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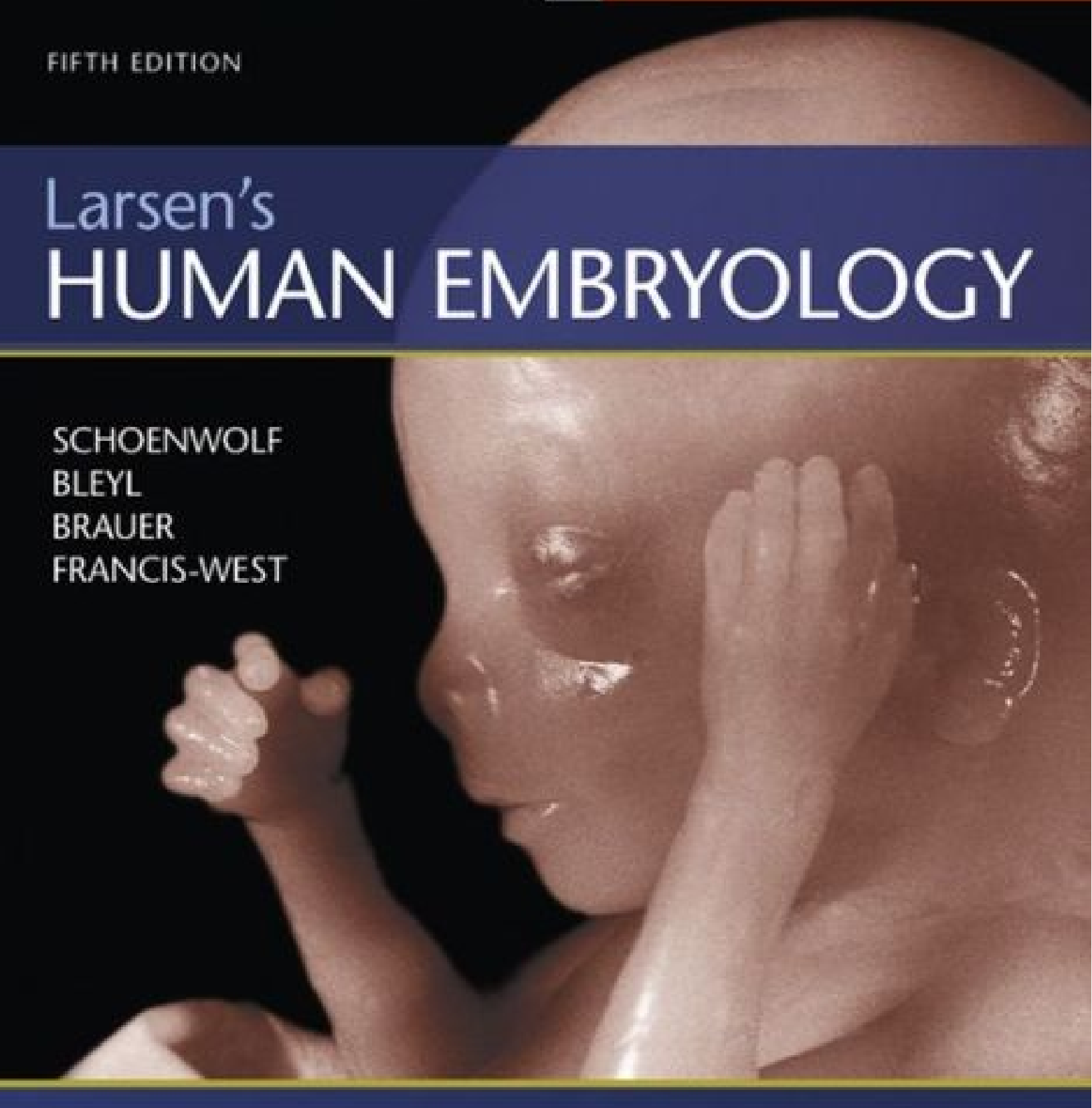

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FIFTH EDITION

Larsen's Human Embryology

GARY C. SCHOENWOLF, PhD
University of Utah School of Medicine
Salt Lake City, Utah

STEVEN B. BLEYL, MD, PhD
University of Utah School of Medicine
Salt Lake City, Utah

PHILIP R. BRAUER, PhD
Creighton University School of Medicine
Omaha, Nebraska

PHILIPPA H. FRANCIS-WEST, PhD
King's College London Dental Institute
London, United Kingdom

CHURCHILL
LIVINGSTONE

ELSEVIER
SAUNDERS

1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

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Content Strategist: Meghan Ziegler
Senior Content Development Manager: Rebecca Gruliow
Publishing Services Manager: Patricia Tannian
Senior Project Manager: Claire Kramer
Design Direction: Julia Dummitt

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The fifth edition of Larsen's Human Embryology *is proudly dedicated to the children who live with birth defects every day, hour, minute, and second of their lives and to their families who provide loving support and care. It is our hope that the information we have assembled here will help the next generation of physicians and scientists make new discoveries, resulting in better prevention, diagnosis, and treatment of birth defects.*

Content Experts

RICHARD ANDERSON
University of Melbourne, Australia

PARKER B. ANTIN
University of Arizona, USA

CAMMON ARRINGTON
University of Utah, USA

SPENCER BEASLEY
University of Otago, New Zealand

BRIAN L. BLACK
University of California at San Francisco, USA

JANICE L.B. BYRNE
University of Utah, USA

JON CLARKE
King's College London, England

MARTYN COBOURNE
King's College London, England

SIMON J. CONWAY
Indiana University School of Medicine, USA

ANDREW COPP
University College London, England

GEORGE P. DASTON
Procter & Gamble, USA

MARK DAVENPORT
King's College Hospital, England

JAMIE DAVIES
University of Edinburgh, Scotland

ELAINE DZIERZAK
Erasmus University Medical Center, The Netherlands

DARRELL J.R. EVANS
Brighton and Sussex Medical School, England

JOHN F. FALLON
University of Wisconsin Madison, USA

RICHARD H. FINNELL
The University of Texas at Austin, USA

ADRIANA GITTENBERGER-DEGROOT
Leiden University Medical Center, The Netherlands

ROBERT G. GOURDIE
Medical University of South Carolina, USA

ANNE GRAPIN-BOTTON
Swiss Institute for Experimental Cancer Research, Switzerland

ANNE GREENOUGH
King's College Hospital, England

BARBARA F. HALES
McGill University, Canada

HIROSHI HAMADA
Osaka University, Japan

CHRISTINE HARTMANN
Institute of Molecular Pathology, Austria

TAKAYUKI INAGAKI
University of Utah, USA

ROBYN JAMIESON
University of Sydney, Australia

CHAYA KALCHEIM
Hebrew University of Jerusalem, Israel

MATTHEW KELLEY
National Institute on Deafness and Other Communication Disorders/National Institutes of Health, USA

THOMAS KNUDSEN
U.S. Environmental Protection Agency, USA

CATHERINE E. KRULL
University of Michigan, USA

RALPH MARCUCIO
University of California at San Francisco, USA

ANTOON F. MOORMAN
Academic Medical Centre Amsterdam, The Netherlands

GUILLERMO OLIVIER
St. Jude Children's Research Hospital, USA

DAVID M. ORNITZ
Washington University, USA

MAURIZIO PACIFICI
The Children's Hospital of Philadelphia, USA

ROGER K. PATIENT
University of Oxford, England

ALAN O. PERANTONI
Frederick National Lab, USA

THEODORE PYSHER
University of Utah, USA

MARIA A. ROS
University of Cantabria, Spain

YUKIO SAIJOH
University of Utah, USA

RAMESH A. SHIVDASANI
Dana Farber Cancer Institute and Harvard Medical School, USA

JANE C. SOWDEN
University College of London and Institute of Child Health and Great Ormond Street Hospital for Children, National Health Service Trust, England

NANCY A. SPECK
University of Pennsylvania, USA

RAJANARAYANAN SRINIVASAN
St. Jude Children's Research Hospital, USA

MICHAEL R. STARK
Brigham Young University, USA

DAVID K. STEVENSON
University of Utah, USA

XIN SUN
University of Wisconsin Madison, USA

CHERYLL TICKLE
University of Bath, England

GIJS VAN DEN BRINK
Academic Medical Centre Amsterdam, The Netherlands

VALERIE WALLACE
University of Ottawa, Canada

JAMES M. WELLS
University of Cincinnati, USA

ARNO WESSELS
Medical University of South Carolina, USA

HEATHER M. YOUNG
University of Melbourne, Australia

Preface

The fifth edition of *Larsen's Human Embryology,* like the fourth edition, has been extensively revised.

