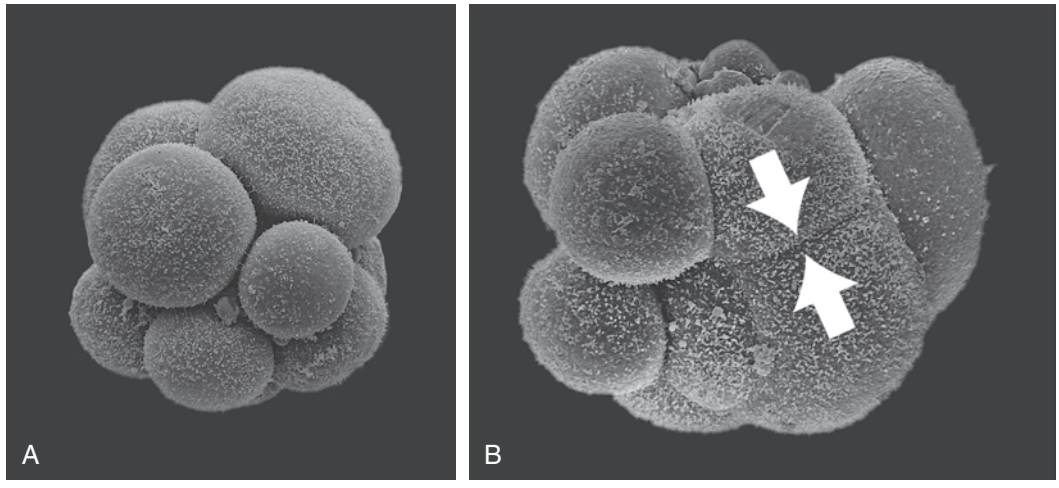
p0460 Within 24 hours after fertilization, the zygote initiates a rapid series of mitotic cell divisions called **cleavage** (Fig. 1-16). These divisions are not accompanied by cell growth, so they subdivide the large zygote into many smaller daughter cells called **blastomeres**. The embryo as a whole does not increase in size during cleavage and remains enclosed in the zona pellucida. The first cleavage division divides the zygote to produce two daughter cells. The second division, which is complete at about 40 hours after fertilization, produces four equal blastomeres. By 3 days, the embryo consists of 6 to 12 cells, and by 4 days, it consists of 16 to 32 cells. The embryo at this stage is called a **morula** (from Latin *morum*, mulberry).

s0280 ## Segregation of Blastomeres into Embryoblast and Trophoblast Precursors

p0470 The cells of the morula will give rise not only to the embryo proper and its associated extraembryonic membranes but also to part of the placenta and related structures. The cells that will follow these different developmental paths become segregated during cleavage. Starting at the 8-cell stage of development, the originally round and loosely adherent blastomeres begin to flatten, developing an inside-outside polarity that maximizes cell-to-cell contact among adjacent blastomeres (Fig. 1-17). As differential adhesion develops, the outer



f0160 **Figure 1-16.** Cleavage and transport down the oviduct. Fertilization occurs in the ampulla of the oviduct. During the first 5 days, the zygote undergoes cleavage as it travels down the oviduct and enters the uterus. On day 5, the blastocyst hatches from the zona pellucida and is then able to implant in the uterine endometrium.



f0170 **Figure 1-17.** Compaction. *A*, Scanning electron micrograph of 10-cell human embryo before compaction. Note deep intercellular clefts. *B*, Scanning electron micrograph of 10-cell human embryo during process of compaction. Note absence of deep intercellular clefts between some of the blastomeres (arrows). The zona pellucida was mechanically removed from both embryos.

surfaces of the cells become convex and their inner surfaces become concave. This reorganization, called **compaction**, also involves changes in the blastomere cytoskeleton.

p0480 With compaction, some blastomeres segregate to the center of the morula and others to the outside. The centrally placed blastomeres are now called the **inner cell mass**, whereas the blastomeres at the periphery constitute the **trophoblast**. Because the inner cell mass gives rise to the embryo proper it is also called the **embryoblast**. The **trophoblast** is the primary source of the fetal component of the placenta (discussed in Ch. 2).

s0290 ## Morula Develops a Fluid-filled Cavity and Is Transformed into a Blastocyst

b0060 ### IN THE RESEARCH LAB

WHAT DETERMINES WHETHER A BLASTOMERE WILL FORM INNER CELL MASS OR TROPHOBLAST?

