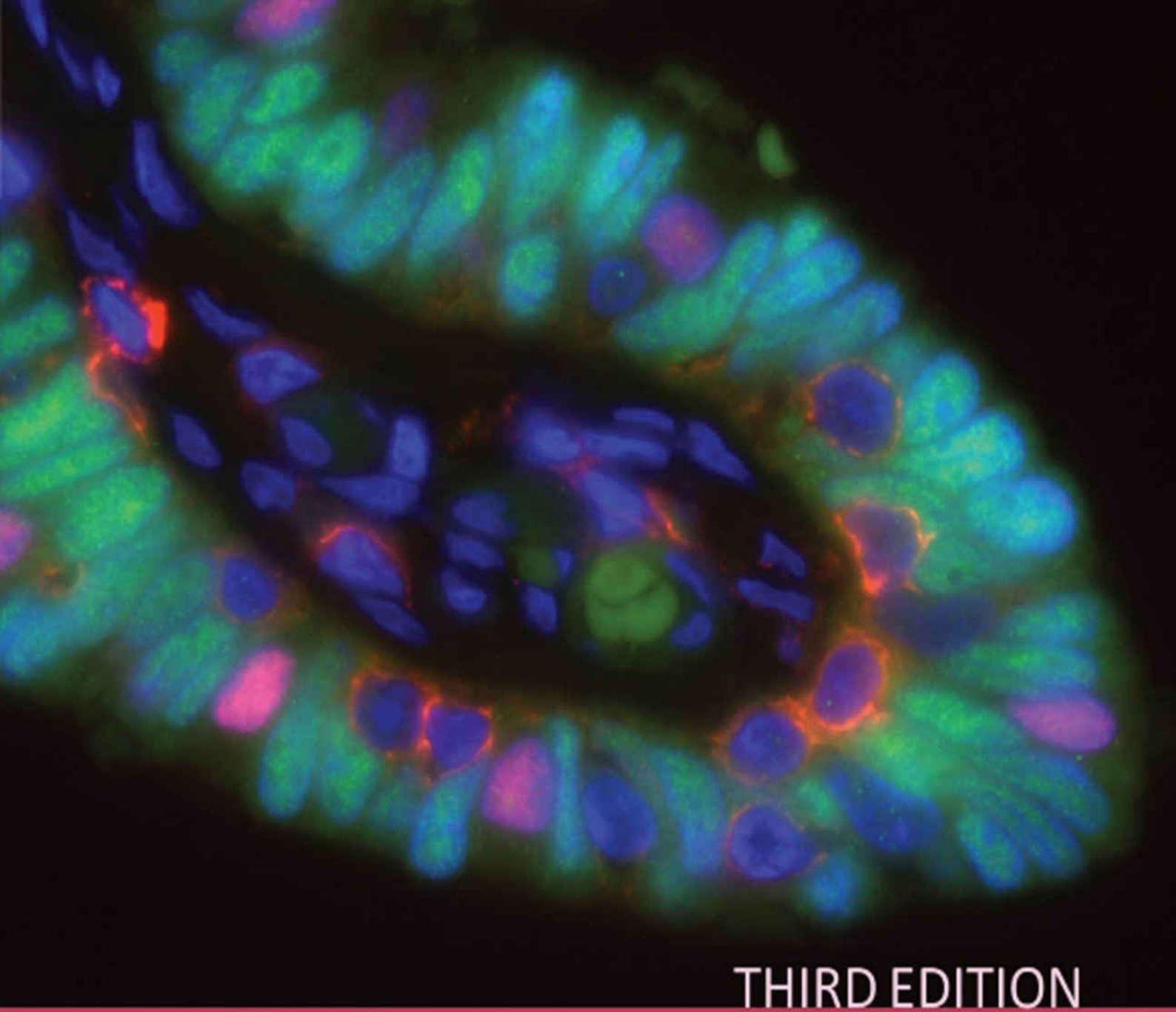

THIRD EDITION

Diagnostic Gynecologic *and* Obstetric Pathology

Crum • Nucci • Howitt • Granter • Parast • Boyd
Haefner • Peters

ELSEVIER

Diagnostic Gynecologic *and* Obstetric Pathology

THIRD EDITION

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Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods,

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Dedication

To Beckett, Spencer, Piper, and Stella

Christopher P. Crum

To my husband, Branch; my two beautiful sons, Julian and Cole; my mother, Dr. Maria Bergamo-Nucci; and in memory of my father, Dr. Cyrus Nucci, a surgeon who secretly wanted to be a pathologist

Marisa R. Nucci

To Michael and Elijah

Brooke E. Howitt

For my Students, Residents, and Fellows

Scott R. Granter

To Mark and Jasmine

Mana M. Parast

To my centers of gravity: Wes, Naomi, Ariana, Anaïs, and Emerson

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Preface

Each new edition of a textbook must confront changes in the discipline that have transpired and push the field forward with new ideas. Some are informed by dramatic discoveries that alter our perspective of a given disease, including the approach to diagnosis, therapy, and prevention. Others are driven by the relentless critical attention to existing dogma or are noteworthy in their own right, but must endure a lag time while their impact is realized.

This edition addresses the changing field of gynecology and obstetrics from the viewpoint of the pathologist, summarizing new information to clarify old conundrums. Interrogation of large tumor databases by next generation technologies have uncovered biologic events or markers that have reclassified tumors previously cataloged only by descriptive pathology with the hope that these strategies will lead to successful targeted therapies. The identification of unique sites or cells of tumor origin, such the cervical squamo-columnar junction or distal fallopian tube, raise hopes for additional opportunities to lower the death rate from cancer through prevention. Highly sensitive molecular assays applied to body fluids hold the potential to replace less effective screening tools.

These are ideals, but the pathologist must wrestle with an avalanche of published material to separate ideal from reality, and despite great advances in the field, we will continue to make decisions based on visual information for the foreseeable future. Two of the greatest challenges include making the correct diagnosis in day-to-day practice and being up to date on the potential impact on patient management. A multiplicity of therapeutic outcomes exists for a single tumor and hinge on diagnostic precision and facility with ancillary tests. Once simple tumor classifications are now sufficiently partitioned to keep the most confident pathologist questioning his or her opinion. It is not enough to be bright; one has to be experienced. It is not enough to be both if one gets

overconfident. One must avoid coming to conclusions too quickly or assuming that yesterday's diagnostic coup guarantees a successful outcome with the next case.

There is no prophylaxis for these aforementioned hazards, but pathology is a visual art with a long memory. A pathologist may not recall where he or she left his or her car keys but she or he will instantly recognize a case when he or she sees the slide again. Unfortunately, some mistakes are avoided only because they were made before. One goal of this book is to recount as many of these pitfalls as possible so that the reader can anticipate them in practice. A broader goal is to address conceptual changes that are altering our perceptions of diseases of the reproductive tract and our role in the management of women who are affected. In the final analysis, it is experience—whether through error or preemptive scholarship—that eases our daily visits to a sometimes uncertain landscape. The practice of pathology has its price, but it is gladly paid by its participants who dwell at the nexus of discovery and patient care.

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CHAPTER 1

Female Genital Tract Development and Disorders of Childhood

Mariko Horii, Theonia K. Boyd, Bradley J. Quade, Christopher P. Crum, Mana M. Parast

Abstract

Female reproductive tract development is a complex process intricately tied to the patterning of the male (Wolffian) reproductive tract and renal anlage. Development starts from undifferentiated mesoderm known as the genital ridge, with germ cells migrating to this location from the yolk sac. The müllerian duct begins as an invagination of the coelomic epithelium at the top of the genital ridge and elongates by active cell proliferation using the Wolffian duct as a guide. Many genes have been linked to female reproductive tract development, but only a few have been directly implicated by animal knockout models; this is because the simplex female reproductive tract pattern (i.e., one with a single cervix and uterus, with two separate fallopian tubes) is limited to humans and other primates. Most recently, genomic sequencing has identified more genes, mostly transcription factors and extracellular signaling molecules, and mutations in those genes, associated with malformations of the female reproductive tract. The most common congenital abnormality of the human female genital tract occurs when the paired müllerian ducts fail to fuse or the subsequent septum fails to resorb, yielding a spectrum of uterine anomalies, including uterus didelphys and bicornuate uterus. External female genital tract development requires both the absence of a key male determining factor (SRY) and presence of its antagonist (WNT4). Virilization of genetically female fetuses is due to excessive androgens from congenital adrenal hyperplasia or maternal blood.

