# 14. GONADAL DEVELOPMENT

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**RECOMMENDED READING:** Larsen, Human Embryology, 3rd Edition, pp 276-293.

**OVERVIEW:** The male and female reproductive tracts are derived from the same embryonic/fetal tissue. The gonads and internal and external genetalia begin as bipotential tissues. The differentiation of male gonad is dependent on the expression of SRY (sex reversal Y) = TDF (testes determining factor). This gene is expressed in the Sertoli cells of the male in a cell autonomous fashion. Its expression results in a cascade of events leading to the development of seminiferous tubules. The Sertoli cells of the seminiferous tubules secrete AMH which stimulates the differentiation of Leydig cells (testosterone secreting). We shall discuss in lecture how these two hormones regulate the further events of male differentiation. The absence of SRY expression results in the differentiation of the presumptive gonad into an ovary. DAX-1 is a gene normally expressed in both ovarian and testicular tissue but is down regulated in the latter. DAX-1 downregulates the effectiveness of SRY or downstream elements, resulting in an ovary. Over expression of DAX-1 in XY individuals causes sex reversal. Development of the internal and external genetalia in the male are dependent on the gonad (testes). Development of female internal and external structures are gonad independent.

## GLOSSARY:

**5α reductase:** Converts testosterone to dihydrotestosterone. If mutated, the external genitalia of XY male are feminized. The degree of feminization depends on enzymatic activity (if any) remaining.
**AMH:** (anti-müllerian hormone) = MIS (Müllerian Inhibitory Substance). Made by the Sertoli cells of the testes and causes degeneration (apotosis) of the Müllerian ducts in males.
**DAX:** Expressed normally in presumptive gonadal tissue but down regulated in male. Expression predates SRY expression by 10 days in humans. DAX duplication in XY individuals can result in sex reveral.
**DHT:** dihydrotestosterone, derived from testosterone by the action of 5 α reductase.
**Hypospadias:** incomplete closure of the urethral folds resulting in inappropriate urinary openings that occur on the inferior aspect of the penis.
**Leydig cells:** of the testes that secrete testosterone.
**Müllerian** = paramesonephric ducts. Retained in female, forms oviduct, uterus, and upper 2/3 of vagina.
**SF-1:** Steroidogenic factor-1. A transcription factor essential in gonadal ridge determination.
**SOX 9** – a gene closely related to SRY structurally, also involved in testes differentiation in humans. Mutations in this gene can lead to sex reversal.

**SRY** – sex reversal Y. A gene on the Y chromosome that initiates the cascade resulting in male sexual differentiation. Gene absent in XY (human) females. Can be translocated to X during meiosis resulting in sex-reversal.
**Wolffian ducts** = mesonephric ducts. Retained in male due to secretion of testosternone by Leydig cells. Forms efferent ductules, vas deferns.
**WT-1:** Wilm's Tumor 1, a transcription factor essential in urogenital ridge determination.

## LEARNING OBJECTIVES:

You should be able to:

1. Discuss the central role of SRY
2. Explain how testicular development (or absence of testes) results in the male/female patterns of differentiation of the internal and external genitalia. What are the hormones involved and which cells produce them?
3. Describe the switch from bipotential gonads, internal ducts and external genitalia into the male or female structure.
4. Discuss the migration of primordial germ cells and the possible mechanism(s) guiding this migration.
5. Discuss the role of DAX-1 (dose sensitive sex-reversal-adrenal congenital hypoplasia on the X chromosome) in sex determination.

### Origin of components of the gonads

The gonads develop from three sources: the mesothelium (coelomic epithelium) lining the posterior abdominal wall, the underlying mesenchyme (intermediate mesoderm), and the primordial germ cells. The mesothelium proliferates to form the genital ridge, a bulge of tissue medial to the mesonephros. From this epithelum **primary sex cords** penetrate the mesenchyme. The **indifferent gonad** now consists of a medulla and cortex. In XX embryos the ovary will originate from the cortex and the medulla will decline. In the XY embryo the medulla will develop into the testes and the cortex regress.

### Primordial Germ Cells (Figs.14-1; 14-2)

The primordial germ cells (PGCs) are committed in the epiblast and have been identified in the mouse early in gastrulation at a position posterior to the primitive streak. PGCs migrate to the extraembryonic mesoderm at the angle of the amniotic membrane (Fig. 14-2A). From here the cells migrate to the base of the allantois and into the yolk sac endoderm (Fig. 14-2A). During hindgut folding they migrate through the hindgut endoderm, up the dorsal mesentery to the genital ridge (Fig. 14-2B-E).

PGCs (50-80 cells) leave the hindgut and extend long (40 um) processes linking one to the other. Once the cells aggregate in the genital ridge they lose these processes and become immobile. The PGCs proliferate by mitosis during their migration to approximately 30,000 when they reach the genital ridge. Two mutant mice lack primordial germ cells ("white spotted" w/w and "steel" sl/sl) . **sl** encodes a growth factor - Stem Cell Factor (SCF) - and **w** encodes the receptor for SCF, a tyrosine kinase receptor (c-kit). SCF is necessary for the survival of the migratory PGCs. c-kit is expressed on the PGCs and SCF is found along the migratory route and is most concentrated in the developing gonad. SCF induces proliferation and guides migration through a chemoattractant/concentration gradient mechanism. PGC migration is also controlled by TGF-beta 1 which is secreted by the genital ridge. TGFβ1 acts as a chemoattractant but inhibits proliferation, thereby modulating the number of primary germ cells in the gonad.

