DISORDERS OF SEXUAL DIFFERENTIATION

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LEARNING OBJECTIVES:

- List the general cascade of events that lead from genetic complement to the formation of male vs female phenotype
- Classify the disorders of sexual development
- Recognize when a prophylactic gonadectomy is recommended
- List a diagnostic work-up for an infant with ambiguous genitalia.

I. Background

Sexual differentiation is the process by which the inherited genetic complement directs development of the gonads towards either testes or ovaries which in turn produce hormones which lead to masculination or feminization of the internal and external genitalia. Disorders of sexual development (DSDs), frequently called 'intersex', are conditions where the genetic, gonadal and/or phenotypic sex is atypical. It has been estimated that approximately **1 in 4,500 babies are born with significant ambiguous genitalia** and that these abnormalities account for **7.5% of all birth defects.**

The following figures are included as a review of normal sex differentiation and will be discussed briefly in class.

In brief, the presence of the **SRY gene** (sex-determining region of the Y chromosome) leads to the development of testicular tissue which includes **gametes, Sertoli cells and Leydig cells**. Sertoli cells synthesize and secrete anti- mullerian hormone **(AMH)** which leads to regression of the Mullerian structures (uterus,tubes and upper vagina), while the Leydig cells secrete **testosterone** which stimulates full development of the Wolffian structures including the epididymis, vas deferens and seminal vesicles (ie male 'internal structures'). Testosterone is converted to dihydrotestosterone **(DHT)** in peripheral tissues, including the skin, to promote rugation of the scrotum, elongation of the phallus with fusion of the urethra to create a meatus at the tip (ie male external structures).

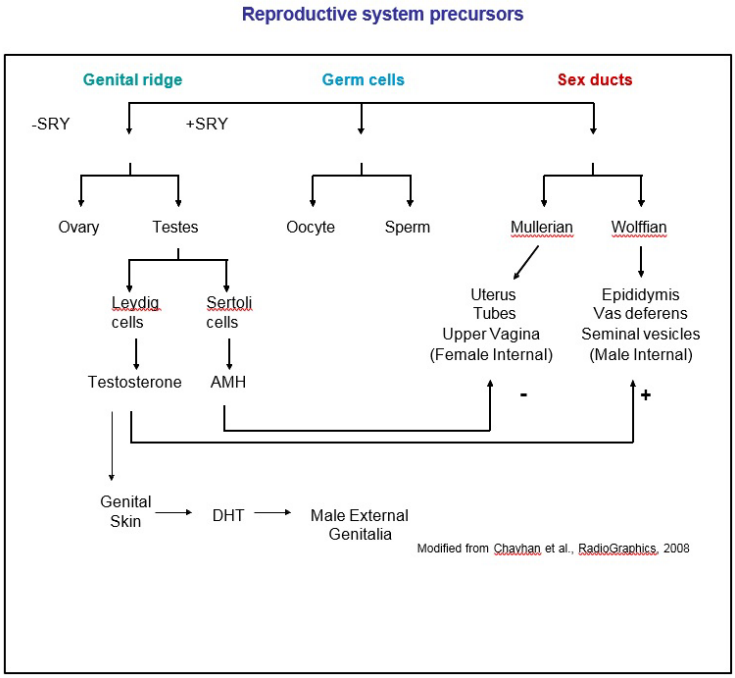

Figure 1: Schematic of sex differentiation

The presence of a normal SRY (sex-determining region, Y chromosome) gene is believed to be necessary for development of the testes; however, it is becoming increasingly clear that numerous additional genes are required for full gonadal development.
Development of the ovary and female phenotype was considered to be **"constitutive", or passive process** (lack of Testosterone, DHT and AMH), but recent studies have identified genes that are importance for normal ovarian development.

Three critical concepts related to in utero development:
1) Expression of genes and hormones must occur in the correct sequence and at the correct time during development/pregnancy (concept of critical developmental windows).
2) All fetuses start out in a 'bipotential/indifferent stage'. Development of internal and external structures develop in response to testosterone, DHT, and AMH or lack thereof.
3) Infants born with ambiguous genitalia have indeterminate genotype (ie you often cannot tell whether they have a male or female genotype by initial physical examination).