Keywords

female reproductive tract development; ambiguous genitalia; uterine malformations; gonadal abnormalities; precocious puberty

CHAPTER OUTLINE

Overview of Reproductive Tract Development

The female genital tract is formed by a complex series of events beginning in the fourth week of development. This process involves the formation of the gonads following germ cell migration from the yolk sac to the dorsal mesentery, formation and fusion of the müllerian ducts to create the uterine corpus and tubes, induction of squamous mucosa in the vagina and cervix, and a series of epithelial-mesenchymal interactions in the introitus and external genital region to model the clitoris and labia. Successful completion of these sequential developmental tasks requires, by definition, the cooperation of concurrent events taking place to form the abdominal wall, separate the rectum from the urogenital sinus, induce urothelial differentiation, and complete rectal and urethral development (Table 1.1; Figs. 1.1 to 1.4).[1,2]

Table 1.1

Timeline of Important Milestones in Genital Tract Development

Postconception Period	Ovaries	Fallopian Tubes, Uterus, and Vagina	Vulva
Week 3	Primordial germ cells appear in the hindgut yolk sac wall.		The primitive streak mesenchyme forms the midline genital tubercle and paired cloacal folds.
Weeks 4 to 6	Germ cells migrate along the dorsal mesentery to invade the urogenital ridge.	Genital ridge mesenchyme folds into columns, which cavitate to form paramesonephric (müllerian) ducts.	The cloacal folds differentiate into urethral and anal folds; labioscrotal swellings form laterally to cloacal folds.
Weeks 7 to 8	Surface coelomic epithelium penetrates mesenchyme to form cortical cords; stromal estradiol production determines ovarian fate.	Cephalad ducts differentiate into paired fallopian tubes and caudal fused ducts differentiate into the uterus, cervix, and vagina; merged uterine and upper vaginal ducts cavitate to form single uterine and vaginal lumens.	The genitalia is indifferent.
Months 4 to 5	Cortical cord cells surround oogonia as	The lower vaginal canal remains solid until the end of month 5 and then	The genitalia is definitive:

	primordial follicles reach maximal number (>7 million).	demarcates the junction between superior müllerian and inferior urogenital sinus origins.	1. Genital tubercle → clitoris 2. Urethral folds → labia minora 3. Labioscrotal swellings → labia majora
Month 7	Oogonia cease proliferation and enter meiotic prophase.		
Term	Germ cell numbers reduced by ~70%.		

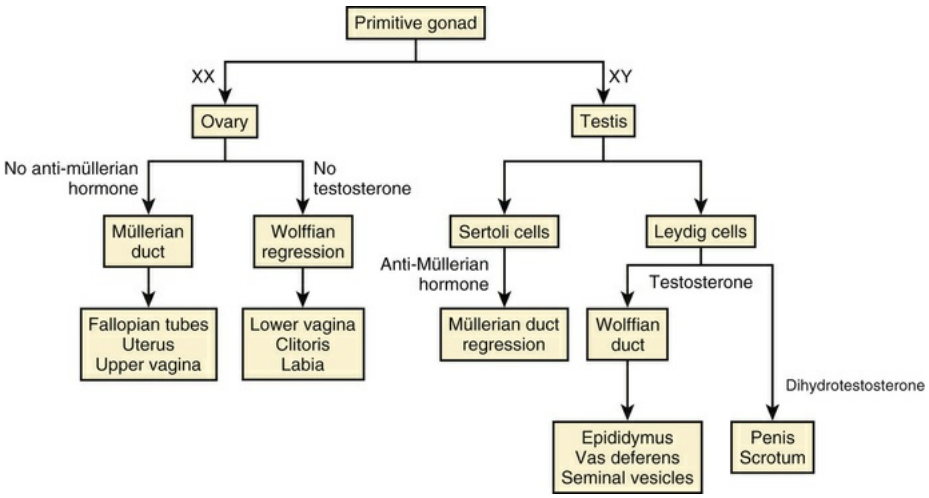

FIG. 1.1 Overview of pathways of müllerian (female) versus Wolffian (male) development. (From Holm I: Ambiguous genitalia in the newborn. In Emans SJ, Laufer MR, Goldstein DP, editors: Pediatric and adolescent gynecology, ed 5, Philadelphia, 2005, Lippincott Williams & Wilkins, p 58, with permission.)

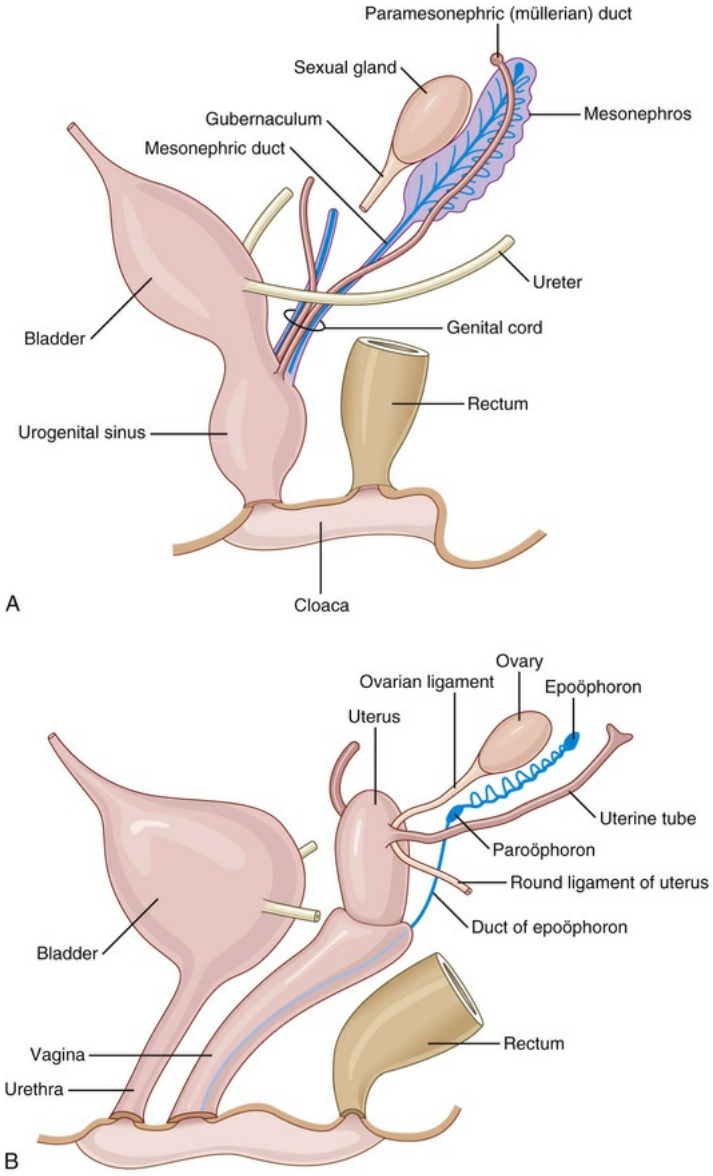

FIG. 1.2 Lower genital tract development before **(A)** and following **(B)** initiation of müllerian tract development.

(Redrawn from Warwick R, Williams PL, editors: Gray's anatomy, ed 35, Philadelphia, 1973, WB Saunders, p 87, with permission.)