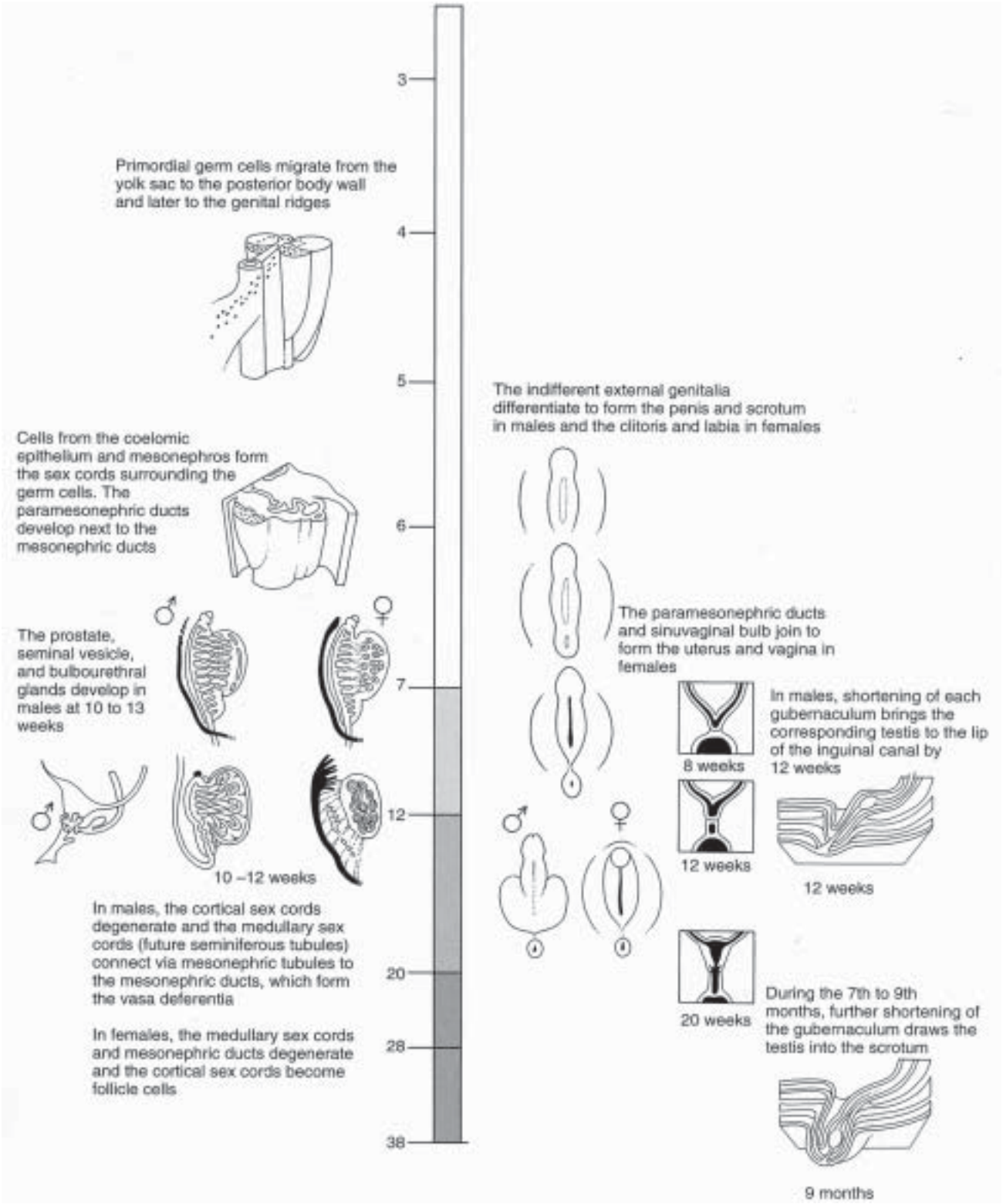


**Figure 14-1**. Summary of time course of development of gonads and reproductive tract in both males and females.

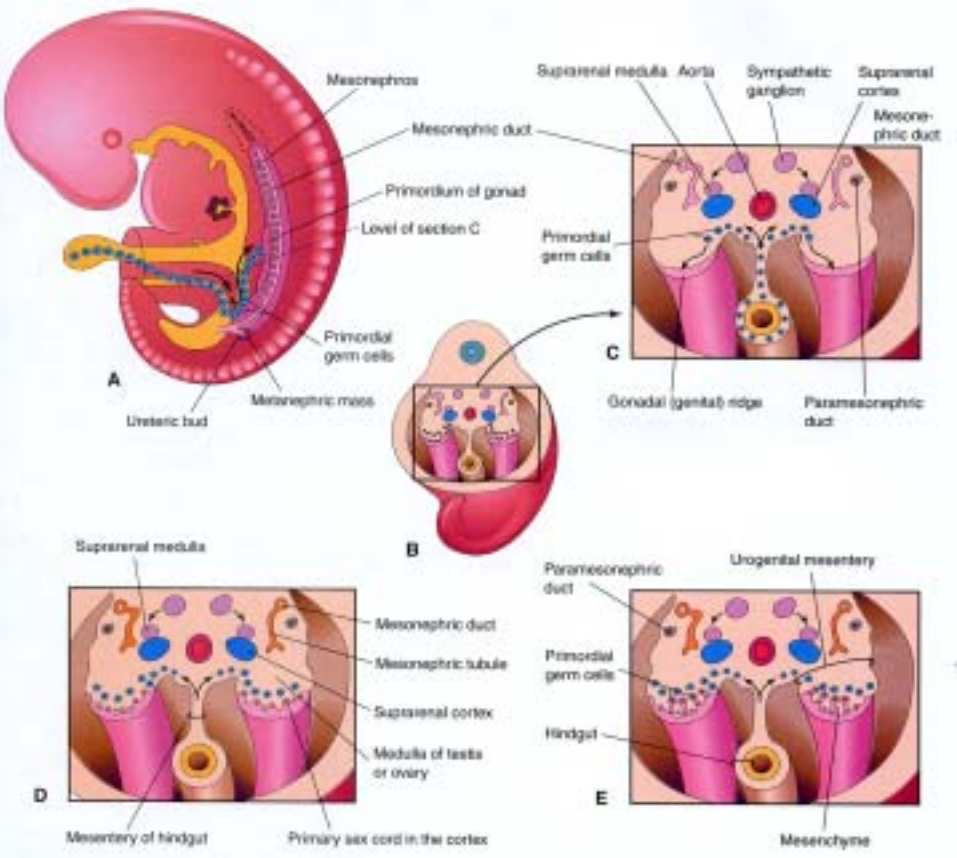


**Fig. 14-2.** A, Sketch of a 5-week embryo illustrating the migration of primordial germ cells from the yolk sac into the embryo. B, Three dimentional sketch of the caudal region of a 5-week embryo, showing the location and extent of the gonadal ridges. C, Transverse section showing the primordium of the suprarenal glands, the gonadal ridges, and the migration of primordial germ cells into the developing gonads. D, Transverse section of a 6-week embryo showing the gonadal cords. E, Similar section at a later stage showing the indifferent gonads and paramesonephric ducts.

