

Gametogenesis: Conversion of Germ Cells into Male and Female Gametes

■ PRIMORDIAL GERM CELLS

Development begins with fertilization, the process by which the male gamete, the **sperm**, and the female gamete, the **oocyte**, unite to give rise to a **zygote**. Gametes are derived from **primordial germ cells** (PGCs) that are formed in the epiblast during the second week, move through the primitive streak during gastrulation, and migrate to the wall of the yolk sac (Fig. 2.1). During the fourth week, these cells begin to migrate from the yolk sac toward the developing gonads, where they arrive by the end of the fifth week. Mitotic divisions increase their number during their migration and also when they arrive in the gonad. In preparation for fertilization, germ cells undergo **gametogenesis**, which includes meiosis, to reduce the number of chromosomes and **cytodifferentiation** to complete their maturation.

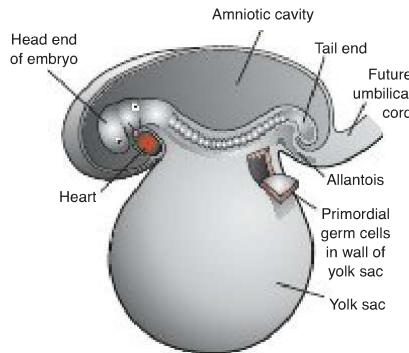


FIGURE 2.1 An embryo at the end of the third week, showing the position of primordial germ cells [PGCs] in the wall of the yolk sac, close to the attachment of the future umbilical cord. From this location, these cells migrate to the developing gonad.

Clinical Correlates

Primordial Germ Cells and Teratomas

Teratomas are tumors of disputed origin that often contain a variety of tissues, such as bone, hair, muscle, gut epithelia, and others. It is thought that these tumors arise from pluripotent stem cells that can differentiate into any of the three germ layers or their derivatives. Some evidence suggests that PGCs that have strayed from their normal migratory paths could be responsible for some of these tumors (Fig. 2.2). Another source may be epiblast cells that give rise to all three germ layers during gastrulation (see p. 66 and Fig. 5.9, p. 67).



FIGURE 2.2 Oropharyngeal teratoma. These tumors may arise from PGCs or from epiblast cells [see Chapter 5], both of which are pluripotent. Tissues within the tumors include derivatives of all three germ layers and may include gut, bone, skin, teeth, and so forth.

■ THE CHROMOSOME THEORY OF INHERITANCE

Traits of a new individual are determined by specific genes on chromosomes inherited from the father and the mother. Humans have approximately 23,000 genes on 46 chromosomes. Genes on the same chromosome tend to be inherited together and so are known as **linked genes**. In somatic cells, chromosomes appear as 23 **homologous** pairs to form the **diploid** number of 46. There are 22 pairs of matching chromosomes, the **autosomes**, and one pair of **sex chromosomes**. If the sex pair is XX, the individual is genetically female; if the pair is XY, the individual is genetically male. One chromosome of each pair is derived from the maternal gamete, the **oocyte**, and one from the paternal gamete, the **sperm**. Thus, each gamete contains a **haploid** number of 23 chromosomes, and the union of the gametes at **fertilization** restores the diploid number of 46.

Mitosis

Mitosis is the process whereby one cell divides, giving rise to two daughter cells that are genetically identical to the parent cell (Fig. 2.3).

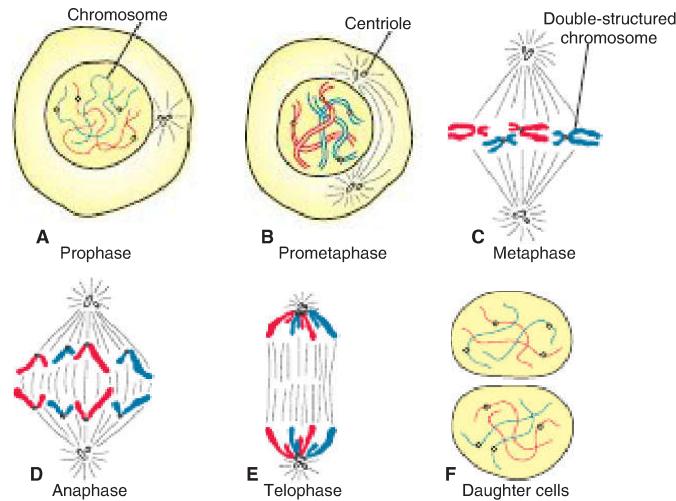


FIGURE 2.3 Various stages of mitosis. In prophase, chromosomes are visible as slender threads. Doubled chromatids become clearly visible as individual units during metaphase. At no time during division do members of a chromosome pair unite. Blue, paternal chromosomes; red, maternal chromosomes.

Each daughter cell receives the complete complement of 46 chromosomes. Before a cell enters mitosis, each chromosome replicates its DNA. During this replication phase, chromosomes are extremely long, they are spread diffusely through the nucleus, and they cannot be recognized with the light microscope. With the onset of mitosis, the chromosomes begin to coil, contract, and condense; these events mark the beginning of **prophase**. Each chromosome now consists of two parallel subunits, **chromatids**, that are joined at a narrow region common to both called the **centromere**. Throughout prophase, the chromosomes continue to condense, shorten, and thicken (Fig. 2.3A), but only at prometaphase do the chromatids become distinguishable (Fig. 2.3B). During metaphase, the chromosomes line up in the equatorial plane, and their doubled structure is clearly visible (Fig. 2.3C). Each is attached by **microtubules** extending from the centromere to the centriole, forming the **mitotic spindle**. Soon, the centromere of each chromosome divides, marking the beginning of anaphase, followed by migration of chromatids to opposite poles of the spindle. Finally, during telophase, chromosomes uncoil and lengthen, the nuclear envelope reforms, and the

cytoplasm divides (Fig. 2.3D–F). Each daughter cell receives half of all doubled chromosome material and thus maintains the same number of chromosomes as the mother cell.

Meiosis

Meiosis is the cell division that takes place in the **germ cells** to generate male and female gametes, sperm and egg cells, respectively. Meiosis requires two cell divisions, **meiosis I** and **meiosis II**, to reduce the number of chromosomes to the haploid number of 23 (Fig. 2.4). As in mitosis, male and female germ cells (**spermatocytes** and **primary oocytes**) at the beginning of meiosis I replicate their DNA so that each of the 46 chromosomes is duplicated into sister chromatids. In contrast to mitosis, however, **homologous chromosomes** then align

themselves in pairs, a process called **synapsis**. The pairing is exact and point for point except for the XY combination. Homologous pairs then separate into two daughter cells, thereby reducing the chromosome number from diploid to haploid. Shortly thereafter, meiosis II separates sister chromatids. Each gamete then contains 23 chromosomes.