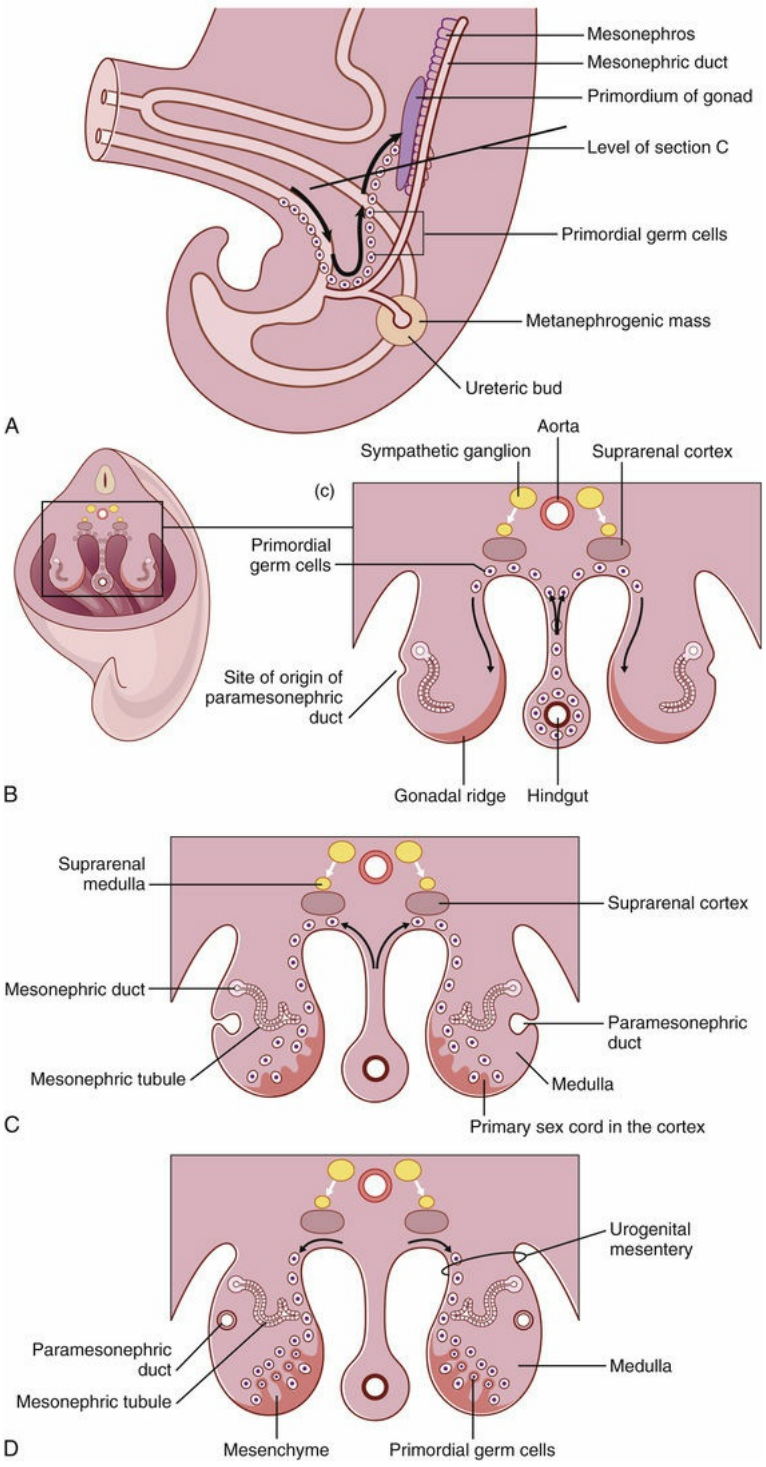

FIG. 1.3 Development of the genital ridge and early ovarian development. (Redrawn from Moore KL: Before we are born. In Moore KL: Basic embryology and birth defects, Philadelphia, WB Saunders, 1974, p 149.)

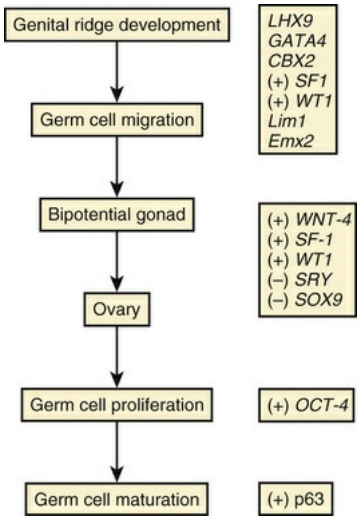

FIG. 1.4 Schematic of genes involved in forming the genital ridge, gonadal development, and sex determination.

These events can be subdivided into four segments involving development of the genital ridge, ovary, uterus and vagina, and external genitalia. Each of these events is influenced, directly or indirectly, by the expression of a range of transcription factors, X chromosome integrity, germ cell development, and secretion of sex steroid hormones. Relative input from these influencing factors ultimately determines the internal and external sexual organ phenotype.

The Genital Ridge

The genitourinary system begins to develop by the fifth week postfertilization as a longitudinal ridge of undifferentiated mesenchymal cells that extend bilaterally, flanking the mesenteric root (see Fig. 1.2). Excluding the bladder and external genitalia, the remainder of the genitourinary system ultimately evolves from this mesenchymal thickening. The undifferentiated mesenchyme in this area comprises the genital ridge and will ultimately give rise to the medulla of the ovary, whereas the coelomic epithelium becomes the ovarian cortex and ovarian surface epithelium (see Fig. 1.3). Genital ridge development is under the control of homeobox gene family members, transcription factors, and wingless family signaling factors that are integral to genital ridge development, and mouse knockouts affecting these genes will nullify

genital ridge development and that of the adjacent kidneys and adrenals.[3,4] In particular, several genes (*LHX1 [LIM1], EMX2, PAX2, WNT4, WNT7a, HNF1β [TCF2],* and *DACH1/2*) are critical to the formation of the urogenital anlagen before sexual differentiation (see Fig. 1.4).[5-9]

Ovary Development and Sex Determination

Human germ cells enter the genital ridges between 4 and 6 weeks' gestation. In the presence of a male genotype containing the sex-determining region gene *(SRY)* on the Y chromosome, or in the event of an XX genotype in which the *SRY* region has been retained via translocation, the embryo will develop into a male. The primary target of *SRY* is *SOX9*, a homeobox gene that has been shown to "rescue" the male phenotype in some XX individuals.[10,11] In the absence of *SRY*, granulosa cells form single layers that invest the primitive oocytes. These primordial follicles rapidly multiply to more than 7 million by the 22nd week. At this point, cell division ceases and the cell population drops by more than two-thirds at birth and by another 90% by puberty, when the average number of oocytes in the ovary is approximately 300,000.[12] In the genetically female fetus, the gonad is distinguished by the end of the second month due to estradiol production from the ovarian stroma.[13] The primitive germ cells proliferate and differentiate to oogonia, beginning in the center of the ovary and moving toward the periphery over time. The oogonia become invested with a single layer of follicular cells derived from coelomic epithelium of mesonephric origin,[14] become oocytes, and form primordial follicles (Fig. 1.5). The earliest follicles develop by 15 weeks. By the end of the seventh month of gestation, all the germ cells have ceased to proliferate and have entered meiotic prophase, where they will remain until ovulation. In contrast to the testis, germ cells in the ovary are critical to the development of their supporting stromal cells. In their absence, the prefollicular cells are not sustained, and a streak gonad will eventuate.[15]

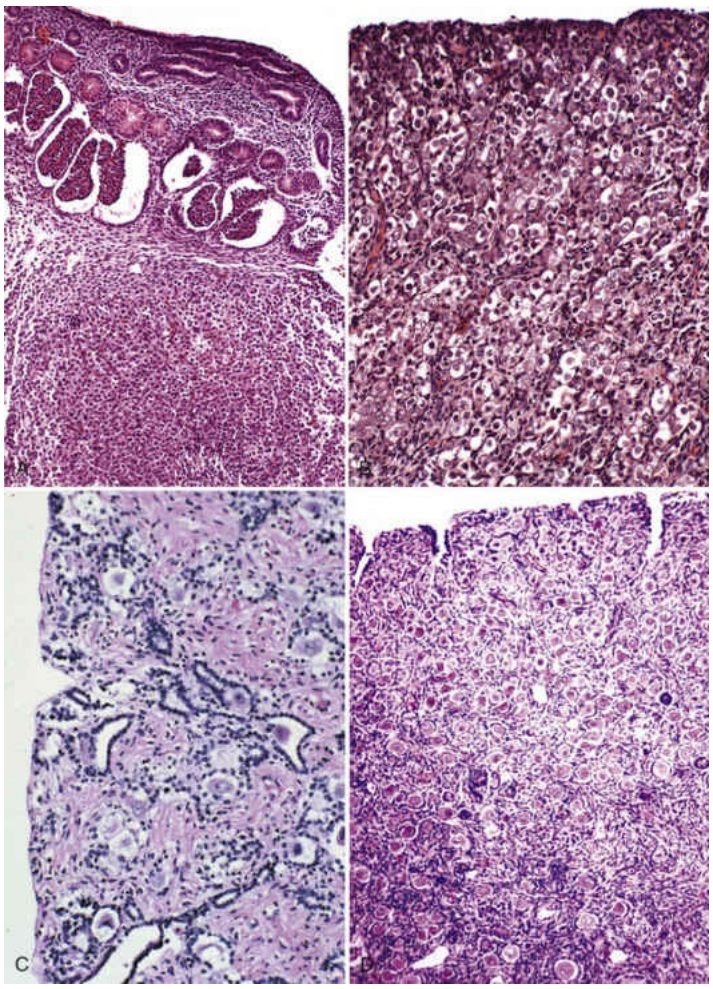

FIG. 1.5 Human ovarian development. **A,** Indifferent gonad at 6 weeks with primitive germ cells *(lower).* The metanephros is above. **B,** Germ cells at 19 weeks. Primordial follicles are not yet conspicuous. **C,** In midgestation, surface coelomic epithelium invaginates to invest oogonia, resulting in primordial follicles. **D,** At term, the cortex is filled with primordial follicles.

Germ cells exhibit brisk proliferative activity in weeks 15 to 20, with high levels of Ki-67 expression in the cortex and medulla (Fig. 1.6A). Concurrent with proliferation is the expression of *OCT4,* a transcription factor that is expressed early in embryogenesis and has been demonstrated to be integral to maintaining viability of the primordial germ cell mass (see Fig. 1.6B).[16] *OCT4* staining is concentrated primarily along the outer rim of the primitive ovary at this point. In the center, a gradually increasing population of enlarged oocytes is seen, and these cells display strong nuclear staining for p63 (see Fig. 1.6C). Between

week 20 and term, the percentage of germ cells staining for *OCT4* progressively declines at the periphery and, as germ cells decline in number, a progressively increasing proportion become p63-positive. Postnatally, all identifiable oocytes show intense p63 nuclear positivity (see Fig. 1.6D).

