Measurement of Unbound Testosterone Levels Most circulating testosterone is bound to SHBG and to albumin; only 0.5–3% of circulating testosterone is unbound, or "free." The unbound testosterone concentration can be measured by equilibrium dialysis or calculated from total testosterone, SHBG, and albumin concentrations. Recent research has shown that testosterone binding to SHBG is a multistep process that involves complex homoallostery within the SHBG dimer; a novel allosteric model of testosterone binding to SHBG dimers provides good estimates of free testosterone concentrations. The previous law of mass action equations based on linear models of testosterone binding to SHBG have been shown to be erroneous. Tracer analogue methods are relatively inexpensive and convenient, but they are inaccurate. *Bioavailable testosterone* refers to unbound testosterone plus testosterone that is loosely bound to albumin; it can be determined by the ammonium sulfate precipitation method.

hCG Stimulation Test The hCG stimulation test is performed by administering a single injection of 1500–4000 IU of hCG intramuscularly and measuring testosterone levels at baseline and 24, 48, 72, and 120 h after hCG injection. An alternative regimen involves three injections of 1500 units of hCG on successive days and measuring testosterone levels 24 h after the last dose. An acceptable response to hCG is a doubling of the testosterone concentration in adult men. In prepubertal boys, an increase in testosterone to >150 ng/dL indicates the presence of testicular tissue. No response may indicate an absence of testicular tissue or marked impairment of Leydig cell function. Measurement of MIS, a Sertoli cell product, is also used to detect the presence of testes in prepubertal boys with cryptorchidism.

SEMEN ANALYSIS

Semen analysis is the most important step in the evaluation of male infertility. Samples are collected by masturbation following a period of abstinence for 2–3 days. Semen volumes and sperm concentrations vary considerably among fertile men, and several samples may be needed before concluding that the results are abnormal. Analysis should be performed within an hour of collection. Using semen samples from over 4500 men in 14 countries, whose partners had a time-to-pregnancy of less than 12 months, the World Health Organization (WHO) has generated the following one-sided reference limits for semen parameters: semen volume, 1.5 mL; total sperm number, 39 million per ejaculate; sperm concentration, 15 million/mL; vitality, 58% live; progressive motility, 32%; total (progressive + nonprogressive) motility, 40%; morphologically normal forms, 4.0%. Some men with low sperm counts are nevertheless fertile. A variety of tests for sperm function can be performed in specialized laboratories, but these add relatively little to the treatment options.

TESTICULAR BIOPSY

Testicular biopsy is useful in some patients with oligospermia or azoospermia as an aid in diagnosis and indication for the feasibility of treatment. Using local anesthesia, fine-needle aspiration biopsy is performed to aspirate tissue for histology. Alternatively, open biopsies can be performed under local or general anesthesia when more tissue is required. A normal biopsy in an azoospermic man with a normal FSH level suggests obstruction of the vas deferens, which may be correctable surgically. Biopsies are also used to harvest sperm for ICSI and to classify disorders such as hypospermatogenesis (all stages present but in reduced numbers), germ cell arrest (usually at primary spermatocyte stage), and Sertoli cell–only syndrome (absent germ cells) or hyalinization (sclerosis with absent cellular elements).

DISORDERS OF SEXUAL DIFFERENTIATION

See Chap. 410.

DISORDERS OF PUBERTY

The onset and tempo of puberty varies greatly in the general population and is affected by genetic and environmental factors. Although some of the variance in the timing of puberty is explained by heritable factors, the genes involved remain unknown.

PRECOCIOUS PUBERTY

Puberty in boys before age 9 is considered precocious. *Isosexual precocity* refers to premature sexual development consistent with phenotypic sex and includes features such as the development of facial hair and phallic growth. Isosexual precocity is divided into gonadotropin-dependent and gonadotropin-independent causes of androgen excess (Table 411-1). *Heterosexual precocity* refers to the premature development of estrogenic features in boys, such as breast development.

Gonadotropin-Dependent Precocious Puberty This disorder, called *central precocious puberty* (CPP), is less common in boys than in girls. It is caused by premature activation of the GnRH pulse generator, sometimes because of central nervous system (CNS) lesions such as hypothalamic hamartomas, but it is often idiopathic. CPP is characterized by gonadotropin levels that are inappropriately elevated for age. Because pituitary priming has occurred, GnRH elicits LH and FSH responses typical of those seen in puberty or in adults. Magnetic resonance imaging (MRI) should be performed to exclude a mass, structural defect, infection, or inflammatory process. Mutations in *MKRN3*, an imprinted gene encoding makorin ring-finger protein 3, which is expressed only from the paternally inherited allele, have been associated with CPP.

Gonadotropin-Independent Precocious Puberty In gonadotropin-independent precocious puberty, androgens from the testis or the adrenal are increased, but gonadotropins are low. This group of disorders includes hCG-secreting tumors; congenital adrenal hyperplasia; sex steroid–producing tumors of the testis, adrenal, and ovary; accidental or deliberate exogenous sex steroid administration; hypothyroidism; and activating mutations of the LH receptor or $G_s\alpha$ subunit.

TABLE 411-1 CAUSES OF PRECOCIOUS OR DELAYED PUBERTY IN BOYS

- I. Precocious puberty
  - A. Gonadotropin-dependent
    - 1. Idiopathic
    - 2. Hypothalamic hamartoma or other lesions
    - 3. CNS tumor or inflammatory state
  - B. Gonadotropin-independent
    - 1. Congenital adrenal hyperplasia
    - 2. hCG-secreting tumor
    - 3. McCune-Albright syndrome
    - 4. Activating LH receptor mutation
    - 5. Exogenous androgens
- II. Delayed puberty
  - A. Constitutional delay of growth and puberty
  - B. Systemic disorders
    - 1. Chronic disease
    - 2. Malnutrition
    - 3. Anorexia nervosa
  - C. CNS tumors and their treatment (radiotherapy and surgery)
  - D. Hypothalamic-pituitary causes of pubertal failure (low gonadotropins)
    - 1. Congenital disorders (see Table 411-2)
    - 2. Acquired disorders
      - a. Pituitary tumors
      - b. Hyperprolactinemia
  - E. Gonadal causes of pubertal failure (elevated gonadotropins)
    - 1. Klinefelter's syndrome
    - 2. Bilateral undescended testes
    - 3. Orchitis
    - 4. Chemotherapy or radiotherapy
    - 5. Anorchia
  - F. Androgen insensitivity

Abbreviations: CNS, central nervous system; GnRH, gonadotropin-releasing hormone; hCG, human chronic gonadotropin; LH, luteinizing hormone.