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Review: Hormonal Disturbance in Ambiguous Patients

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Abstract: Sex development in humans is divided into two sequential steps: sex determination and sex differentiation. Sex determination refers to the expression of gene networks that direct the development of undifferentiated bipotential gonads into either testes or ovaries. Once developed, testes and ovaries secrete hormones that promote further sex differentiation of the body throughout embryonic development and adulthood. Mutations have been identified in genes that control both steps, leading to Disorders of sex development-DSD. A thorough history, physical examination, and appropriate diagnostic testing are needed to identify the underlying etiology. DSD are congenital conditions in which development of chromosomal, gonadal, or anatomic sex is atypical. DSD are chronic medical conditions collectively affecting ~1% of the population, frequently requiring life-long care by multiple specialists, and carrying a significant public health burden. Some of DSD are associated with life-threatening events, such as adrenal crises in congenital adrenal hyperplasia. DSD are also associated with increased infertility, cancer, gender dysphoria risks, psychosocial distress, and pervasive challenges to health-related quality of life for patients and families. DSD are broadly classified into three categories: sex chromosome DSD, 46, XY DSD, and 46, XX DSD and are further classified according to the type of gonad found in the patient (ovary, testis, ovotestis). Currently, known etiologies include disorders of gonadal development and disorders in androgen synthesis or action, and are considered Mendelian. Ambiguous genitalia is defined as a condition in which there is difficulty in assigning sex of an individual based on the appearance of external genitalia. Possible genital presentations are: male or female regular, males with ambiguous features and hypovirilization (micropenis, lack of scrotal fusion, incomplete testicular descent, hypospadia) or females with ambiguous features and virilization (clitoromegaly, labio-scrotal fusion).

Keywords: Ambiguous, DSD, hormonal disturbance, sex determination, sex differentiation.

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Introduction:

Disorder of sex development (DSD) is a group of rare conditions that are generally characterized by an abnormality of the chromosomal, gonadal or phenotypic features that typically define sex development. Such conditions usually present with atypical genitalia in the newborn period or as delayed puberty in an adolescent (1). Ambiguous genitalia are a term to describe how a baby's genitals look different than the genitals of most other boys and girls (2).

It has been estimated that in around 1 in every 4500 live births, babies have genitalia that is ambiguous enough to not be able to assign a sex immediately (3), but some types of genital abnormalities may occur in as many as 1 in every 300 births .True cases of absolute ambiguous genitalia are rare, but it is likely that the pediatric nurse will come across a case in their career (4). In order to understand the wide variety of genital ambiguity it is crucial

to be aware of the regular sexual differentiation process. The differentiation into each gender is dependent on the exact sequence of events occurring at specific time points, dependent on chromosomal, hormonal and hormonal receptor function. While the genetic sex and the gonadal differentiation are determined by the sex chromosomes, the phenotype is dependent on the influence of the hormonal secretions from the testes (5). Families of ambiguous child are anxious about the sex of their child, and societal pressure for gender identification is great. Lingering doubts of gender identity may affect the parent-infant relationship significantly. Evaluation and diagnosis must proceed rapidly to determine the sex of rearing and to initiate necessary medical interventions. From the moment a child who has ambiguous genitalia is born, consultation with the pediatrician, pediatric endocrinologist, geneticist and surgeon should ensue (6).

Evaluating a newborn with ambiguous genitalia can be challenging and requires prompt investigation to determine the underlying cause. A multidisciplinary team approach including neonatology, endocrinology, gynecology, urology, genetics, ethics, social work, and psychology is recommended when evaluating infants with ambiguous genitalia (7). As already mentioned it is important that a newborn with ambiguous genitalia be evaluated in a timely manner by a multi-disciplinary team, preferably one with experience in DSD evaluation and treatment. The reason for this is twofold: (1) to assign an appropriate sex of rearing to the infant based on the etiology of the condition and associated medical and psychosexual outcomes, and (2) to detect any underlying life-threatening disorder if present. The etiology underlying genital ambiguity in newborns impacts several aspects of management including recommendations for sex of rearing, assessing risk for gonadal malignancy, and the need to replace hormones such as cortisol, aldosterone and/or steroid hormones. Thus, it is important to initiate an appropriate work-up expeditiously (8). Surgical intervention (gonadoplasty, vaginoplasty, gonadectomy) must be delayed until a clear diagnosis is established and should be performed only by an experienced surgeon. The benefits of any surgical or medical procedure must clearly outweigh the risks, and any unnecessary procedures should be delayed until the child is old enough to make an informed decision. Once a sex-assignment decision is made, the child and family will need long-term follow-up with medical providers who are experienced with DSD. Ongoing education is essential, because many of these patients will require surgery and hormonal therapy. It is important to assess the patient's and parents' satisfaction with the sex-assignment decision. Psychological assessment at regular intervals is recommended to screen for mental health issues such as gender dysphoria and to provide support for families (9).