- The number of chapters has been expanded from eighteen to twenty. This was done to organize the material better and to incorporate new information efficiently and logically.
- The text was heavily edited to increase clarity and avoid ambiguity, to improve accuracy, and to include many new scientific and medical advances since the last edition.
- Building on the success of the section called "Clinical Tasters," which was added in the fourth edition to introduce the clinical relevance of the material covered in each chapter, we added a new section—called "Embryology in Practice"—to close each chapter. The title of this section is a bit of a play on words; *practice* refers to both clinical practice and a chance for the reader to practice being a clinician and to use the material presented in the text to "walk through" a clinical scenario. As with the "Clinical Tasters," the "Embryology in Practice" section focuses on the impact of birth defects on the lives of children and their families. Although fictitious scenarios, they reflect real-life stories encountered in clinical practice with real problems that patients and their families face.
- Many new illustrations have been added; these additions reflect research advances and their clinical relevance. Many previous illustrations were thoroughly revised to facilitate student understanding. Although admittedly biased, we believe that the fifth edition of *Larsen's Human Embryology* contains the best compilation in any one textbook of illustrations on human three-dimensional descriptive embryology, animal model experimental embryology, and human birth defects.
- About fifty full-color animations have been linked directly to relevant sections of the text. These help the student understand not only the three-dimensional structure of human embryos, but also their four-dimensional structure as its complexity morphs and increases over time.
- As with the fourth edition, new Content Experts have been chosen to partner with the authors in producing the fifth edition of *Larsen's Human Embryology*. More than fifty new Content Experts are listed. With roughly the same number participating in the fourth edition, the textbook has now been critically evaluated by about 100 experts in their respective areas. Although that strengthens the book tremendously, it still does not make the book perfect, an impossible task in a complex and ever-changing field. Hence, we greatly appreciate input we receive for further improvement from students and faculty. Please continue to send your comments to schoenwolf@neuro.utah.edu.

Acknowledgments

Without students there would be no need for textbooks. Thus the authors thank the many bright young students with whom we have been fortunate enough to interact throughout our careers, as well as those students of the future, in eager anticipation of continuing fruitful and enjoyable interactions. For us as teachers, students have enriched our lives and have taught us at least as much as, if not more than, we have taught them.

For this edition, we are especially grateful to the more than fifty Content Experts who were integral partners in the preparation of the fifth edition and who, like our students, also have taught us much. Each of the Content Experts read one or more chapters, offered numerous suggestions for revision, and in some cases even provided new text and illustrations. We have pondered their many suggestions for revision, but in the end, rightly or wrongly, we chose the particular direction to go. The authors share a captivation for the embryo and have sought to understand it fully, but, of course, we have not yet accomplished this objective; thus our studies continue (we all are active researchers). Nevertheless, we took faith when writing this edition in a quote from one of the great scientific heroes, Viktor Hamburger: "Our real teacher has been and still is the embryo, who is, incidentally, the only teacher who is always right."

Finally, we must thank the many authors, colleagues, patients, and families of patients who provided figures for the textbook. Rather than acknowledging the source of each figure in its legend, we have clustered these acknowledgments into a Figure Credits section. This was done not to hide contributions but rather to focus the legends on what was most relevant.

Chapter 1

Gametogenesis, Fertilization, and First Week

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 fourth to sixth 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 happen in the developing embryo is that the germ line is set aside 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 (i.e., when the embryo's paternal and maternal grandmothers were pregnant with the embryo's father and mother).

From the wall of the yolk sac, PGCs actively migrate between the sixth and twelfth 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 twenty-three pairs of chromosomes (for a total of forty-six 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 twenty-three chromosomes (one of each pair); therefore, they are **haploid**. The developing gametes also undergo cytoplasmic differentiation, resulting in the production of mature **spermatozoa** in the male and **definitive oocytes** in the female.

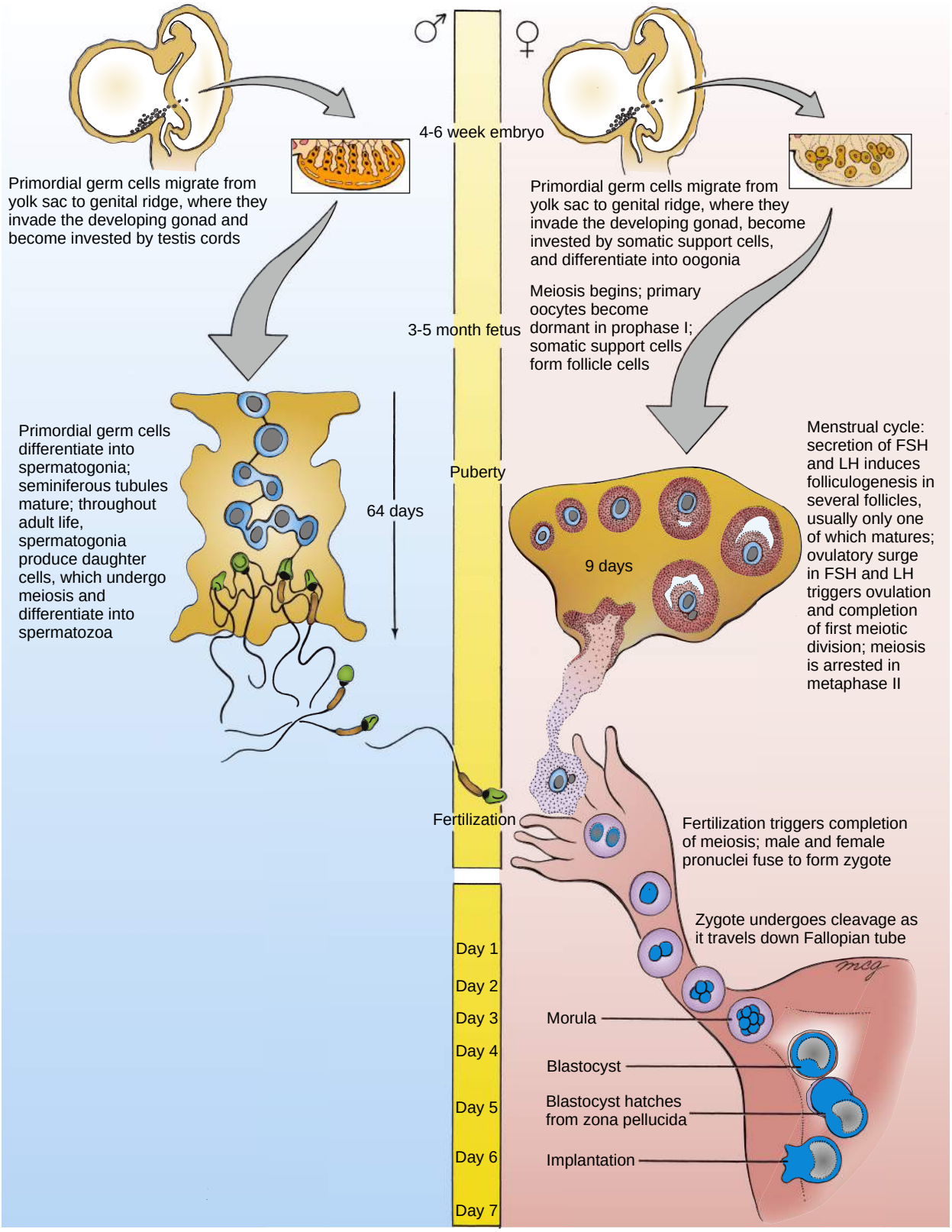
In the male, spermatogenesis takes place in the seminiferous tubules of the testes and does not occur until **puberty**. In contrast, in the female, oogenesis is initiated during fetal life. Specifically, between the third and fifth months of fetal life, oogonia initiate the first meiotic division, thereby 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 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. Cleavage subdivides 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 eight- to sixteen-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 thirty-cell stage, the embryo begins to form a fluid-filled central cavity, the **blastocyst cavity**. By the fifth to sixth day of development, the embryo is a hollow ball of about one-hundred cells, called a **blastocyst**. At this point, it enters the uterine cavity and begins to implant into the endometrial lining of the uterine wall.