## Male Gonad (Fig. 14-3)

Regulated by SRY (see below), the primary sex cords enter the medulla and differentiate into the seminiferous cords. These are the precursors to the seminiferous tubules where sperm will be produced. The parts of the primary sex cords that extend deepest into the medulla form the rete testes, the first in a series of structures by which sperm leave the testes in adulthood. As the seminiferous tubules are forming, the PGC enter the gonad and associate with the tubules. The PGC will give rise to sperm (after puberty), the cords give rise to the "sustentacular cells" of the tubules, the Sertoli cells.

In the presence of Sertoli cells, the germ cells remain in meiotic arrest and are inactive spermatogonia until puberty. This phenomenon is due to the secretion by the Sertoli cells of anti-müllerian hormone (AMH; see Fig. 14-4 and below). AMH also signals for mesenchymal cells (intermediate mesoderm) to differentiate into Leydig cells which secret testosterone (see Fig.14-4).

## Female gonad (Fig. 14-3)

The ovary develops more slowly than the testes. Although the primary sex cords enter the genitial ridge at the same time the ovary cannot be distinguished histologically until the 10-11th week. The primary sex cords degenerate and the secondary sex cords (also called cortical cords) extend from the surface epithelium (mesothelium). As these cords increase in size the PGC are incorporated into them. At about 16 weeks these cords break up into isolated clusters called primordial follicles. Each follicle consists of an oogonium (from the PGC) and a single layer of flattened cells (follicular cells) derived from the cords. Oogonia undergo a period of rapid proliferation. In the absence of SRY and AMH, PGC undergo the first prophase of meiosis.

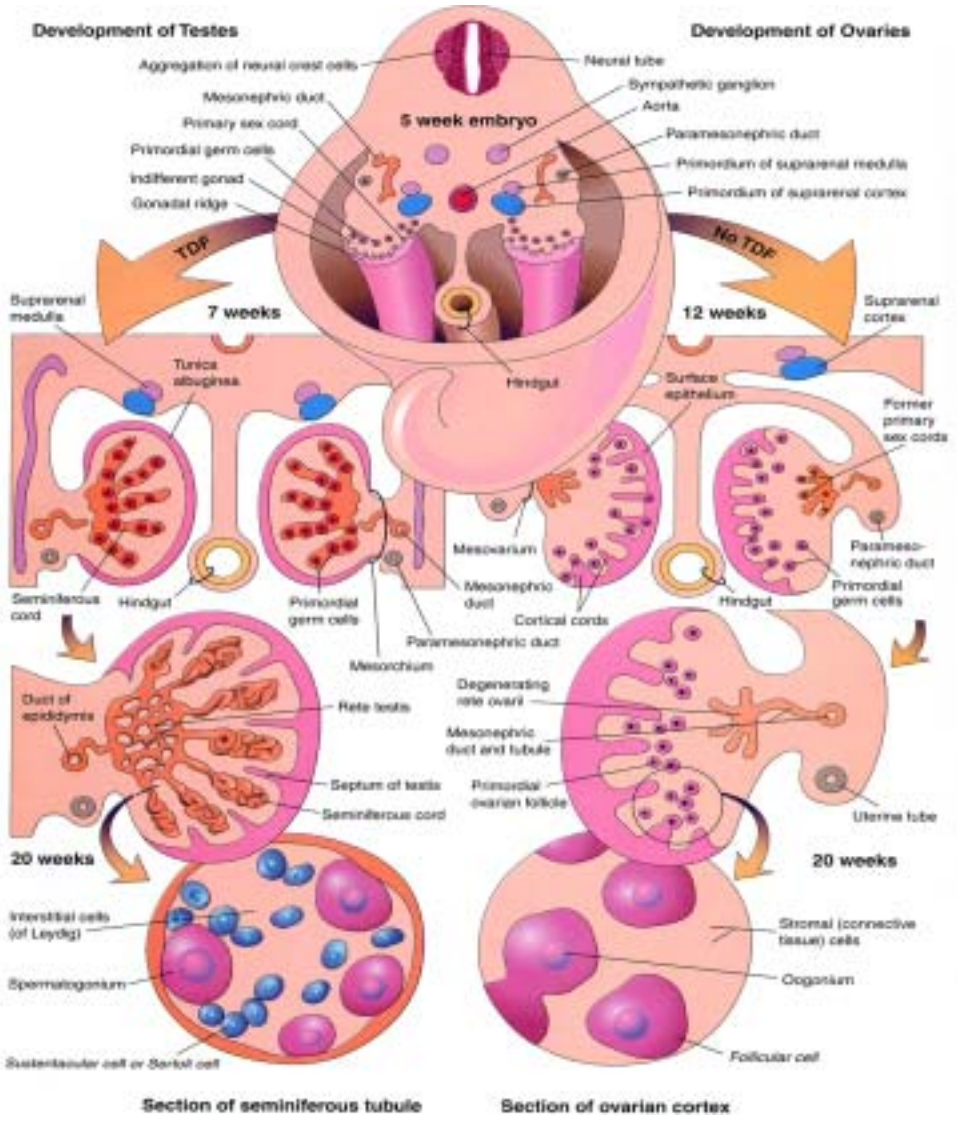


**Fig. 14-3:** Time course of differentiation of the bipotential gonad.

## Chromosomal Sex

The discovery of sex chromosomes was first discovered in 1923 in insects. The relationship of the **human** X and Y chromosomes to genital differentiation was not made until 1959. Karyotype analysis of Turner's syndrome (XO=female) and Klinefelter's syndrome (XXY=male) patients provided the necessary data on the role of the chromosomes in gonadal differentiation with the female being XX and the male having XY. These genetic factors initially exert their influence upon the bipotential gonad.

## Genes Determining Development of the Bipotential Gonad

The gonad is bipotential, capable of forming either a testis or an ovary until about the sixth week of gestation. The initial formation of the bipotential gonad (from uncommitted urogenital ridge) requires the function of Wilm's tumor (WT1) and steroidogenic factor (SF-1). Both are transcription factors (see glossary) and key in both gonadal and kidney development.