Normal Sexual Differentiation:

Critical to understanding ambiguous genitalia and to determining the sex of rearing is a basic knowledge

of sexual differentiation. The innate tendency of the bipotential fetus is to develop as a female (10). Without influence from a cascade of events initiated by the testes-determining factor, now known as the sex determining region of the Y chromosome (SRY), the internal and external genitalia will be female(11). Complete newborn male differentiation and development requires:

1) The action of the SRY and downstream genes.
2) Testicular production of both Antimullerian hormone (AMH), also called mullerian inhibiting substance (MIS) and testosterone.
3) Normal gonadotropin production by the hypothalamic-pituitary axis (during the second and third trimester).
4) Conversion of testosterone to dihydrotestosterone (DHT) by *5 a* reductase.
5) end organ response to androgens.

The bipotential gonad in humans begins its development close to the mesonephros (primitive kidney). By approximately day 42 of gestation, the germ cells that originate in the yolk sac endoderm migrate into the gonadal ridge: the bipotential gonad then is formed from three sources: mesodermal cells of the coelomic epithelium, mesodermal cells from the underlying mesenchyma and germ cells originating in the yolk sack or endoderm (12). The primordial germ cells are the progenitors of the spermatogonia of the testis and the oogonia of the ovary. The mesodermal cells from the coelomic epithelium form the Sertoli cells in the male and the granulosa cells in the female while the mesenchyma gives rise to the Leydig cells in the male and the theca and Stroma cells in the female (13). The Sertoli (AMH-producing) and Leydig (testosterone-producing) cells are found in the testis: granulosa and theca cells are found in the ovary. By day 43 to 50 of gestation, the bipotential gonad differentiates into a testis in the presence of SRY: in its absence, an ovary is formed (14). The SRY gene product (a transcription factor) binds to specific DNA sequences that regulate transcription of other genes affecting testicular differentiation by day 43 to 50 of gestation, the developing fetus contains both female (mullerian) and male (wolffian) genital ducts making chromosomal male and female fetuses phenotypically indistinguishable (15). The mullerian ducts have the potential to develop into fallopian tubes, uterus, and the upper one third of the vagina: wolffian ducts differentiate into the vas deferens, epididymis, and seminal vesicles (Figure 1). Testicular secretion of AMH leads to regression of the female mullerian structures, and secretion of testosterone leads to full differentiation and stabilization of the male internal and external genitalia (16). First trimester testicular testosterone production is primarily under the control of placentally derived human chorionic gonadotropin (HCG): fetal pituitary gonadotropins play a central role in the second and third trimesters (16). Testosterone is converted by 5 *a* reductase in the genital skin to its more active metabolite DHT, which is responsible for forming the scrotum and penis from the labioscrotal and urethral folds by the end of the first trimester (Figure 2).

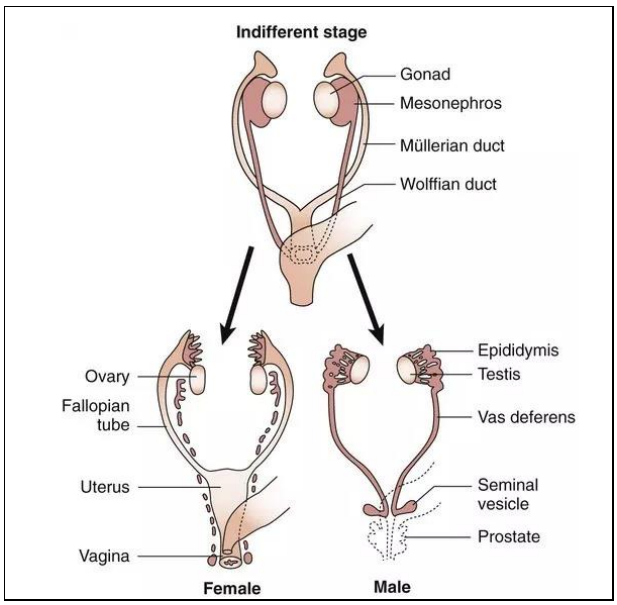

Figure (1): Diffreiitiationof of internal genitalia (16).

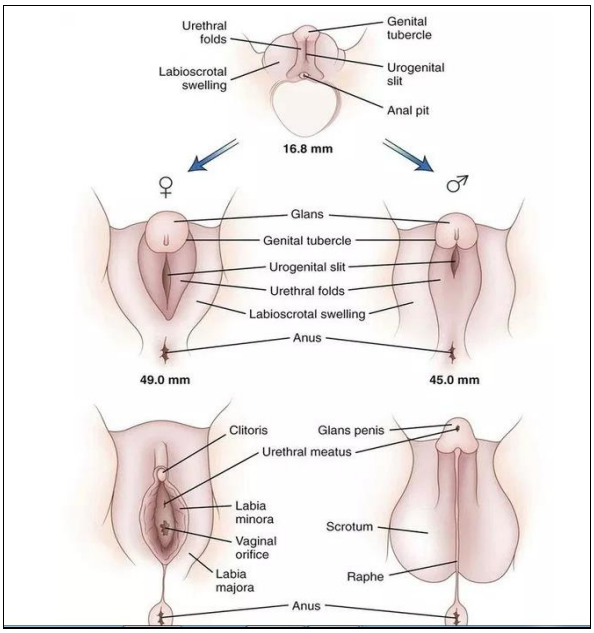

Figure (2): Differentiation of external genitalia (16).