Crossover

Crossovers, critical events in meiosis I, are the **interchange of chromatid segments** between paired homologous chromosomes (Fig. 2.4C). Segments of chromatids break and are exchanged as homologous chromosomes separate. As separation occurs, points of interchange are temporarily united and form an X-like structure, a **chiasma** (Fig. 2.4C). The approximately

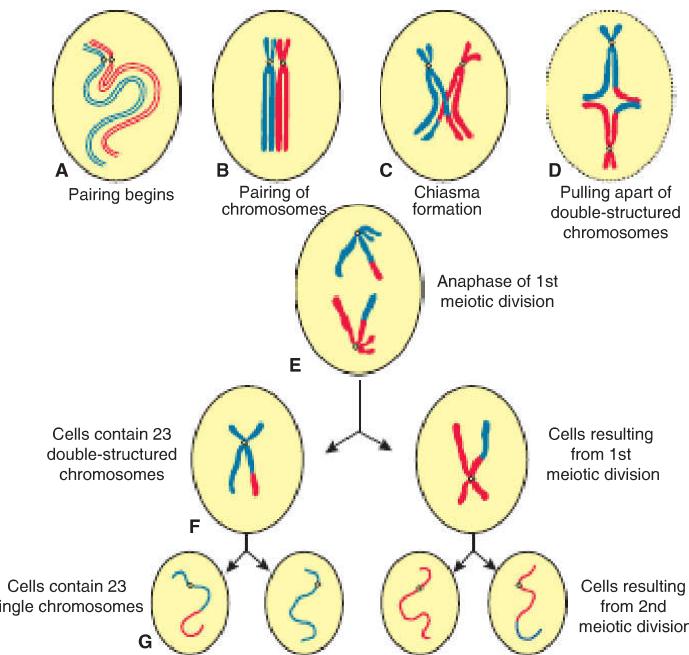


FIGURE 2.4 First and second meiotic divisions. **A**, Homologous chromosomes approach each other. **B**, Homologous chromosomes pair, and each member of the pair consists of two chromatids. **C**, Intimately paired homologous chromosomes interchange chromatid fragments (crossover). Note the chiasma. **D**, Double-structured chromosomes pull apart. **E**, Anaphase of the first meiotic division. **F,G**, During the second meiotic division, the double-structured chromosomes split at the centromere. At completion of division, chromosomes in each of the four daughter cells are different from each other.

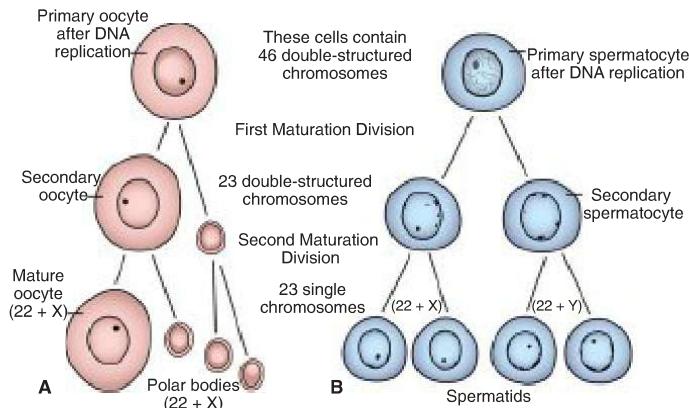


FIGURE 2.5 Events occurring during the first and second maturation divisions. **A**, The primitive female germ cell [primary oocyte] produces only one mature gamete, the mature oocyte. **B**, The primitive male germ cell [primary spermatocyte] produces four spermatids, all of which develop into spermatozoa.

30 to 40 crossovers (one or two per chromosome) with each meiotic I division are most frequent between genes that are far apart on a chromosome.

As a result of meiotic divisions:

- **Genetic variability** is enhanced through
 - Crossover, which redistributes genetic material
 - Random distribution of homologous chromosomes to the daughter cells
- Each germ cell contains a haploid number of chromosomes so that at fertilization, the diploid number of 46 is restored.

Polar Bodies

Also during meiosis, one primary oocyte gives rise to four daughter cells, each with 22 plus 1 X chromosome (Fig. 2.5A). Only one of these develops into a mature gamete, however, the oocyte; the other three, the **polar bodies**, receive little cytoplasm and degenerate during subsequent development. Similarly, one primary spermatocyte gives rise to four daughter cells, two with 22 plus 1 X chromosomes and two with 22 plus 1 Y chromosomes (Fig. 2.5B). In contrast to oocyte formation, however, all four develop into mature gametes.

Clinical Correlates

Birth Defects and Spontaneous Abortions: Chromosomal and Genetic Factors

Chromosomal abnormalities, which may be **numerical** or **structural**, are important causes of birth defects and spontaneous abortions. It is estimated that 50% of conceptions end in spontaneous abortion and that 50% of these abortuses have major chromosomal abnormalities. Thus, approximately 25% of conceptuses have a major chromosomal defect. The most common chromosomal abnormalities in abortuses are 45,X [Turner syndrome], triploidy,

and trisomy 16. Chromosomal abnormalities account for 10% of major birth defects, and **gene mutations** account for an additional 8%.

Numerical Abnormalities

The normal human somatic cell contains 46 chromosomes; the normal gamete contains 23. Normal somatic cells are **diploid**, or **2n**; normal gametes are **haploid**, or **n**. **Euploid** refers to any exact multiple of **n** (e.g., diploid or triploid). **Aneuploid** refers to any chromosome number that is not euploid; it is usually applied when an extra chromosome is present (**trisomy**)

or when one is missing (**monosomy**). Abnormalities in chromosome number may originate during meiotic or mitotic divisions. In **meiosis**, two members of a pair of homologous chromosomes normally separate during the first meiotic division so that each daughter cell receives one member of each pair (Fig. 2.6A). Sometimes, however, separation does not occur (**nondisjunction**), and both members of a pair move into one cell (Fig. 2.6B,C). As a result of nondisjunction of the chromosomes, one cell receives 24 chromosomes, and the other receives 22 instead of the normal 23. When, at fertilization, a gamete having 23 chromosomes fuses with a gamete having 24 or 22 chromosomes, the result is an individual with either 47 chromosomes (trisomy) or 45 chromosomes (monosomy). Nondisjunction, which occurs during either the first or the second meiotic division of the germ cells, may involve the autosomes or sex chromosomes. In women, the incidence of chromosomal abnormalities, including nondisjunction, increases with age, especially at 35 years and older.

Sometimes, chromosomes break, and pieces of one chromosome attach to another. Such **translocations** may be **balanced**, in which

case breakage and reunion occur between two chromosomes, but no critical genetic material is lost and individuals are normal; or they may be **unbalanced**, in which case part of one chromosome is lost, and an altered phenotype is produced. For example, unbalanced translocations between the long arms of chromosomes 14 and 21 during meiosis I or II produce gametes with an extra copy of chromosome 21, one of the causes of Down syndrome (Fig. 2.7). Translocations are particularly common between chromosomes 13, 14, 15, 21, and 22 because they cluster during meiosis.

TRISOMY 21 (DOWN SYNDROME)

Down syndrome is caused by an extra copy of **chromosome 21** (**trisomy 21**) (Fig. 2.8). Features of children with Down syndrome include growth retardation; varying degrees of intellectual disability; craniofacial abnormalities, including upward slanting eyes, epicanthal folds (extra skin folds at the medial corners of the eyes), flat facies, and small ears; cardiac defects; and hypotonia (Fig. 2.9). These individuals also have an increased chance of developing leukemia, infections, thyroid dysfunction, and premature aging.

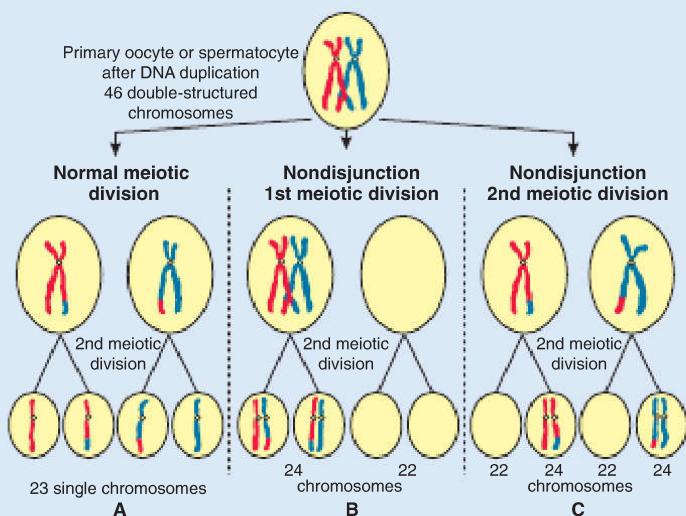


FIGURE 2.6 **A.** Normal maturation divisions. **B.** Nondisjunction in the first meiotic division. **C.** Nondisjunction in the second meiotic division.

