Development of the Male Gonad

6

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Learning Objectives

- Normal sexual development
- Gonads and sex differentiation
- Testicular descent
- Stages of errors in development

6.1 Introduction

Sexual development is a complex process which begins with fertilization and stops at gamete formation. The phenotypic gender as first apparent at birth seems to be a simple phenomenon, but, in fact, the differences between a male and female are strikingly complex (Wilhelm et al. 2007). The purpose of sexual differentiation is to provide the needful anatomy and physiology for reproduction. The sexual fate is determined following gonadal development. All secondary sexual dimorphisms pursue from the gonadal differentiation and their acquirement of endocrine function.

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A. Kumar, M. Sharma (eds.), *Basics of Human Andrology*,
DOI 10.1007/978-981-10-3695-8_6

6.2 Normal Sexual Development

According to the Jost Paradigm, sex is determined through:

(a) Chromosomal or genetic sex: influenced by the presence of Y chromosome
(b) Gonadal sex or primary sex: influenced by the presence of testis-determining factor (TDF)
(c) Phenotypic sex or secondary sexual characters: influenced by hormones produced by gonads (Jost 1979)

The chromosomal sex determines the gonadal sex. The gonadal sex in turn decides the fate of internal ducts and type of phenotypic appearance that leads to the gender identity.

6.2.1 Chromosomal or Genetic Sex

The male chromosomal pattern is 46, XY, while the female pattern is 46, XX. During initial development, all embryos are similar with regard to sexual differentiation having bipotential gonads, Mullerian and Wolffian ducts; and bipotential external genitalia.

6.2.2 Gonadal Sex

The gonadal/genital ridge develops during the first week of gestation and becomes a bipotential gonad. The gonadal differentiation is guided by the TDF. During second month of gestation, in the presence of TDF, the indifferent gonad undergoes testicular development. On the contrary, it develops into an ovary in absence of this factor.

6.2.3 Phenotypic Sex

The gonad exerts paracrine effect on the internal duct and influences its development. In the absence of testicular tissue, female internal duct or Mullerian duct develops, and development of female phenotype occurs. Similarly, in the presence of testicular tissue along with adequate testosterone and Mullerian inhibiting substance (MIS), male internal duct or Wolffian duct develops, and phenotype becomes masculine. MIS is produced by Sertoli cells of testes during eighth week of gestation. In a male fetus with normal testicular functions, testosterone stimulates development of Wolffian duct, and MIS represses Mullerian duct development. As a result, the epididymis, vas deferens, and seminal vesicles are formed (see Fig. 6.1; Wilhelm et al. 2007). In contrast to testes, the follicular cells of ovary are not active in steroid production before puberty.