The "inside-outside" hypothesis explains the differentiation of blastomeres based on their position into either inner cell mass or trophoblast—more central cells of the morula become inner cell mass, and cells on the outside of the morula become trophoblast. But how does this differentiation occur? In the morula stage, two transcription factors (transcription factors are discussed in Ch. 5) are expressed uniformly throughout all blastomeres: *Oct4* (discussed earlier in the chapter) and *Nanog* (a homeobox-containing transcription factor). As the inner cell mass and trophoblast form, *Oct4* and *Nanog* expression is maintained in the inner cell mass, but both are turned off in the trophoblast. Loss-of-function experiments show that commitment of cells to the lineage of the inner cell mass requires the expression of these two transcription factors. Another transcription factor, *Cdx2* (like *Nanog*, also a homeobox-containing transcription factor), is expressed in the trophoblast as it is forming, as is the T box–containing transcription factor *Eomes* (also known as *Eomesodermin*). Loss-of-function experiments show that expression of these factors is required to downregulate expression of *Oct4* and *Nanog*. Collectively, these studies demonstrate that both expression of *Oct4* and *Nanog* in the inner cell mass and repression of expression of these two transcription factors in the trophoblast is required for the first overt differentiation event that occurs in the morula. Finally, the inner cell mass also expresses *Sox2*, an HMG box–containing factor highly related to SRY (discussed in Ch. 15). Experiments have shown that *Sox2/Oct4* regulate expression of *Fgf4* protein in the inner cell mass, which is required for differentiation of the trophoblast. Thus, cell interactions occur between these two nascent populations of cells that are essential for specifying their fate.

p0490 By 4 days of development, the morula, consisting now of about 30 cells, begins to absorb fluid. Several processes seem to be involved. First, as the trophoblast differentiates it assembles into an epithelium in which adjacent cells are tightly adherent to one another. This adhesion results from the deposition on lateral cell surfaces of **E-CADHERIN**, a calcium-dependent cell adhesion molecule, and the formation of intercellular junctions, specifically, **tight junctions, gap junctions, adherens junctions**, and **desmosomes.** Second, forming trophoblast cells express a basally polarized membrane sodium/potassium ATPase (an energy-dependent ion-exchange pump), allowing them to transport and regulate the exchange of metabolites between the outside of the morula (i.e., the maternal environment of the oviduct) and the inside of the morula (i.e., toward the inner cell mass). The sodium/potassium ATPase pumps sodium into the interior of the morula, and water follows through osmosis to become blastocoelic fluid. As the hydrostatic pressure of the fluid increases, a large cavity called the **blastocyst cavity (blastocoel)** forms within the morula (see Fig. 1-16). The embryoblast cells (inner cell mass) then form a compact mass at one side of this cavity, and the trophoblast organizes into a thin, single-layered epithelium. The embryo is now called a **blastocyst**. The side of the blastocyst containing the inner cell mass is called the **embryonic pole** of the blastocyst, and the opposite side is called the **abembryonic pole.**

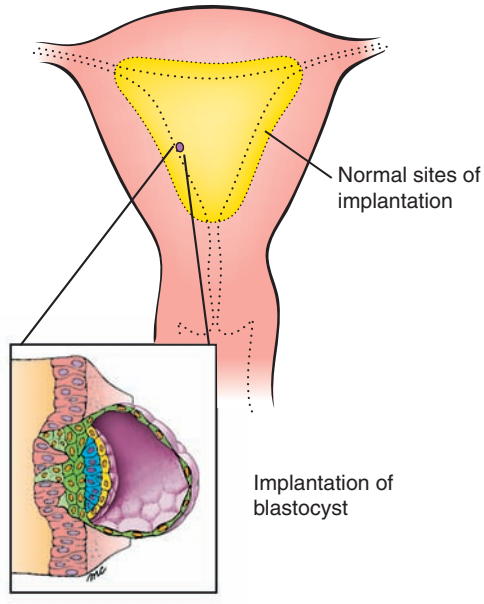
End of First Week: Initiating Implantation

s0300

Blastocyst Hatches from Zona Pellucida before Implanting

s0310

p0500 The morula reaches the uterus between 3 and 4 days of development. By day 5, the blastocyst hatches from the clear zona pellucida by enzymatically boring a hole in it and squeezing out (see Fig. 1-16). The blastocyst is now naked of all its original investments and can interact directly with the endometrium.