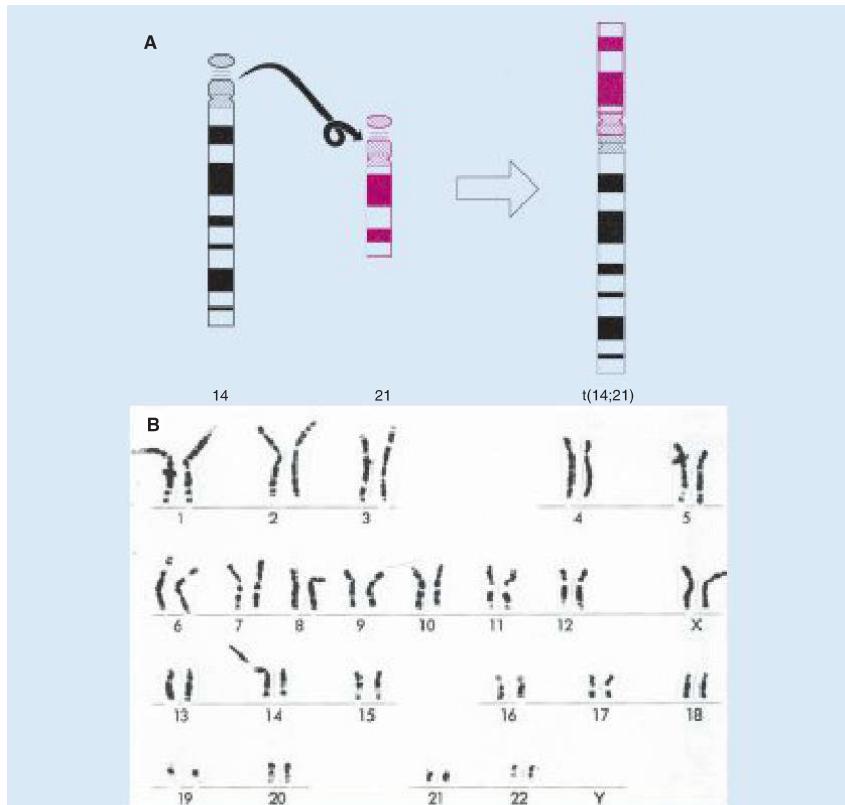


FIGURE 2.7 **A.** Translocation of the long arms of chromosomes 14 and 21 at the centromere. Loss of the short arms is not clinically significant, and these individuals are clinically normal, although they are at risk for producing offspring with unbalanced translocations. **B.** Karyotype of translocation of chromosome 21 onto 14, resulting in Down syndrome.

Furthermore, an increased frequency and earlier onset of Alzheimer disease is observed among persons with Down syndrome. In 95% of cases, the syndrome is caused by trisomy 21 resulting from meiotic nondisjunction, and in 75% of these instances, nondisjunction occurs during **oocyte formation**. The incidence of Down syndrome is approximately 1 in 2,000 conceptuses for women under age 25 years. This risk increases with maternal age to 1 in 300 at age 35 years and 1 in 100 at age 40 years.

In approximately 4% of cases of Down syndrome, there is an unbalanced transloca-

tion between chromosome 21 and chromosomes 13, 14, 15, or 21 (Fig. 2.7). The final 1% is caused by mosaicism resulting from a trisomic conception followed by the loss of the extra chromosome in some cells during mitosis. These individuals have **mosaicism**, with some cells having a normal chromosome number and some having trisomy. They may exhibit few or many of the characteristics of Down syndrome.

TRISOMY 18

Patients with **trisomy 18** show the following features: intellectual disability, congenital

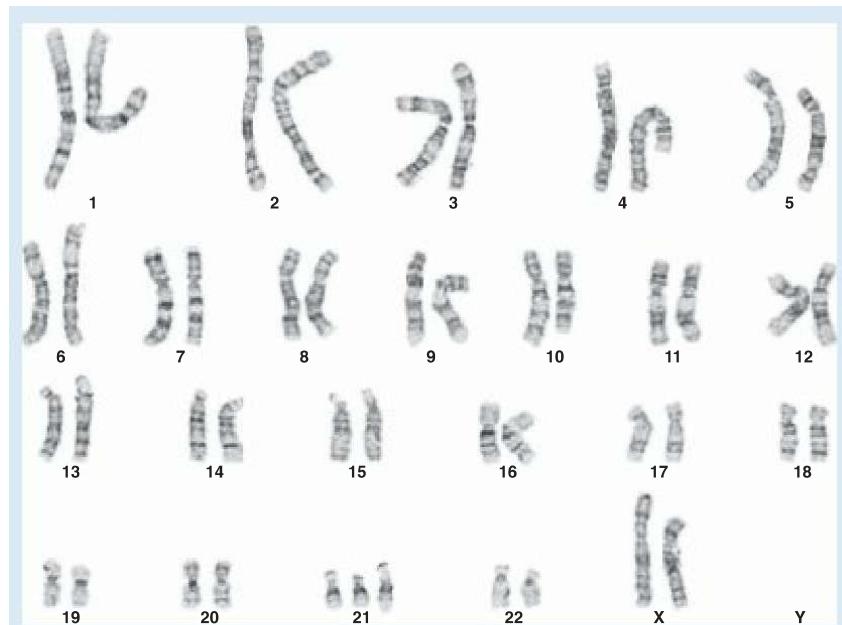


FIGURE 2.8 Karyotype of trisomy 21, Down syndrome.

heart defects, low-set ears, and flexion of fingers and hands [Fig. 2.10]. In addition, patients frequently show micrognathia, renal anomalies, syndactyly, and malformations of the skeletal system. The incidence of this

condition is approximately 1 in 5,000 newborns. Eighty-five percent are lost between 10 weeks of gestation and term, whereas those born alive usually die by 2 months of age. Approximately 5% live beyond 1 year.



FIGURE 2.9 A. Child with Down syndrome. Note the flat broad face, oblique palpebral fissures, and protruding tongue. Children with Down syndrome usually have some degree of intellectual disability and many have cardiac defects. B. Another characteristic of these children is a broad hand with a single transverse [simian] crease.



FIGURE 2.10 Child with trisomy 18. Note the low-set ears, small mouth, deficient mandible [micrognathia], flexion of the hands, and absent and/or hypoplasia of the radius and ulna.



FIGURE 2.11 Child with trisomy 13. Note the bilateral cleft lip, the sloping forehead, and anophthalmia.

X chromosomes is normally inactivated]. The incidence is approximately 1 in 500 males. Nondisjunction of the XX homologues is the most common causative event. Occasionally, patients with Klinefelter syndrome have 48 chromosomes: 44 autosomes and 4 sex chromosomes [48,XXXY]. Although intellectual disability is not generally part of the syndrome, the more X chromosomes there are, the more likely there will be some degree of cognitive impairment.