Fusion of the labioscrotal folds to form a scrotum occurs during the critical first trimester. In the female fetus, because there is no AMH, the mullerian ducts complete their development: the wolffian genital ducts undergo involution (17). In the absence of DHT, the labioscrotal folds do not fuse and the clitoris does not enlarge. Even in the absence of SRY, the presence of two intact X chromosomes is a prerequisite to the formation of normally differentiated ovaries (18) . Infact X chromosome deletion analysis has demonstrated that deletions on either the long or short arm may lead to abnormal gonadal differentiation germ cell loss and oocyte degeneration

ultimately yielding streak gonads (Turner syndrome). Despite the gonadal abnormalities and characteristic phenotypic appearance of patients who have Turner syndrome. The external and internal genitalia are completely normal demonstrating the critical active role that SRY, AMH, alid testosterone play in directing male genital differentiation (16).

Male, Female Genitalia and Ambiguous Genitalia:

To appreciate the various genital phenotypes associated with ambiguity, it is important to first define typical genitalia for males and females, respectively. A full-term male infant is expected to possess bilateral testicles that are descended, complete formation of scrotal folds including midline fusion, a typical size penis (average penile length is 3.5 ± 0.4 cm, for a full term infant (19) including well-formed corporal bodies and a urethral meatus located at the tip. An infant with bilateral cryptorchoidism, bifid scrotum and hypospadias, or isolated penoscrotal hypospadias, should be investigated for DSD. Micropenis by itself, if both testes are descended and normal in size is not considered a presentation of ambiguous genitalia (Table 1). Similarly, distal hypospadias with no other atypical genital features in males is not usually indicative of DSD (20). In contrast, a full-term female infant is expected to have bilateral separation of the labial folds, no palpable gonads, and separate urethral and vaginal openings. The average clitoral length and width for a full term infant girl born in U.S. is 4.0 ± 1.24 mm and 3.32 ± 0.78 mm, respectively (19).Labial fusion or palpable gonads in what appears to otherwise be typical female external genitalia should be investigated further. Perceived clitoromegaly is not usually associated with an underlying DSD if the newborn girl was born prematurely(19).

Table (1): Phenotypic characteristic of normal full term male, female genitalia and ambiguous genitalia.

	Appearance of Genitalia
Full Term Male	Bilateral testicles that are descended, Complete formation of scrotal folds with midline fusion, Average penile length of 3.5 ± 0.4 cm
Full Term Female	Bilateral separation of labial folds ,No palpable gonads, Separate urethral and vaginal openings
Newborn with Ambiguous Genitalia	Bilateral cryptorchidism in a male, Bifid scrotum with hypospadias in a male, Penoscrotal hypospadias in a male, Labial fusion in a female ,Palpable gonads in a female

In fact sex of an individual is determined by a conglomeration of factors such as chromosomal pattern (XX vs. XY), nature of gonads (ovary vs. testis), predominance of circulating sex hormones (estrogen vs. androgen), topographic anatomy of genitalia and secondary sexual characters (21). Usually genital appearance and phenotype are influenced by sex hormones secreted from gonads which in turn are genetically programmed by chromosomal arrangement (22). Therefore harmony between various determinants of sex is presumed and individuals are neatly categorized into

male or female. Problem arises when there is discordance between the various factors. For example, in complete androgen insensitivity syndrome (CAIS) the individual is chromosomally a male with 46XY and has bilateral testes which secrete androgen; but due to receptor deficiency circulating testosterone fails to effect male phenotype, consequently the individual will externally look like a female with fully developed breasts and labial folds (23). Contrastingly, in congenital adrenal hyperplasia (CAH) the individual is genetically a female with 46XX and gonads are typically ovaries; but due to deficiency of steroidogenic enzymes excess testosterone is produced thereby leading to virilization. Therefore, girls with CAH will have fused labia mimicking scrotum and hypertrophied clitoris mimicking penis(24). Permutation of sex determining factors (Table 2) suggests that sex is a spectrum rather than two neatly packed compartments (25). Conventional male and female is at the extremes of the spectrum with innumerable shades of sexes lying between them. Surgical reduction of the enlarged clitoris in CAH and excision of testes in CAIS are basically attempts of trimming the individual to suit one of the two artificial categories.

Table (2): Clinical Manifestation of Discordance among Sex Determinants(25).

Chromosomal sex[1]	Gonadal sex[2]	Morphological sex[3]	Clinical Manifestation
Male	Male	Male	Normal male
Male	Male	Incomplete Male	Aphallia, Exstrophy, Micropenis *
Male	Male	Female	Defective synthesis of androgens (eg. 5-α reductase deficiency, 17β HSD) Receptor insensitivity to androgens (eg. Androgen Insensitivity Syndrome†)
Male	Female	Male	True Hermaphroditism
Male	Female	Female	SRY Deletion Syndrome / 46XY Pure Gonadal Dysgenesis (Swyer Syndrome)
Female	Male	Male	XX Male Syndrome (de la Chapelle syndrome)
Female	Mixed	Female	Ovotesticular DSD
Female	Hypoplastic	Female	46XX pure gonadal dysgenesis / Bilateral Steak Ovaries
Female	Female	Male	Congenital adrenal hyperplasia
Female	Female	Female	Normal female