f0180 **Figure 1-18.** Implantation. On about day 6.5 after fertilization, the trophoblast cells at the embryonic pole of the blastocyst proliferate to produce the syncytiotrophoblast, which is able to invade the uterine lining. The yellow area indicates normal sites of implantation in the uterine wall, and the enlargement shows the implanting blastocyst.

p0510 Very soon after arriving in the uterus, the blastocyst becomes tightly adherent to the uterine lining (Fig. 1-18). The adjacent cells of the endometrial stroma respond to its presence and to the progesterone secreted by the corpus luteum by differentiating into metabolically active, secretory cells called **decidual cells**. This response is called the **decidual reaction** (discussed in Ch. 6; see Fig. 6-2). The endometrial glands in the vicinity also enlarge, and the local uterine wall becomes more highly vascularized and edematous. It is thought that secretions of the decidual cells and endometrial glands include growth factors and metabolites that support growth of the implanting embryo.

p0520 The uterine lining is maintained in a favorable state and kept from sloughing partly by the progesterone secreted by the corpus luteum. In the absence of an implanted embryo, the corpus luteum normally degenerates after about 13 days. However, if an embryo implants, cells of the trophoblast produce the hormone **human chorionic gonadotropin (hCG)**, which supports the corpus luteum and thus maintains the supply of progesterone **(maternal recognition of pregnancy)**. The corpus luteum continues to secrete sex steroids for 11 to 12 weeks of embryonic development, after which the placenta itself begins to secrete large amounts of progesterone and the corpus luteum slowly involutes, becoming a corpus albicans.

Implantation in Abnormal Site Results in Ectopic Pregnancy

s0320

p0530 Occasionally, a blastocyst implants in the peritoneal cavity, on the surface of the ovary, within the oviduct, or at an abnormal site in the uterus. The epithelium at these abnormal sites responds to the implanting blastocyst with increased vascularity and other supportive changes, so that the blastocyst is able to survive and commence development. These **ectopic pregnancies** often threaten the life of the mother because blood vessels that form at the abnormal site are apt to rupture as a result of growth of the embryo and placenta. Typically, ectopic pregnancy is revealed by symptoms of abdominal pain and/or vaginal bleeding. Drug (methyltrexate, which blocks rapid division) or surgical intervention is usually required to interrupt the pregnancy.

b0070

IN THE CLINIC

CONTRACEPTION

Human Reproductive Efficiency Is Very High

An average couple who does not practice contraception and has intercourse twice a week (timed randomly with respect to ovulation) has a better than 50% chance of fertilizing any given oocyte. Because (as discussed above) about half of all embryos undergo spontaneous abortion, the chance that 1 month's intercourse will produce a term pregnancy is thus better than 25%. Healthy humans have astounding reproductive efficiency; it is not rare for couples who do not practice contraception to produce 10 to 20 offspring in a reproductive lifetime.

Contraception has played an important role in family planning for much of human history. Some of the oldest forms are simple **barrier contraceptives**, and these methods remain among the most frequently used today. Current contraceptive research focuses on developing strategies that interfere with many of the physiological mechanisms discussed earlier in this chapter that are required for successful conception.

Barrier Contraceptives Prevent Spermatozoa from Reaching Egg

One of the oldest types of contraceptive device is the **male condom**, originally made of animal bladders or sheep cecum and now made of latex rubber and often combined with a chemical spermicide. The male condom is fitted over the erect penis just before intercourse. The **female condom** is a polyurethane sheath that is inserted to completely line the vagina as well as the perineal area. Use of both the male and female condom can help prevent the spread of **sexually transmitted diseases (STDs).** Other barrier devices, such as the **diaphragm** and **cervical cap**, are inserted into the vagina to cover the cervix and are usually used in conjunction with a spermicide. These must be fitted by a physician to determine the proper size. The **contraceptive sponge** is a spermicide-impregnated disc of polyurethane sponge that also blocks the cervix. Its advantage over the diaphragm and cervical cap is that the sponge does not need to be fitted by a physician because one size fits all.