TURNER SYNDROME

Turner syndrome, with a 45,X karyotype, is the only monosomy compatible with life. Even then, 98% of all fetuses with the syndrome are spontaneously aborted. The few that survive are unmistakably female in appearance [Fig. 2.12] and are characterized by the absence of ovaries [**gonadal dysgenesis**] and short stature. Other common associated abnormalities are webbed neck, lymphedema of the extremities, skeletal deformities, and a broad chest with widely spaced nipples. Approximately 55% of affected females are monosomic for the X and chromatin body negative because of nondisjunction. In 80% of these females, nondisjunction in the male

TRISOMY 13

The main abnormalities of **trisomy 13** are intellectual disability, holoprosencephaly, congenital heart defects, deafness, cleft lip and palate, and eye defects, such as microphthalmia, anophthalmia, and coloboma [Fig. 2.11]. The incidence of this abnormality is approximately 1 in 20,000 live births, and more than 90% of the infants die in the first month after birth. Approximately 5% live beyond 1 year.

KLINEFELTER SYNDROME

The clinical features of **Klinefelter syndrome**, found only in males and usually detected by amniocentesis, are sterility, testicular atrophy, hyalinization of the seminiferous tubules, and usually gynecomastia. The cells have 47 chromosomes with a sex chromosomal complement of the XXY type, and a **sex chromatin (Barr) body** is found in 80% of cases. (**Barr body**: formed by condensation of an inactivated X chromosome; a Barr body is also present in normal females because one of the



FIGURE 2.12 Patient with Turner syndrome. **A.** At birth. Note the loose skin at the posterior of the neck caused by the remains of a cystic hygroma (fluid-filled cyst), the short neck, malformed ears, and swelling in the hand (**B**) and the foot (**C**) caused by lymphedema. **D.** At 6 years of age, the webbed neck is prominent, and the nipples are widely spaced with a broad chest.

gamete is the cause. In the remainder of females, structural abnormalities of the X chromosome or mitotic nondisjunction resulting in mosaicism is the cause.

TRIPLE X SYNDROME

Patients with **triple X syndrome** (47,XXX) often go undiagnosed because of their mild physical features. However, these girls frequently have

problems with speech and self-esteem. They have two sex chromatin bodies in their cells.

Structural Abnormalities

Structural chromosome abnormalities, which involve one or more chromosomes, usually result from chromosome breakage. It has been suggested that breaks are caused by environmental factors, such as viruses, radiation,

and drugs, but the evidence is inconclusive. The result of breakage depends on what happens to the broken pieces. In some cases, the broken piece of a chromosome is lost, and the infant with **partial deletion** of a chromosome is abnormal. A well-known syndrome, caused by partial deletion of the short arm of chromosome 5, is the **cri-du-chat syndrome**. Affected infants have a cat-like cry, microcephaly (small head), intellectual disability, and congenital heart disease. Many other relatively rare syndromes are known to result from a partial chromosome deletion.

Microdeletions, spanning only a few **contiguous genes**, may result in **microdeletion syndrome** or **contiguous gene syndrome**. Sites where these deletions occur, called **contiguous gene complexes**, are usually identified by **fluorescence in situ hybridization (FISH)** (see p. 24). An example of a microdeletion occurs on the long arm of chromosome 15 [15q11–15q13]. [Note: Chromosomes have a long arm, designated “q,” and a short arm, designated “p,” based on the position of the centromere.] When the microdeletion occurs on the maternal chromosome, it results in **Angelman syndrome**, and the children have intellectual disability, cannot speak, exhibit poor motor development, and are prone to unprovoked and prolonged periods of laughter [Fig. 2.13]. If the microdeletion occurs on the paternal chromosome, **Prader-Willi syndrome** results. Affected individuals are characterized by hypotonia, obesity, intellectual disability, hypogonadism, and undescended testes [Fig. 2.14]. Characteristics that are differentially expressed depending upon whether the genetic material is inherited from the mother or the father are examples of **genomic imprinting**. Other contiguous gene syndromes may be inherited from either parent, including **Miller-Dieker syndrome** (lissencephaly, developmental delay, seizures, and cardiac and facial abnormalities resulting from a deletion at 17p13) and most cases of **22q11 syndrome** (palatal defects, conotruncal heart defects, speech delay, learning disorders, and schizophrenia-like disorder resulting from a deletion in 22q11).

Fragile sites are regions of chromosomes that demonstrate a propensity to separate or break under certain cell manipulations. For example, fragile sites can be revealed by culturing lymphocytes from a patient in folate-deficient medium. Although numerous



FIGURE 2.13 Patient with Angelman syndrome resulting from a microdeletion on maternal chromosome 15. If the defect is inherited on the paternal chromosome, Prader-Willi syndrome occurs (Fig. 2.14).

fragile sites have been defined and consist of **CGG repeats**, only those in the **FMRI** gene on the long arm of the X chromosome (Xq27) have been correlated with an altered phenotype that is called the **fragile X syndrome**. Greater than 200 repeats occur in the promoter region of the gene in affected individuals compared to 6 to 54 repeats in normal subjects. Fragile X syndrome is characterized by intellectual disability, large ears, prominent jaw, and large testes. The syndrome occurs in 1 per 5,000 individuals, and because it is an X-linked condition, males are affected almost exclusively, which may account for the preponderance of males among the cognitively impaired. Fragile X syndrome is second only to Down syndrome as a cause of intellectual disability due to genetic abnormalities.

Gene Mutations

Many congenital malformations in humans are inherited, and some show a clear Mendelian pattern of inheritance. Many birth defects are

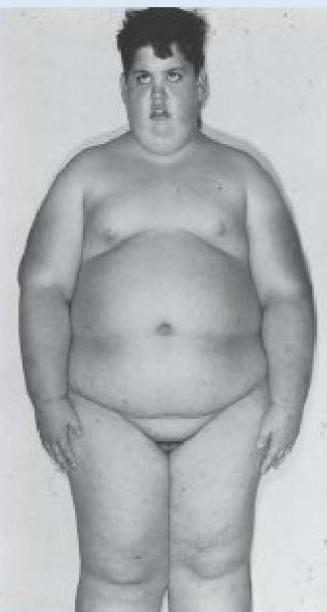


FIGURE 2.14 Patient with Prader-Willi syndrome resulting from a microdeletion on paternal chromosome 15. If the defect is inherited on the maternal chromosome, Angelman syndrome occurs [Fig. 2.13].

directly attributable to a change in the structure or function of a single gene, hence the name **single gene mutation**. This type of defect is estimated to account for approximately 8% of all human malformations.

With the exception of the X and Y chromosomes in the male, genes exist as pairs, or **alleles**, so that there are two doses for each genetic determinant: one from the mother and one from the father. If a mutant gene produces an abnormality in a single dose, despite the presence of a normal allele, it is a **dominant mutation**. If both alleles must be abnormal (double dose) or if the mutation is X-linked (occurs on the X chromosome) in the male, it is a **recessive mutation**. Variations in the effects of mutant genes may be a result of **modifying factors**.

In some cases, mutations occur in a cell as an embryo is developing. If the mutation occurs in a somatic cell, the individual will have **mosaicism** (having more than one genetically distinct cell

line) with some cells having the mutation and some not. If the mutation occurs in a germ line cell (egg or sperm), the result is germ line mosaicism. In this case, the parent does not express an abnormality or disease because his or her somatic cells are normal. However, the parent can transmit the defect to multiple offspring.

The application of molecular biological techniques has increased our knowledge of genes responsible for normal development. In turn, genetic analysis of human syndromes has shown that mutations in many of these same genes are responsible for some congenital abnormalities and childhood diseases. Thus, the link between key genes in development and their role in clinical syndromes is becoming clearer.

In addition to causing congenital malformations, mutations can result in **inborn errors of metabolism**. These diseases, among which **phenylketonuria**, **homocystinuria**, and **galactosemia** are the best known, may be accompanied by or cause various degrees of intellectual disability if proper diets and medical care are not instituted.