Epidemiology of DSD:

There are no clear estimates of the incidence rate of subjects presenting with ambiguous genitalia at birth, and only a proportion of them present a major challenge regarding male or female assignment (26). However, it has been estimated to be approximately 1 in 4,500–5,500. Data are not available to determine the exact frequency of specific DSDs, while only a small fraction of those with DSDs require extensive multidisciplinary assessment to reach a recommendation for gender assignment (27). The incidence rate among subjects with 46, XY to have a DSD has been estimated to be 1 in 20,000 births. Ovotesticular DSDs have been estimated to occur in 1 of 100,000

live births. The frequency of testicular or mixed gonadal dysgenesis is estimated at 1: 10,000. The worldwide incidence of 46,XX DSD, consisting primarily of CAH – mostly 21-hydroxylase deficiency –, has been estimated to be 1 in 14,000–15,000 live births, but it varies by regions because of ethnic differences in gene mutation frequency (28). CAH and mixed gonadal dysgenesis constitute about half of all DSD patients presenting with genital ambiguity. When all congenital genital anomalies are considered, including cryptorchidism and hypospadias, the rate may be as high as 1: 200 to 1: 300 (29). Among patients with hypospadias and cryptorchidism, currently the diagnosis of specific DSD conditions is generally limited to those with proximal hypospadias with cryptorchidism. The overall incidence estimations also include those with Klinefelter syndrome (estimated in 1: 500 to 1: 1000 live births) and Turner syndrome (about 1: 2,500 live births). These known estimates hopefully provide a useful perspective (30).

Classification of DSD:

The current system for the classification of DSD was introduced in the Chicago Consensus in 2005 (19). There are three broad groups: 46, XX DSD, 46, XY DSD and sex chromosome DSD.

2.4.1.46, XX DSD:

46, XX DSD encompasses disorders of gonadal development, such as gonadal dysgenesis and disorders secondary to androgen excess. Androgen excess during pregnancy may be endogenous (secondary to an adrenal adenoma, dermoid cyst, Sertoli-Leydig tumor, sex cord stromal tumor or metastatic carcinoma) or exogenous (secondary to danazol, progestins or potassium sparing diuretics (31).Exogenous steroids taken during pregnancy can also cause posterior fusion of the labia, clitoral enlargement, and increased degrees of androgenization (32). The commonest known genetic condition that leads to 46, XX DSD is CAH due to 21α-hydroxylase deficiency and this occurs in approximately one in 10,000 to one in 14,000 infants. Rarer conditions include 46, XX testicular DSD which refers to a male with testes and male genitalia, and 46, XX Ovotesticular DSD which refers to individuals that have both ovarian and testicular tissue in the gonads, usually as ovotestes, but less commonly as separate gonads (33).

The 46, XX DSDs can be subdivided into either disorders of androgen excess or disorders of ovarian development. These conditions are summarized in Table 3 (34). The most common cause of genital ambiguity in a 46, XX female is congenital adrenal hyperplasia. The CAH is an autosomal recessive condition with a defect in the synthesis of cortisol. The most common cause of CAH is 21-hydroxylase deficiency, which accounts for 95% of cases; however, 11- hydroxylase deficiency and 3b-hydroxysteroid dehydrogenase (HSD) deficiency can also cause virilization of a female infant. The block in the production of cortisol leads to shunting of cortisol precursors toward the androgen pathway, which leads to virilization of the external genitalia. 46, XX individuals with CAH have normal

female internal genital development (35). Females born with CAH can present with a wide range of genital ambiguity, from mild clitoromegaly to phenotypicappearing males with empty scrotal sacs. Infants with classic 21-hydroxylase deficiency also have deficient aldosterone production and can present with a salt wasting crisis (hyponatremia, hyperkalemia, hypoglycemia, hypovolemia, shock). Aromatase deficiency is a condition in which patients are unable to convert androgen precursors to estrogen. Aromatase deficiency in the placenta leads to virilization of both the mother and the fetus (36).

Maternal causes of androgen excess that can lead to virilization of a 46, XX infant include ingestion of androgens or progestins, virilizing adrenocortical tumors, ovarian tumors, or Luteomas. Disorders of ovarian development in a 46, XX individual that can present with ambiguous genitalia include ovotesticular DSD and 46, XX testicular DSD. In ovotesticular DSD, the gonad contains both ovarian and testicular tissue. Patients with ovotesticular DSD can present with a spectrum of genital ambiguity as well as both male and female internal duct structures (37). In 46, XX testicular DSD, both of the gonads develop into testes, and these patients do not have ovarian or müllerian components. These patients can present with mild genital abnormalities including hypospadias and undescended testes, though in many cases they are phenotypically normal males who present later in life because of infertility (9).

Table (3): 46, XX Disorder of Sexual Development (DSD) That Can Present With Ambiguous Genitalia (9, 34).

