Ambiguous Genitalia in the Newborn

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Drs Chi, Chong Lee, and Neely did not disclose any financial relationships relevant to this article.

Objectives

After completing this article, readers should be able to:

1. Describe the implications of genital ambiguity in a newborn.
2. Explain the differences in hormone production of the gonads in developing male and female fetuses.
3. Determine the assumptions that should be made during the initial evaluation of a newborn who has palpable or nonpalpable gonads.

Abstract

Efficient and accurate evaluation of the newborn who has ambiguous genitalia is required to provide appropriate medical therapy and assuage parental anxiety. Genital ambiguity usually is due to virilization of genetic females or undervirilization of genetic males who have normal gonads. Congenital adrenal hyperplasia is the most common condition leading to inappropriate virilization in females. Defects in testosterone production, metabolism, or peripheral action can lead to ambiguous genitalia in males. In any condition involving ambiguous genitalia or question of sex assignment, a karyotype should be obtained within 24 hours of delivery. Parents should be apprised of the situation in a professional manner with the appropriate level of detail; sex assignment should be withheld until sufficient data are gathered to make an accurate diagnosis. Families can be counseled with the latest available information and resources to make the best decisions for their individual situations.

Introduction

The evaluation of a newborn who has ambiguous genitalia can present a diagnostic challenge to the physician. An efficient and accurate evaluation is needed to provide appropriate medical therapy to the infant and to assuage parental anxiety. In general, newborns who have ambiguous genitalia require input from a multidisciplinary team consisting of the primary physician, pediatric endocrinologist, geneticist, surgeon, and social worker. Parents should be updated frequently as information becomes available and provided with psychological support throughout the hospitalization.

Genital ambiguity usually is due to virilization of genetic females or undervirilization of genetic males who have normal gonads (Fig. 1). Less common are disorders of sexual differentiation that involve gonadal dysgenesis (Table). In females, congenital adrenal hyperplasia (CAH), specifically 21-hydroxylase deficiency, is the most common condition leading to inappropriate virilization. CAH newborn screening is now standard in most of the United States. In males, defects in testosterone production, metabolism, or peripheral action can lead to ambiguous genitalia. It is important to differentiate undervirilization in the newborn male from an isolated urogenital defect or syndrome of multiple congenital anomalies.

Development of the Reproductive System

Sexual differentiation begins at 6 to 7 weeks of gestation. After the first trimester, internal genital tracts are unresponsive to hormonal stimulation, and midline fusion and external

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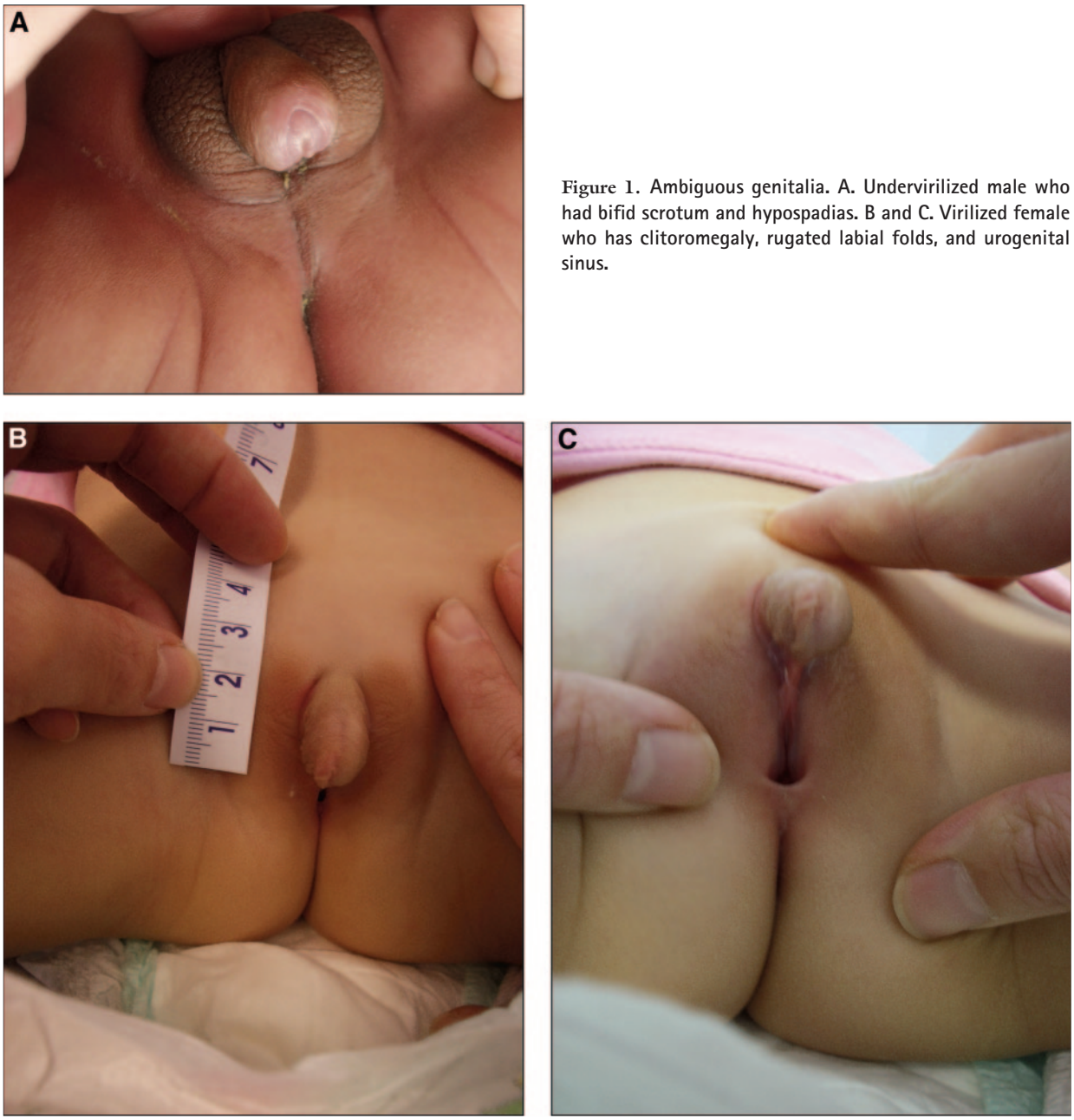