Diagnostic Techniques for Identifying Genetic Abnormalities

Cytogenetic analysis is used to assess chromosome number and integrity. The technique requires dividing cells, which usually means establishing cell cultures that are arrested in metaphase by chemical treatment. Chromosomes are **Giemsa-stained** to reveal light and dark banding patterns (G-bands; Fig. 2.7) unique for each chromosome. Each band represents 5 to 10×10^6 base pairs of DNA, which may include a few to several hundred genes. Recently, **high-resolution metaphase banding techniques** have been developed that demonstrate greater numbers of bands representing even smaller pieces of DNA, thereby facilitating diagnosis of small deletions.

Molecular techniques, such as **fluorescent in situ hybridization (FISH)**, use specific DNA probes to identify ploidy for a few selected chromosomes and for detecting microdeletions. Fluorescent probes are hybridized to chromosomes or genetic loci using cells on a slide, and the results are visualized with a fluorescence microscope [Fig. 2.15].

Microarrays use spots of specific DNA sequences (probes) attached to a solid surface, usually glass or silicon (Affymetrix chips). These probes may be a short sequence from a gene or other DNA element that are used to

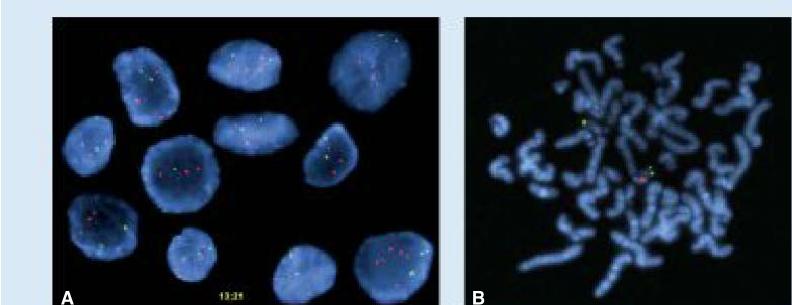


FIGURE 2.15 **A.** FISH, using a probe for chromosome 21 [red dots]. Note that there are three red dots in each cell, indicating trisomy 21 [Down syndrome]. The green dots represent a control probe for chromosome 13. Two cells are superimposed on the lower right, giving the impression of the presence of multiple probes. **B.** FISH analysis of 22q11 deletion syndrome. The green signals identify chromosome 22; the red signal represents FISH probe N25, which is in the q11 region. It is present on only one of the pairs of chromosome 22 indicating the other has the 22q11 deletion.

hybridize a cDNA or cRNA sample (the target sample). Hybridization of probe-target sequences are detected and quantified using fluorescence or other reporter techniques. Results can detect single nucleotide polymorphisms, mutations, and changes in expression levels. Some companies now offer such techniques commercially for anyone who wants their genome tested or sequenced.

Exome sequencing represents a new approach to finding **mutations** and **polymorphisms** (single nucleotide changes [SNPs] in a DNA sequence) responsible for birth defects and diseases. With this technique, only the coding regions (exons) in the genome are sequenced. Together, these coding regions make up the **exome** and represent only 1% of the entire human genome, thereby making sequencing them more practical than trying to sequence all of the genome. Because most genetic variants lie within the coding regions for proteins, the technique is an efficient

way to discover these differences. The technique is also superior to older approaches that relied on linkage studies followed by positional cloning (searching for candidate genes in specific regions of chromosomes) because these techniques required large numbers of affected individuals within a family and were not applicable to studying affected individuals from different families. In contrast, exome sequencing can find a causative mutation in a single affected individual if the exomes from both parents can also be sequenced. Even sequencing-affected individuals from different families regardless of kinship can be successful. It must be remembered, however, that exome sequencing can only identify variants in the coding regions of genes that alter proteins. Other genetic causes of birth defects that lie outside the coding region will have to be identified by whole genome sequencing, but for now, the expense and time required to conduct these studies is prohibitive.

■ MORPHOLOGICAL CHANGES DURING MATURATION OF THE GAMETES

Oogenesis

Oogenesis is the process whereby oogonia differentiate into mature oocytes.

Maturation of Oocytes Begins Before Birth

Once PGCs have arrived in the gonad of a genetic female, they differentiate into **oogonia**

(Fig. 2.16A,B). These cells undergo a number of mitotic divisions, and by the end of the third month, they are arranged in clusters surrounded by a layer of flat epithelial cells (Figs. 2.17 and 2.18). Whereas all of the oogonia in one cluster are probably derived from a single cell, the flat epithelial cells, known as **follicular cells**, originate from surface epithelium covering the ovary.

The majority of oogonia continue to divide by mitosis, but some of them arrest their cell division in prophase of meiosis I and form **primary**

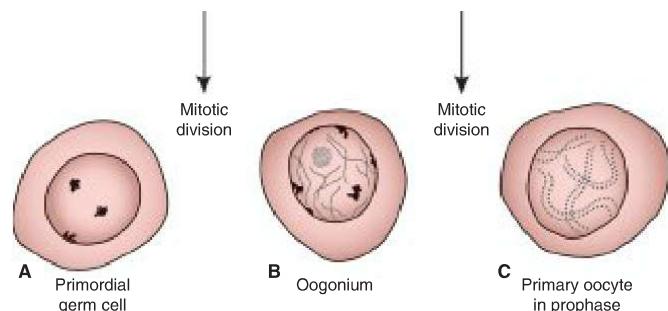


FIGURE 2.16 Differentiation of PGCs into oogonia begins shortly after their arrival in the ovary. By the third month of development, some oogonia give rise to primary oocytes that enter prophase of the first meiotic division. This prophase may last 40 or more years and finishes only when the cell begins its final maturation. During this period, it carries 46 double-structured chromosomes.

oocytes (Figs. 2.16C and 2.17A). During the next few months, oogonia increase rapidly in number, and by the fifth month of prenatal development, the total number of germ cells in the ovary reaches its maximum, estimated at 7 million. At this time, cell death begins, and many oogonia as well as primary oocytes degenerate and become **atretic**. By the seventh month, the

majority of oogonia have degenerated except for a few near the surface. All surviving primary oocytes have entered prophase of meiosis I, and most of them are individually surrounded by a layer of flat follicular epithelial cells (Fig. 2.17B). A primary oocyte, together with its surrounding flat epithelial cells, is known as a **primordial follicle** (Fig. 2.18A).

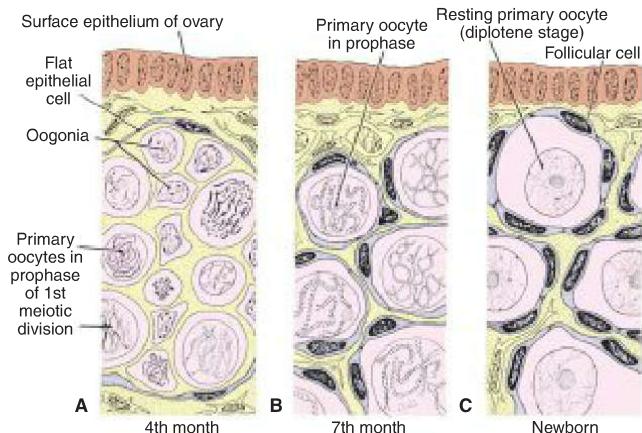


FIGURE 2.17 Segment of the ovary at different stages of development. **A.** Oogonia are grouped in clusters in the cortical part of the ovary. Some show mitosis; others have differentiated into primary oocytes and entered prophase of the first meiotic division. **B.** Almost all oogonia are transformed into primary oocytes in prophase of the first meiotic division. **C.** There are no oogonia. Each primary oocyte is surrounded by a single layer of follicular cells, forming the primordial follicle. Oocytes have entered the diplotene stage of prophase, in which they remain until just before ovulation. Only then do they enter metaphase of the first meiotic division.