Disorders of androgen excess	
Congenital adrenal hyperplasia	Can range from mild clitoromegaly to phenotypicappearing males with empty scrotal sacs
21-hydroxylase deficiency	May present with salt-wasting crisis
11-b hydroxylase deficiency	Hypertension is a feature of 11b-HSD
3b-hydroxysteroid dehydrogenase deficiency	
Aromatase deficiency	Virilization of both mother and fetus
Maternal causes • Ingestion of androgens or progestins • Virilizing adrenocortical tumors • Ovarian tumors • Luteomas	Varying degrees of virilization, important to take a thorough maternal history
Disorders of ovarian development	
Ovotesticular DSD	Spectrum of genital ambiguity Can have both male and female internal ductal structures
XX testicular DSD	Typically normal phenotypic males, although can present with mild ambiguity such as hypospadias or cryptorchidism

46, XY DSD:

46, XY DSD has three broad categories: disorders of gonadal development, disorders in androgen synthesis or action and other causes, including hypogonadotropic hypogonadism, cryptorchidism, and

isolated hypospadias (38). Girls with 46, XY DSD will most likely have androgen insensitivity syndrome (AIS), gonadal dysgenesis or a biochemical disorder of androgen synthesis (19). 46, XY DSDs can be classified further into disorders of testicular development and disorders of androgen synthesis or action. These conditions are summarized in (Table 4) (9).

Table (4): 46, XY Disorder of Sexual Development (DSD) That Can Present With Ambiguous Genitalia (9, 19,39).

Disorders of testicular development	
Gonadal dysgenesis	Wide range of undervirilization depending on degree of dysgenesis
Gonadal regression/vanishing testes	Wide range of undervirilization depending on the timing of gonadal regression in utero
XY Ovotesticular DSD	Typically present with ambiguous genitalia or severe hypospadias
Disorders of androgen action	
Androgen insensitivity syndrome	Wide range of phenotypes depending on the degree of tissue responsiveness to androgens
Disorders of androgen synthesis	
Testosterone biosynthesis enzyme defects	Wide range of undervirilization/ambiguity depending on the specific enzyme deficiency
7-dehydrocholesterol reductase deficiency	
STAR protein/P450scc deficiency	Can present with salt-wasting crisis depending on the specific enzyme defect
3b-HSD type 2 deficiency	
17a-hydroxylase/17,20 lyase deficiency	
17b-hydroxysteroid dehydrogenase type 3 deficiency	
Leydig cell hypoplasia/aplasia	Undervirilization depends on degree of Leydig cell hypoplasia
5a-reductase deficiency	Ambiguous genitalia, micropenis, hypospadias؛ spontaneous virilization can occur at puberty
Other	
• Hypogonadotropic hypogonadism	Can present with micropenis and other pituitary hormone deficiencies

Disorders of testicular development include gonadal dysgenesis, gonadal regression or vanishing testes syndrome and XY Ovotesticular DSD (40). In 46, XY complete gonadal dysgenesis (Swyer syndrome), no testicular development occurs and patients appear as phenotypic females with bilateral streak gonads and normal müllerian structures (41). Patients with 46, XY partial gonadal dysgenesis have some degree of testicular development and can present with variable internal and external phenotypes depending on how functional the testes are. Several syndromes, including camptomelic dysplasia, Frasier syndrome, and Denys-Drash syndrome, are associated with XY gonadal dysgenesis. Vanishing testes or testicular regression syndrome is a condition in which testes are absent in a 46, XY individual (42). These individuals are thought to have disappearance or regression of the testes in utero after an insult such as torsion or vascular thrombosis (43). The

appearance of the external genitalia depends on the timing of the testicular regression in relation to sexual development: testicular regression before 8 weeks of gestation results in a phenotypic female, loss between 8 and 10 weeks results in ambiguous genitalia, and loss after 12 weeks will result in normal male external genitalia (44). XY ovotesticular DSD is a condition in which an XY individual has both ovarian and testicular tissue. Patients with XY ovotesticular DSD most commonly present with ambiguous genitalia or severe hypospadias. Disorders of androgen action include complete or partial androgen insensitivity (45). These conditions result from mutations in the androgen receptor gene. The phenotype of patients with androgen insensitivity depends on the degree of tissue responsiveness to androgen activity. Patients with complete androgen insensitivity have female external genitalia with a blind vaginal pouch(46). These individuals typically present in adolescence with primary amenorrhea, though in some instances they are identified earlier because of inguinal or labial swellings containing testes.Patients with partial androgen insensitivity can present with a wide range of phenotypes, from ambiguous genitalia to phenotypic males who present with infertility in adulthood(45,46). Disorders of androgen synthesis include testosterone biosynthesis enzyme defects, Leydig cell aplasia or hypoplasia, and 5a-reductase deficiency. Enzymatic defects in the adrenal gland or testes can lead to decreased production of testosterone and cause undervirilization in 46, XY individuals. The adrenal biosynthesis pathway is shown in (Figure 3) (47).