Figure 1. Ambiguous genitalia. A. Undervirilized male who had bifid scrotum and hypospadias. B and C. Virilized female who has clitoromegaly, rugated labial folds, and urogenital sinus.

genital formation are complete, except for continued phallic responsiveness to androgen. During embryogenesis, the fetus contains both female (müllerian) and male (wolffian) genital ducts. Müllerian ducts give rise to the fallopian tubes, uterus, and the upper one third of the vagina; wolffian ducts develop into the vas deferens, epididymis, and seminal vesicles. The "default" pathway of the bipotential gonad and internal structures is female. However, the presence of the sex-determining region Y gene (*SRY*) in males activates a cascade of events that culminates in differentiation of the gonad as a testis. Two key hormones, testosterone and müllerian inhibiting substance (MIS), also called anti-müllerian hormone, are produced by the testis and stimulate wolffian duct differentiation and müllerian duct regression, respectively. Testicular testosterone production initially is driven by placental human chorionic gonadotropin (hCG), which subsequently is replaced by fetal pituitary gonadotropins

Table. Causes of Ambiguous Genitalia

Virilization of Female Infant
Excessive Androgen Production
• Congenital adrenal hyperplasia (CAH)
21-alpha hydroxylase deficiency
11-beta hydroxylase deficiency
3-beta hydroxysteroid dehydrogenase deficiency
Defects in Androgen Metabolism
• Placental aromatase deficiency
Maternal Hyperandrogenism
• Maternal androgen production
Luteoma of pregnancy
Adrenal tumor
Untreated CAH
• Progestational agents
Undervirilization of Male Infant
Defects in Testosterone Production
• Leydig cell hypoplasia/agenesis
• Defects in testicular and adrenal steroidogenesis
Steroid acute regulatory (StAR) protein deficiency
3-beta hydroxysteroid dehydrogenase deficiency
17-alpha hydroxylase/17,20 lyase deficiency
17-beta hydroxysteroid dehydrogenase (ketosteroid reductase) deficiency
Defects in Testosterone Metabolism
• 5-alpha reductase deficiency
Defects in Testosterone Action
• Androgen insensitivity syndrome
Exogenous Estrogen/Progestin Exposure
Genetic Disorders of Sexual Differentiation
Gonadal Dysgenesis
• 45,XO (streak ovaries)
• 46,XX gonadal dysgenesis
• 46,XY complete and partial gonadal dysgenesis
• 45,X/46,XY mixed gonadal dysgenesis
• 47,XXY (seminiferous tubular dysgenesis)
True Hermaphroditism
Sex Reversal
• XX males ± SRY
• XY female ± SRY
Smith-Lemli-Opitz Syndrome
DAX1 mutations
WT1 mutations

after the first trimester, both acting through the luteinizing hormone (LH) receptor. Local conversion of testosterone to dihydrotestosterone (DHT) by the enzyme 5-alpha reductase leads to fusion of the labioscrotal folds and formation of the scrotum and penis, both critical events occurring in the first trimester.