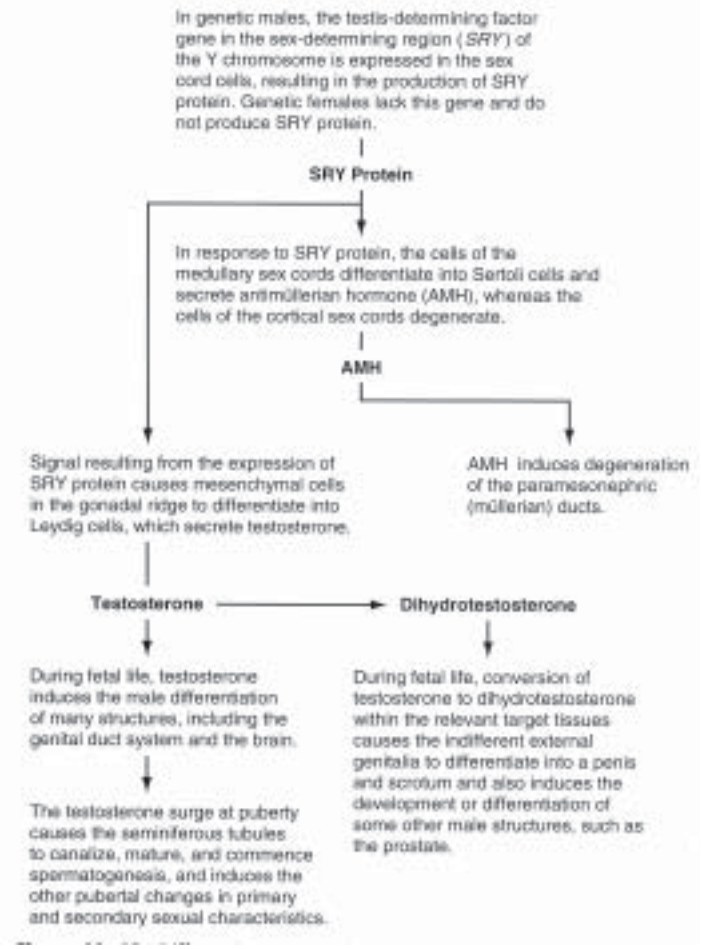


**Fig. 14-4.** Differentiation cascade of male genital system development.

## Genes Affecting Testicular and Ovarian Differentiation

Once the bipotential gonad is formed the next pivotal occurrence in sexual determination is the presence or absence of a gene located on the short arm of the Y chromosome termed the sex-determining region (SRY). It is the activity of SRY and its interplay with SOX-9 and DAX-1 that determines if the fetus develops a testis or an ovary.

## Sex Determining Region of the Y Chromosome- SRY Gene Testicular Determination

In the developing fetus, SRY is expressed in the Sertoli cells, consistent with the hypothesis that germ cells are originally bipotential. Clinically, three important observations have documented that SRY determines whether or not a testis is formed. (1) Microinjection of SRY gene into an XX fertilized ovum results in offspring with testes and male genitalia; (2) 15 to 20 % of human XY females have mutations of SRY gene (gonadal dysgenesis of female internal and external genitalia, see below) and (3) the majority of XX males are found to carry SRY gene. SRY is a high mobility group (HMG) transcription factor that regulates gene expression by bending DNA. One of its immediate downstream targets is SOX-9, another HMG type. SOX-9 is originally expressed within the urogenital ridge in both sexes before the appearance of SRY. SRY expression up-regulates SOX-9 in the male while DAX-1 (see below) down-regulates this gene in the female. Patients with mutations in SOX-9 have campomelic dysplasia (severe thoracic and limb skeletal defects) with gonadal abnormalities. SOX-9 is also expressed in Sertoli cells and with SRY, up-regulates AMH (Fig. 14-4).

## Ovarian Determination (DAX-1)

According to classic Jostarian teaching, the removal of both testes from the male fetus results in the development of female external and internal genitalia, i.e., the female phenotype is gonad independent. This concept, although true for the phenotypic differentiation of the internal and external genitalia, is not true for the development of the gonads. DAX-1, a member of the nuclear hormone receptor family, is found on the short arm of the X-chromosome. If translocated to the X chromosome in an XY individual it can lead to complete XY sex reversal. (It's first name was DDS; double dosage sex.) It is postulated based on its expression by both X chromosomes, that DAX-1

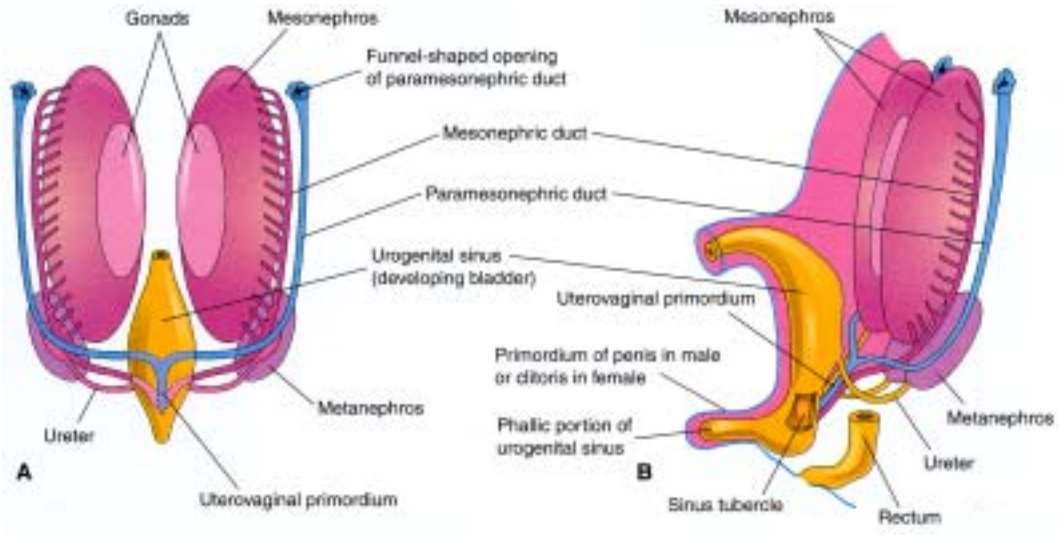


**Fig. 14-5.** A, Sketch of a ventral view of the posterior abdominal wall of a 7-week embryo showing the two pairs of genital ducts present during the indifferent state of sexual development. B, Lateral view of a 9-week fetus showing the sinus tubercle (muller tubercle) on the posterior wall of the urogenital sinus. It becomes the hymen in females and the seminal colliculus in males. The colliculus is an elevated part of the urethral crest on the posterior wall of the prostatic urethra.

inhibits SRY directly or inhibits SRY's upregulation of SOX-9. DAX-1 is orignally expressed in both male and female gonad, but persists only in the latter. Figure 14-4 outlines the cascade of male differentiation. Figure 14-3 outlines gonadal development for both males and females.