Clinical Taster

A couple, both in their late thirties, is having difficulty conceiving a child. Early in their marriage, about ten years ago, they used birth control pills and condoms thereafter, but they stopped using all forms of birth control more than two 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 the 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 thirteen. The man had a varicocele, which was corrected when he was nineteen; the urologist who performed the surgery assured him that there would be no subsequent adverse effects 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 (sixty million sperm per ejaculate),



Time line. Gametogenesis and first week of development.

sperm mobility (vigorous motility and forward progression [i.e., straight swimming movement]), sperm morphology (70% with an oval head and a tail seven to fifteen times longer than the head), and semen volume (3.5 mL with a normal fructose level) are within 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 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 heartbeats. This is confirmed two months later by ultrasonography. Early in the ninth month of gestation, two healthy babies are delivered—a 6-pound 2-ounce girl and a 5-pound 14-ounce boy.

PRIMORDIAL GERM CELLS

PRIMORDIAL GERM CELLS RESIDE IN YOLK SAC

Cells that give rise to **gametes** in both males and females can be identified during the fourth 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 (covered in Chapter 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 rounded shape (Fig. 1-1*B, C*), and because they can be specifically labeled with a number of molecular markers.

PRIMORDIAL GERM CELLS MIGRATE INTO DORSAL BODY WALL

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 (see Fig. 1-1*A*, *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 (epithelial) lining of the coelomic cavity. Most PGCs populate the region of the body wall at the level that will form the gonads (covered in Chapter 16). 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*D*, *E*).

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, the most common tumors in newborns, occur in 1 in 20,000 to 70,000 births (Fig. 1-1*D, E*). 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 the fact that they can give rise to a variety of definitive anatomic structures, including hair, teeth, pituitary gland, and even a fully formed eye.

PRIMORDIAL GERM CELLS STIMULATE FORMATION OF GONADS

Differentiation of the gonads is described in detail in Chapter 16. 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*F*; see also Figs. 16-1*D* and 16-5). Proliferation of the somatic support cells creates a swelling just medial to each mesonephros (embryonic kidney) on both 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. Somatic support cells in the male quickly assemble into epithelial cords called **testis cords**.

In the Research Lab

ORIGIN OF PGCS

Although the exact time and place of origin of PGCs in humans are 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; covered in Chapters 2 and 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.

Migration of PGCs to the developing gonads involves processes shared by migrating neural crest cells (see Chapter 4),

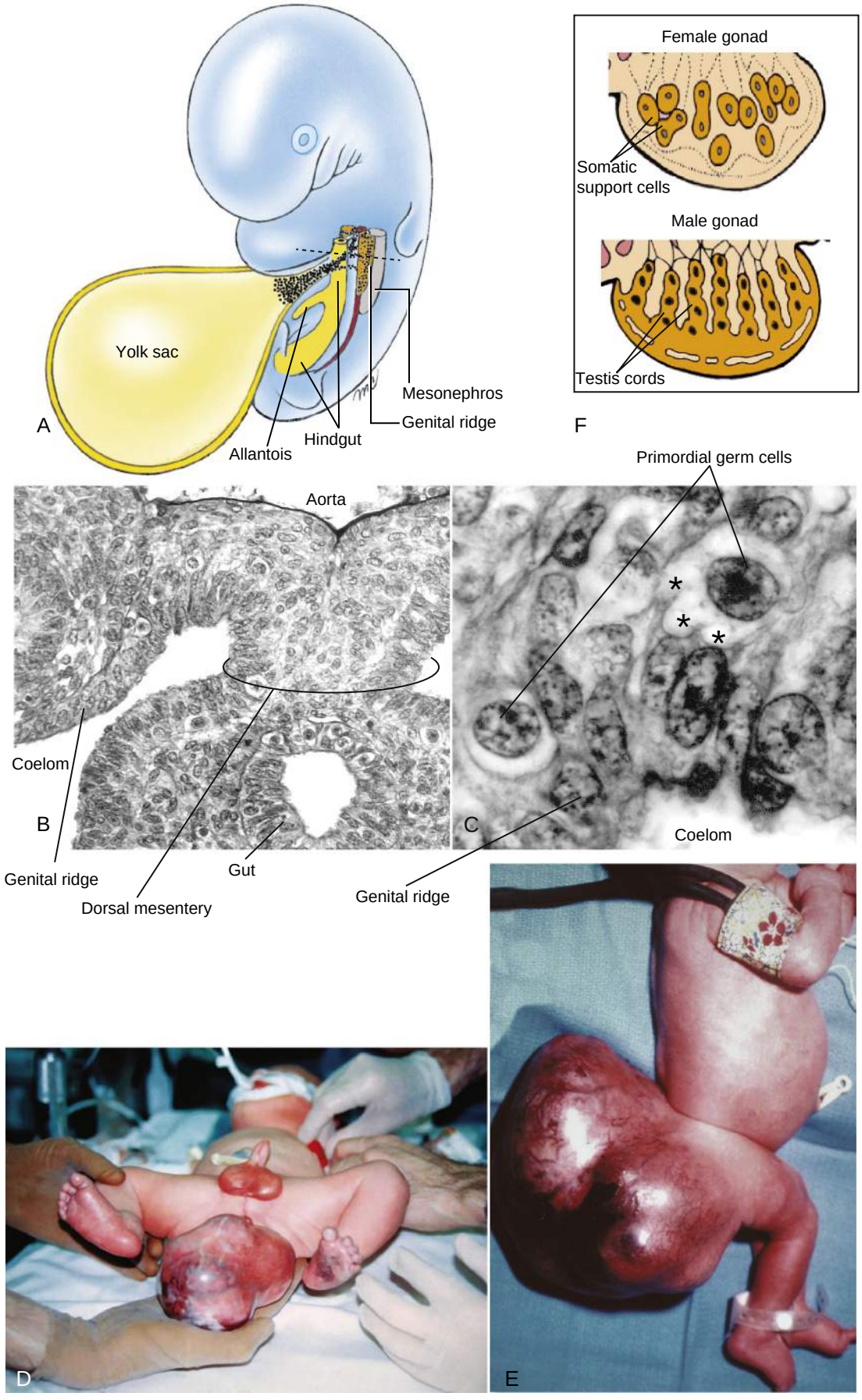


Figure 1-1. Yolk-sac origin of primordial germ cells and their migration during normal development, and formation of teratomas. *A*, Primordial germ cells (PGCs) reside in the endodermal layer of the caudal side of the yolk sac during four to six weeks of development. *B*, *C*, PGCs then migrate to the dorsal body wall. Asterisks indicate three pseudopodia on a migrating PGC. *D*, *E*, Infants with large sacrococcygeal teratomas. *F*, Between six and twelve weeks, PGCs stimulate formation of the genital ridges in the dorsal body wall. Somatic support cells differentiate and invest PGCs. In females, somatic support cells become ovarian follicle cells; in males, somatic support cells assemble in testis cords and ultimately become Sertoli cells of the seminiferous tubules.

neuronal processes (see Chapters 9 and 10), and developing blood and lymphatic vessels (see Chapter 13). These include intrinsic motility programs involving cytoskeletal dynamics (note pseudopods on one of the PGCs shown in Fig. 1-1C), adhesive substrates (such as tenascin C, β2 integrin, and laminin, all of which seem to be required for PGC migration), and extracellular attractive and repulsive cues. As covered in Chapter 10, **chemokines** (a type of **cytokine**) and their receptors direct the migration of sympathetic precursor cells. Similarly, chemokines play important roles in PGC migration by acting as **chemotropic signals** (i.e., attractive signals produced by the developing gonads) to regulate PGC homing. Such chemokines include the ligand Sdf1 (stromal cell–derived factor-1, also known as 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. Moreover, factors involved in the migration of melanocytes (covered in Chapter 4) also are involved in PGC migration. These include steel factor (also known as stem cell factor), the c-kit ligand, and its receptor c-kit.