Birth Control Pill Prevents Ovulation

Knowledge of the endocrine control of ovulation led to the introduction of the birth control pill ("the Pill") in the early 1960s. These early pills released a daily dose of estrogen, which inhibited ovulation by preventing secretion of the gonadotropic hormones FSH and LH from the pituitary. In modern pills, the estrogen dosage has been reduced, the progesterone analog **progestin** has been added, and the doses of estrogen and progestin are usually varied over a 21-day cycle. Although the normal function of progesterone is to support pregnancy through its effect on the endometrium, it also interferes with the release of FSH and LH, thus preventing ovulation. In addition, it prevents the cervical mucus from entering its midcycle phase of becoming thin and watery (which would allow spermatozoa to pass through it more readily) and the endometrium from thickening (in preparation for implantation), and it may also interfere with oocyte transport down the oviduct or with sperm capacitation.

Injected or Implanted Sources of Progesterone Deliver a Chronic Antiovulatory Dose

A **depot preparation** of medroxyprogesterone acetate (Depo-Provera) can be injected intramuscularly and will deliver antiovulatory levels of the hormone for 2 to 3 months. Alternatively, rods or capsules have been developed (Norplant or Implanon) that are implanted subdermally and release a synthetic form of progesterone (progestin) for a period of one to five years. Another alternative is the hormone patch (Ortho Evra), which can stay in place for a week, delivering both progesterone and estrogen transdermally. Other devices act by releasing the hormone into the female reproductive tract rather than the bloodstream. Progesterone-containing **intrauterine devices** (**IUDs**) emit low levels of progesterone for a period of 1 to 4 years. **Vaginal rings** are inserted and removed by the user and when in place around the cervix, release progestins continuously for 3 months.

Nonmedicated IUDs May Interfere with Conception through Effects on Both Sperm and Egg

The mechanism by which nonmedicated loop-shaped or T-shaped IUDs prevent conception when inserted in the uterus is unclear. Originally, they were thought to act by irritating the endometrium, resulting in an inflammatory reaction that prevented implantation of the conceptus. Because some people believe that preventing an embryo from implanting is an abortion (whereas others believe that an abortion involves removing an embryo that is already implanted), this potential mechanism of action creates ethical concerns for some people. It is now thought that IUDs act mainly by inhibiting sperm migration, ovum transport, and fertilization, rather than preventing implantation.

Antiprogesterone Compound RU-486 Is an Abortifacient

RU-486 (mifepristone) has potent antiprogesterone activity (its affinity for progesterone receptors is 5 times greater than that of endogenous progesterone) and may also stimulate prostaglandin synthesis. When taken within 8 weeks of the last menses, an adequate dose of RU-486 will initiate menstruation. If a conceptus is present, it will be sloughed along with the endometrial decidua. A large-scale French study in which RU-486 was administered along with a prostaglandin analog yielded an efficacy rate of 96%.

Sterilization Is Used by About One Third of American Couples

Sterilization of the male partner **(vasectomy)** or female partner **(ligation of the fallopian tubes)** is an effective method of contraception and is often chosen by people who do not want additional children. However, both methods involve surgery, and neither is reliably reversible.

How Effective is Contraception?

Sterilization and the use of hormonal contraceptives (such as the pill) have an annual pregnancy probability of from less than 1% to about 5%, whereas barrier contraception is less effective: the use of the male condom has an annual pregnancy probability of about 15%—equivalent to practicing the rhythm (natural family planning) method in which the couple practices abstinence in the days before, during, and after the expected time of ovulation; and the use of the diaphragm has an annual pregnancy probability of about

25%—equivalent to practicing the withdrawal method (coitus interruptus).