**Reproductive Ducts (Figs. 14-5; 14-6)**

At the end of the seventh week the gonads are sex determined (Fig. 14-3), but the **other parts of the reproductive tract are still bipotential** (Figs. 14-5, 14-6). The gonads are in contact with the mesonephros. Although the mesonephros regresses (see Chapter 13), the mesonephric tubules grow into the gonadal ridge and these remain connected to the mesonephric (Wolffian) duct (Fig. 14-5).

A second duct system, the paramesonephric (Müllerian) duct forms as an invagination of the coelomic epithelium on the lateral aspect of the gonadal ridge (Figs. 14-1, 14-2, 14-3). The Müllerian ducts are independent of the mesonephros and meet and fuse at the end of the eighth week, contacting the urogenital sinus (see Figs.14-5, 14-6).

At the end of the eighth week these two sets of ducts are present (Figs. 14-5, 14-6). This represents the indifferent duct stage. The direction of differentiation of the internal reproductive tract depends on the secretions of the testes. **Unilateral** gonadectomy of male and female fetal rabbits were used to illustrate the pattern of duct differentiation. In the male, the side **with a testes** retains the Wolffian duct. The Müllerian ducts degenerate on both sides. In the **unilaterally** gonadectomized female, the Wolffian ducts degenerate bilaterally and both paramesonephric ducts survive. Why?

In males, testosterone secreted by Leydig cells acts **locally** to ensure the survival of the mesonephric tubules and Wolffian ducts ipsilaterally to the remaining testes. The AMH from the Sertoli cells acts via the **general** circulation to actively cause degeneration of the paramesonephric (Müllerian) duct system.

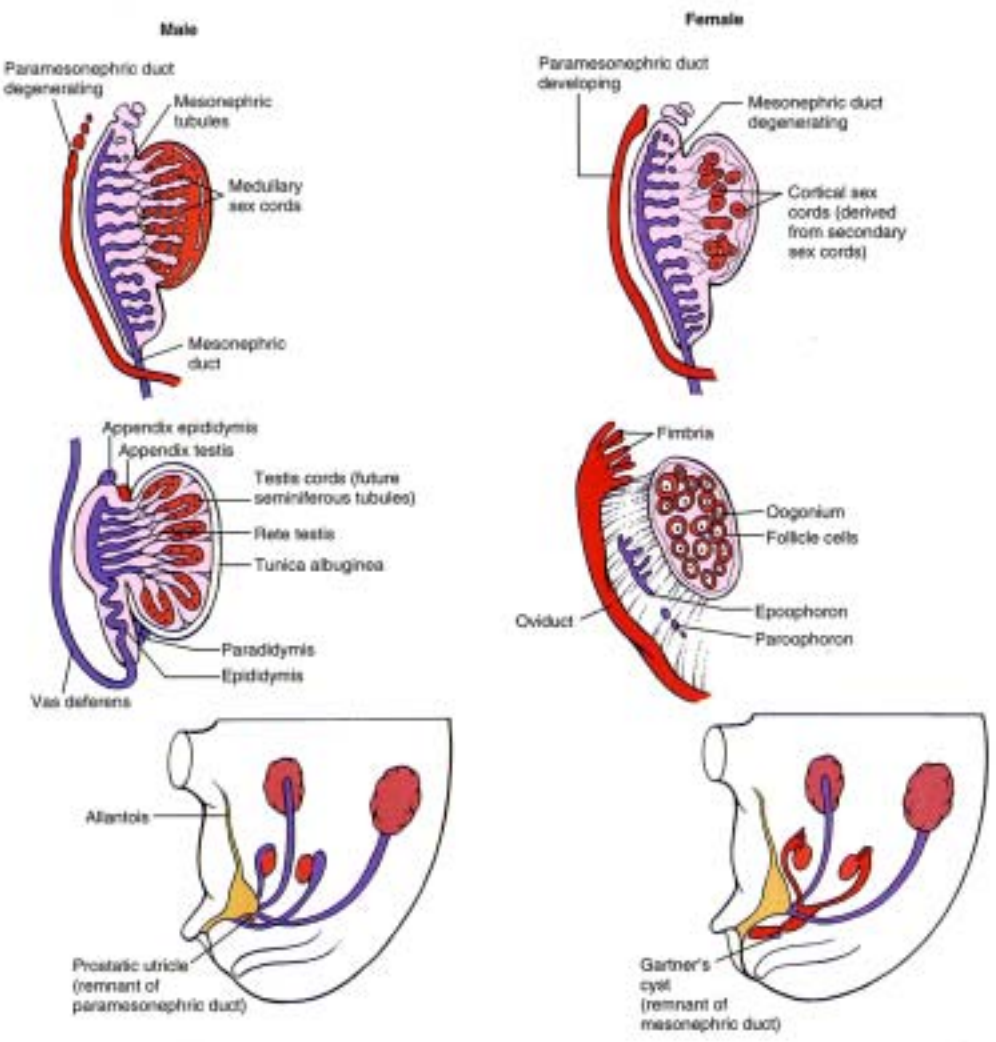


**Fig. 14-6.** Male gonadal development compared with that of the female. The male and female genital systems are virtually identical through the seventh week. In the male, SRY protein produced by the pre-Sertoli cells causes the medullary sex cords to develop into presumptive seminiferous tubules and rete testis tubules and causes the cortical sex cords to regress. Antimullerian hormone produced by the Sertoli cells then causes the paramesonephric ducts to regress. Leydig cells also develop, which in turn produce testosterone, the hormone that stimulates development of the male genital duct system, including the vas deferens and the presumptive efferent ductules.