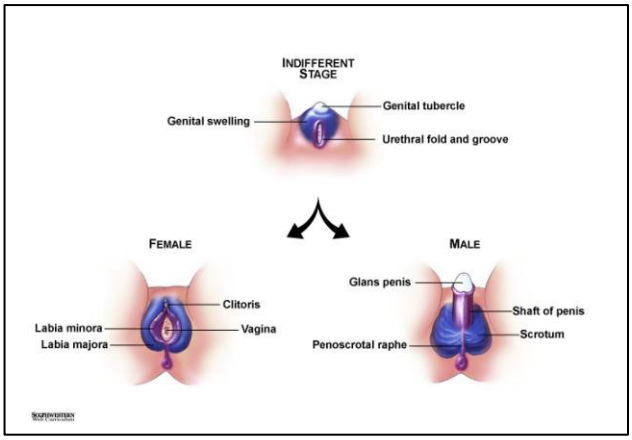

Figure 2: Development of the external genitalia

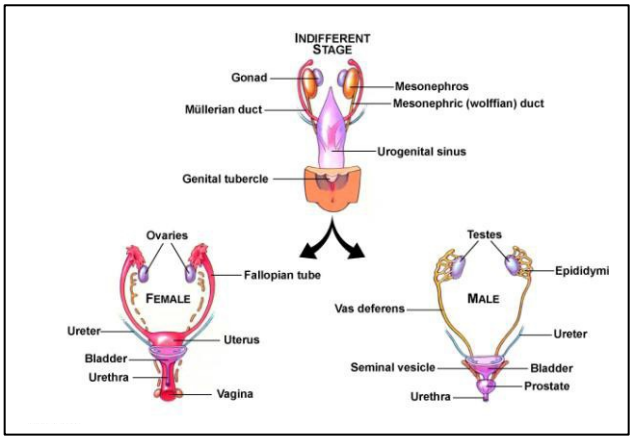

Figure 3: Development of the internal genitalia

Final sexual differentiation occurs at puberty. Activation of the hypothalamic-gonadal axis results in stimulation of gonadal function. Under the influence of luteinizing hormone (LH), the testes secrete markedly increased amounts of testosterone (and other androgens) stimulating an array of changes including sperm production, an increase in size of the testes and phallus, an increase in muscle mass, and deepening of the voice. Activation of adrenal sex steroid production (primarily DHEA-S – Dehydroepiandrosterone sulfate) is responsible for the development of the so-called sexual hair pattern (axillary and pubic hair) in both the male and female and does not depend on functioning gonads. In the male, testosterone/DHT likely are responsible for development of facial and chest hair. In females, ovarian estrogen production is important for breast development, an increase in uterine size and endometrial growth which ultimately allows for menstruation in response to cyclic ovulation.

Estrogen is critical for healthy bone development in both males and females. Yes - males have some estrogen and females have some androgens – at lower amounts.

II. Classification

Table 1. Classification of disorders of sex differentiation (DSD)

	Old classification	New classification
Genetic Male (Undervirilized male to female phenotype)	Male pseudohermaphrodite	46,XY DSD
Genetic Female (Over virilized female to male phenotype)	Female pseudohermaphrodite	46,XX DSD
Variable Genotype and Phenotype	True Hermaphrodite	Ovotesticular DSD

* The term pseudohermaphrodite has recently been changed to account for a better understanding of the causative genetic and hormonal etiologies in these patients. The old and new classifications are provided here as many texts still use the old classification.

Disorders of sex development can be divided into:

1) Gonadal dysgenesis
   - A) Abnormal karyotype
     - 45,X (Turner's)
     - 47,XXY (Klinefelter's)
     - 45,X/46,XY (mixed gonadal dysgenesis)
   - B) Normal karyotype
     - 46,XX or 46,XY (pure gonadal dysgenesis)

2) 46,XX DSD (previously female pseudohermaphroditism)
3) 46,XY DSD (previously male pseudohermaphroditism)
4) Ovotesticular DSD (previously true hermaphrodite)

Note: The clinical presentation of many of these disorders may be highly variable. For example, patients may have varying degrees of testosterone production in the case of 46,XY gonadal dysgenesis. As another example, many patients with Turners syndrome are mosaics (45,X/46,XX) and may have differing phenotypes depending on the number and tissue distribution of the chromosomally abnormal cells.

III. Questions to ask yourself

A few simple questions can be very useful when developing a differential diagnosis for the cause of abnormal sex differentiation.

1. Were the relevant hormones (T, DHT, AMH) synthesized and secreted in utero?
2. Were they made at the right time during development?
3. Were the relevant hormones (T, DHT, estrogen, DHEA-S) synthesized and secreted at puberty?
4. Should they have been made? (eg., elevated androgens in female with CAH)
5. Were they made in the right amounts? (ie., low testosterone in Klinefelter's)?
6. Were the target organs able to respond (ie., are hormonal receptors present and functional)?