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 functions 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 fruit fly, worm, and frog, **maternal effect genes** (covered in Chapter 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 gene vasa 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, in which 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 covered in Chapter 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 covered in Chapter 5). One such signal is provided by bone morphogenetic proteins (Bmps). In chimeric mouse embryos (mouse injection chimeras are covered in Chapter 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 unknown, as knock-outs of neither gene affect PGC cell specification.

In contrast, two other genes have been identified that are lacking in Bmp signaling mutants and when knocked out result in the loss of PGCs. One, B-lymphocyte–induced maturation protein 1, Blimp1, is a master regulator of plasma cell differentiation from B cells during development of the immune system. The other, Prdm14, has less defined roles. Both of these genes are essential for PGC differentiation.

Proliferation and survival of PGCs are 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 gene nanos (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.

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 covered 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 is named after the mutation in which the gene is inactivated and the germ line is lost.

GAMETOGENESIS

TIMING OF GAMETOGENESIS IS DIFFERENT IN MALES AND FEMALES

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, the timing of these processes differs in the two sexes (see Timeline for this chapter). In males, PGCs (usually now called **gonocytes**) remain dormant from the sixth 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.

In contrast, in females, PGCs (again, usually now called **gonocytes**) undergo a few more mitotic divisions after they become invested by the somatic support cells. They then differentiate into **oogonia**. By the fifth 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 fifty years of age. The process of gametogenesis in the male and female (called **spermatogenesis** and **oogenesis**, respectively) is covered in detail later in this chapter.

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 the 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 support of this, Tet1, a member of the Tet family of proteins, was recently shown to be required for the activation of meiosis in female mice. Although it is unclear how Tet1 functions, Tet proteins play a role in erasing epigenetic marks in DNA—a critical event in the development of PGCs, as covered in Chapter 2.

In males, the genital ridge prevents prenatal entry into meiosis, and 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

Although the timing of meiosis is very different between males and females, the basic chromosomal events of the process are the same in the two sexes (Fig. 1-2). Like all normal somatic (non-germ) cells, PGCs contain twenty-three pairs of chromosomes, or a total of forty-six 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 forty-six chromosomes, twenty-two 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 chromosomes, 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 covered in Chapter 2; mechanisms underlying sex determination are covered in detail in Chapter 16).

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 the N number of a cell do not always coincide. Somatic cells and PGCs have two copies of each kind of chromosome; hence, they are called **diploid**. In contrast, mature gametes 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 so are **2N**. However, during earlier phases of meiosis or mitosis, each chromosome of a diploid cell has two molecules of DNA, and so the cell is **4N**.

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, non-functional, haploid **polar bodies**.

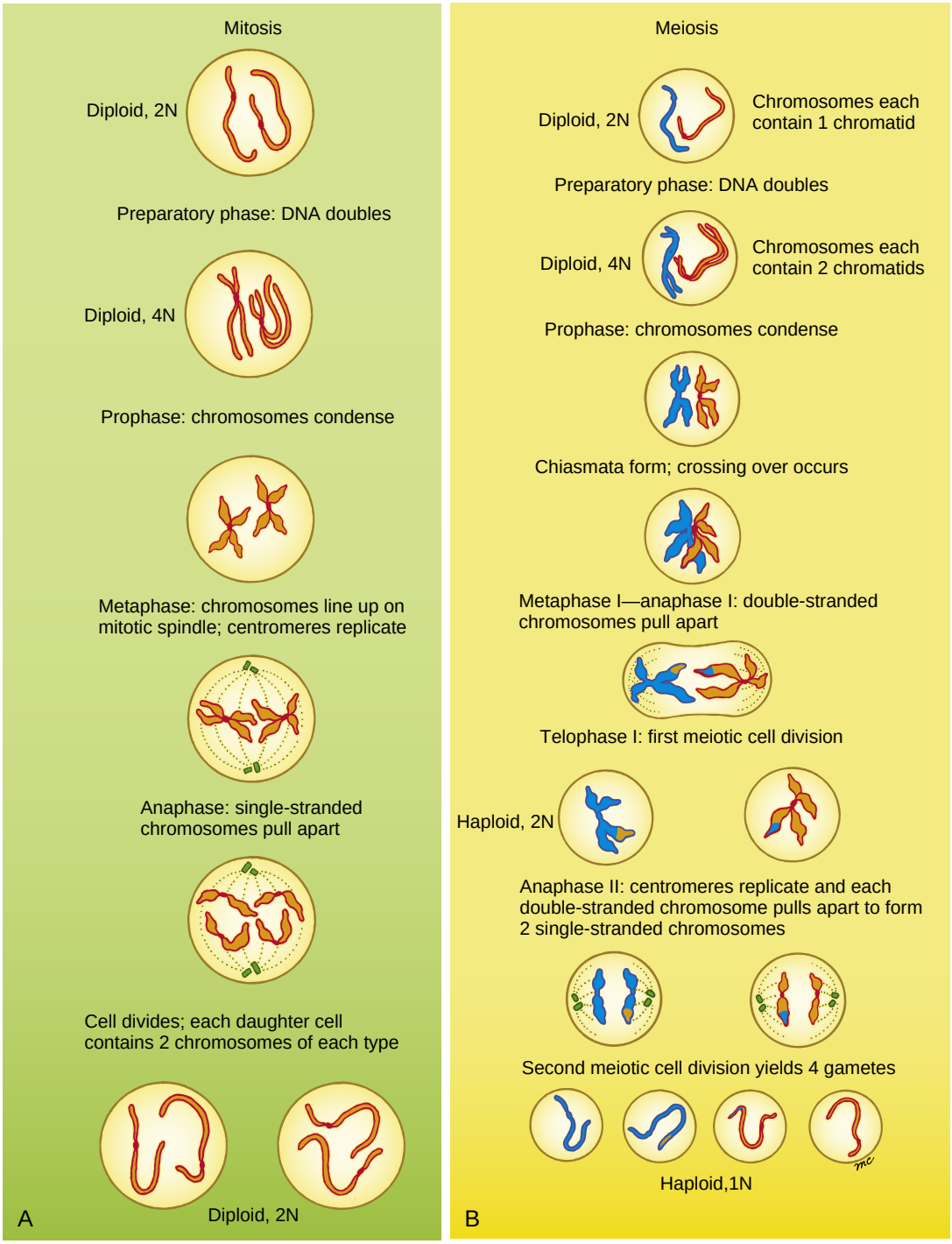


Figure 1-2. Types of cell division. *A,* Mitosis. *B,* Meiosis. See Table 1-1 for a description of the stages.

First Meiotic Division: DNA Replication and Recombination, Yielding Two Haploid, 2N Daughter Cells

The steps of meiosis are illustrated in Figure 1-2*B* and are 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; do not confuse DNA double strands with the two chromatid strands composing each chromosome).

In the next step, called **prophase**, the chromosomes condense into compact, double-stranded structures (i.e.,

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 two 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; two chromosomes of each homologous pair align at the centromeres to form a four-limbed chiasma; recombination by crossing over occurs		
Metaphase	Chromosomes align along the equator; *centromeres do not replicate*		
Anaphase and telophase	One 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 two 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

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; therefore, it increases the genetic variability of future gametes. As mentioned earlier, the primary oocyte enters a phase of meiotic arrest during the first meiotic prophase.