As shown in Fig. 14-6, the germ cell deficient region of the medullary cords becomes the rete testes. This structure joins the five to twelve resident mesonephros tubules (under the influence of testosterone) to form the efferent ductules. These then drain into the mesonephric duct which develops into the epidymis and vas deferens. The male duct system is therefore continuous with the seminiferous tubules.

In females, in the absence of testosterone, the mesonephric tubules and ducts degenerate and the paramesonephric ducts (in the absence of AMH) form the fallopian tubes, uterus and upper part of the vagina. This occurs in the **presence or absence of ovaries** and therefore the internal female reproductive tract is <u>independent of gonadal secretions</u>, and is cell autonomous.

Paramesonephric distal tips adhere and contact the posterior wall of the pelvic urethra (Figs. 14-6, 14-7). This wall forms a slight thickening, the sinusal tubercle. After contact with the sinusal tubercle, the Müllerian ducts fuse from caudal to cranial, forming the uterovaginal (genital) canal.

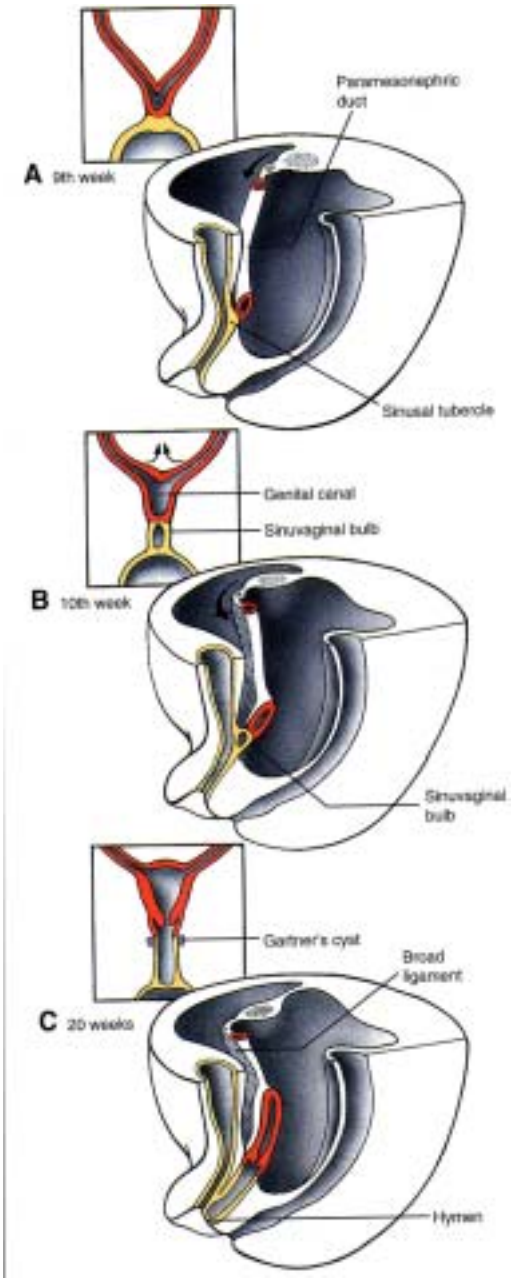


**Fig. 14-7.** Formation of the uterus and vagina. A, The uterus and superior end of the vagina begin to form as the paramesonephric ducts fuse together near their attachment to the posterior wall of the primitive urogenital sinus. B, C, The ducts then zipper together in a superior direction between the third and fifth months. As the paramesonephric ducts are pulled away from the posterior body wall, they drag a fold of peritoneal membrane with them, forming the broad ligaments of the uterus. A-C, The inferior end of the vagina forms from the sinuvaginal bulbs on the posterior wall of the primitive urogenital sinus.

The superior aspect of the canal contributes to the vagina and forms the uterus. The unfused cranial portion of the paramesonephric ducts develops into the oviduct. The endoderm of the sinusal tubercle continues to thicken forming the sinusal bulbs, separating the vagina and urinary system. The bulbs become the lower aspect of the vagina. The inferior aspect of the uterovaginal canal occludes with the vaginal plate (origin unknown). The latter elongates during the third-fifth month and canalizes to form the vaginal lumen. There is no contact between the developing duct system and the ovarian follicles during development and therefore at maturity, the released egg must be caught by the fimbria of the fallopian tube.

## Formation of the External Genitalia

The development of external genitalia also goes through an indifferent or bipotential phase (Fig.14-8). The external genetalia form from a pair of labrioscrotal folds, a pair of genital folds and an anterior genital tubercle and are similarly structured in males and females until ~8th week of development.

**Male:** Elongation of the genital tubercle begins in the 10th week in the male. As shown in Fig 14-8, the genital folds fuse and, with the elongation of genital tubercle, form the shaft and glans of the penis. Fusion of the urogenital folds, encloses the definitive urogenital sinus, forming the penile urethra. A small invagination of ectoderm covering the glans penis forms the distal most urethra.
The scrotum is formed from the labioscrotal folds. All of these morphological changes are dependent on the hormone, **DHT**. This hormone is derived locally from testosterone by the action of the enzyme, 5 alpha reductase.

**Female:** In the absence of androgen, the genital tubercle does not elongate and becomes the clitoris. The genital folds do not fuse and become the labia minora. The labioscrotal (genital) swellings become the labia majora.