As shown in the figure, the synthesis of testosterone begins with cholesterol. Deficiency of 7-dehydrocholesterol reductase results in impaired synthesis of cholesterol and leads to Smith-Lemli-Opitz syndrome(48). This syndrome is characterized by variable clinical features including cardiac defects, cleft palate, syndactyly, polydactyly, and genital ambiguity (49). The conversion of cholesterol to pregnenolone depends on the steroidogenic acute regulatory (StAR) protein and P450scc; hence, defects in these 2 enzymes can lead to undervirilized males. These patients typically appear as phenotypic females with undescended testes. Individuals with StAR mutations have adrenal insufficiency and lipid accumulation and enlargement of their adrenal glands(50). Patients with P450scc have a phenotype similar to that of individuals with StAR deficiency. 3b-HSD type II is the enzyme that converts dehydroepiandrostenedione to androstenedione. Deficiencies in 3b-HSD type II can cause hypospadias, micropenis, or more severe undervirilization/ ambiguity in addition to cortisol and mineralocorticoid deficiency (51). Mutations in the CYP17 gene can lead to deficiency in the activities of 17a-hydroxylase and 17, 20 lyase. XY individuals with 17a-hydroxylase/17, 20 lyase deficiencies can present with varying degrees of undervirilization ranging from micropenis, perineal hypospadias, and cryptorchidism to a complete female external phenotype (52).

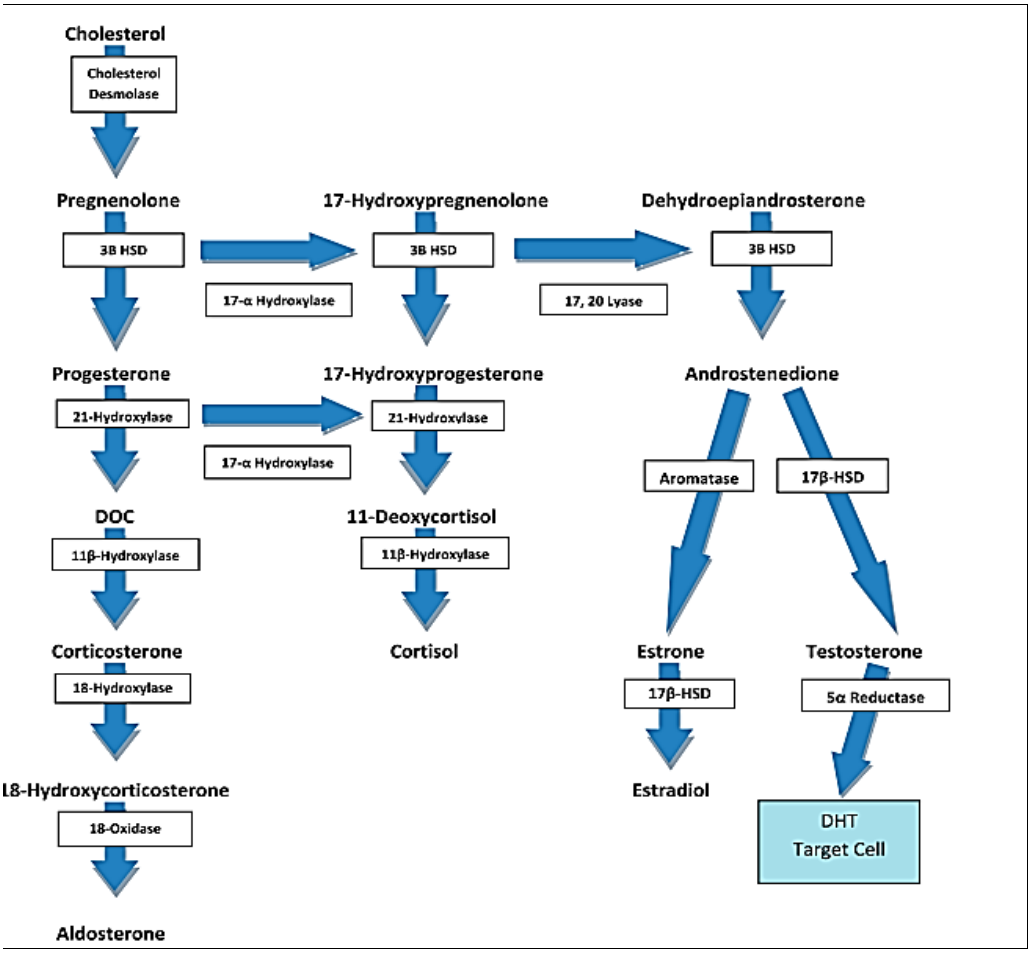

Figure (3): The adrenal biosynthesis pathway (47,53).

7b-HSD type III deficiency leads to an inability to convert androstenedione to testosterone. These XY individuals can have either a female or ambiguous external phenotype (54). Mutations in the luteinizing hormone (LH)/human chorionic gonadotropin (HCG) receptor can lead to testicular Leydig cell aplasia or hypoplasia. Without normal production of testosterone by the Leydig cells, undervirilization of the XY male will occur (55). The extent of undervirilization depends on the degree of Leydig cell hypoplasia. 5a-reductase deficiency leads to a defect in the conversion of testosterone to DHT. Without adequate levels of DHT to virilize the external genitalia, XY individuals can present with ambiguous genitalia, micropenis, hypospadias, or female external genitalia. These patients can have spontaneous virilization during puberty because of the increased testosterone levels (9).