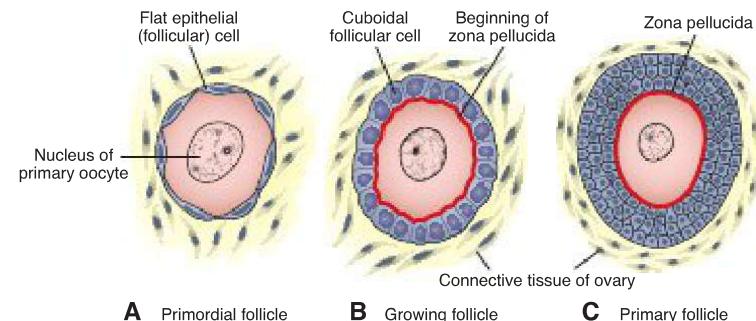


FIGURE 2.18 **A.** Primordial follicle consisting of a primary oocyte surrounded by a layer of flattened epithelial cells. **B.** Early primary or preantral stage follicle recruited from the pool of primordial follicles. As the follicle grows, follicular cells become cuboidal and begin to secrete the zona pellucida, which is visible in irregular patches on the surface of the oocyte. **C.** Mature primary [preantral] follicle with follicular cells forming a stratified layer of granulosa cells around the oocyte and the presence of a well-defined zona pellucida.

Maturation of Oocytes Continues at Puberty

Near the time of birth, all primary oocytes have started prophase of meiosis I, but instead of proceeding into metaphase, they enter the **diplotene stage**, a resting stage during prophase that is characterized by a lacy network of chromatin (Fig. 2.17C). Primary oocytes remain arrested in prophase and do not finish their first meiotic division before puberty is reached. This arrested state is produced by **oocyte maturation inhibitor (OMI)**, a small peptide secreted by follicular cells. The total number of primary oocytes at birth is estimated to vary from 600,000 to 800,000. During childhood, most oocytes become atretic; only approximately 40,000 are present by the beginning of puberty, and fewer than 500 will be ovulated. Some oocytes that reach maturity late in life have been dormant in the diplotene stage of the first meiotic division for 40 years or more before ovulation. Whether the diplotene stage is the most suitable phase to protect the oocyte against environmental influences is unknown. The fact that the risk of having children with chromosomal abnormalities increases with maternal age indicates that primary oocytes are vulnerable to damage as they age.

At puberty, a pool of growing follicles is established and continuously maintained from the supply of primordial follicles. Each month, 15 to 20 follicles selected from this pool begin to mature. Some of these die, whereas others begin to

accumulate fluid in a space called the **antrum**, thereby entering the **antral or vesicular stage** (Fig. 2.19A). Fluid continues to accumulate such that, immediately prior to ovulation, follicles are quite swollen and are called **mature vesicular follicles** or **graafian follicles** (Fig. 2.19B). The antral stage is the longest, whereas the mature vesicular stage encompasses approximately 37 hours prior to ovulation.

As primordial follicles begin to grow, surrounding follicular cells change from flat to cuboidal and proliferate to produce a stratified epithelium of **granulosa cells**, and the unit is called a **primary follicle** (Fig. 2.18B,C). Granulosa cells rest on a basement membrane separating them from surrounding ovarian connective tissue (stromal cells) that form the **theca folliculi**. Also, granulosa cells and the oocyte secrete a layer of glycoproteins on the surface of the oocyte, forming the **zona pellucida** (Fig. 2.18C). As follicles continue to grow, cells of the theca folliculi organize into an inner layer of secretory cells, the **theca interna**, and an outer fibrous capsule, the **theca externa**. Also, small, fingerlike processes of the follicular cells extend across the zona pellucida and interdigitate with microvilli of the plasma membrane of the oocyte. These processes are important for transport of materials from follicular cells to the oocyte.

As development continues, fluid-filled spaces appear between granulosa cells. Coalescence of these spaces forms the **antrum**, and the follicle is

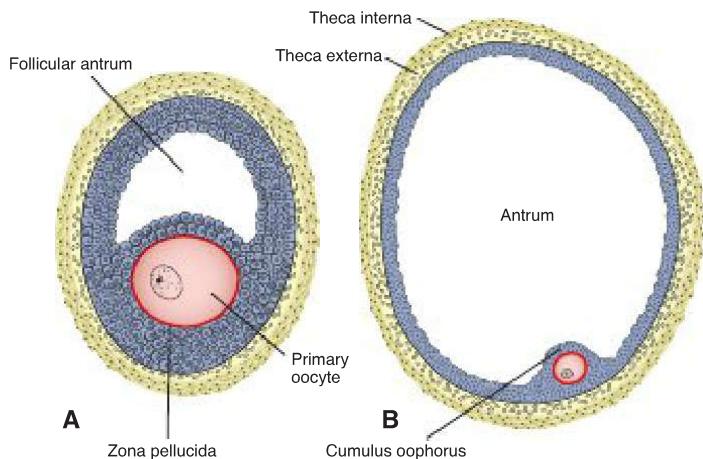


FIGURE 2.19 **A.** Vesicular [antral] stage follicle. The oocyte, surrounded by the zona pellucida, is off center; the antrum has developed by fluid accumulation between intercellular spaces. Note the arrangement of cells of the theca interna and the theca externa. **B.** Mature vesicular (graafian) follicle. The antrum has enlarged considerably, is filled with follicular fluid, and is surrounded by a stratified layer of granulosa cells. The oocyte is embedded in a mound of granulosa cells, the cumulus oophorus.

termed a **vesicular or an antral follicle**. Initially, the antrum is crescent-shaped, but with time, it enlarges (Fig. 2.19). Granulosa cells surrounding the oocyte remain intact and form the **cumulus oophorus**. At maturity, the **mature vesicular (graafian) follicle** may be 25 mm or more in diameter. It is surrounded by the theca interna, which is composed of cells having characteristics of steroid secretion, rich in blood vessels, and

the theca externa, which gradually merges with the ovarian connective tissue (Fig. 2.19).

With each ovarian cycle, a number of follicles begin to develop, but usually, only one reaches full maturity. The others degenerate and become atretic. When the secondary follicle is mature, a surge in **luteinizing hormone (LH)** induces the preovulatory growth phase. Meiosis I is completed, resulting in formation

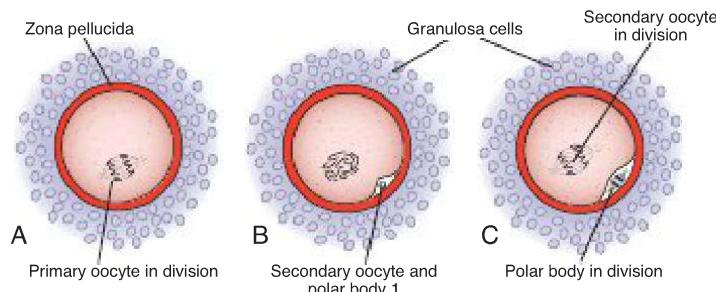


FIGURE 2.20 Maturation of the oocyte. **A.** Primary oocyte showing the spindle of the first meiotic division. **B.** Secondary oocyte and first polar body. The nuclear membrane is absent. **C.** Secondary oocyte showing the spindle of the second meiotic division. The first polar body is also dividing.

of two daughter cells of unequal size, each with 23 double-structured chromosomes (Fig. 2.20A,B). One cell, the **secondary oocyte**, receives most of the cytoplasm; the other, the **first polar body**, receives practically none. The first polar body lies between the zona pellucida and the cell membrane of the secondary oocyte in the perivitelline space (Fig. 2.20B). The cell then enters meiosis II but arrests in metaphase approximately 3 hours before ovulation. Meiosis II is completed only if the oocyte is fertilized; otherwise, the cell degenerates approximately 24 hours after ovulation. The first polar body may undergo a second division (Fig. 2.20C).