In females, the labioscrotal folds remain unfused, and the clitoris does not enlarge without DHT. Absence of testosterone and MIS leads to involution of the wolffian ducts and differentiation of the müllerian ducts into female internal genitalia. Both X chromosomes are needed to develop normally differentiated and functional ovaries. Females who have X chromosome deletion (Turner syndrome) have abnormal gonadal differentiation and oocyte loss, leading to streak gonads.

Virilization of the Female Infant

The clinical manifestation of virilization in the female newborn varies from mild clitoromegaly to complete labial fusion and urogenital sinus, depending on the time, duration, and severity of testosterone exposure. Virilization during the first trimester results in some degree of labial fusion (ratio of the distance from anus to fourchette/anus to base of clitoris >0.5). Testosterone exposure throughout or toward the end of pregnancy leads to further enlargement of the clitoris. Internal genital tracts develop into normal female structures due to the absence of MIS.

The most common form of CAH is 21-hydroxylase deficiency, which leads to increased androgen production and decreased cortisol and aldosterone synthesis (Fig. 2). In classic salt-losing 21-hydroxylase deficiency, female infants are born with ambiguous genitalia and may appear to be males with undescended testes. Unless identified and treated appropriately, such infants present with life-threatening salt-wasting crisis at about 2 to 3 weeks after birth. Most newborn screening programs include a 17-hydroxyprogesterone measurement to screen for 21-hydroxylase CAH in an effort to decrease mortality associated with acute adrenal insufficiency. Less common enzyme deficiencies are of 11-alpha-hydroxlyase, which results in hypertension without salt loss, and 3-beta-hydroxysteroid dehydrogenase.

Placental aromatase deficiency is a rare autosomal recessive disorder leading to an elevated androgen concentration in the female fetus. Aromatase normally converts testosterone to estradiol and androstenedione to estrone, limiting the concentration of androgens in the fetus (Fig. 2). Deficiency of aromatase activity in the placenta results in both progressive maternal virilization during pregnancy and severe virilization of the female newborn. Other uncommon causes of virilization include maternal adrenal or ovarian tumor and maternal exposure to progestational agents or synthetic androgens during pregnancy.