Sex chromosome DSD:

Sex chromosome DSD includes conditions such as 47, XXY (Klinefelter syndrome and variants), 45, X (Turner syndrome and variants), 45, X/46, XY (mixed gonadal dysgenesis) and 46, XX/46, XY (chimerism) (56). These are often identified antenatally, frequently as an incidental finding; with confirmation of the diagnosis after

birth(57). Antenatal diagnosis allows for focused evaluation of the other complications often associated with these disorders, for example, cardiac anomalies in Turner syndrome. It also provides the opportunity to offer counseling to families prior to the birth(9).

After initial examination, first line investigations should include a karyotype, ultrasound examination for Müllerian structures, and serum levels of AMH and 17-hydroxyprogesterone. Ideally, results of these tests should be available within 48 hours to allow for sex assignment as early as possible (58). Additional investigations should include serum testosterone, cortisol, androstenedione, gonadotropins, and urinalysis. Serial measurements of urea and electrolytes are useful, in order to identify cases of CAH with salt wasting, although this may present later (59). The requirements for further investigations will be guided by the results of these initial tests (60). The internal anatomy of any patient with ambiguous genitalia should be evaluated with pelvic/abdominal ultrasonography or magnetic resonance imaging. Imaging can identify the presence or absence of müllerian structures (uterus) and the location of gonadal tissue. Gonadal biopsy may be necessary to determine the type of gonadal tissue in cases of suspected ovotesticular DSD or gonadal dysgenesis. The prenatal and post natal steps summarized in (Table 5, 6) (61).

Table (5): Laboratory Tests and Diagnostic Findings in Infants with DSD (61).

Test	Diagnostic Findings
17-hydroxyprogesterone level	Elevation is suggestive of CAH
11-deoxycortisol and 11-deoxycorticosterone levels	Both are elevated in 11-b-hydroxylase deficiency and depressed in 21-hydroxylase deficiency associated with CAH
Testosterone-to dihydrotestosterone ratio*	A ratio of more than 20:1 is indicative of a 5a-reductase deficiency
Human chorionic gonadotropin stimulation	Nonresponse (i.e, absence of increase in the testosterone level) is indicative of nonfunctioning Leydig cells, anorchia, or luteinizing hormone receptor defect
Antimullerian hormone and inhibin B levels	Normal values in the postnatal period are suggestive of normal Sertoli cell function and the presence of at least one testis

***The ratio is determined when testosterone levels are normal.**

Ambiguous genitalia and hormonal disturbance:

For patients with ambiguous genitalia and non-palpable gonads, there should be a high suspicion of CAH (62). Initial evaluation should include; - measurement of serum electrolytes to look for salt wasting, serum glucose that may be low in cases of cortisol deficiency, 17-hydroxyprogesterone to evaluate for 21-hydroxylase deficiency and a corticotropin (ACTH)-stimulation test may be necessary to evaluate the cortisol response and enzymatic deficiencies that can cause CAH. It can be done by obtaining baseline and ACTH-stimulated levels of cortisol, 17-hydroxyprogesterone, 17-hydroxypregnenolone, progesterone, androstenedione, dehydroepiandrosterone,

deoxycorticosterone, 11-deoxycortisol, and testosterone. The ACTH-stimulation test should not delay treatment in cases of suspected CAH(63). In any suspected case of CAH, renin and aldosterone levels also should be obtained. The normal value is summarized in (Table5) (61).

Additional testing includes baseline gonadotropins ((LH) and follicle-stimulating hormone(FSH)), which may be low in cases of hypogonadotropic hypogonadism and indicative of possible pituitary deficiency (64). Hypogonadotropic hypogonadism can be a cause of micropenis in 46, XY individuals. AMH level is a reliable marker of the presence and function of testicular tissue and can be helpful in evaluating undervirilized XY individuals (65). AMH levels will be low in cases of vanishing testes, XY gonadal dysgenesis, or persistent müllerian duct syndrome. AMH levels can be elevated in cases of androgen insensitivity and hypogonadotropic hypogonadism. HCG-stimulation test is used to evaluate the production of testosterone from testicular tissue (66). The hCG-stimulation test requires measuring baseline and stimulated levels of testosterone and DHT.The ratio of testosterone to DHT will be elevated in cases of 5a-reductase deficiency. An inadequate response to HCG stimulation in an XY individual can be indicative of gonadal dysgenesis, ovotesticular DSD, LH receptor defect, orhypogonadotropic hypogonadism(61). High testosterone levels in an undervirilized 46, XY patient should raise suspicion for androgen insensitivity, and genetic testing should be considered. Hormone measurements need to be interpreted in relation to the specific assay characteristics and to normal values for gestational and chronological age (67). In some cases serial measurements or stimulation tests may be needed. An extensive investigation is required when the external genitalia are sufficiently ambiguous to hamper sex assignment or inconsistent with the results of prenatal tests (68). Serum 17-OHP is usually unreliable before the age of 36 hours, and in the salt-losing form of CAH.Serum electrolyte levels usually do not become abnormal before day for of life. Steroid hormone determination should be performed after an extraction or chromatography to avoid concerns of analytical specificity (69, 70).

Serum levels of testosterone are low in the normal male newborn during the first 7–14 days of life, and increase progressively thereafter until the age of 2–3 months, thus results should be interpreted in that context (70).