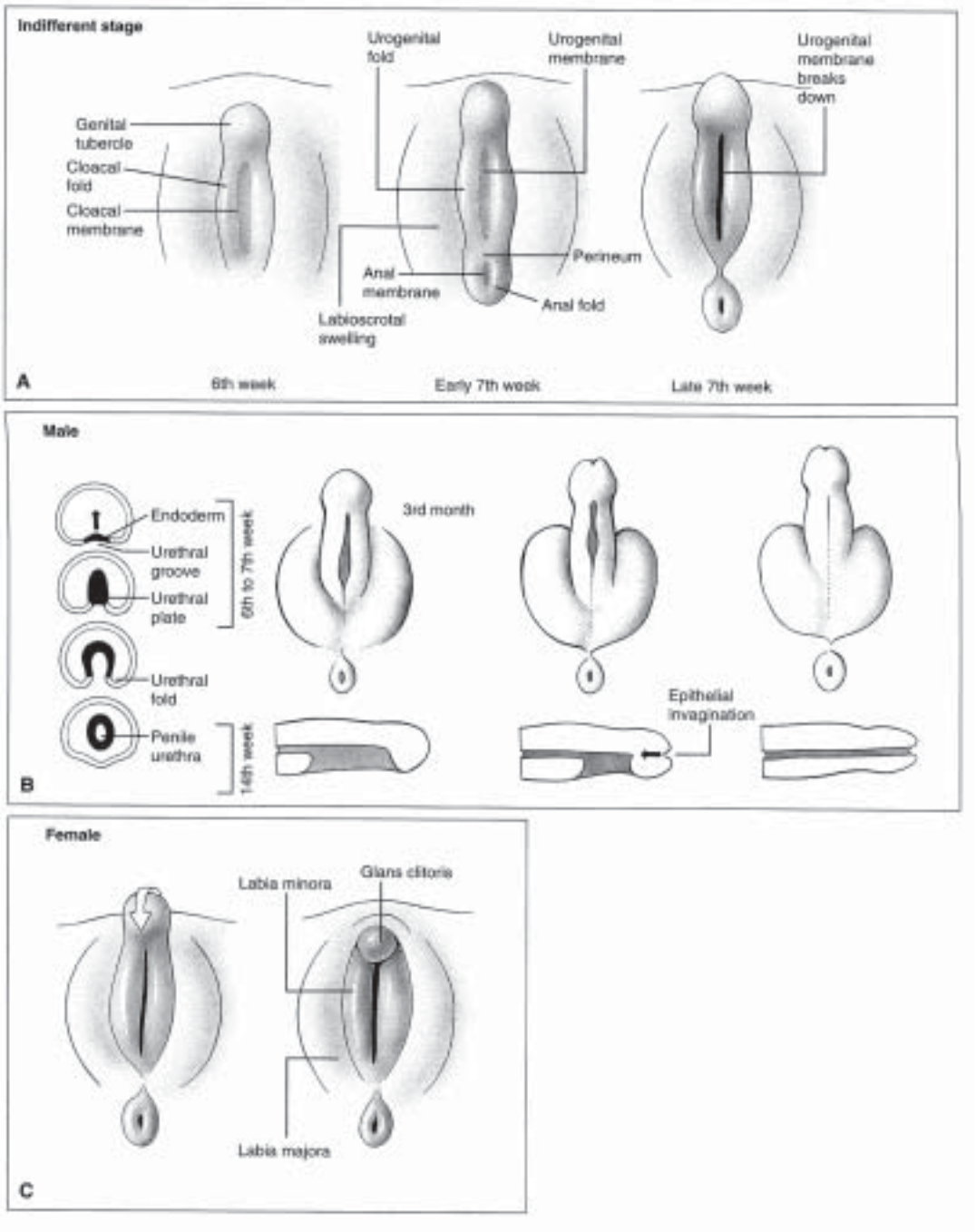


**Fig. 14-8.** Formation of the external genitalia in males and females. A, The external genitalia form from a pair of labioscrotal folds, a pair of urogenital folds, and an anterior genital tubercle. Male and female genitalia are morphologically indistinguishable at this stage. B, In males, the urogenital folds fuse, and the genital tubercle elongates to form the shaft and glans of the penis. Fusion of the urogenital folds encloses the definitive urogenital sinus to form most of the penile urethra. A small region of the distal urethra is formed by the invagination of ectoderm covering the glans. The labioscrotal folds give rise to the scrotum. C, In females, the genital tubercle bends inferiorly to form the clitoris, and the urogenital folds remain separated to form the labia minora. The labioscrotal folds form the labia majora.

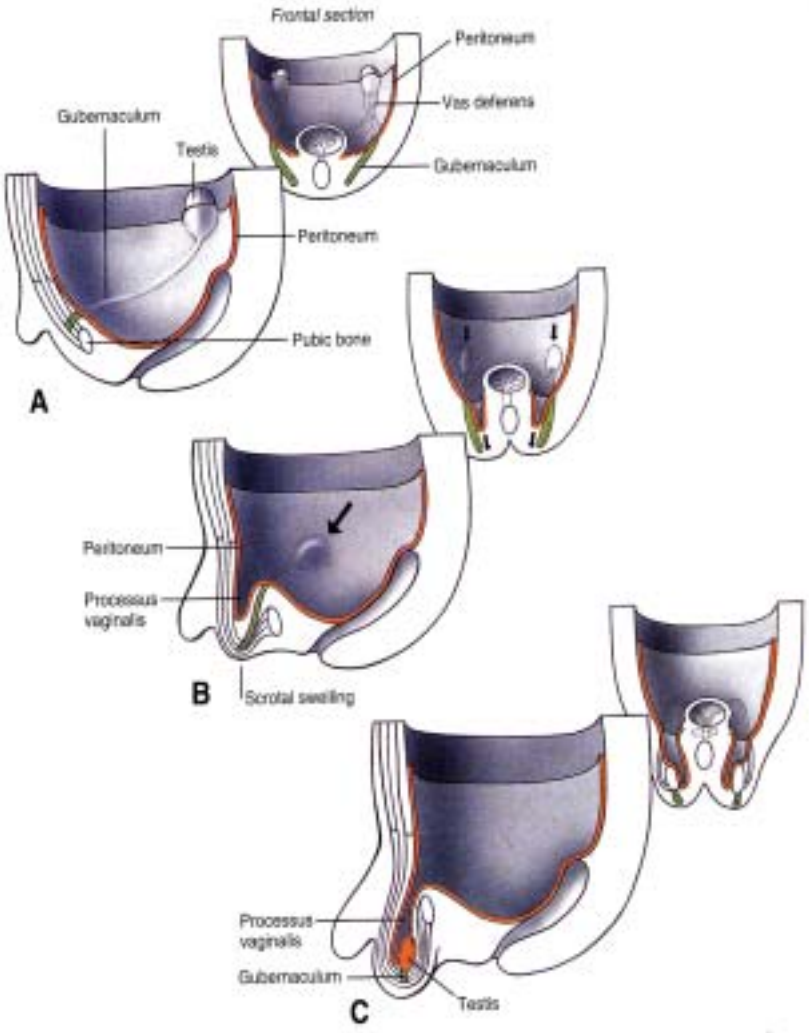


**Fig. 14-9.** Descent of the testes. A-C, Between seventh week and birth, shortening of the gubernaculum testis causes the testes to descend from the 10th thoracic level into the scrotum. The testes pass through the inguinal canal in the anterior abdominal wall.