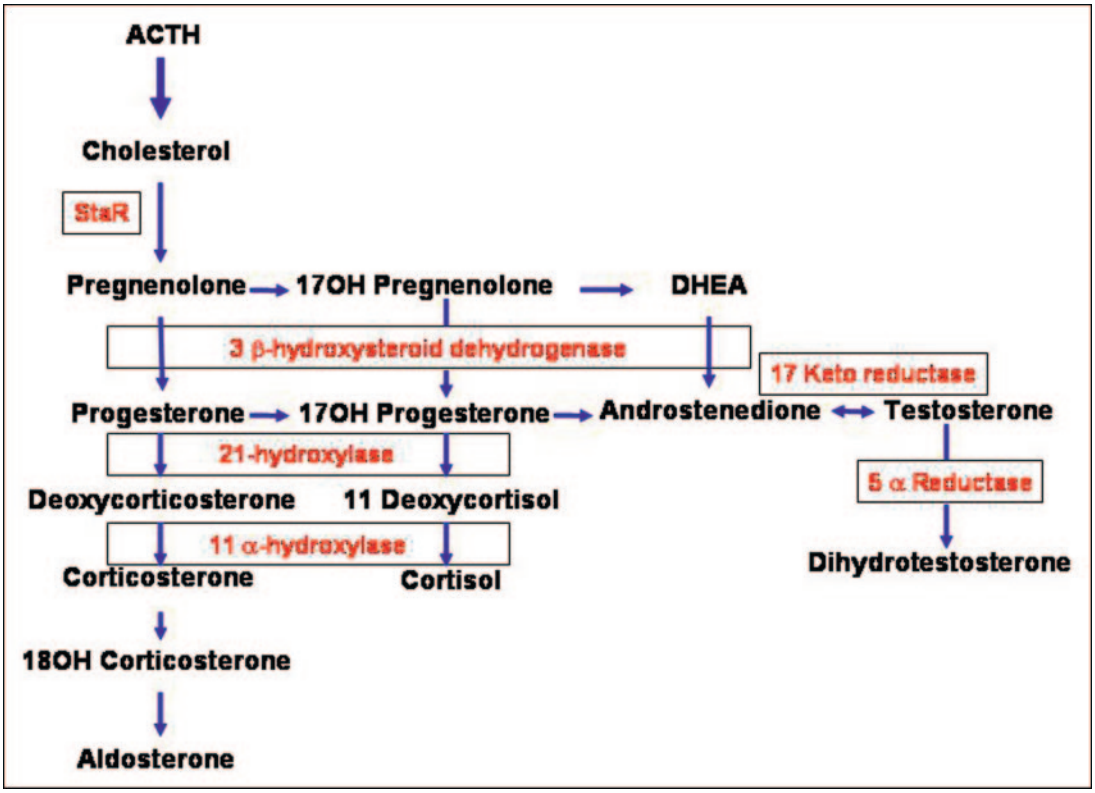

Figure 2. Adrenal and gonadal steroidogenesis pathway.

Undervirilization of the Male Infant

Incomplete masculinization of the male infant occurs during the critical stages of sexual differentiation and is due to defects in testosterone production, decreased testosterone metabolism, or insensitivity to testosterone action (Table). The critical period for external virilization is within the first 12 weeks of gestation. In the first trimester, testosterone production from the Leydig cell is driven by placental hCG. Fetal pituitary LH secretion increases during the second trimester and stimulates phallic enlargement and testicular descent. Absence of testosterone or defects in testosterone action leads to a wide array of undervirilized phenotypes. Diagnosis of ambiguous genitalia in the male newborn usually is more complicated than in the female because of difficulty in differentiating isolated urogenital defects from a hormonal disorder.

Palpation of one or both testes in the scrotum or inguinal region generally indicates a male karyotype and should guide the evaluation toward an undervirilized male. DHT is the primary hormone responsible for differentiation of male external genitalia and is converted from testosterone by 5-alpha reductase. Decreased 5-alpha reductase activity leads to varying degrees of ambiguity, ranging from a blind vaginal pouch to mild hypospadias with bifid scrotum. Similarly, partial androgen resistance results in a spectrum of genital ambiguity. The androgen receptor is located on the X chromosome and, therefore, transmission is X-linked. Family members who have the same androgen receptor mutation can have different phenotypes, which makes partial androgen insensitivity syndrome challenging to diagnose. Mutations that lead to complete androgen insensitivity syndrome, however, result in normal female external genitalia. Müllerian structures (eg, the uterus) typically are absent, and testes may be intra-abdominal or in the inguinal canal. Complete androgen insensitivity syndrome often is not diagnosed until late puberty in the context of primary amenorrhea and the absence of evidence of androgen activity (eg, pubic hair).

Decreased testosterone production generally is attributed to an enzymatic defect leading to impaired steroidogenesis in the testis and adrenal gland. A rarer cause is hypoplasia or agenesis of the Leydig cells of the testis caused by mutations of the LH/hCG receptor. Major enzymatic blocks leading to insufficient testosterone production are shown in Figure 2. To differentiate the remaining enzymatic defects, steroid profiles are needed

to determine which precursors are elevated or diminished. Classic 3-beta hydroxysteroid dehydrogenase deficiency leads to impaired conversion of delta-5 intermediates—pregnenolone, 17-OH pregnenolone, and DHEA—to progesterone, 17-OH progesterone, and androstenedione, respectively. Increased concentrations of aldosterone precursors in the setting of decreased cortisol and testosterone production suggests 17-alpha hydroxylase/17,20 lyase deficiency. The enzyme 17-beta hydroxysteroid dehydrogenase, also known as 17-ketosteroid reductase, is expressed primarily in the testis and is responsible for conversion of androstenedione to testosterone. StAR protein is responsible for transporting cholesterol across the outer mitochondrial membrane and converting it to pregnenolone. Therefore, deficiency of the StAR protein results in decreased production of all adrenal and gonadal steroids. Affected male infants are born with female external genitalia and a blind vaginal pouch. Internal reproductive structures are still masculine, and müllerian remnants are absent due to unaffected MIS production. Abdominal imaging shows enlarged, lipid-laden adrenal glands caused by cholesterol buildup.