By 2020, about 16% of the world's population, or about 1.2 billion people, will enter their childbearing years, raising the issue that better contraceptive methods may need to be developed. Although new approaches are being tested, tough government regulations and concerns about liability and profitability (especially where the greatest demand for products will be in poor countries) is preventing most companies from striving to develop new contraceptive products. Contraceptive research had its heyday in the 1950s and 1960s, which resulted in a major breakthrough, the development of the birth control pill. However, similar breakthroughs have not occurred since, and contraceptive choices remain highly limited. For example, at the time the pill was introduced, men had only two choices for birth control: condoms and vasectomy. Some 50 years later, these are still the only choices.

ASSISTED REPRODUCTIVE TECHNOLOGY

About 1 in 6 couples have difficulty conceiving on their own. In about 30% of the cases the female is infertile, in about 30% the male is infertile, and in about 30% both the male and female are infertile. In another 10% of the cases whether the male or female (or both) is infertile is unknown. It is estimated that about 90% of the infertile couples can conceive with medical intervention. A variety of medical options are available to help couples conceive, including artificial insemination (AI) and hormonal therapies, which are the most common procedures. In vitro techniques also can be used to assist reproduction. These techniques are referred to as **assisted reproductive technology (ART)**, and they consist of **in vitro fertilization (IVF)** and **embryo transfer, intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer (GIFT)**, and **zygote intrafallopian transfer (ZIFT)**. Improved tissue culture techniques, including the use of defined culture media, have made it possible to maintain human gametes and cleavage-stage embryos outside the body. Gametes and embryos also can be successfully frozen **(cryopreserved)** and stored for later use, adding to the options for assisted reproduction.

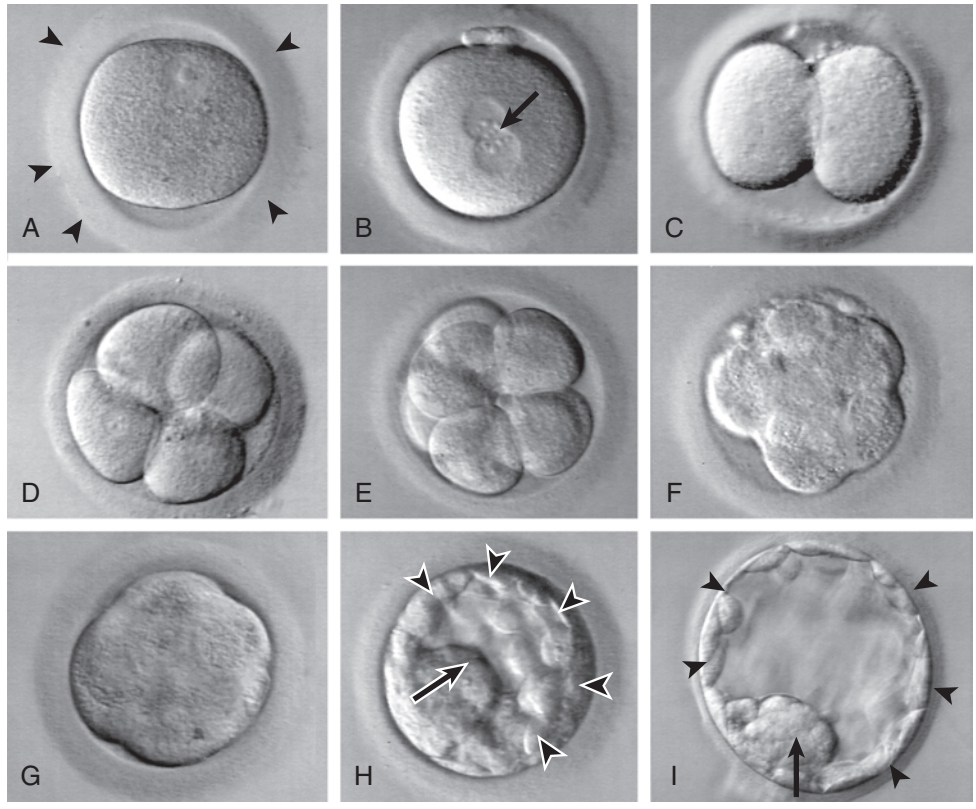
Oocytes Can Be Fertilized In Vitro and Then Implanted in Uterus