Although AMH is expressed by both testicular Sertoli cells and ovarian granulosa cells, AMH is detectable at birth at much higher circulating concentrations in boys than in girls (70).

In 46, XX newborns, elevated 17-OHP and androgen levels are distinctive of CAH, with hyponatremia and hyperkalemia in salt-wasting variants. With the availability of genotyping, a salt-losing crisis is no longer required for the diagnosis of this variant (68). When androgen and AMH values are above the female range, ovotesticular DSD is likely, whereas when androgen values are elevated but AMH is in the normal female range, aromatase deficiency should be suspected. If androgen levels decrease progressively, together with the degree of virilization,

maternal virilizing tumors could be the source (71).

In Y chromosome bearing newborns, low AMH and androgen levels are indicative of dysgenetic gonads, low androgen values and normal/high AMH suggest steroid production defects, and normal/high AMH and androgen values are characteristic of androgen insensitivity or nonendocrine malformative DSDs(71,72).

Gonadotropin levels may also be helpful, since they are usually very high in dysgenetic DSDs and normal or only slightly elevated in steroid synthesis defects and partial androgen insensitivity. They can even be low in patients with complete AIS (73).

In addition to repeated measurements of basal AMH and androgen levels, decision-making algorithms include HCG and ACTH stimulation tests to assess testicular and adrenal steroid biosynthesis and urinary steroid analysis by LC-MS/MS, together with imaging studies and a biopsy of gonadal tissue (74). Basal AMH and androgen levels are indicative of the mass of functional Sertoli and Leydig cells. Their levels may range from very low in XY patients with severely dysgenetic gonads or XX patients with ovotesticular DSDs with predominant ovarian tissue to normal male values in mildly dysgenetic DSDs or ovotesticular DSDs with abundant testicular tissue (75). Since AMH and androgens levels are normally low in the male newborn and increase progressively after the third week of life repeated measurements may be needed. ACTH test may help when a steroidogenic defect affecting both the gonads and the adrenals is suspected(76) (Table 5).

Growth failure is a problem for virtually all individuals with Turner syndrome (TS), with untreated individuals achieving an average adult stature 20 cm shorter than that of their peers. GH therapy is now standard of care for girls with TS. Gonadotropin levels may be useful for predicting future gonadal function as well as determining appropriate timing and dosing of hormonal replacement therapy(77).

Radetti *et al*. studied 478 females with TS, mean age of 15.5 yr, and found that 22.2% had positive thyroid autoantibodies, of which 27% (29/106) were hypothyroid and 3% were thyrotoxic (78). Data on serum testosterone and estradiol in healthy prepubertal children which collected from our children hospitals (Welfare teaching hospital / medical city-Baghdad) are scanty, and there are no studies investigating sex steroids secretion in Klinefelter syndrome during infancy. Serum T concentrations tend to fall to the mid-low range in the young adult with KS in accordance to the appearance and/or worsening of hypo gonadal signs and symptoms (79). However, the age of onset of hypogonadism is extensively variable. In literature, lower than normal serum T concentrations (<12 nmol/L) is found in variable percentages (65–85 %) of adults with KS, although serum T can sometimes be in the normal range. Hypogonadism is always coupled with elevated gonadotropins (hypergonadotropic hypogonadism) and the latter are usually higher than normal even in patients with serum testosterone still in the normal range (9) (Table 6).

Table (6): Laboratory findings in example of patients with ambiguous genitalia /at welfare teaching hospital / medical city . Baghdad.

	Age	Gender	17-hydroxy progesterone ng/ml	S.cortisol AM nmol/l	ACTH ng/ml	S.ca mg/ dl	S.Na mEq /L	S.CL mEq /L	S.k mEq /L	b. urea levelm/ dl	DHEA-S µg/dl	Diagnosis
1	6 M	F	170.1	65.5		10.2	131	95	7.8			CAH
2	2 D	M					131	99	5.7	48		CAH
3	1 M	M	1.31	150	43		136	101	5.8			CAH
4	1 M	F		64.23	18.8		137	102	6			CAH
5	1 M	F	5.41	2.72	581		137		4.5			CAH
6	1 M	F	27.7	341.5	1408		134	101	5.4			CAH
7	10M	F	19.8		300		137	101	4.4			CAH
8	4 M	F				9.4	137		5.1	10.1		CAH
9	1 M	M	4.0	157.6	389.1		134		6.5		75.1	CAH
10	1 M	M	128.8	296.3			128	93	6.8	29.1		CAH
11	2 D	F		78.5	174.4		141		4.3			CAH
12	12D	F	6.11				109		7.1	22	218.8	CAH
13	1 M	M					133		4			CAH
14	3 M	F		581.9			114		5.4	150		CAH
15	1 W	F	3062		124.8		142	104	4.1	21		CAH
16	1 D	F	2.92	708			140	104	4.2			CAH
17	1 M	F	24.4	308	48		132	98	5.5	17		CAH
18	1 D	M					136	103	5.5			CAH
19	18D	F				10.3	133	98	5.3		320.5	CAH
20	2M	F	21	65.3	89.7	2.2	138	105	5.2			CAH

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