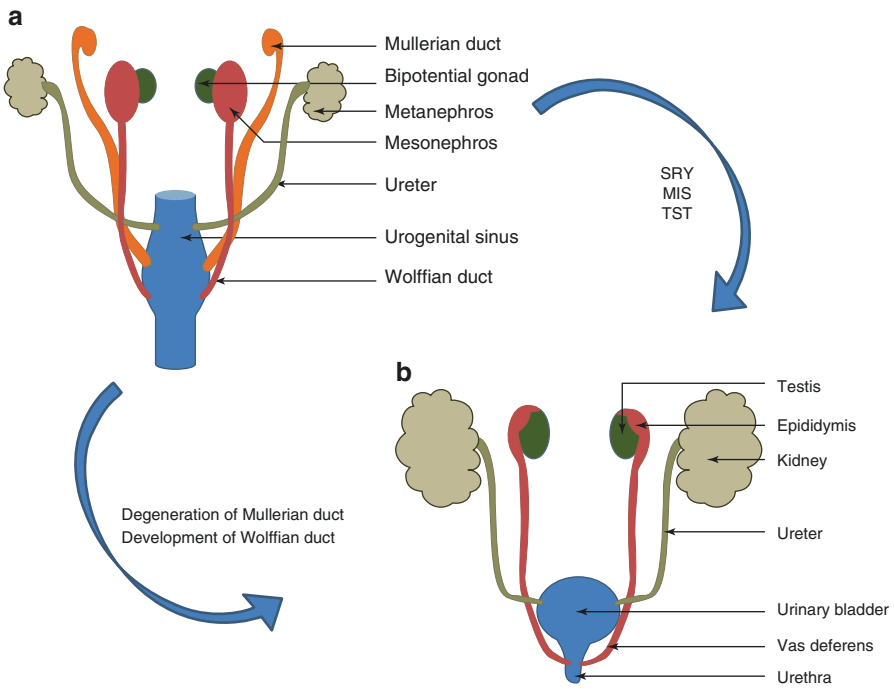

Fig. 6.1 Schematic diagram showing development of the male internal genitalia from the Wolffian duct (**a**) and degeneration of Mullerian duct (**b**); *MIS* Mullerian inhibiting substance, *SRY* sex-determining region Y, *TST* testosterone

The external genitalia are bipotential till 7 weeks of intrauterine life. Without androgen effect, the external genitalia appear phenotypically female. In the absence of MIS, normal vagina is formed.

If dihydrotestosterone (DHT) is sufficient from seventh to eighth week of gestation until birth and fetal response is normal, the bipotential genitalia develop into male type (Siitera and Wilson 1974). The genital tubercle forms the glans. The urethral folds from both sides fuse in midline to form urethral meatus at the glans tip. The scrotum is formed by fusion of two genital swellings. The prostate develops from urogenital sinus. The development of prostate gland has been described in the chapter, The Prostate Gland.

6.3 Gonads and Sex Differentiation

Various genes including some autologous ones are involved in sexual differentiation. The sex-determining region Y (SRY) gene gives rise to a protein product; the presence of which in the somatic cells leads to male development. This step is the primary sex determination. Testosterone production then determines the further sexual differentiation. The gonads are derived from the following embryonic structures:

(a) Mesothelium
(b) Underlying mesenchyme
(c) Primordial germ cells (PGCs)

PGCs originate in the epiblast and migrate through the primitive streak. The PGCs migrate during the fourth week along the dorsal mesentery of the hindgut and arrive at the genital ridge in the fifth week. At the fifth week of gestation, on the medial side of the mesonephros, a thickened mesothelium develops. Proliferation of the mesothelial cells and condensation of underlying mesenchyme produces a bulge called genital ridge. The factors involved in increased proliferation and migration of cells from adjacent mesonephros include hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and neurotrophin-3 (NT-3). PGCs lose their motility in the genital ridge, and they invade genital ridges during the sixth week. PGCs are thus bipotential (Hilscher et al.1974).

Just before arrival of PGCs, genital ridge epithelium proliferates, and epithelial cells penetrate the underlying mesenchyme. Finger-like primitive epithelial sex cords are formed. This is the state of bipotential gonad. The bipotential gonad consists of an outer cortex and an inner medulla. In females (XX), cortex normally differentiates into an ovary and medulla regresses. In males (XY), medulla differentiates into testis and cortex regresses (Wilhelm et al. 2007).

In females, germ cells proliferation continues till they enter meiosis at 13.5 days postconception (dpc). In female embryos, XX PGCs develop as oocytes, and in male embryos, XY PGCs develop as prospermatogonia (Palmer and Burgoyne 1991b). Certain processes occur only in males after SRY expression onset (Capel et al. 1999). Sertoli cells get polarized and reside within the testis. The size of gonads increases.

The female gonad continues to appear indifferent and does not express SRY. The primitive sex cords are ill-defined. The in-growth of coelomic and mesonephric epithelial cells is followed by their condensation in the more cortical regions. The mesenchymal cells secrete outer membrana propria. The mesenchymal cells give rise to the granulosa cells of follicles, while the oogonia give rise to oocytes. During the 20th–25th week, primordial follicles give rise to the primary follicles. Each follicle contains a single germ cell. The Mullerian duct differentiates into the oviducts, uterus, cervix, and upper vagina, while the Wolffian duct degenerates in females.