IV.

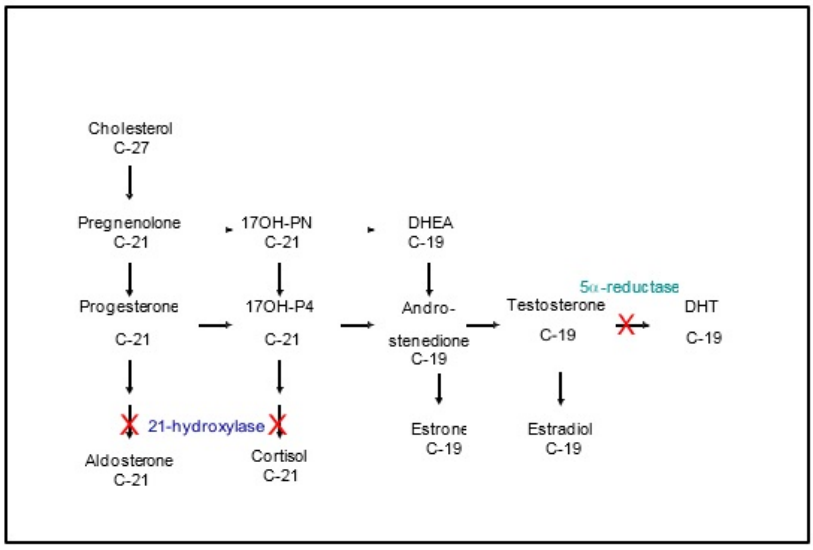

Figure 4. Steroidogenic pathway. Mutations in the 21-hydroxylase (CYP21) gene cause 46,XX DSD while mutations in the 5α-reductase gene result in 46,XY DSD.

V. Gonadal dysgenesis: Abnormal karyotype

By definition, the term **gonadal dysgenesis** describes an ovary or testes which is functionally and often anatomically abnormal. Dysgenetic gonads can be observed in patients with normal or abnormal karyotypes. The two most common causes of chromosomally abnormal gonadal dysgenesis are patients with either Turner or Klinefelter syndrome.

A. Turner Syndrome (45,X)

Turner syndrome can be considered to be a form of gonadal dysgenesis (ie., the ovaries did not develop properly) due to an abnormal karyotype (gonadal dysgenesis can also occur in the presence of a normal karyotype as described below) The incidence is **one in 2-5,000 girls** at birth. These patients are characterized by their **short stature** (typically less than 5 feet tall), webbed neck, shield like chest, hearing loss, and renal and cardiac anomalies, as well as an array of additional anomalies. It is believed that these patients start out with the full complement of oocytes, but that these undergo accelerated atresia such that no follicles are present at the time of puberty. The small amount of connective tissue remaining is termed a **"streak gonad".** These patients lack a surge in estrogen production at puberty and therefore the uterus remains prepubertal (small) and these patients lack breast development. The internal genitalia and the external genitalia are female (ie lack of T, DHT and AMH).

Adrenarche occurs normally as determined by measurement of adrenal steroids, but axillary and pubic hair are scant if present at all. The reason for this is observation is not clear.
The classical Turner patient has a **45,X** genotype, although many patients are mosaics, frequently with a 45,X/46,XX or 45,X/46,XY phenotype. Mosaics are likely to have a small amount of normal ovarian function and may actually menstruate for a short time and even conceive spontaneously.

Diagnosis: These patients are often diagnosed in infancy due to their physical stigmata. Some mosaic Turner patients may not be discovered until after puberty with premature ovarian failure.

Treatment: They may require treatment for various associated medical conditions and will need hormonal replacement for bone development and maintenance.

Fertility: Many can undergo IVF with donor egg but this is often not advised in the face of an elevated risk of cardiac anomaly/complications.

B. Klinefelter Syndrome (47,XXY)

Klinefelter syndrome is the most common major abnormality of sex differentiation with an incidence of **1 in 500 males**. The classic **genotype is 47,XXY** although less common variants include 48,XXXY and 46,XY/47,XXY (common characteristic is an extra X chromosome).