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

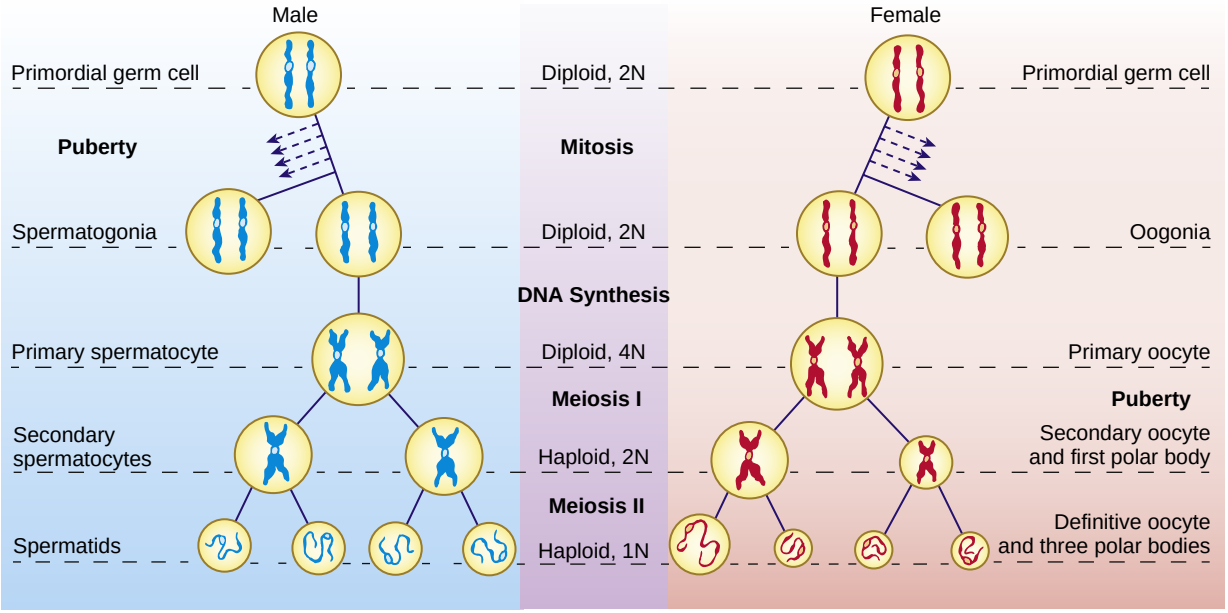


Figure 1-3. Nuclear maturation of germ cells in meiosis in the male and female. In the male, primordial germ cells (PGCs) remain dormant until puberty, when they differentiate into spermatogonia and commence mitosis. Throughout adulthood, spermatogonia produce primary spermatocytes, which undergo meiosis and spermatogenesis. Each primary spermatocyte divides to form two secondary spermatocytes, each of which forms two spermatozoa. Thus, each primary spermatocyte yields four functional gametes. In the female, PGCs differentiate into oogonia, which undergo mitosis and then commence meiosis during fetal life as primary oocytes. The primary oocytes remain arrested in prophase I until stimulated to resume meiosis during a menstrual cycle. Each primary oocyte has the potential to form a secondary oocyte and first polar body. Moreover, each secondary oocyte has the potential to form a definitive oocyte and another polar body, and the first polar body has the potential to form two polar bodies. Thus, each primary oocyte has the potential to yield a single functional gamete and three polar bodies.

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; 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).

Second Meiotic Division: Double-Stranded Chromosomes Divide, Yielding Four Haploid, 1N Daughter Cells

No DNA replication occurs during the second meiotic division. The twenty-three 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).

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.

SPERMATOGENESIS

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.

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*). Adjacent Sertoli cells are interconnected between the pockets by **tight junctions**, which help establish

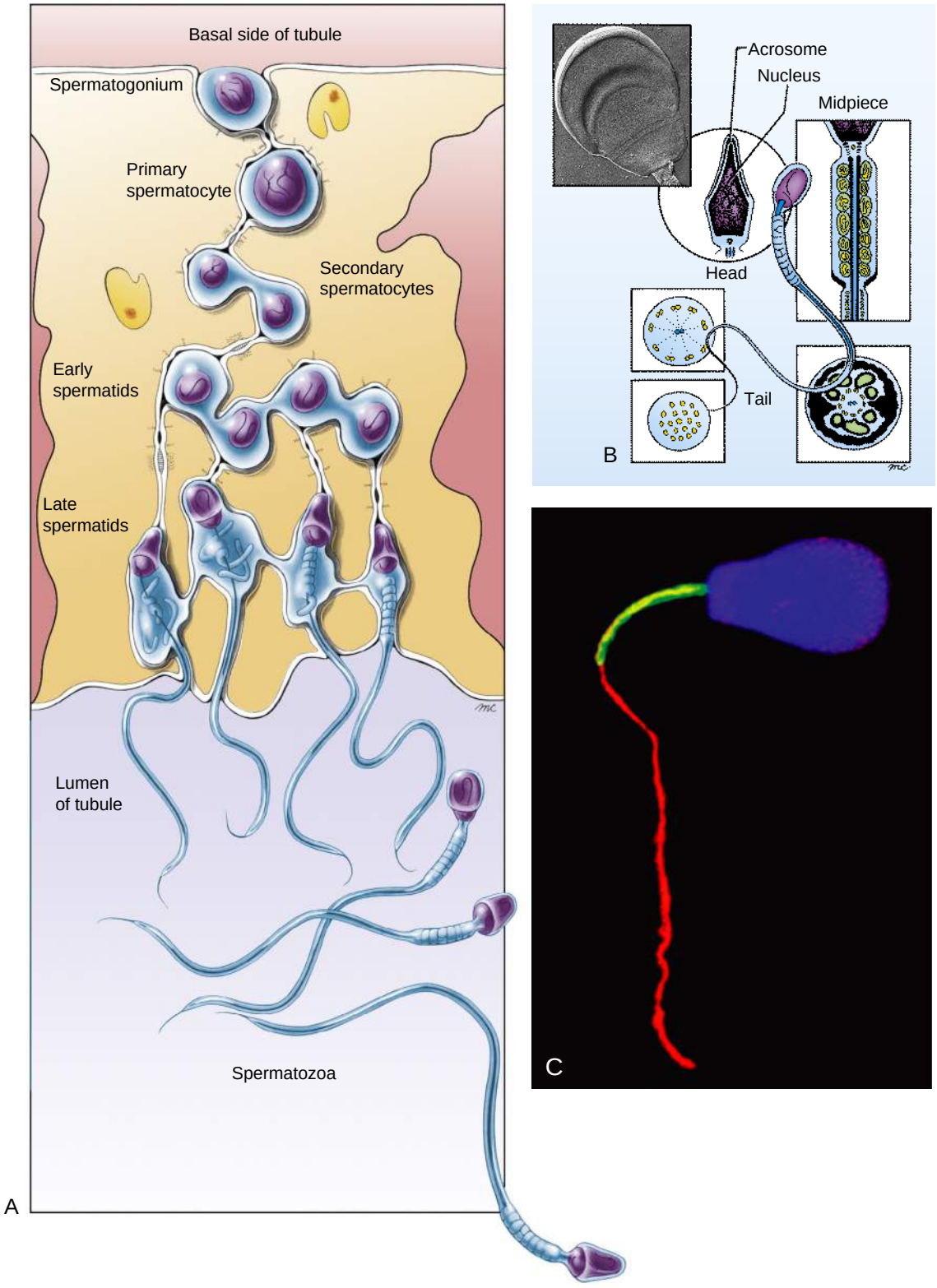


Figure 1-4. Spermatogenesis and spermiogenesis. *A*, Schematic section through the wall of the seminiferous tubule. Spermatogonium just under the outer surface of the tubule wall (basal side) undergoes mitosis to produce daughter cells, which may continue to divide by mitosis (thus renewing the spermatogonial stem cell population) or may 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. *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.

a **blood-testis barrier**. Thus, developing spermatogonia reside within an immune privileged site during their development in the testes.

MALE GERM CELLS ARE TRANSLOCATED TO SEMINIFEROUS TUBULE LUMEN DURING SPERMATOGENESIS

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

Sertoli cells participate intimately in 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 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**.

As shown in Figure 1-4*B*, *C*, a spermatozoon consists of a **head**, a **midpiece**, and a **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

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.

CONTINUAL WAVES OF SPERMATOGENESIS OCCUR THROUGHOUT SEMINIFEROUS EPITHELIUM

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. This 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 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.

In the human male, each cycle of spermatogenesis takes about sixty-four days. Spermatogonial mitosis occupies about sixteen days, the first meiotic division takes about eight days, the second meiotic division takes about sixteen days, and spermiogenesis requires about twenty-four days.