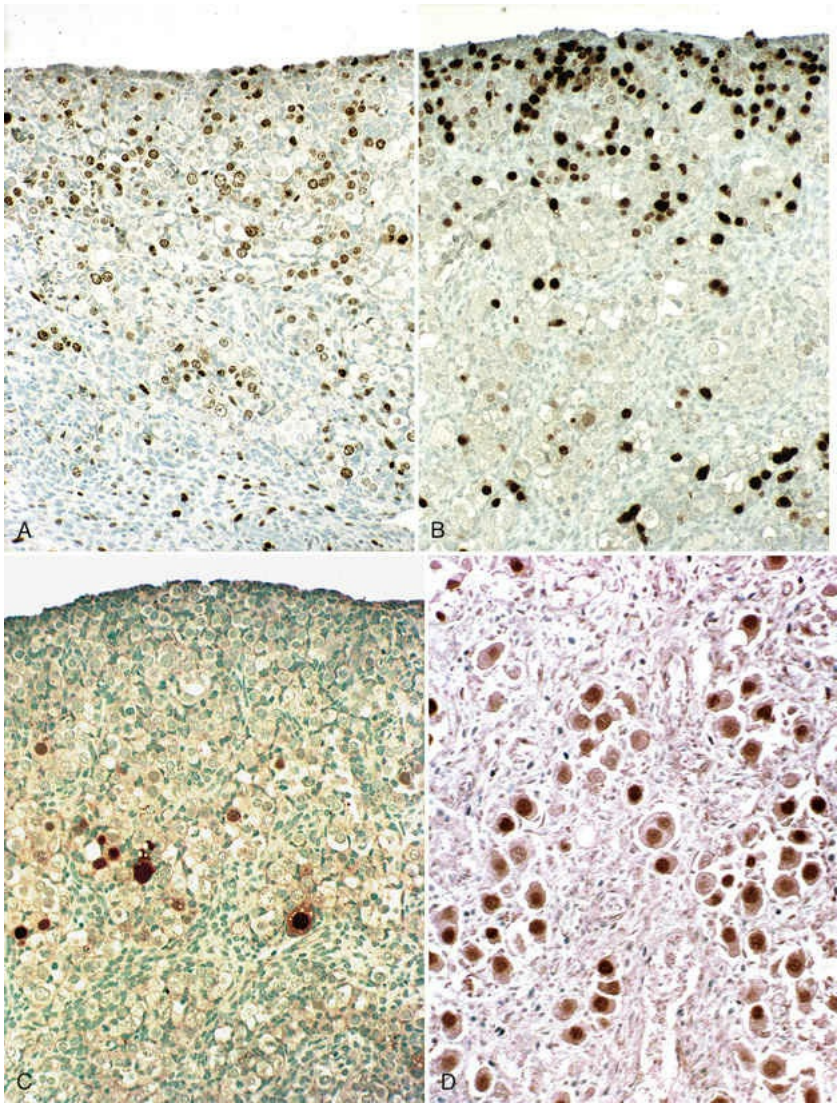

FIG. 1.6 Expression of OCT3/4, Ki-67, and TAp63 at 19 weeks' gestation. **A,** Ki-67 is expressed in immature germ cells and stromal cells, predominantly in the cortex. **B,** Expression of OCT4 predominates in the immature cortical germ cells. **C,** Expression of full-length p63 (TAp63) is limited to the maturing oocytes in the primordial follicles. **D,** At birth, TAp63 identifies a high

percentage of oocytes in the ovarian cortex. Ki-67 and OCT3/4 are not expressed at this stage. (Courtesy F. McKeon, MD and C.P. Crum, MD.)

This sequence of immunostaining patterns is consistent with the role of *OCT4* in maintaining primordial germ cells through the proliferative phase in the first two trimesters of pregnancy. Unknown factors result in the programmed cell death of a large number of the remaining germ cells in the last trimester. The preservation of a discrete subset is coincident with the expression of p63, which is expressed subsequently throughout the life of the oocyte in the ovarian cortex. Studies in mice have identified p63, and specifically the TA isoform, as the "guardian of the female germ line," required for the process of cell death induced by DNA damage,[17] similar to the function served by p53 in somatic cells.

The preceding process is under control of several genes at critical points. Expression of *LHX9* appears to be necessary for the development of supporting ovarian stroma. In mouse models lacking this gene, germ cell migration is normal, but the somatic cells of the genital ridge fail to proliferate, with failure of gonad formation.[18,19] *LHX9* mutants do not display other disorders, making this gene an attractive candidate for isolated gonadal dysgenesis. However, mutations in this gene have yet to be identified in humans.[20] Mutations in another gene, *CBX2,* have been identified in 46,XY females with normal genitalia and ovaries.[21] Disruption of expression of *CBX2,* a component of the Polycomb group complex of regulatory proteins, leads to delayed development of the genital ridge and male to female sex reversal.[22,23] This phenotype has been linked to *CBX2*'s regulation of *Sry* gene expression.[24] Yet another gene, *GATA4,* has also been linked to disorders of gonadal development in human.[25] Unlike other genes involved in growth and maintenance of the genital ridge, this gene has been implicated in gonadal initiation as the coelomic epithelium in the *GATA4*-conditional knockout mouse fails to thicken, remaining as a morphologically undifferentiated monolayer.[26]

Maintenance of ovarian germ cells is dependent on an intact X chromosome. X chromosomal abnormalities, such as monosomy X, are associated with accelerated follicular atresia.[27] These include deletions of Xp11, Xq13, and specific genes such as *ZFXA* and *DAZLA*.[28]

The Uterus and Vagina

Concurrent with genital ridge formation and germ cell migration during the fourth postfertilization week is invagination and tubal extension of coelomic epithelium in the transition area between the pronephros and mesonephros to form the paramesonephric (müllerian) ducts.[29] Rostrally, the paramesonephric ducts lie lateral to the mesonephric (Wolffian) ducts; caudally, their paths cross the mesonephric ducts ventrolaterally to meet in the midline, where they fuse by the eighth week of gestation. It is now believed that the müllerian duct grows by active epithelial proliferation using the mesonephric ducts as a guide.[29] The extracellular signaling molecule *Wnt9b* is required for müllerian duct elongation, presumably because the guiding mesonephric ducts fail to develop in *Wnt9b*$^{-/-}$embryos.[30] *LHX1* is the first transcription factor to be identified as essential in epithelial progenitor cells of the müllerian duct, with its lack of expression leading to uterine hypoplasia and complete loss of the endometrium.[31]

Following midline fusion, the müllerian ducts merge and cavitate to form the uterovaginal cavity, which is destined to form the uterus and upper third of the vaginal canal. The more caudal portion remains solid and merges with an ingrowth of solid endoderm from the urogenital sinus, called the sinovaginal bulb. These solid formations undergo canalization by the 20th week to form the vagina, with the hymen demarcating the junction of paramesonephric (cephalad) and endodermal (caudad) tissue origins. The upper vagina, which is derived from the distal end of the müllerian duct, is flanked at its superior pole by recesses of mesonephric duct origin, which will eventually form the vaginal fornices (Fig. 1.7).

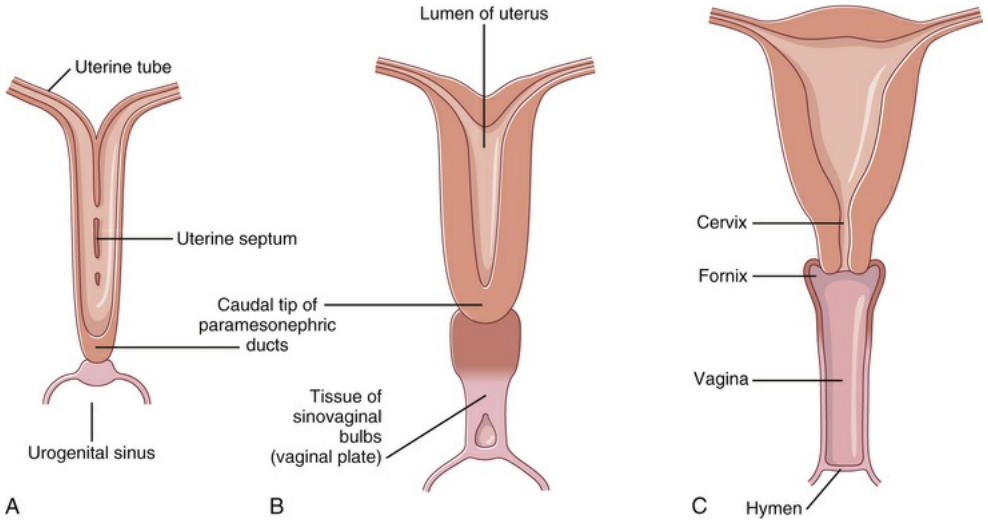

FIG. 1.7 Schematic of uterine and vaginal development.

(Redrawn from Sadler TW: Langman's medical embryology, ed 5, London, 1985, Williams & Wilkins, with permission.)