Because SRY is present in all forms of Klinefelter syndrome, testes develop and the patients undergo normal male sex differentiation in utero. These patients are indistinguishable at birth from normal males with the characteristic phenotype becoming evident during puberty. The fetal testes is normal in these patients, however, there is a loss of spermatogonia during childhood with progressive **hyalinization of the seminiferous tubules** which results in **small firm testes** with poor testosterone production and **low to no sperm production.**

These patients frequently develop **gynecomastia** (increased breast tissue). As testosterone is known to counteract estrogen-dependent breast development, the low levels of testosterone in these patients is the likely cause for this clinical sign. These patients are frequently **tall** with disproportionate length of the extremities due to delayed fusion of the epiphyses. The low levels of circulating testosterone result in inadequate local levels of estrogen at the bone to cause epiphyseal fusion.

Diagnosis: Often made at puberty when testes fail to grow normally. Milder forms may not be diagnosed until a couple undergoes infertility evaluation and low to no sperm count is found.

Treatment: Testosterone replacement (usually skin patch or gel)

Fertility: If there are some sperm, IVF may be attempted. If no sperm, (azoospermia), they will need donor sperm vs adoption.

C. Mixed Gonadal Dysgenesis

Unlike Klinefelter and Turner patients, these patients have **asymmetric** gonadal development, classically a **single testis on the right and a streak gonad on the left.** Presentation ranges from partial virilization and ambiguous genitalia at birth to patients

with a completely male or female phenotype. The majority have some mullerian development due to poor AMH production.

V. Gonadal dysgenesis: Normal karyotype

Dysgenetic gonads may also be found in patients with a normal 46,XX or 46,XY karyotype, also said to have 'pure gonadal dysgenesis'. These patients are presumed to have specific gene defects, many of which are beginning to be elucidated. Another term for pure 46XY gonadal dysgenesis is 'Swyer Syndrome'. These individuals are born with **female external and internal genitalia** (lack of testosterone/DHT as well as AMH). They do not feminize at puberty because they lack normal ovaries/estrogen.

Remember: Any dysgenetic gonad with Y-containing cell line is at increased risk for tumor development (dysgerminoma, gonadoblastoma), and the incidence is found in some studies up to 30%. These patients MUST have their streak/dysgenetic gonad(s) surgically removed. Examples: Swyer Syndrome (46 XY gonadal dysgenesis), Turner Syndrome IF they are mosaic with Y – ie 45X/46XY. Note: Patients with 46XX gonadal dysgenesis do not need gonadectomy.

VI. 46,XX Disorder of Sex Differentiation (46,XX DSD)

A. Congenital adrenal hyperplasia (CAH).

CAH is the most common cause of ambiguous genitalia in the newborn, and constitutes 60% of all DSDs. It is an **autosomal recessive disorder** most commonly due to a mutation in the gene encoding the enzyme **21-hydroxylase enzyme** which leads to shunting of the steroidogenic pathway away from aldosterone and cortisol and toward androgens. The incidence is **one out of every 10,000-15,000 children.** Less commonly, CAH may be due to mutations in the 11-hydroxylase or 3 –beta hydroxysteroid dehydrogenase enzymes. The more severe the enzymatic defect, the more severe the androgen exposure and consequent virilization of the external genitalia. The phenotype can range from clitoromegaly with labial fusion to the appearance of a normal phenotypic male. Note: the labia/scrotum will not contain a gonad as ovaries rarely descend. These patients will have normal internal female genitalia (uterus and fallopian tubes) and will therefore not have secreted AMH, and they have ovaries.

Of note: The severe form of CAH is considered a **medical emergency at birth** and if not treated promptly can lead to 'salt wasting' , with hyperkalemia, hyponatremia, metabolic acidosis, and death typically within 10-20 days of life. Fortunately, Texas law requires newborn screening for over 50 disorders (CAH being one of them) and CAH is now most often discovered early and treated promptly within the first week of life.

At puberty, these patients will undergo the expected breast development and even onset of menses, although they may have oligomenorrhea.

Females with the milder form (nonclassic, or late onset CAH), may present at puberty with a picture like polycystic ovarian syndrome (irregular periods and hyperandrogenism).

B. If presented with a patient with 46,XX DSD it is always important to rule out exposure to high levels of **exogenous androgens** during development either through maternal intake of exogenous androgens, ovarian androgen producing tumor (such as luteoma), or placental aromatase deficiency.

VII. 46,XY Disorder of Sex Differentiation (46,XY DSD)

These 46,XY patients have bilateral testes but express a female, or ambiguous phenotype. The phenotypic presentation of patients with abnormalities in androgen synthesis can vary widely depending on the specific enzyme affected and the degree of the defect. The details of these abnormalities are beyond the scope of this lecture.