Genetic Disorders of Sexual differentiation

A complete review of genetic disorders of sexual differentiation is beyond the scope of this article. We focus primarily on gonadal dysgenesis, including the diagnosis of true hermaphroditism. The two most common forms of gonadal dysgenesis, nonmosaic Turner syndrome (45,XO) and Klinefelter syndrome (47,XXY), are not associated with ambiguous genitalia. Girls who have chromosomal makeup 45,XO have phenotypic characteristics, including short stature, webbing of the neck, widely spaced nipples, aortic coarctation, low posterior hairline, and cubitus valgus. Although affected girls have normal external female genitalia, absence of the second X chromosome leads to streak gonads and pubertal failure requiring estrogen supplementation. Primary gonadal failure is also a component of Klinefelter syndrome in which boys have one or more additional X chromosome(s). Boys virilize at puberty but have delayed pubertal progression. The characteristic findings are small testes, moderate androgen deficiency, and azoospermia. Testicular failure is progressive due to hyalinization of the seminiferous tubules. 45,X/46,XY mosaicism results in a wide spectrum of phenotypes, including males with mixed gonadal dysgenesis. Affected patients typically have a unilateral testis paired with a streak gonad.

True hermaphroditism is defined by the presence of both testicular and ovarian tissue. External genitalia are often ambiguous, although rarely a newborn may appear structurally female or male. Ovaries maintain their intra-abdominal anatomic location; testes and ovotestes may descend. Most cases have a 46,XX karyotype with undetectable Y chromosomal material. The diagnosis may be suspected from biochemical evidence showing functional testicular tissue despite the possible presence of müllerian structures on imaging. Definitive diagnosis is based on histologic evidence showing the presence of seminiferous tubules and ovarian follicles on biopsy.

Diagnosis

The assessment and treatment of newborns who have ambiguous genitalia requires urgency and sensitivity. The baby should be transferred to a referral center that has experience and resources to manage intersex conditions. Expeditious diagnosis is important for medical reasons, namely, to treat glucocorticoid deficiency and salt wasting if present, and for the psychological well-being of the family. A detailed history should be obtained from the parents, including family history, exposure to medications during pregnancy, and infant deaths in the family. Consanguinity may be a factor in autosomal recessive conditions such as CAH. In unexplained cases of masculinized females, the mother should be evaluated for signs of virilization.

A complete physical examination should be performed to assess for any dysmorphic features. The genital examination should involve careful palpation for gonads. Palpation of the scrotum/labial area and along the inguinal canal should be carried out by sliding two to three fingers with varying pressure along the groin. A frog-leg position may facilitate palpation. External genitalia should be inspected. Phallic length should be measured while fully stretched, with the ruler pressed against the pubic ramus to the tip of the glans. Lengths less than 2.5 cm in a term male infant may be considered micropenis. The penis also should be examined carefully for hypospadias. A clitoris that appears large can be assessed by width, with those having a width less than 6 mm considered normal. The presence of a vaginal orifice indicates the absence of androgen effect. Masculinization of the vaginal orifice can vary from labial fusion to the formation of a common urogenital sinus.

In any condition involving ambiguous genitalia or question of sex assignment, a karyotype should be obtained within 24 hours of delivery. The cytogenetics laboratory should be informed of the urgent nature of determining sex; high-resolution studies are not necessary in this situation. Testosterone concentration, along with an extra sample of blood that can be used for future studies, should be obtained in the first 24 hours after

delivery. Although rectal examination has been suggested as a means of locating the uterus, this may be avoided by performing pelvic ultrasonography. Ultrasonography is fast, noninvasive, and readily available at most institutions. In addition to the uterus, ultrasonography may assist in locating gonadal tissue, although it is not a definitive test in either case.

If gonads are not palpable, the evaluation should be directed toward the diagnosis of a virilized female. The newborn screen and biochemical panel for CAH should be obtained. In addition, renin should be measured. While waiting for confirmatory diagnosis, electrolytes should be monitored to rule out salt wasting. Testing for the primary enzymatic defects of CAH should be conducted after 24 hours, including measurement of 17-OH progesterone, 17-OH pregnenolone, progesterone, androstenedione, DHEA, deoxycorticosterone, 11-deoxycortisol, testosterone, and cortisol. A cosyntropin stimulation test also should be performed, measuring cortisol concentrations before and after administration. 17-OH progesterone values are somewhat higher in preterm infants. In the context of a stable preterm infant who has no electrolyte abnormalities or abnormal genitalia, an abnormal newborn screen result can be followed by repeated 17-OH progesterone measurements without therapy.