The procedure of **in vitro fertilization (IVF)** and **embryo transfer** is widely used in cases in which scarring of the oviducts (a common consequence of pelvic inflammatory disease [PID], a serious complication of sexually transmitted diseases such as gonorrhea) prevents either the sperm from reaching the ampulla of the oviduct or the fertilized oocyte from passing to the uterus. In IVF, the woman's ovaries first are induced to **superovulate** (develop multiple mature follicles) by administration of an appropriate combination of hormones, usually human menopausal gonadotropin (hMG) or FSH, sometimes combined with clomiphene citrate—a drug that blocks the ability of hypothalamic cells to detect estrogen in the blood. In the presence of clomiphene citrate, hypothalamic cells respond to the perceived deficiency of estrogen by signaling the pituitary to release high levels of FSH, which stimulates follicles' growth and their secretion of estrogen. Once estrogen levels rise sufficiently, the pituitary gland rapidly releases LH, triggering maturation of oocytes. Sometimes to ensure that maturation of oocytes occurs, hCG is also given when follicles have attained optimal growth (determined by ultrasound examination of the ovaries and plasma estradiol concentration measurements).

Maturing oocytes are then harvested from the follicles, usually by using an ultrasonography-guided needle inserted via the vagina (transvaginal ultrasound–guided aspiration). Once retrieved, oocytes are allowed to mature in a culture medium to the second meiotic metaphase and are then fertilized with previously obtained and capacitated sperm (if obtained from the woman's partner, they are collected 2 hours before egg retrieval; if obtained from a sperm donor, they are obtained from a previously collected frozen aliquot). The resulting zygotes are allowed to develop in culture for about 48 hours and are then inserted (usually one or two) into the uterine cavity. IVF has increased our understanding of the earliest stages in human development, as embryos can be readily observed as they develop in vitro (Fig. 1-19).

Before the embryo is inserted into the uterine cavity, **assisted hatching** can be preformed in cases where the zona pellucida is tougher than normal and, consequently, make it more difficult for embryos to hatch. The zona pellucida (''shell'') can be tougher in woman older than 40 or in younger woman who have a paucity of eggs. Assisted hatching involves making a small tear in the zona pellucida using acid tyrode solution, laser ablation, or mechanical means.

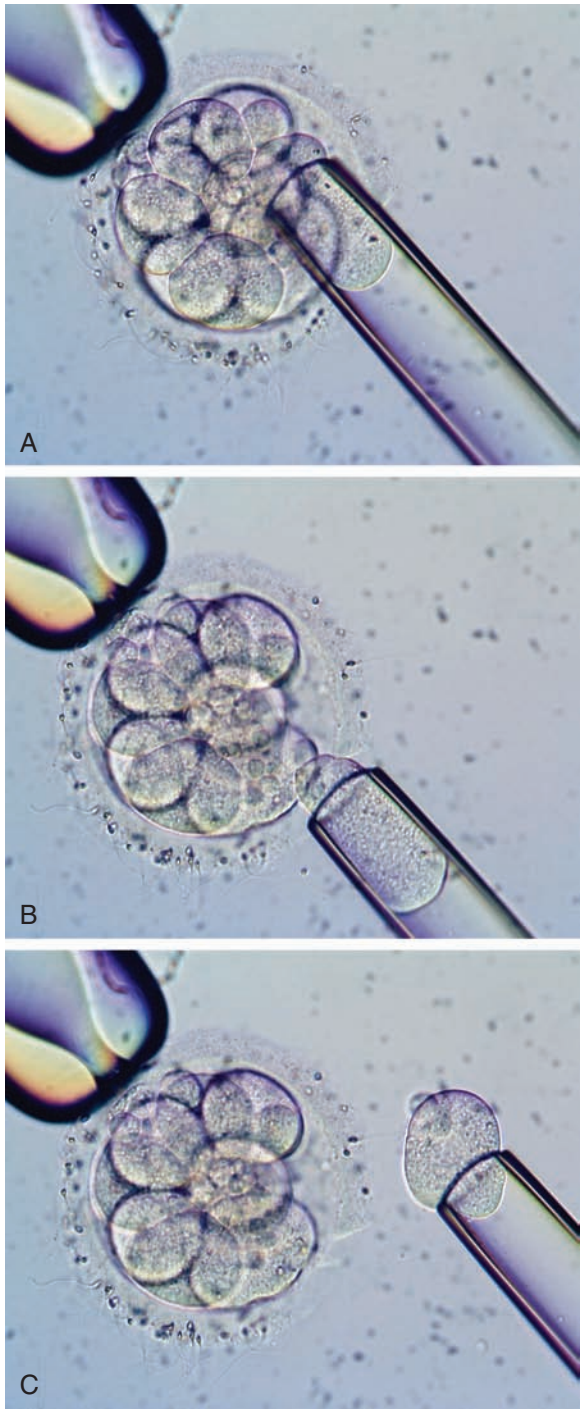
The first successful case of IVF occurred in 1978 with the birth of Louise Brown, the world's first ''test-tube'' baby. By the time of her 20th birthday, 300,000 IVF children had been born worldwide. By 2005, that number reached more than 1 million. On the average, IVF results in the delivery of a live baby in about 30% to 35% of the attempts (i.e., live births per egg retrieval; thus, to have 300,000 IVF children required about 1 million IVF conceptions). The success rate of IVF is remarkable considering that (as discussed above) for a normal healthy couple practicing unprotected intercourse, the successful pregnancy rate is about 25% per monthly cycle.