A) Complete Androgen insensitivity syndrome (CAIS) is a failure of end organ (external genitalia and skin) to respond to DHT. It is inherited in a sex-linked fashion because the AR (androgen receptor) is on the X chromosome. Patients with complete androgen insensitivity will not have Mullerian duct structures (testes produced AMH), will not have Wolffian duct structures (could not respond to testosterone in utero), and will have normal female external genitalia (cannot respond to DHT). They will often have a small "vaginal dimple" as the lower vagina is derived from the urogenital sinus. At puberty, **they will develop breasts** due to conversion of testosterone to estrogen but **will not develop pubic hair.** Some patients with AIS have a complete lack of androgen receptor function (**CAIS** – ie complete) and have the above findings, yet others with have a more incomplete lack of function (**PAIS** ie partial) and can exhibit ambiguous genitalia at birth.

Testicular removal at diagnosis to prevent the development of a testicular tumor was standard of care, but this is now controversial. The testes in patients with AIS are not 'dysgenetic', they are more normal yet cryptorchid (non-descended) and the risk of malignancy is much lower (<5%). Therefore, gonad removal by laparoscopy is often deferred until after pubertal breast development, and some patients choose to retain the gonads and have them serially monitored with ultrasound or MRI throughout their life. If they choose to have a bilateral gonadectomy, they will need hormone replacement (estrogen to prevent osteoporosis).
Diagnosis: They may not be diagnosed until puberty when they undergo a work-up for primary amenorrhea. Alternatively, some are discovered when an amniocentesis reveals a 46XY karyotype and a female phenotypic child is born.

B) 5α-reductase gene mutation prevents the conversion of testosterone to DHT (see Figure 4). It is **autosomal recessive**, and has been seen more frequently in cultures with consanguinity. These mutations are generally incomplete so patients will make low levels of DHT, allowing for some degree of external masculinization. The internal genitalia are normal male in response to high testosterone production. At puberty, the high testosterone levels may be converted to DHT and result in further masculinization of the external genitalia.

Diagnosis: Often at birth with a workup due to ambiguous genitalia.

Treatment: Controversial, many have been raised a females, undergoing gonadectomy and feminizing surgery. Many who are raised girls without treatment will sex reassign to male during puberty.

Have you read the book **Middlesex** by Jeffrey Eugenides? It is a Pulitzer Prize-winning novel and bestseller published in 2002. It depicts a child and then adult in the 1960s and 70s with 5 alpha reductase deficiency.

"I was born twice: first, as a baby girl, on a remarkably smogless Detroit day of January 1960; and then again, as a teenage boy, in an emergency room near Petoskey, Michigan, in August of l974.

C) Mutations in Anti-mullerian Hormone (AMH) or the AMH receptor
('Persistent Mullerian Duct Syndrome', or 'hernia uteri inguinale')
Although very rare, 46XY males have been reported with mutations in either AMH or AMH receptor. They are born with a male phenotype and have **male internal and external genitalia**. Due to a lack of normal AMH function, **they also have mullerian structures** (uterus and fallopian tubes). Diagnosis: They often present with cryptorchidism and inguinal hernia which is found to contain mullerian structures.

VIII. Ovotesticular DSD (formerly 'true hermaphrodite')
This is a rare disorder characterized by **both ovarian and testicular tissue**, most often with an ovary on the left and and ovotestes or testes on the right. There is almost always a uterus (failure of adequate AMH production), but the internal ductal system may be complex depending on local levels of testosterone production. The karyotype may be 46,XX or 46,XX/46,XY with 80% of the 46,XX patients expressing the SRY gene. Despite this, many of these patients menstruate and may become pregnant. These patients are proof that our understanding of sex differentiation is incomplete.

XI. **The time at presentation** can be broadly divided into: 1) the newborn period in the infant with ambiguous genitalia and, 2) puberty with failure of final sexual maturation, 3) infertility evaluation

1) Ambiguous Genitalia
2) **Primary Amenorrhea**
3) **Azoospermia**

1) Ambiguous genitalia
Infants born with ambiguous genitalia represent a medical and social emergency. **The most common diagnosis is CAH** (75% will present with the severe 'salt-wasting' type).
Male infants with CAH due to 21 hydroxylase deficiency are phenotypically normal and are often discovered on routine prenatal screening. A team-oriented approach is crucial and parents are understandably overwhelmed and confused. The team includes neonatologists, endocrinologists, geneticists, urologists, gynecologists, nurses, counselors and social workers.