Palpable gonads suggest the presence of testicular tissue, and evaluation should be directed toward the diagnosis of an undervirilized male, a situation involving inadequate testosterone production or androgen receptor insensitivity. Further laboratory testing includes measurement of testosterone, DHT, LH, and follicle-stimulating hormone in the first 24 hours; inhibin B and MIS to evaluate for testicular function; and fluorescence in situ hybridization for *SRY*. If the testosterone value is normal or high, genetic testing for androgen insensitivity should be considered. Low DHT values in the face of normal testosterone concentrations suggest 5-alpha reductase deficiency. Magnetic resonance imaging of the pelvis or gonadal biopsy may be indicated if there is suspicion of gonadal dysgenesis.

Management

When ambiguous genitalia are recognized after delivery, parents should be apprised of the situation in a professional manner with the appropriate level of detail. The physical examination findings can be shown objectively, and the physician can inform the parents at the same time of the importance of further testing and consultation for medical reasons. Based on initial findings, the next step should involve consultation with appropriate pediatric specialists, including endocrinology, urology, and genetics.

Often the first question asked by friends and family after birth is the sex of the baby. Accordingly, there is pressure for the parents and medical team to know how to address this question. Nevertheless, sex assignment should be withheld until enough data have been gathered to make a well-informed decision. One piece of data, even karyotype, may not be sufficient for an accurate diagnosis. Therefore, sex assignment should be deferred until the initial diagnostic evaluation has been completed and clarified the condition. A suggested approach is to tell the parents, "The genitalia are incomplete in their development, and we need some time to perform tests to help us to assess the sex of your baby." Naming of the baby also should be deferred while undergoing diagnostic evaluation. Although a diagnosis may not be possible until a few days after delivery, a policy of sharing available information generally is appreciated by the family.

Social workers can assist families during the stressful time of diagnostic evaluation. In some situations, a psychiatrist or psychologist may aid the family in coping. It is also important to inform and educate other staff members, such as nurses taking care of the baby, so families receive appropriately sensitive care.

Genitoplasty remains controversial and includes surgery of various structures. Current surgical techniques in clitoroplasty allow for sparing of nerves and erectile tissue to preserve later function. Vaginoplasty can be performed in the newborn period, but some groups advocate waiting until puberty, when vaginal dilatations are more feasible, to prevent stenosis. Labioplasty is performed at the time of vaginoplasty to create normal-appearing female external genitalia.

Once a diagnosis has been established, male or female sex can be assigned to most babies. However, in a minority of patients, particularly those who have partial androgen insensitivity, a question of appropriate sex assignment may remain. This controversial area is still under discussion by the medical community. Individual families can be counseled with the latest available information and resources to make the best decisions for their situation. Following diagnosis, genetic counseling is another important aspect of care that should be provided to the family.

Suggested Reading

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NeoReviews Quiz

8. Sexual differentiation begins at 6 to 7 weeks of gestation in humans. Of the following, the cascade of events that culminate in the differentiation of the gonad as a testis is initiated by:

   A. 5 alpha-reductase.
   B. Human chorionic gonadotropin.
   C. Müllerian inhibiting substance.
   D. Sex-determining region Y gene.
   E. Testosterone.

9. Genital ambiguity usually results from virilization of genetic females or undervirilization of genetic males who have normal gonads. Of the following, the *most* common cause of virilization of genetic females is:

   A. Fetal congenital adrenal hyperplasia.
   B. Luteoma of pregnancy.
   C. Maternal adrenal tumor.
   D. Maternal exposure to progestational agents.
   E. Placental aromatase deficiency.

10. Undervirilization of genetic males who have normal gonads usually results from defective testosterone production, decreased testosterone metabolism, or insensitivity to testosterone action. Of the following, the feature that is commonly *not* seen in undervirilization of genetic males is:

    A. Hypospadias.
    B. Cryptorchidism.
    C. Bifid scrotum.
    D. Müllerian duct remnants.
    E. Blind vaginal pouch.

11. A term newborn presents with ambiguous genitalia characterized by micropenis (phallic length of 0.8 cm) and bilateral undescended testes. You suspect a hormonal defect. Of the following, stimulation of phallic enlargement and testicular descent is *most* attributed to:

    A. Fetal growth hormone.
    B. Fetal pituitary luteinizing hormone.
    C. Maternal androstenedione.
    D. Maternal medroxyprogesterone.
    E. Placental human chorionic gonadotropin.

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