SPERMATOZOA UNDERGO TERMINAL STEP OF FUNCTIONAL MATURATION CALLED CAPACITATION

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 fifteen- to twenty-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 covered further in Chapter 16). As many as three hundred 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 ampulla region. In the **ampulla** of the oviduct, sperm survive and retain their capacity to fertilize an oocyte for one to three days.

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

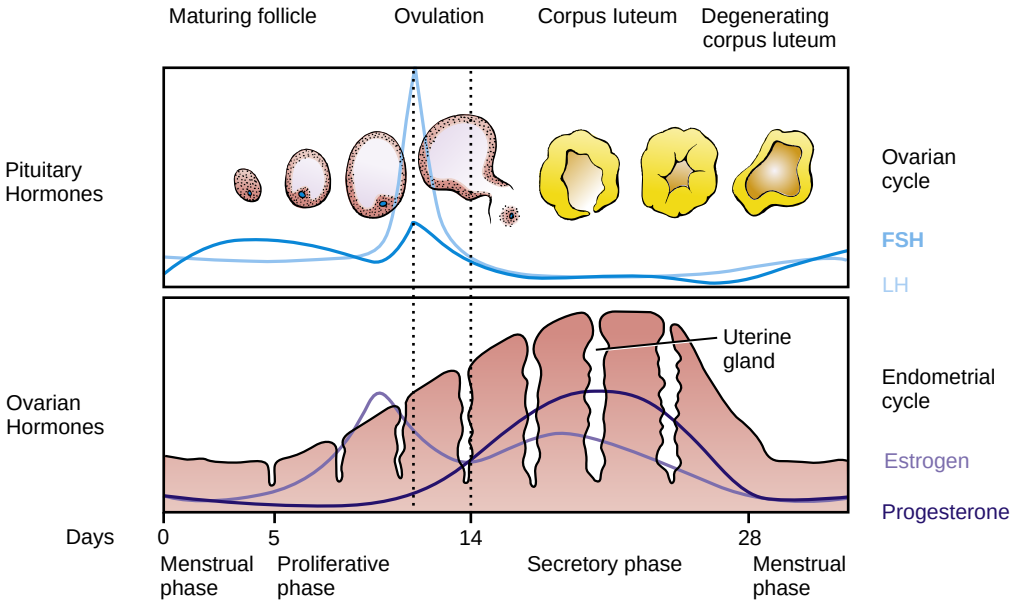


Figure 1-5. Ovarian, endometrial, and hormonal events of the menstrual cycle. Pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) directly control the ovarian cycle and also control production of estrogen and progesterone by responding follicles and corpus luteum of the ovary. These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

reproduction in humans (assisted reproduction technology, or ART, is covered later in the chapter in an "In the Clinic" entitled "Assisted Reproductive Technology").

OOGENESIS

PRIMARY OOCYTES FORM IN OVARIES BY FIVE MONTHS OF FETAL LIFE

As mentioned earlier, after female germ cells become invested by somatic support cells, they undergo a series of mitotic divisions and then differentiate into oogonia (see Fig. 1-3). By twelve 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.

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** (covered below). By five months, the number of primordial follicles in the ovaries peaks at about seven million. Most of these follicles subsequently degenerate. By birth, only seven hundred thousand to two million remain, and by puberty, only about four hundred thousand.

HORMONES OF FEMALE CYCLE CONTROL FOLLICULOGENESIS, OVULATION, AND CONDITION OF UTERUS

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 twenty-eight–day cycle consists of the following:

- Monthly maturation of (usually) a single oocyte and its enclosing follicle
- Concurrent proliferation of the uterine endometrium
- Process of ovulation by which the oocyte is released from the ovary
- Continued development of the follicle into an endocrine corpus luteum
- Sloughing of the uterine endometrium and involution of the corpus luteum (unless a fertilized ovum implants in the uterus and begins to develop)

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 fifth day of the cycle (the fifth 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.

ABOUT FIVE TO TWELVE PRIMARY FOLLICLES RESUME DEVELOPMENT EACH MONTH

Before a particular cycle, and independent of pituitary gonadotropins, the follicular epithelium of a small group of primordial follicles thickens, converting the

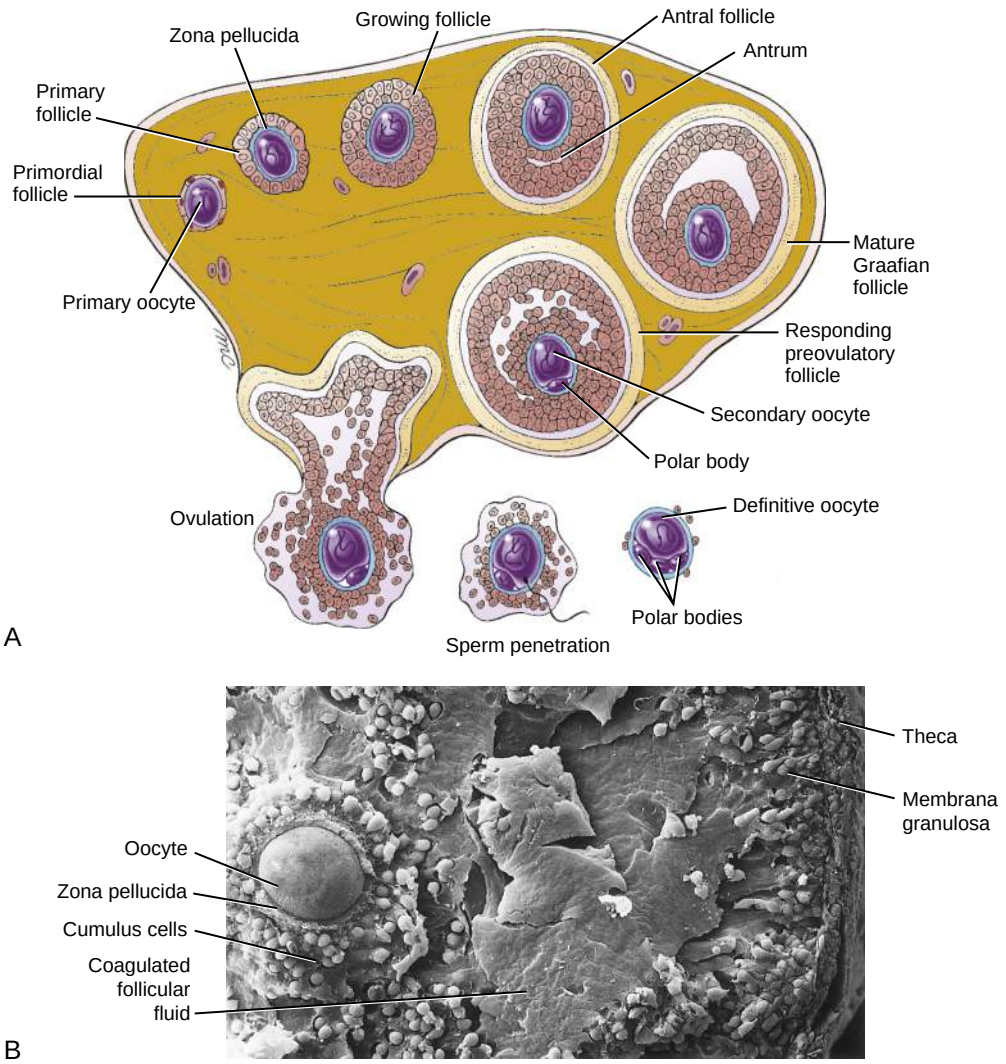


Figure 1-6. Maturation of the egg in the ovary and ovulation. *A*, Schematic depiction of the ovary showing folliculogenesis and ovulation. Five to twelve primordial follicles initially respond to the rising levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), but only one matures. 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.

single-layered follicular epithelium from a layer of 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 the 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