The eventual epithelialization of the vagina and cervix is potentially explicable by two theories. The first resolves the presence of squamous mucosa by the ingrowth of squamous epithelium from the introitus. The second holds that the squamous mucosa of the vagina and cervix emerges via induction of basal cells in the müllerian epithelium of the cervix and vagina. This latter hypothesis is supported by several lines of evidence.[32,33] First, p63 expression is seen along the full length of not only the vagina but also the urethra during this interval, indicating that urothelium and squamous epithelium develop via the same process (Fig. 1.8). Second, although the newborn human vagina is fully covered by squamous epithelium, the vagina of the newborn mouse is lined by mucus-secreting, endocervical-type columnar epithelium, beneath which lies a single row of reserve cells. With the onset of estrus, the reserve cells expand and undergo squamous differentiation, after which the vagina is permanently lined by mature squamous epithelium. This process of basal cell induction fails in the p63 null mouse, and the vagina remains lined by a combination of müllerian and primitive urogenital sinus epithelium.[32,33]

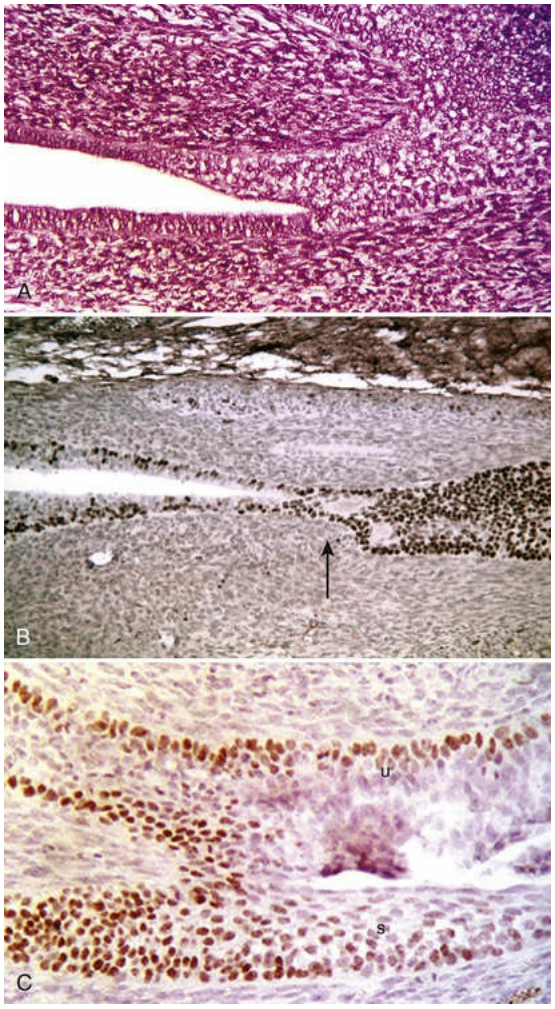

FIG. 1.8 **A,** Junction of müllerian *(left)* and vaginal squamous *(right)* epithelium in the mouse embryo. **B,** p63 expression highlights the induction of squamous differentiation in the latter *(right)*. **C,** At high power, both the urethra *(upper left)* and vagina *(lower left and right)* in the fetal mouse express p63 during urothelial (u) and squamous (s) differentiation, respectively.

The final development of the müllerian duct can be divided into three separate phases: (1) regional specification of tissue and organ identity; (2) partitioning and expansion of distinct endometrial and myometrial compartments; and (3) endometrial adenogenesis. Several of the homeobox gene family members have been identified as genetic factors required for assignment of tissue and organ identity along the length of the müllerian duct.[8,34] From such studies, an idealization of the plan for segmentalization can be inferred. In such schema, *HOXA9* expression

defines the future oviduct. *HOXA10* (rostral) and *HOXA11* (caudal) expression is needed for uterine fundal development, and *HOXA11* also is needed for cervical development. Finally, *HOXA13* is expressed in the cervical and upper vaginal anlage. In null mutants, their patterns of segmental or partially overlapping expression along the müllerian duct (from the rostral to caudal ends) is disturbed and results in homeotic transformations. The downstream details of *HOX* gene activity on regionalization of the müllerian duct have yet to be elucidated. In addition, the developmental genetics of endomyometrial partitioning and endometrial adenogenesis have not been defined, in part reflecting the differences between the human female genital tract and those of lower, more commonly studied mammals.

The External Genitalia

The hindgut and urogenital sinus open into a common cloaca prior to the seventh week of gestation. At this point, a ridge of mesenchyme—the urorectal septum—migrates caudad toward the cloacal membrane and separates the genitourinary system from the rectum. An external midline protuberance develops at this point, the genital tubercle, which is flanked dorsolaterally by the genital folds, which are in turn laterally cuffed by the labioscrotal swellings. The genital tubercle ultimately becomes the clitoris, the genital folds eventually form the labia minora, and the labioscrotal swellings differentiate into the labia majora (Fig. 1.9).

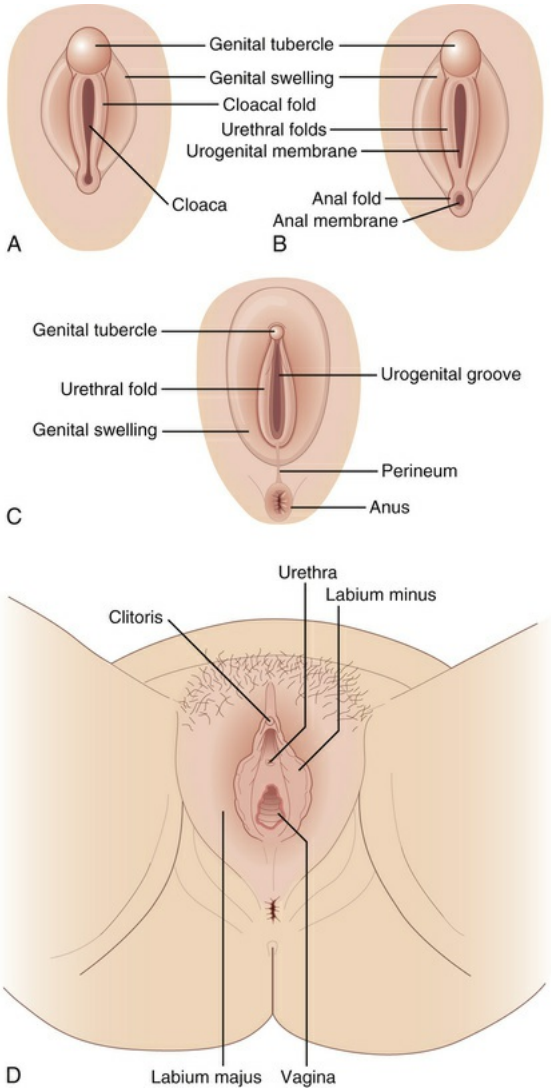

FIG. 1.9 Schematic of the development of the external genitalia. **A,** Early development before completion of the urorectal septum. **B,** Separation of the anus from the urogenital sinus lined by the urethral folds. **C,** Development of the genital swelling. **D,** Completion of the vagina, labia, and clitoris.

The factors involved in the modeling of the external genitalia are multiple and involve sequential expression of regulatory genes and the induction of gene expression by specific epithelial-stromal interactions. The sonic hedgehog gene *(SHH)* has been shown to regulate genes expressed in mesenchyme, including patched 1 *(PTCH1),* bone morphogenetic protein-4 *(BMP4), HOXD13,* and fibroblastic growth factor *(FGF10)*. The absence of *SHH* (in *SHH* null mice) is associated with agenesis of the genital tubercle, accelerated cell death, diminished cell

growth, and an abnormal shift in expression of *BMP4* from the mesenchyme to the epithelium. Thus, *SHH* is considered to increase outgrowth and differentiation of the genital tubercle.[35] Predictably, maintenance of the mucocutaneous genital mucosal epithelium is critical to the development of the appropriate mesenchymal response; p63 null mice, which are devoid of skin and squamous mucosa, fail to complete the urorectal septum, exhibit abnormalities in bladder and phallic development, and are born with a common cloaca.[32] Humans with p63 mutations have defects in the growth of hair, teeth, and distal limb development. They exhibit less conspicuous genital anomalies but exhibit genital hypoplasia similar to that seen in the external genitalia of the p63 null mouse. The conclusion from study of these models is that the integrity of the squamous mucosa is also critical for normal caudal, urogenital, mesenchymal development.

The female genital phenotype was once considered a default phenotype, resulting from the absence of interference by androgenic hormones, despite inactivated estrogen receptor proteins or aromatases.[36,37] *SRY*, the sex-determining gene located on the short arm of the Y chromosome, is critical in gonadal sex differentiation, such that the absence of *SRY* confers (or permits) female gonadal differentiation. However, current evidence also indicates that generation of the female phenotype occurs actively, via autosomal genes such as *WNT4* on chromosome 1p, which functions along the pathway of *DAX1*, an X chromosome gene, to antagonize *SRY* expression.[38] Alternative pathways independent of genetic gonadal regulation, as with in utero diethylstilbestrol (DES) exposure, lead to inappropriate expression of estrogens, resulting in vaginal adenosis and structural malformations of the uterus and cervix.[39]

Common Disorders of Gonadal and Genital Tract Development

The Ovary and Fallopian Tube

Developmental Abnormalities

Developmental abnormalities of the fallopian tubes and ovaries arise via three mechanisms: (1) disturbance in müllerian duct development; (2) disturbance in gonadal development; and (3) abnormal sex chromosomes.