Under the influence of SRY gene, there is proliferation of primitive sex cords that penetrate deeper into the medulla to form the medullary or testis cords. With further differentiation, tubules of rete testis are formed. A dense layer of fibrous connective tissue or tunica albuginea is formed, which separates the testis cords from surface epithelium. During the fourth month, testis cords become continuous with those of the rete testis. The testis cords are composed of two types of cells – Sertoli cells and primitive germ cells. Both cell types are derived from the surface epithelium of the gonad. Leydig cells develop shortly after the beginning of the differentiation of testis cords and are derived from the original mesenchyme of the genital ridge. The mesenchyme is present between the testis cords. Leydig cells produce testosterone.

Testes are able to influence differentiation of the genital ducts and external genitalia by testosterone; the secretion of which starts around the eighth week.

At puberty, testis cords acquire lumen and transform into the seminiferous tubules. The seminiferous tubules join rete testis which enters efferentes ductules. The efferent ductules link rete testis and the Wolffian duct, which becomes the ductus deferens (Wilhelm et al. 2007).

6.3.1 Descent of Testes

During intrauterine life, testes descend from the initial intra-abdominal site at tenth thoracic vertebral level to scrotal location at around 33–35 weeks of gestation or just before birth in humans. In abdomen, the mesonephric gonadal complex is held in position by cranial suspensory ligament and gubernaculum (see Fig. 6.2). Cranial suspensory ligament runs from cranial portion of the gonad to the diaphragm. Gubernaculum is mesenchymal in origin and is attached to the caudal portion of the gonad and extends to the peritoneal floor where it is attached to the fascia between

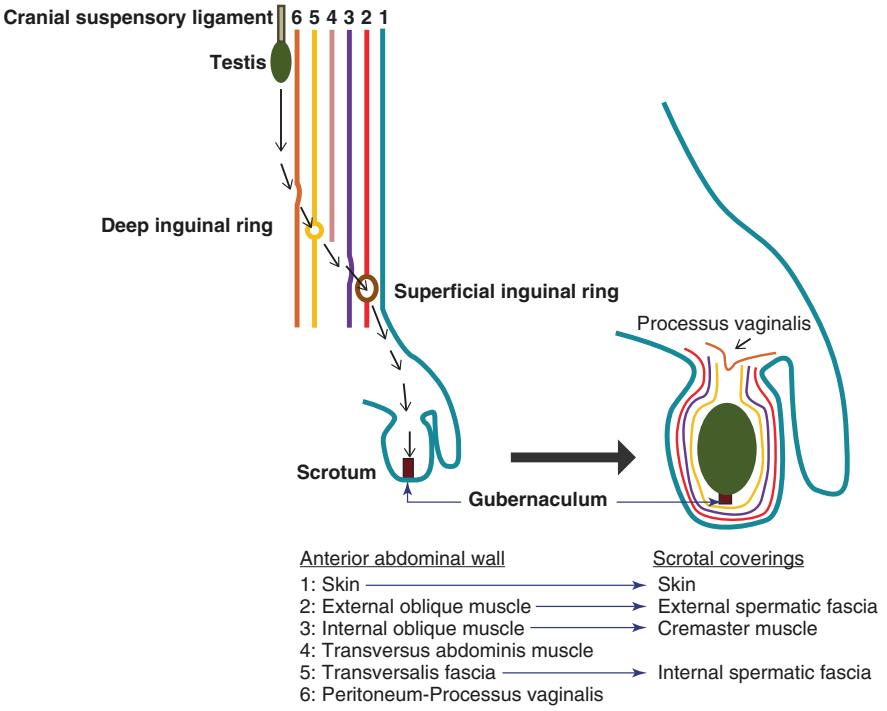

Fig. 6.2 Schematic diagram showing descent of testis

the developing external and internal oblique abdominal muscles in the region of labioscrotal swellings (Hunter 1762).