## Gonadal Descent

During embryonic and fetal life both the testes and the ovaries descend from their original position in the thorax to a much lower level. The testes ultimately descends into the scrotum and the ovaries remain in the pelvis. In both sexes, the descent depends upon a structure called the gubernaculum. Fig. 14-9 depicts the descent of the testis between seven weeks and forty weeks of gestation. The action of both testosterone and dihydrotestosterone (DHT) are important in the function of the gubernaculum and this is described in detail in your text. Lack of descent of the testes is a condition called cryptorchism (undescended testes). Many times these children are treated with HCG (human chorionic gonadotropin) which, much like LH in the normal situation, stimulates the Leydig cells to produce testosterone causing the testes to descend. This is physiologically significant as normal sperm production in the male is dependent on the cooler temperature found in the scrotum compared to the abdominal cavity.

## SUMMARY:

The development of a normal reproductive tract in the human fetus is a chronological process which begins with the distribution of sex chromosomes to the pronucleus of the fertilized egg. The expression of specific genes on these chromosomes and the migration and proliferation of specific cells provides the regulatory mechanisms for subsequent genital differentiation. The growth of gonads along the posterior body walls is mediated by the interaction of sex-determining factors, their downstream targets and embryonic cells localized in the region. Gonadal development and differentiation provides autosomal regulatory factors which control the growth and distribution of the rest of the reproductive tract, including the external genitalia. In both males and females complete sexual maturity is not realized until puberty when another cascade of hormone regulated events induces the capacity to reproduce.

**Clinical correlations**

**I. Female sex reversal or adrenal genital syndrome (AGS)**

The most common intersexuality problem by far is due to AGS. **Adrenal Genital Syndrome ot Congenital Adrenal Hyperplasia (CAH)** occurs in 46 XX genetic females who are virilized. AGS is the only type of ambiguous genitalia in which surgery is not required for diagnosis. This can be done with **karyotyping** and biochemical testing.

**CAH is caused by an interruption in the biosynthetic pathway of glucocorticoids and mineralocorticoids** (this will be discussed later in SBPM/D). Four basic enzymes can go awry. The most common defect is **21-hydroxylase deficiency**, resulting in the increased synthesis and secretion of **17-hydroxyprogesterone**, which is diagnostic of CAH. Early diagnosis of 21-hydroxylase deficiency is important since it leads to profound dehydration due to salt loss, resulting from decreased levels of glucocorticoids and mineralocorticoids. Deficiencies in any of the four basic enzymes result in increased levels of **androstenedione**, a powerful virilizing hormone. These females become virilized, the degree to which is related to the levels of androstenedione. Androstenedione can be converted either to testosterone or to estradiol. Depending on the degree of the enzyme malfunction, individuals with CAH can appear to be anything from a normal female (at low levels of blockage) to a normal male (at high blockage levels). In addition, low blockage levels show no salt wasting.

In cases of complete blockage, the child looks perfectly normal with a rugated scrotum, but has bilateral **non-palpable** gonads, since there are o**varies not testes**. In one of these cases, the baby presented 10 days after birth with severe salt wasting and coma before the diagnosis of CAH was made.

**A take home message**: When we see a baby in the newborn nursery with undescended testes or bilateral non-palpable testes and any form of phallic development, that baby must be evaluated for Congenital Adrenal Hyperplasia. The karyotype can be done by a buccal smear.

The gender assignment in these cases should be female (hormones at puberty will be female, absence of testes results in survival of internal female genitalia). Hormonal management, glucocorticoid and mineralocorticoid replacement, clitoral recession, and vaginoplasty are required. Some 46 XX gender-assigned males do exist, but life is difficult for these children because they are actually females.

**II. Male Sex Reversal**

Male pseudohermaphroditites are 46 XY individuals who are poorly virilized for one of three reasons: (A) insufficient testosterone production, (B) inability to convert testosterone to DHT (5 alpha-reductase deficiency), (C) diminished response to DHT at the end organ.

(A) In the case of insufficient testosterone production, or **androgen insufficiency syndrome**, any one of the enzymes involved in the production of testosterone may be at fault. Androgen insufficiency syndrome cases are very rare, and are due to an autosomal recessive genetic pattern. If a 46 XY male presents with androgen insufficiency syndrome, and an accurate diagnosis is established, the child can immediately be given testosterone and DHT and will go on to lead a normal life. In addition, the child's testes are brought down and an initial repair of hypospadias is perfomed.

(B) The inability to convert testosterone (T) to DHT is the result of **5 alpha-reductase deficiency**. This deficiency, which is an autosomal recessive, results in 46 XY individuals who are phenotypically female. At puberty, when the testes start producing testosterone, these individuals develop male secondary sex characteristics both physiologically and behaviorally. This can cause problems if the individual is gender-assigned female. In some cultures (where this mutation is common), this is recognized early and these children are designated as "other" instead of male or female.

**testosterone** —(**5 alpha-reductase**)→ **dihydrotestosterone**

(C) Diminished response to T or DHT at the end organ is also known as **end organ insensitivity syndrome**. This is also known as androgen insensitivity syndrome and testicular feminization syndrome –there are varying degrees of end organ insensitivity to DHT. The lack of response to dihydrotestosterone causes the genital folds, genital swellings, and genital tubercle of the 46 XY male to appear female. As the person can convert testosterone to estrogen (see below), secondary sex characteristics at puberty can be female. These individuals have testes and normal Wolffian duct structures (only need T), but their end organ was not able to respond to DHT, so their external genitalia appear to be those of a female. It is interesting to note that T and DHT use the same "androgen" receptor but tissues respond in tissue specific manner.

**testosterone** —(**Aromatase**)→ **estradiol**