Ovarian Hypoplasia

Pathogenesis

The classic example of ovarian hypoplasia is associated with Turner syndrome (45,X karyotype, also designated monosomy X). The primordial germ cells make their way to the genital ridge but fail to induce follicle development and degenerate postnatally, producing an ovary with no germ cells by toddlerhood. Because the Y chromosome is absent, normal genitouterine development takes place. However, because the ovary is not capable of promoting folliculogenesis and producing estrogens, external sexual characteristics remain infantile. Patients with a pure 45,X genotype are not at increased risk for gonadal neoplasia; it is the phenotypic female Turner syndrome patients with a Y chromosome constitution harboring the *SRY* gene who are at risk for gonadal neoplasia—namely, gonadoblastoma and dysgerminoma (seminoma).[40]

Histopathology

The typical ovary in monosomy X is a streak gonad. Grossly, the ovary is a small, flat, ovoid structure; microscopically it consists principally of scant ovarian cortical stroma, which may contain scattered primitive sex cord structures. Oocytes are not present (Fig. 1.10).

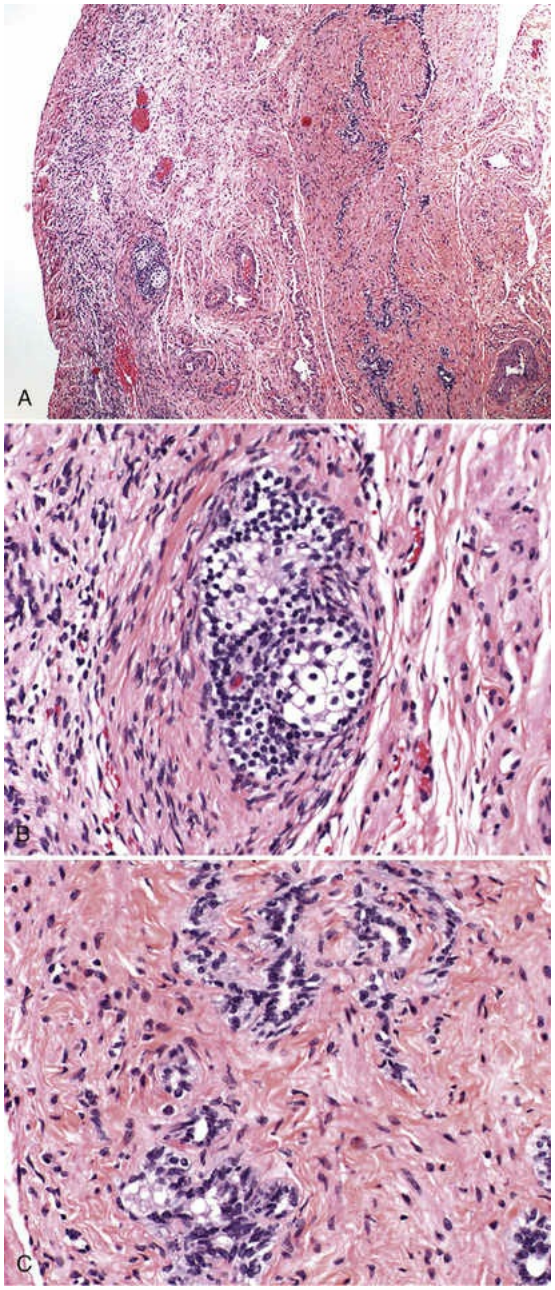

FIG. 1.10 Streak gonad from an XO genotype. **A**, Rudimentary cortex, with focal islands of sex cord derivatives **(B). C,** Attenuated Wolffian remnants.

Agenesis and Dysgenesis of the Ovary

Pathogenesis

This disorder is not associated with a chromosomal anomaly; patients are therefore karyotypically normal (46,XX). The primordial germ cells

neither develop nor migrate; therefore, the gonad does not develop. Maternal-placental estrogens complete development of the external genitalia and the müllerian ducts, but the external phenotypic stigmata of Turner syndrome are not seen in such cases.

Histopathology

The ovaries are streak gonads, indistinguishable from the Turner ovarian histologic phenotype.

Androgen Insensitivity (Testicular Feminization) Syndrome

Pathogenesis

These patients have a normal 46,XY male karyotype, do not respond to testosterone, and therefore are externally phenotypic females. Internally, the paramesonephric duct system is suppressed, uterine development is blunted or absent, and the vagina ends in a blind pouch. The testes do not descend and remain in the abdomen or inguinal region, where they are at risk of germ cell tumorigenesis, a risk that increases over time.[41]

Histopathology

The testes may exhibit at least four distinct features: Sertoli cell–only tubules, Leydig cell hyperplasia, nodular Sertoli cell masses, and germ cell atypia and neoplasia (Figs. 1.11 to 1.13). The background pattern consists of Sertoli tubules devoid of spermatogonia, admixed with abundant Leydig cells (see Fig. 1.12A and B). Within this background, discrete nodular masses of Sertoli tubules are often seen, which are variably described as hyperplastic, neoplastic, or hamartomatous (see Fig. 1.11A and B). Scattered, enlarged hyperchromatic intratubular germ cells may be present, with positive immunostaining for placental alkaline phosphatase (see Fig. 1.13A–C), suggesting a predisposition toward development of germ cell neoplasia (gonadoblastoma) that may evolve to or be associated with germ cell tumors, particularly seminoma.[42]

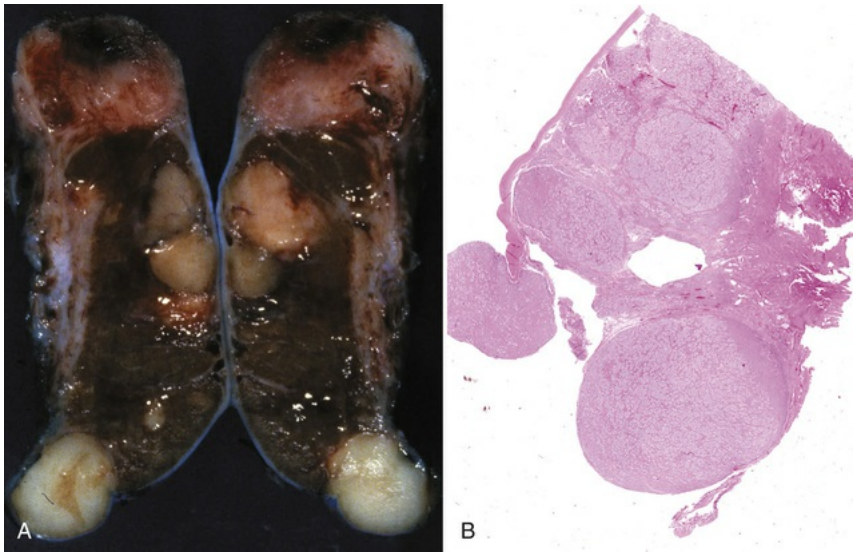

FIG. 1.11 Testes from two cases of androgen insensitivity syndrome containing gross **(A)** and nodular **(B)** masses.

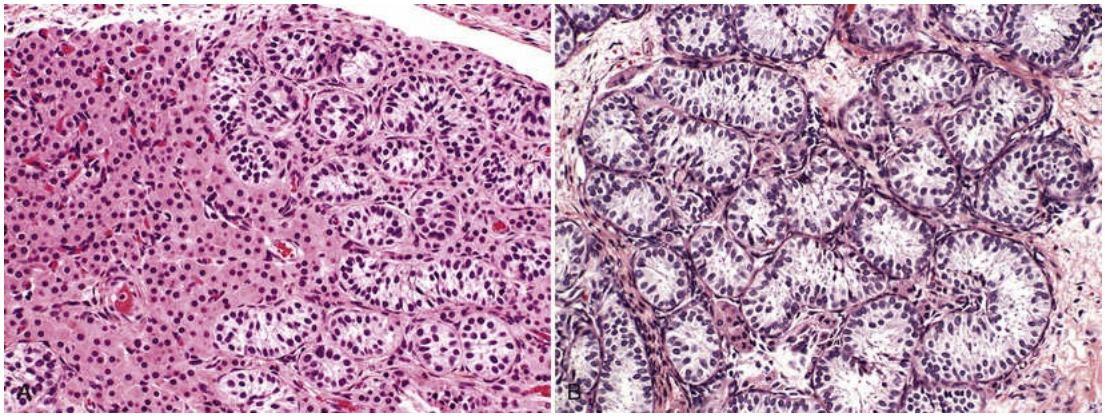

FIG. 1.12 Androgen insensitivity syndrome. **A,** Mixture of Sertoli tubules *(right)* and Leydig cell hyperplasia *(left).* **B**, Pure Sertoli cell differentiation within a discrete nodule.