Spermatogenesis

Maturation of Sperm Begins at Puberty

Spermatogenesis, which begins at puberty, includes all of the events by which **spermatogonia** are transformed into **spermatozoa**. At birth, germ cells in the male infant can be recognized in the sex cords of the testis as large, pale cells

surrounded by supporting cells (Fig. 2.21A). Supporting cells, which are derived from the surface epithelium of the testis in the same manner as follicular cells, become **sustentacular cells** or **Sertoli cells** (Fig. 2.21B).

Shortly before puberty, the sex cords acquire a lumen and become the **seminiferous tubules**. At about the same time, PGCs give rise to spermatogonial stem cells. At regular intervals, cells emerge from this stem cell population to form **type A spermatogonia**, and their production marks the initiation of spermatogenesis. Type A cells undergo a limited number of mitotic divisions to form clones of cells. The last cell division produces **type B spermatogonia**, which then divide to form **primary spermatocytes** (Figs. 2.21B and 2.22). Primary spermatocytes then enter a prolonged prophase (22 days) followed by rapid completion of meiosis I and formation of **secondary spermatocytes**. During the second meiotic division, these cells immediately begin to form haploid **spermatids** (Figs. 2.21B, 2.22, 2.23). Throughout this series of events, from the time type A cells leave the

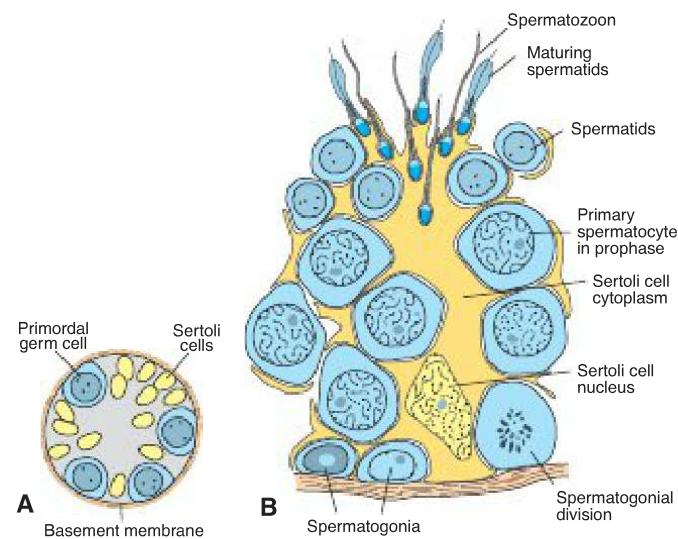


FIGURE 2.21 **A.** Cross section through primitive sex cords of a newborn boy showing PGCs and their supporting Sertoli cells. **B.** Cross section through a seminiferous tubule at puberty. Note the different stages of spermatogenesis and that developing sperm cells are embedded in the cytoplasmic processes of a supporting Sertoli cell.

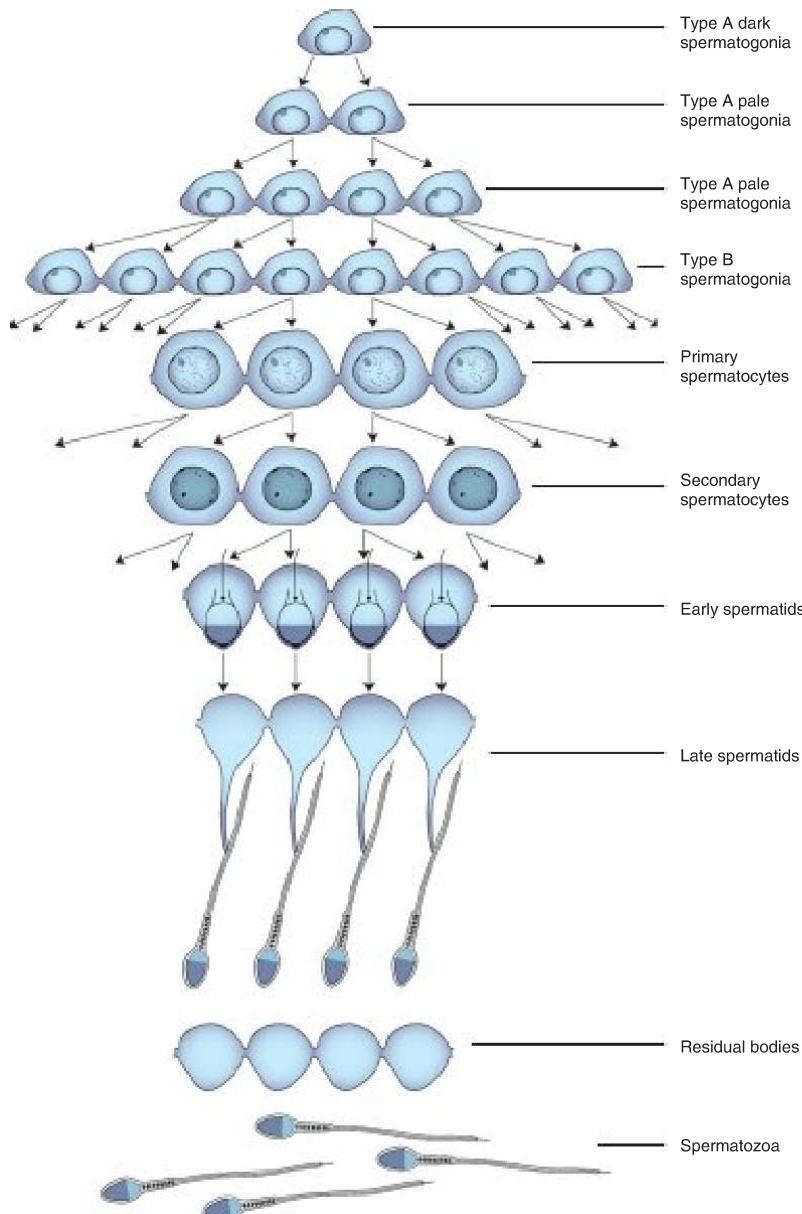


FIGURE 2.22 Type A spermatogonia, derived from the spermatogonial stem cell population, represent the first cells in the process of spermatogenesis. Clones of cells are established, and cytoplasmic bridges join cells in each succeeding division until individual sperm are separated from residual bodies. In fact, the number of individual interconnected cells is considerably greater than depicted in this figure.

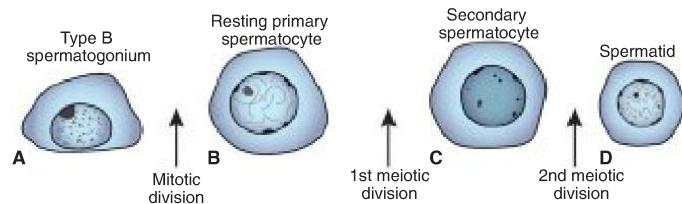


FIGURE 2.23 The products of meiosis during spermatogenesis in humans.

stem cell population to formation of spermatids, cytokinesis is incomplete so that successive cell generations are joined by cytoplasmic bridges. Thus, the progeny of a single type A spermatogonium form a clone of germ cells that maintain contact throughout differentiation (Fig. 2.22). Furthermore, spermatogonia and spermatids remain embedded in deep recesses of Sertoli cells throughout their development (Fig. 2.21B). In this manner, Sertoli cells support and protect the germ cells, participate in their nutrition, and assist in the release of mature spermatozoa.