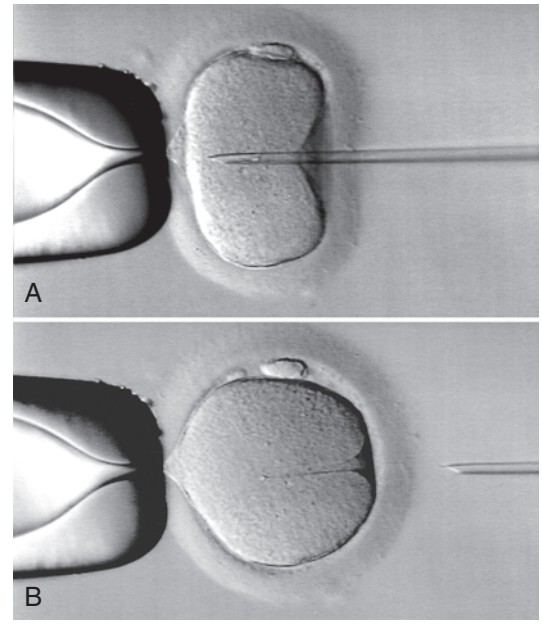
f0190 **Figure 1-19.** Human development in vitro. *A*, Ovulated secondary oocyte prior to introduction of sperm and fertilization. The oocyte containing its germinal vesicle is surrounded by the zona pellucida (arrowheads). *B*, Shortly after in vitro fertilization (IVF) the male and female pronuclei (arrow) have formed. *C*, Two-cell stage. *D*, Four-cell stage. *E*, Eight-cell stage. *F*, Morula initiating compaction. *G*, Compacted morula. *H*, Early blastocyst, with trophoblast (arrowheads) and inner cell mass (arrow). Hatching from the zona pellucida has not occurred. *I*, Hatched blastocyst, with trophoblast (arrowheads) and inner cell mass (arrow).

With IVF, preimplantation diagnosis of genetic conditions **(preimplantation genetic diagnosis, PGD)** can be performed using first or second polar bodies or blastomeres. These can be removed during IVF (Fig. 1-20), presumably without harm to further development, and then screened for aneuploidy or translocations with standard karyotypic analysis or FISH, and for mutations with techniques like the **polymerase chain reaction (PCR)**. PCR can be used to amplify DNA from a single cell, producing many copies for sequence analysis (eggs and embryos are stored until the diagnosis is made). Polar body diagnosis, unlike blastomere diagnosis, provides information about maternal contributions to the zygote but not paternal contributions, as polar bodies contain only maternal genes (i.e., they are formed by meiotic divisions of the oocyte). Hence, they are used only when the mother is at risk for transmitting a disease-causing mutation. If the mutation is found in a polar body, the assumption is made that the oocyte does *not* contain the mutation (if the rationale for this assumption is unclear, review meiosis). PGD offers the major advantage that it can be used to select only unaffected embryos for implanting, avoiding the later possibility of a selective termination of an affected pregnancy following prenatal diagnosis.