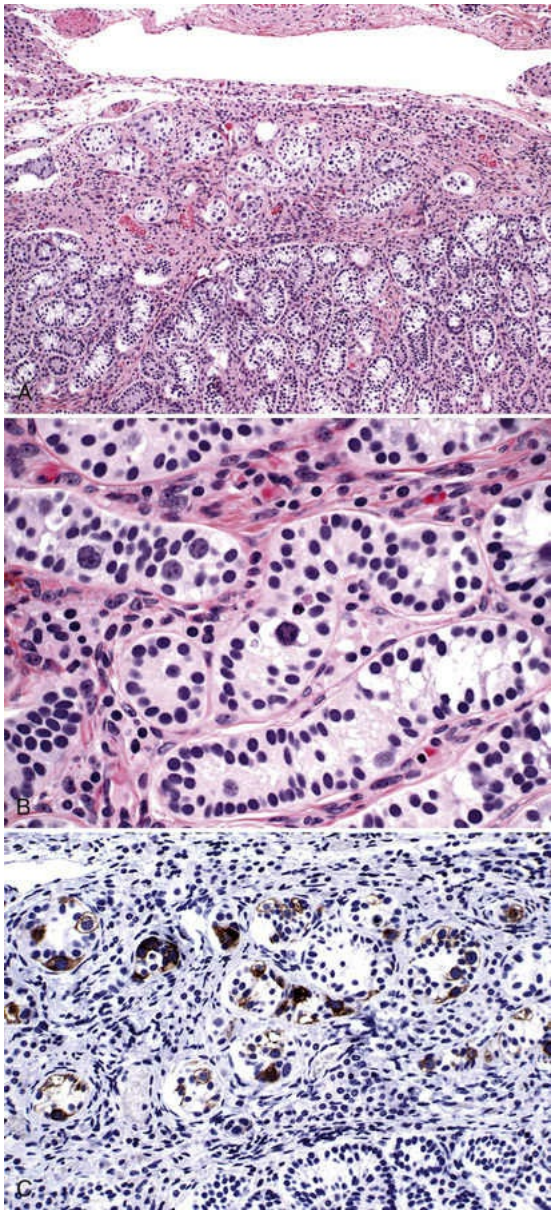

FIG. 1.13 Androgen insensitivity syndrome with germ cells. **A,** Focus of germ cells with atypia (gonadoblastoma; *upper*) adjacent to uninvolved Sertoli tubules *(lower).* **B,** Discrete nuclear enlargement in the Sertoli tubules characterizes germ cell differentiation. **C,** Following staining for placental alkaline phosphatase.

Other Abnormalities in Sex Determination (Disorders of Sexual Differentiation; Intersex)

Hermaphrodites and Pseudohermaphrodites

By definition, true hermaphrodites have external genitalia and gonads of both genders, whereas pseudohermaphrodites have external phenotypes opposite the genotype but internal genitalia consistent with the genotype.[43,44] Hermaphrodites are extremely rare and present with one of the following types of gonad(s): (1) bilateral ovotestes; (2) an ovotestis paired with an ovary; or (3) testes and a unilateral ovary–contralateral testis. The diagnosis of hermaphroditism is based entirely on the gonads; the sexual organ phenotype is variable and ranges from entirely female (Bergman type 1) to entirely male (type 5). Intermediate phenotypes characterize ambiguous genitalia (Box 1.1).

Box 1.1

Ambiguous Genitalia and Their Causes

Female pseudohermaphrodism

Congenital adrenal hyperplasia (excess fetal androgens)

- 21-hydroxylase deficiency
- 11-beta-hydroxylase deficiency
- 3-beta-hydroxysteroid dehydrogenase deficiency

Exogenous androgens

- Maternal ingestion of androgens, progestogens
- Maternal congenital adrenal hyperplasia
- Virilizing adrenal or ovarian tumor

Excess placental androgen production

- Placental P450 aromatase deficiency

Iatrogenic fetal virilization

Female pseudohermaphrodism with associated congenital malformations

Idiopathic

Male pseudohermaphrodism

- Impaired Leydig cell activity
- Defects in testosterone biosynthesis
- Leydig cell hypoplasia; luteinizing hormone receptor defect
- 20,22-desmolase (congenital lipoid adrenal hyperplasia)
- 3-beta-hydroxysteroid dehydrogenase
- 17,20-hydroxylase (17,20-desmolase)
- 17-beta-hydroxysteroid dehydrogenase (17-ketosteroid reductase)

Defects of testis development or maintenance

- XY gonadal dysgenesis
- Mixed gonadal dysgenesis
- Rudimentary testis syndromes

End-organ resistance to androgens (androgen insensitivity syndrome)

- Complete
- Partial
- Defects in the intracellular metabolism of testosterone (5-alpha-reductase deficiency)

Others

- Persistent müllerian duct syndrome
- Iatrogenic male pseudohermaphrodism
- Idiopathic male pseudohermaphrodism

True hermaphrodism

The gonads of hermaphrodites vary widely in their appearance. The ovary and testis may be juxtaposed in a single gonad, represented by Sertoli cells arranged in tubules within an ovarian cortex, accompanied by Leydig cells and Wolffian remnants (Fig. 1.14). In general, the ovary is more prominent, and graafian follicles with corpora lutea may develop. By contrast, the testicular tissue is usually less developed, and spermatogenesis is rare. However, based on external phenotype, two-thirds of true hermaphrodites are raised as males, despite the presence of an XX karyotype in at least half of them. Other karyotypes are 46,XX/46,XY and 46,XY.

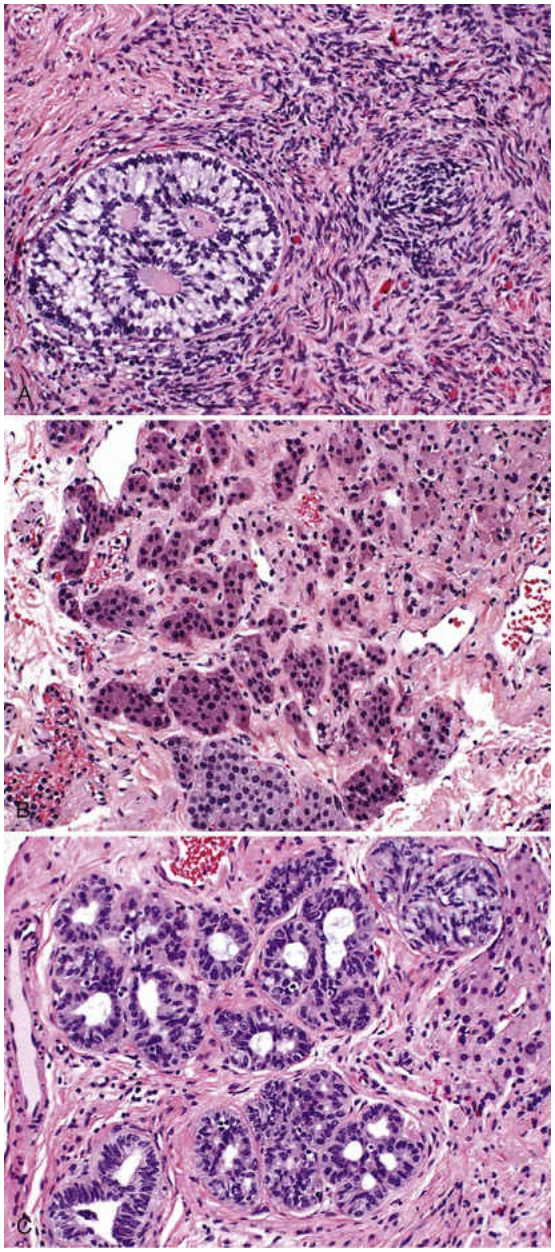

FIG. 1.14 Gonad from a true hermaphrodite. **A,** Sertoli cell differentiation is present within ovarian cortical stroma. **B,** Leydig cell hyperplasia in the ovarian hilus. **C,** Well-developed Wolffian remnants.

Pseudohermaphrodites exhibit ambiguous genitalia and possess one type of gonad, either testis (male pseudohermaphrodite) or ovary (female pseudohermaphrodite), that coincides with the genotype. Examples of the latter include adrenogenital syndrome (congenital adrenal hyperplasia), in which genotypically female (46,XX) individuals present with masculinized external genitalia, ranging from clitoral hypertrophy

to labial fusion. Male pseudohermaphrodites have a male genotype (46,XY) and variably feminized external sex characteristics, depending on the strength of androgenic hormone stimulation. Types of ambiguous genitalia are illustrated in Fig. 1.15, and the differential diagnosis is outlined in Fig. 1.16. An example of a male pseudohermaphrodite is shown in Fig. 1.17.[45,46]

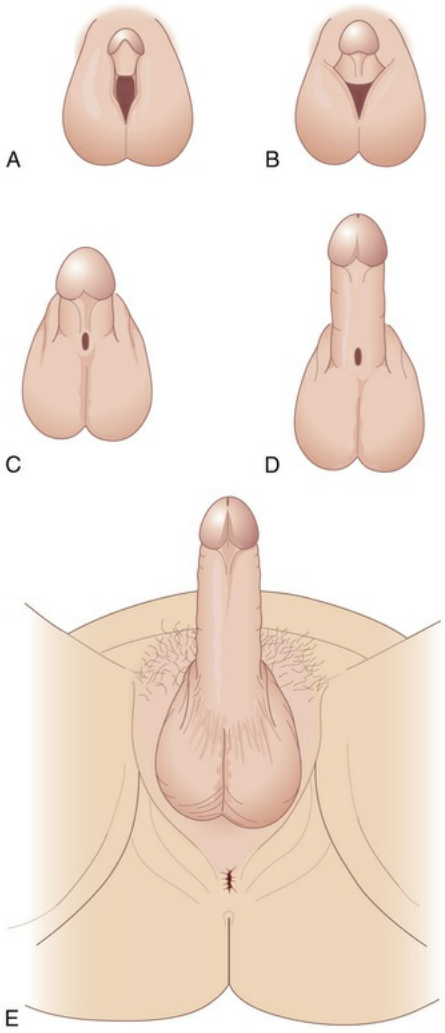

FIG. 1.15 Five stages of ambiguous genitalia made up of clitoromegaly **(A)**, clitoromegaly with labial fusion **(B)**, further phallic enlargement with complete labioscrotal fusion and urogenital sinus (C), complete scrotal fusion with urogenital sinus at the base of the phallus **(D),** and normal male genitalia **(E).**[45] (Redrawn from Reiner WG: Assignment of sex in neonates with ambiguous genitalia. Curr Opin Pediatr 11:363–365, 1999.)