The physical exam in patients with a suspected disorder of sexual differentiation focuses on the genitalia (penile length, clitoral size, presence of gonads, urethral opening, labial fusion). A mass in the inguinal canal or scrotum is almost always a testes (ie., ovaries rarely descend). Additional evaluation will generally include:

1. Karyotype, evaluation for SRY
2. Imaging of pelvic structures
3. Measurement of 17-OH-P (17 hydroxyprogesterone) in the neonate to rule-out CAH (life-threatening), electrolytes

2) Primary amenorrhea
The definition of primary amenorrhea is lack of menses by age 14 (if lack of secondary sex characteristics) or by age 16 (with secondary sex characteristics). Patients with pure gonadal dysgenesis - 46XX or 46XY will present with lack of breast development/menses (no ovaries/estrogen) and they have mullerian structures (lack of AMH). Patients with CAIS (normal female phenotype except lack of pubic hair) will present with lack of menses, lack of mullerian

structures (they have AMH) and a blind vaginal pouch. The latter may need vaginal creation (usually with serial dilation) for intercourse.

3) **Azoospermia**
Patients with Klinefelter Syndrome often have azoospermia, a very low sperm count, or a few sperm that can be retrieved by testicular sperm extraction. The latter is a process of local anesthesia, removal of testicular tissue, and microscopic examination.

XII. Treatment

The treatment of these disorders is highly variable due to the range of potential defects. In many cases, it is important to include specialists in neonatology, genetics, endocrinology, gynecology, urologists, surgeons, and counselors.

A. Gender assignment.

Newborns with ambiguous genitalia will need to a have a gender assignment (sex-of- rearing). This process can be difficult for the family and current thinking is that **this does not need to be done immediately.**

B. Surgical correction of ambiguous genitalia.

Each child with DSD will have a unique characteristics which will dictate management on a case by case basis. *As it is easier to create female external genitalia, undervirilized males were often reassigned as females.* The timing of this surgery and even whether this is indicated is a current debate among various advocacy groups. Some human rights advocates push for a delay in surgery to allow for DSD individuals to make their own informed decision especially when it consists of irreversible genital surgery.
Surgical excision of streak/dysgenetic gonads in the presence of a Y-containing cell line is indicated due the high incidence (25-30%) of tumor formation.

C. Hormone replacement.

Patients with gonadal dysgenesis lack normal steroidogenesis. At the time of normal puberty, they frequently require treatment with either testosterone or estrogen to develop secondary sex characteristics. 46,XX infants with **complete** congenital adrenal hyperplasia (CAH) will require mineralocorticoid and glucocorticoid replacement emergently after birth.

Example: Swyer Syndrome 46XY gonadal dysgenesis

Female internal (mullerian – no AMH) and external genitalia (no T, DHT)
No ovaries
Streak gonads (dysgenetic) – need to be removed
Hormone replacement (estrogen) for breast development and bone health
Donor egg or adoption for family

Finally: what's in a name?

The term DSD is well accepted in the medical community but the word 'disorder' is seen as pejorative by some. Be on the lookout for another change in nomenclature as this area evolves.

Questions:

1) These facts are true regarding sexual differentiation EXCEPT:
   A. The female phenotype occurs in the absence of testosterone/DHT secretion in utero
   B. AMH, secreted by the Sertoli cells of the fetal testes will cause mullerian structures to regress
   C. Patients with ovotesticular DSD have streak gonads
   D. The classic karyotype for Klinefelter's syndrome is 47,XXY

2) You are called to the NICU to evaluate an infant with ambiguous genitalia. There is marked clitoromegaly/ small phallus, fused labioscrotal folds, and no palpable gonads in those folds or inguinal canals. The most likely diagnosis for this patient is:
   A. Congenital adrenal hyperplasia due to a mutation in the gene for 11-hydroxylase enzyme
   B. Congenital adrenal hyperplasia due to a mutation in the gene for 21-hydroxylase enzyme
   C. Ovotesticular DSD
   D. Androgen Insensitivity Syndrome

3) The hormone thought to be primarily responsible for sexual hair growth is:
   A. Testosterone from the ovaries/testes
   B. Testosterone from the adrenal glands
   C. DHEAS from the ovaries/testes
   D. DHEAS from the adrenal glands

Answers
C
B
D