In cases in which a partner's spermatozoa are unable to penetrate the zona pellucida, a technique called **intracytoplasmic sperm injection (ICSI)** may be used. In this procedure, a single spermatozoon is selected under a microscope, aspirated into a needle, and injected into the oocyte cytoplasm (Fig. 1-21). In one recent study, children born after ICSI were twice as likely to have major congenital anomalies as children conceived naturally. Other risks for these children include an unbalanced chromosome complement (ICSI can damage the meiotic spindle,



f0200 **Figure 1-20.** Human morula undergoing a blastomere biopsy. The temporal sequence is shown in order from top to bottom (*A-C*). The morula is held with a suction pipette and a hole is made in the zona pellucida. A micropipette is used to remove a selected blastomere by aspiration.



f0210 **Figure 1-21.** Intracytoplasmic sperm injection (ICSI). *A*, As the oocyte is held with a suction pipette, a micropipette, containing a single sperm, is used to penetrate the zona pellucida and oocyte. *B*, After pressure injection of the sperm, the micropipette is withdrawn, leaving a tract in the cytoplasm that soon disappears.

potentially leading to aneuploidy) and male infertility. Men with cystic fibrosis (CF), an autosomal recessive disease that affects breathing and digestion, also have congenital absence of the vas deferens and are, therefore, infertile. Using microsurgical epididymal sperm aspiration (MESA), sperm can be removed from the epididymis of CF men for use in IVF. However, such sperm are unable to fertilize an egg because they have not fully matured, a process that is completed during their passage through the epididymis and vas deferens. To overcome this problem ICSI can be used. The children born to fathers with CF using MESA and ICSI are normal CF carriers (to have CF, one must inherit a mutation in both the maternal and paternal chromosomes). Because absence of the vas deferens is associated with a mild form of CF that is otherwise asymptomatic, and tests for CF mutations detect only about 87% of the mutations, it is now recommended that both parents be genetically tested for CF mutations and appropriately counseled before using ICSI in cases in which the vas deferens is congenitally absent.

Gametes or Zygotes Can Be Introduced Directly into Ampulla of Oviduct

If the woman's oviduct is normal and the couple is infertile because of an innate deficiency in spermatozoon motility or for some other reason, a technique called **gamete intrafallopian transfer (GIFT)** is often used. Oocytes are harvested as described earlier and are then placed into a laparoscope catheter along with precapacitated spermatozoa. The oocytes and spermatozoa are introduced together directly into the ampulla of the oviduct, where fertilization takes place. Further development occurs by normal processes. In an alternative technique, **zygote intrafallopian transfer (ZIFT)**, the oocytes are fertilized in vitro, and only fertilized pronuclear zygotes are introduced into the ampulla.

ART in Perspective

In 1998 the following statistics were reported: in the United States, 60,000 births per year resulted from AI, 15,000 resulted from IVF, and at least 1,000 resulted from surrogacy arrangements (a couple arranges for another woman to carry their child to birth following IVF and implantation of an embryo in her uterine cavity). With about 4 million total births in the United States per year, the use of ART (IVF and IVF plus surrogacy) thus accounts for about 0.4% of all births in the United States. An infertile couple can choose to remain childless, undergo medical therapy including ART, or adopt a child. It was also reported in 1998 that only 30,000 healthy children were available for adoption in the United States. ART thus provides new opportunities for couples who choose not to be childless. ART is not without its risks, however: 37% of ART births are multiple as compared with 2% in the general population (risks associated with multiple births are discussed in Ch. 6), and ART increases pregnancy-related risks to woman, including preeclampsia, diabetes mellitus, bleeding, and anemia, as well as a possible risk of ovarian cancer owing to hormonal stimulation during ART. Moreover, ART-associated birth defects occur at a 1.4- to 2-fold higher rate than the overall rate of 3% to 4% of births in general.

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