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**

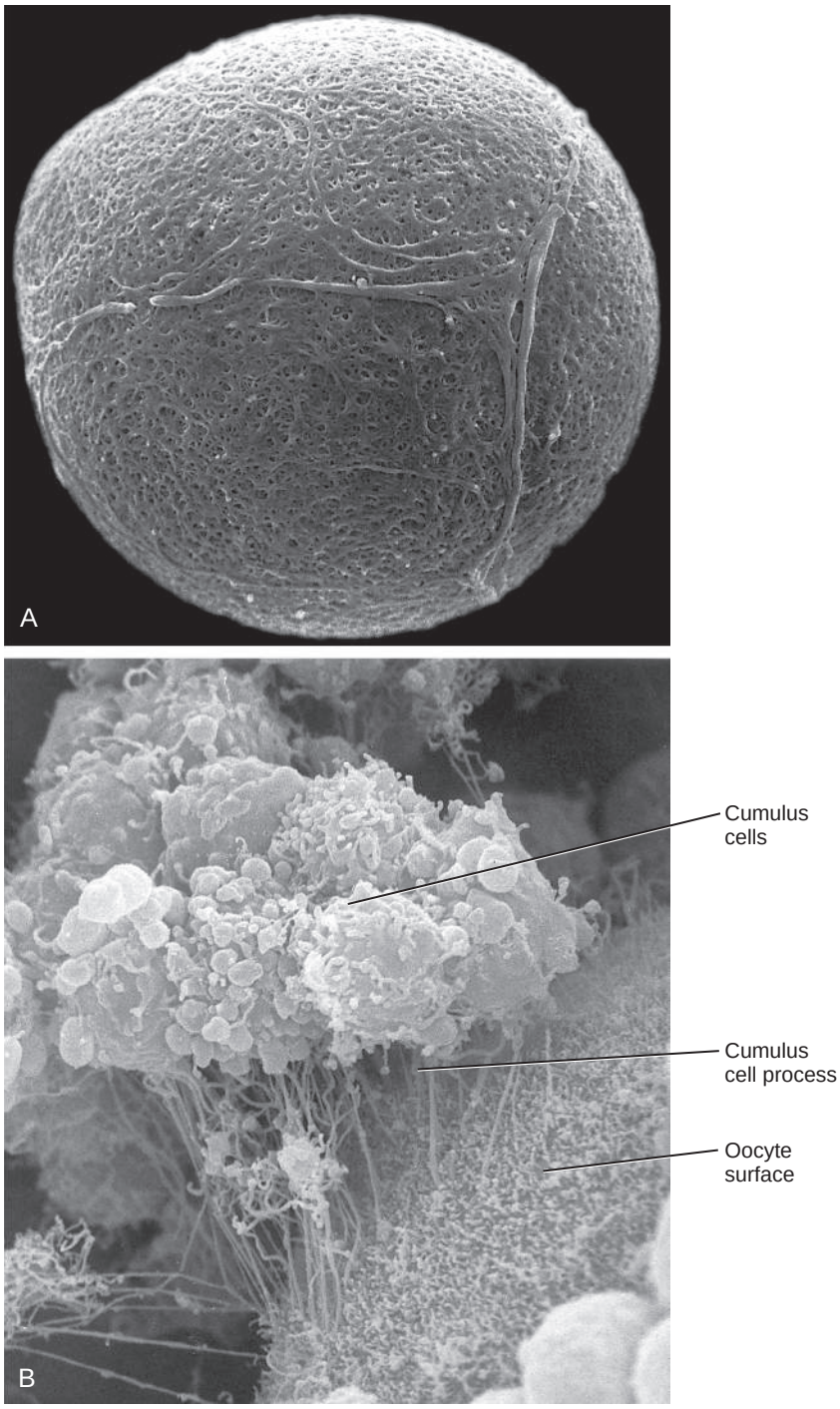


Figure 1-7. The ovulated egg and associated structures. *A*, Scanning electron micrograph of the zona pellucida after removal of the cumulus cells. The zona consists of protein and mucopolysaccharide and forms a barrier that the sperm penetrates by means of its acrosomal enzymes. *B*, Scanning electron 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.

vesicular follicle or a **mature Graafian follicle** (see Fig. 1-6). At this point, the oocyte still has not resumed meiosis.

WHY IS FOLLICULOGENESIS SELECTIVELY STIMULATED IN ONLY A FEW FOLLICLES EACH MONTH?

The reason why only five to twelve primordial follicles commence folliculogenesis each month—and why, of this group, all but one eventually degenerate—is uncertain. One possibility is that follicles become progressively more sensitive to the stimulating effects of FSH as they advance in development. Therefore, follicles that are slightly more advanced simply on a random basis would 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 pituitary and ovarian hormones and growth factors.

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 non-random patterns of developmental abnormalities, that is, **syndromes**. One of these syndromes, Down syndrome, is covered in detail in the following section; others are covered 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 they 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 of 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**. 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 atrial and ventricular septa; covered in Chapter 12), hearing loss, duodenal obstruction, a propensity to develop leukemia, and immune system defects. Trisomy in most Down syndrome individuals is the result of non-disjunction in the mother, usually during the first meiotic division (75% to 80% of 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 non-disjunction 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 non-disjunction in the maternal germ line. Consequently, it is now accepted that only about 5% of 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; 2% to 5% 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 non-disjunction 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 non-disjunction. 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); 2% to 5% 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 translocated regions of chromosome 21, **Down syndrome candidate regions** on chromosome 21 have been identified. Completion of sequencing of chromosome 21 (in May 2000) and the generation of transgenic mice (transgenic mice are covered in Chapter 5) trisomic for these candidate regions are 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 live baby born with Down syndrome at maternal age thirty is 1 in 900. The risk increases to 9 in 1000 by maternal age forty. However, it is not clear whether older women actually produce more oocytes with non-disjunction 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. Trisomy 13 is also called **Patau syndrome**, and trisomy 18, **Edwards syndrome**. Similarly, trisomies and monosomies of sex chromosomes occur (e.g., **Klinefelter** and **Turner** syndromes, two syndromes in which there are extra or decreased numbers of sex chromosomes, respectively; covered in Chapter 16). **Triploid** or **tetraploid** embryos, in which multiple copies of the entire genome are present, can arise by errors in fertilization (covered in Chapter 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**.

As covered above, maternal age is a major factor in the incidence of Down syndrome. Emerging new evidence shows that the rate of new mutations increases with paternal age, with the number of new mutations in a male's germ line doubling about every 16.5 years. Because spermatogonia