Spermatogenesis is regulated by LH production by the pituitary gland. LH binds to receptors on Leydig cells and stimulates testosterone production, which in turn binds to Sertoli cells to promote spermatogenesis. Follicle-stimulating hormone (FSH) is also essential because its binding to Sertoli cells stimulates testicular fluid production and synthesis of intracellular androgen receptor proteins.

Spermiogenesis

The series of changes resulting in the transformation of spermatids into spermatozoa is **spermiogenesis**. These changes include (1) formation of the **acrosome**, which covers half of the nuclear surface and contains enzymes to assist in penetration of the egg and its surrounding layers during fertilization (Fig. 2.24); (2) condensation of the nucleus; (3) formation of neck, middle piece, and tail; and (4) shedding of most of the cytoplasm as residual bodies that are phagocytized by Sertoli cells. In humans, the time required for a spermatogonium to develop into a mature spermatozoon is approximately 74 days, and approximately 300 million sperm cells are produced daily.

When fully formed, spermatozoa enter the lumen of seminiferous tubules. From there, they are pushed toward the epididymis by contractile elements in the wall of the seminiferous tubules. Although initially only slightly motile, spermatozoa obtain full motility in the epididymis.

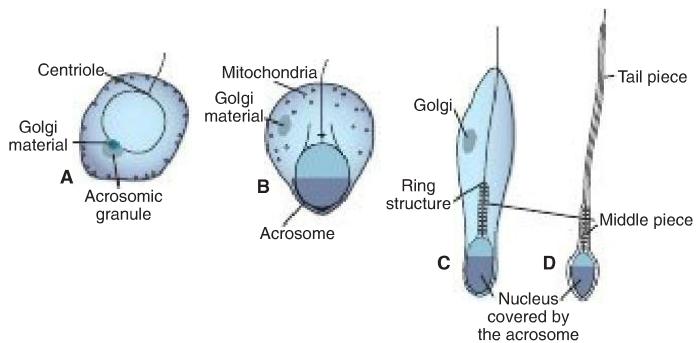


FIGURE 2.24 Important stages in transformation of the human spermatid into the spermatozoon.

Clinical Correlates

Abnormal Gametes

In humans, and in most mammals, one ovarian follicle occasionally contains two or three clearly distinguishable primary oocytes (Fig. 2.25A). Although these oocytes may give rise to twins or triplets, they usually degenerate before reaching maturity. In rare cases, one primary oocyte contains two or even three nuclei (Fig. 2.25B). Such binucleated or trinucleated oocytes die before reaching maturity.

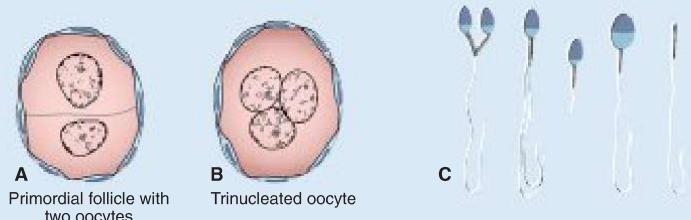


FIGURE 2.25 Abnormal germ cells. **A.** Primordial follicle with two oocytes. **B.** Trinucleated oocyte. **C.** Various types of abnormal spermatozoa.

SUMMARY

Primordial germ cells (PGCs) are derived from the epiblast during gastrulation and migrate to the wall of the yolk sac in the fourth week and then to the indifferent gonad (Fig. 2.1), where they arrive at the end of the fifth week. In preparation for fertilization, both male and female germ cells undergo **gametogenesis**, which includes **meiosis** and **cytodifferentiation**. During meiosis I, **homologous chromosomes pair and exchange genetic material**; during meiosis II, cells fail to replicate DNA, and each cell is thus provided with a **haploid** number of chromosomes and half the amount of DNA of a normal somatic cell (Fig. 2.4). Hence, mature male and female gametes have 22 plus X or 22 plus Y chromosomes, respectively.

Birth defects may arise through abnormalities in **chromosome number or structure** and from **single gene mutations**. Approximately, 10% of major birth defects are a result of chromosome abnormalities, and 8% are a result of gene mutations. **Trisomies** (an extra chromosome) and **monosomies** (loss of a chromosome) arise

In contrast to atypical oocytes, abnormal spermatozoa are seen frequently, and up to 10% of all spermatozoa have observable defects. The head or the tail may be abnormal, spermatozoa may be giants or dwarfs, and sometimes, they are joined (Fig. 2.25C). Sperm with morphologic abnormalities lack normal motility and probably do not fertilize oocytes.

Diagnostic techniques for identifying genetic abnormalities include **cytogenetics** to test for chromosome numbers (**ploidy**) and for **high-resolution metaphase banding** techniques to test for small deletions. **Fluorescent in situ hybridization (FISH)** uses fluorescent DNA probes to identify specific chromosomes or regions of chromosomes to determine deletions, translocations, and ploidy. **Microarrays** employ small sequences of DNA on chips as probes to detect mutations and changes in expression levels of specific genes. **Exome sequencing** sequences the protein coding region of DNA (1% of the total DNA; the **exome**) to identify mutations and polymorphisms responsible for birth defects and diseases. The technique is accurate, timely, and cost-effective compared to sequencing the entire genome.

In the female, maturation from primitive germ cell to mature gamete, which is called **oogenesis**, begins before birth; in the male, it is called **spermatogenesis**, and it begins at **puberty**. In the female, PGCs form **oogonia**. After repeated mitotic divisions, some of these arrest in prophase of meiosis I to form **primary oocytes**. By the seventh month, many oogonia have become atretic, and only primary oocytes remain surrounded by a layer of **follicular cells** derived from the surface epithelium of the ovary (Fig. 2.17). Together, they form the **primordial follicle**. At puberty, a pool of growing follicles is recruited and maintained from the finite supply of primordial follicles. Thus, every month, 15 to 20 follicles begin to grow, and as they mature, they pass through three stages: (1) pri-

mary or preantral, (2) vesicular or antral, and (3) mature vesicular or graafian follicle. The primary oocyte remains in prophase of the first meiotic division until the secondary follicle is mature. At this point, a surge in **luteinizing hormone (LH)** stimulates preovulatory growth: Meiosis I is completed, and secondary oocyte and polar body are formed. Then, the secondary oocyte is arrested in metaphase of meiosis II approximately 3 hours before ovulation and will not complete this cell division until fertilization.

In the male, primordial cells remain dormant until puberty, and only then do they differentiate into **spermatogonia**. These stem cells give rise to **primary spermatocytes**, which through two successive meiotic divisions produce four **spermatids** (Fig. 2.5). Spermatids go through a series of changes (**spermiogenesis**) (Fig. 2.24), including (1) formation of the acrosome; (2) condensation of the nucleus; (3) formation of neck, middle piece, and tail; and (4) shedding of most of the cytoplasm. The time required for a spermatogonium to become a mature spermatozoon is approximately 74 days.

Problems to Solve

- What is the most common cause of abnormal chromosome number? Give an example of a clinical syndrome involving abnormal numbers of chromosomes.
- In addition to numerical abnormalities, what types of chromosomal alterations occur?
- What is mosaicism, and how does it occur?