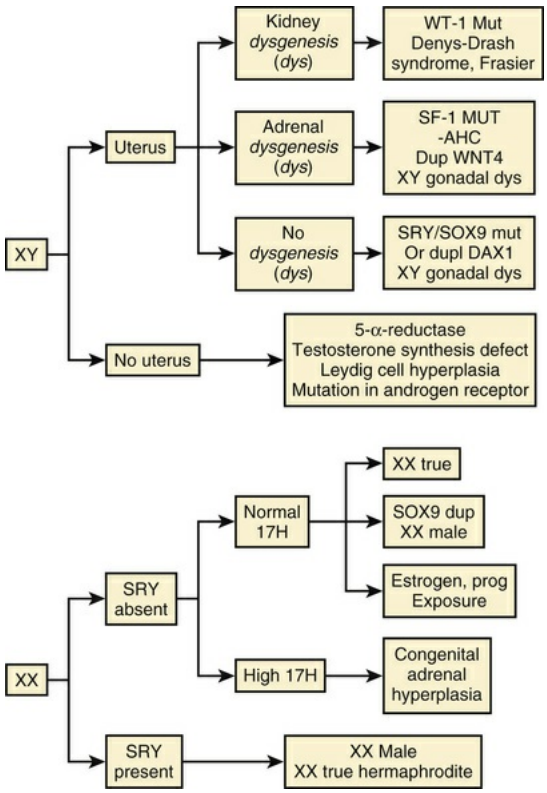

FIG. 1.16 Schematic of the differential diagnoses of intersex. *prog,* Progestogen. (From Dewing P, Bernard P, Vilain E: Disorders of gonadal development. Sem Reprod Med 20:189–197, 2002.)

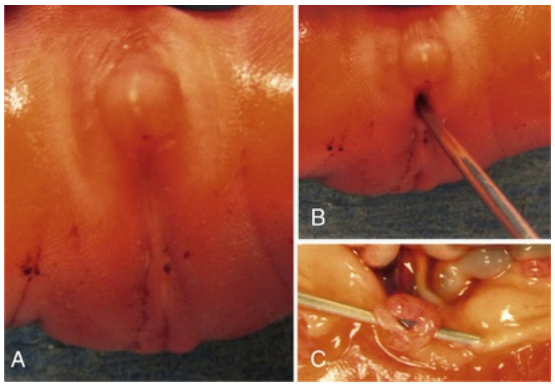

FIG. 1.17 Ambiguous genitalia in a stillborn male with severe intrauterine growth restriction. This phenotype depicts a micropenis with partially fused labioscrotal folds **(A),** with a blind "vaginal" pouch **(B)**; undescended testes **(C)** were identified bilaterally.

Acquired Disorders of Childhood Associated With

Gonadal Abnormalities

Precocious Puberty

Precocious puberty is defined as acquisition of the following characteristics at 8 years of age or younger: (1) breast development; (2) pubic or axillary hair; (3) menarche; (4) acne; and (5) body odor. The incidence is from 1 to 5/10,000 children.[47,48]

Precocious puberty can be stratified into two main categories of causative factors: central, or gonadotropin-dependent, and peripheral, or gonadotropin-independent, precocious puberty (Table 1.2). Central precocious puberty in young girls is largely idiopathic (95%), associated with premature activation of a gonadotropin-releasing hormone (GnRH) pulse generator in the hypothalamus and resulting in pituitary hormone release of follicle-stimulating hormone and luteinizing hormones.[46] Recently, whole exome sequencing has identified mutations in *MKRN3* in a significant proportion of families (33%) with central precocious puberty.[49] This is an imprinted gene, located in the Prader-Willi syndrome critical region of chromosome 15, and is paternally expressed; mutations cause a deficiency of gene expression or protein function and appear to affect puberty in both genders.[49] Less commonly, central precocious puberty is associated with a range of central nervous system disorders or tumors that initiate premature GnRH secretion; rarely, gonadotropin-dependent precocious puberty will be triggered following treatment for a sex steroid–producing tumor, with GnRH release initiated as hormone levels fall.

Table 1.2

Causes of Precocious Puberty

Type	Causative Factors
Gonadotropin-Dependent (Central Precocious Puberty)	
Idiopathic (95%)	
Familial (rare)	
Central nervous system (CNS) disorders	Inflammatory, infectious, radiation, chemotherapy, trauma
CNS tumors	Hypothalamic hamartoma, gliomas, craniopharyngioma, ependymoma, LH-secreting adenoma, pinealoma
Congenital malformations	Arachnoid cyst, suprasellar cyst, phakomatosis, hydrocephalus, septo-optic dysplasia
Dysmorphic syndromes	Williams-Beuren syndrome, Klinefelter syndrome

CNS "priming" by peripheral process	Congenital adrenal hyperplasia; sex steroid/sex hormone disorder/producing tumor
Gonadotropin-Independent (Peripheral Precocious Puberty)	
Ovarian disorders	Granulosa–theca cell tumors
	Theca cell tumor
	Estrogen- or gonadotropin-secreting germ cell tumors (e.g., dysgerminoma, choriocarcinoma, teratoma, embryonal carcinoma)
	Steroid-producing tumors
	Sex cord tumors with annular tubules
	McCune-Albright syndrome
Other neoplasms and conditions	Adrenal adenoma
	Adrenal carcinoma (usually virilizing)
	Pinealoma, hepatoblastoma, choriocarcinoma, teratoma (hCG)
	Congenital adrenal hyperplasia (i.e., transient follicle cysts)
	Exogenous source of steroids (steroid-producing pills, food additives, cosmetic creams)

hCG, Human chorionic gonadotropin; *LH*, luteinizing hormone.

Adapted from Partsch CJ, Sippell WG: Treatment of central precocious puberty. Best Pract Res Clin Endocrinol Metab 16:165–189, 2002.

Peripheral precocious puberty, also termed *precocious pseudopuberty*, can be caused by hormone-producing ovarian neoplasms, including the full range of sex steroid– and gonadotropin-producing tumors (see Table 1.2). Other causes include adrenal cortical tumors and exogenous ingestion of estrogens. The significance of repetitive low levels of exposure to internalized estrogens in accelerating puberty, such as may be found in food or topically applied products, is controversial but has been supported by epidemiologic studies.

Ovarian Neoplasia

Ovarian masses in children are generally benign (Tables 1.3 and 1.4). Neoplasms are more likely for tumors exceeding 10 cm, and most tumors (about two-thirds) are germ cell tumors.[50,51] In one study of 134 patients, 81 (≈60%) ovarian masses were benign functional cysts; of the neoplastic lesions, 27 of 44 were benign cystic teratomas, and only 6 were malignant germ cell tumors (see Table 1.3).[52] The rate of malignancy in pediatric ovarian neoplasms appears to range from 4% to 28% in most studies,[53,54] although in one study of 67 cases, it was as high as 55%.[55]

Table 1.3

Causes of Ovarian Masses in Children

Type	Number	Percentage
Functional cyst	81	60.4
Neoplastic	44	32.8
• Benign cystic teratoma	27	
• Malignant germ cell tumor	5	
• Epithelial neoplasm	6	
• Miscellaneous	6	
Other	9	
Total	134	

From de Silva KS, Kanumakala S, Grover SR, et al: Ovarian lesions in children and adolescents: an 11-year review. J Pediatr Endocrinol Metab 17:951–957, 2004.

Table 1.4

Primary Ovarian Tumors Treated at Boston Children's Hospital Medical Center (1928–1982)

Classification	Number	Percentage
Mature cystic teratoma	78	47
• Cystic	76	
• Solid	2	
Common epithelial tumors	27	16
• Mucinous	12	
• Serous	14	
• Mixed	1	
Sex cord stromal tumors	21	13
• Granulosa cell	10	
• Thecoma	2	
• Fibroma	1	
• Sertoli-Leydig	7	
• Unclassified	1	
Immature teratoma	17	10
Yolk sac carcinoma	14	8
Dysgerminoma	8	5
Choriocarcinoma	1	—
TOTAL	166	100

From Lack E, Goldstein DP: Primary ovarian tumors in childhood and adolescence. Curr Prob Obstet Gynecol 7:8, 1984.

Although epithelial ovarian neoplasms compose most of these tumors