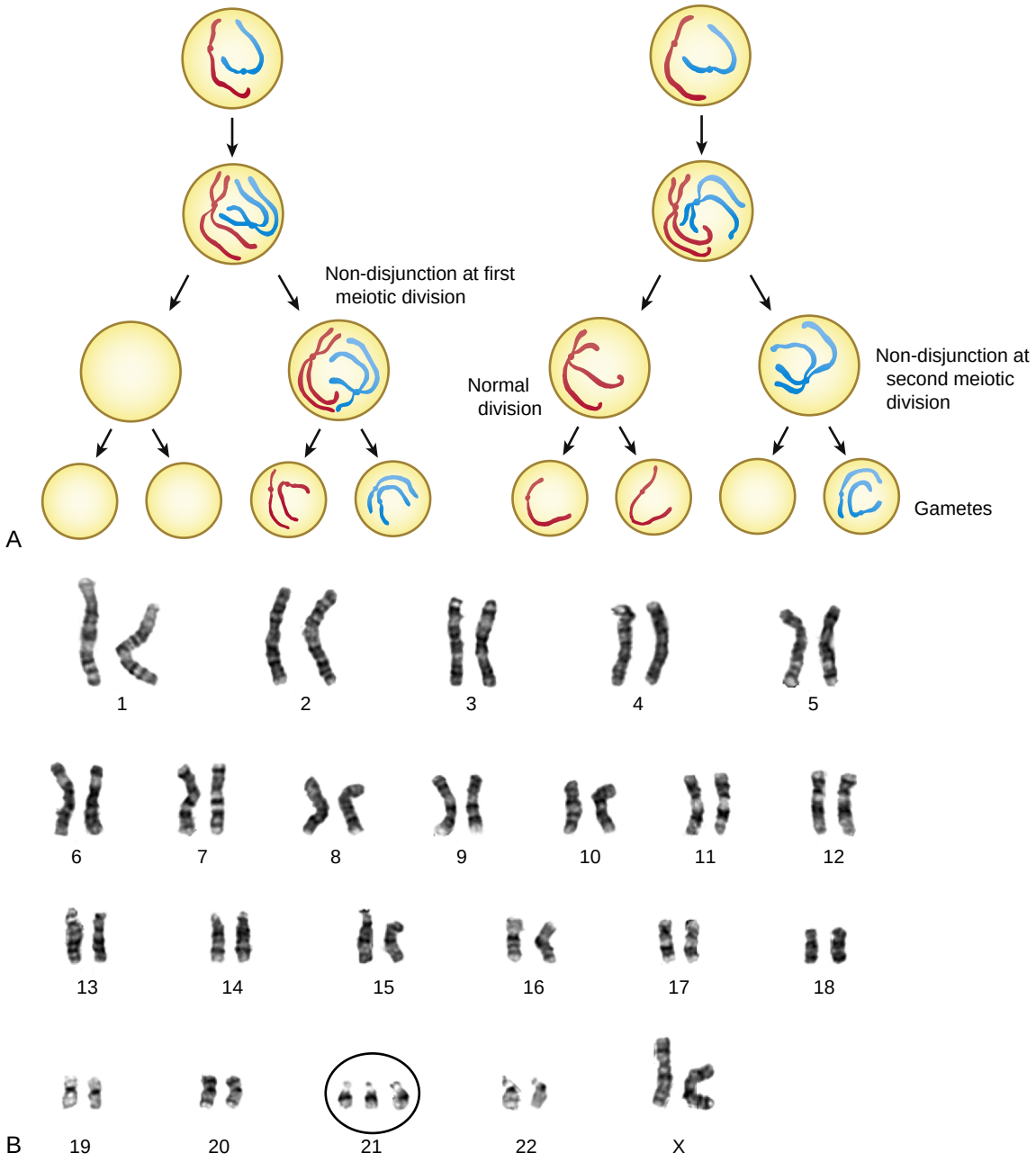


Figure 1-8. Chromosomal non-disjunction in meiosis. *A*, Failure of homologous double-stranded chromosomes to separate before cytokinesis during the first meiotic division (left-hand panel) results in their distribution to only one of the secondary gonocytes (or first polar body). Failure of the two 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.

divide throughout life, replicating more than twenty times per year, they accumulate genetic copying errors such that a seventy-year-old man is about eight times more likely to pass on mutations to his offspring than is a twenty-year-old man. For example, a fifty-year-old man is about twice as likely to pass on mutations that contribute to autism than is a twenty-nine-year old man. Moreover, increased paternal age has been suggested to contribute to higher risk of other neurological disorders, such as schizophrenia, epilepsy, and bipolar disorder.

Chromosome Analysis Can Characterize Defective Genetic Material and Can Guide Diagnosis and 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

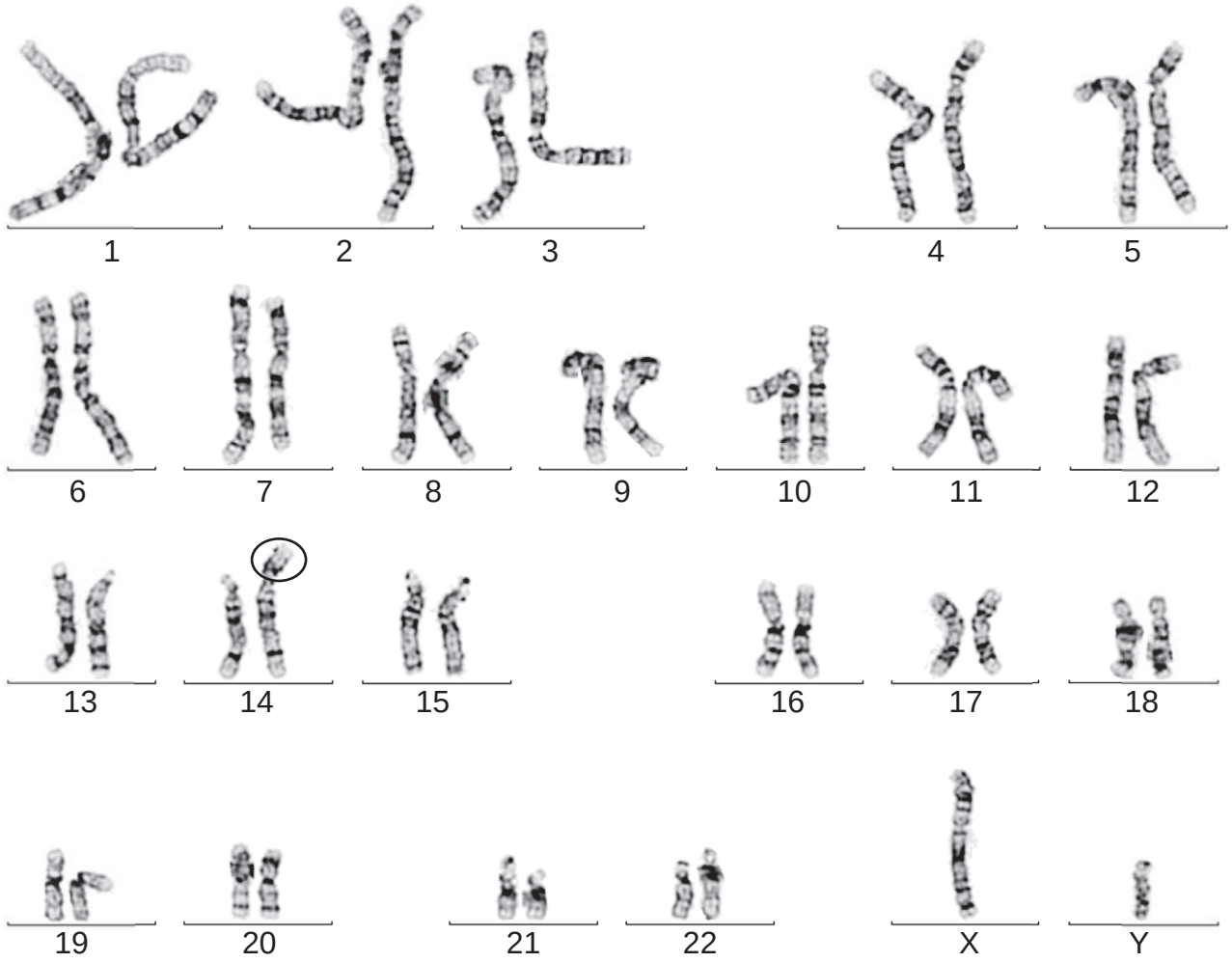


Figure 1-9. Karyotype of a male with Down syndrome caused by translocation of a third chromosome 21 onto one of the chromosomes 14 (circled).

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 from the amniotic fluid (**amniocentesis**) or from the chorionic villi (**chorionic villous sampling**) can be used to detect many disorders early in pregnancy (covered in Chapter 6). Recent advances also allow non-invasive detection of trisomies from maternal serum through analysis of cell-free fetal DNA (see Chapter 6).

Two other molecular approaches are used routinely for chromosomal analysis (Figs. 1-10, 1-11): fluorescence in situ hybridization (FISH) and chromosome microarray (CMA). In both of these techniques, DNA probes linked to fluorescent dyes (i.e., 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. These techniques are particularly useful for detecting changes in chromosome copy number (aneuploidy) and for characterizing chromosomal material involved in translocations when paired with high-resolution chromosome banding. CMA is also useful in detecting inheritance of chromosomal material that is improperly imprinted, as in uniparental isodisomy (where entire or parts of both chromosome pairs are inherited from the same parent).

OVULATION

Animation 1-1: Ovulation.
Animations are available online at StudentConsult.

RESUMPTION OF MEIOSIS AND OVULATION ARE STIMULATED BY OVULATORY SURGE IN FSH AND LH

On about day thirteen or fourteen 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 fifteen 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 twenty hours, the chromosomes are lined up in metaphase. Cell division to form the secondary oocyte and the first polar body rapidly ensues (Fig. 1-12*B*). The secondary oocyte promptly begins the second