Descent of testes occurs in two phases – transabdominal phase followed by inguinoscrotal phase. After 8 weeks of gestation, the peritoneum just ventral to the gubernaculum evaginates forming processus vaginalis. Further evagination of processus vaginalis pushes out extensions of transversalis fascia, internal oblique muscle, and external oblique muscle which eventually form the internal spermatic fascia, cremaster muscles, and external spermatic fascia, respectively, all forming the coverings of the scrotum (see Fig. 6.2).

Due to the evagination of processus vaginalis, inguinal canal is formed which extends from deep inguinal ring to superficial inguinal ring. The deep ring is situated at the point where fascia transversalis is pushed inferiorly by the processus vaginalis. Similarly, superficial ring is formed where external oblique muscle is pushed inferiorly by the processus vaginalis.

The processus vaginalis evaginates up to scrotum after which gubernaculum shortens and pulls gonads through inguinal canal into scrotum. The gubernaculum expresses homeobox gene (HOX A10) and fibroblast growth factor-10 (FGF10) which control gubernacular enlargement by cell proliferation (Nightingale et al. 2008). Gubernacular swelling has an additional role of enlarging inguinal canal. The increased abdominal pressure created by the growth of abdominal viscera also assists movement of testes through the canal.

During the time of inguinoscrotal descent of testes, the androgen receptors have been found to be located in the inguinoscrotal fat pad which is supplied by the genitofemoral nerve whose cell bodies are located in the dorsal root ganglion at first and second lumbar spinal nerves (Hutson et al. 2015). Possibly, androgens bind to androgen receptors in inguinoscrotal fat pad which produces neurotrophins that regulate secretion of calcitonin gene-related peptide (CGRP) by genitofemoral nerve. CGRP acts as a chemoattractant that guides the path of migration of the gubernaculum.

Once testis reaches the scrotum, the proximal end of the processus vaginalis disintegrates. The distal remnant of processus vaginalis left is called tunica vaginalis. Some proximal remnants of processus vaginalis may remain, and these and the tunica vaginalis may fill with serous fluid, forming testicular hydrocoeles in pathologic conditions or subsequent to injury. If the proximal end of the processus vaginalis does not disintegrate, abdominal contents may herniate through the inguinal canal into the scrotum causing congenital inguinal hernia. The condition where one or both testes fail to reach bottom of the scrotum is called cryptorchidism.

6.3.2 Genes Involved in Bipotential Gonad

Mutation analysis in humans and animal experiments has helped to identify some genes relevant in the initial formation of indifferent genital ridge (Wilhelm et al. 2007).

Wt1 (Wilms' tumor suppressor 1) The sites of its location are the mesonephros, urogenital ridge, and developing gonads. Wt1 is expressed in the developing granulosa cells in females and Sertoli cells in males (Pritchard-Jones et al. 1990; Hanley et al. 1999; Hammes et al. 2001).

SF1 (Steroidogenic Factor 1) SF1 is present in the developing urogenital ridge, hypothalamus, and the anterior pituitary gland. After sexual differentiation, it is detected in the testes, in both Leydig and Sertoli cells (Hanley et al. 1999; El-Khairi and Achermann 2012; Suntharalingham et al. 2015).

Lhx9 (LIM homeobox gene 9) Lhx9 is present in the developing urogenital ridge and is involved in protein-protein interactions. The gene has a nucleic acid-binding homeobox domain (Birk et al. 2000).

Emx2 (Empty-spiracles homeobox gene 2) Emx2 is expressed in the developing dorsal telencephalon and in the epithelial parts of the urogenital system. In Emx2-/- mutants, the Mullerian duct never develops (Miyamoto et al. 1997).

6.4 Stages of Errors in Development

6.4.1 Chromosomal or Genetic Sex

The SRY is an important gene locus for testicular development (Swain and Lovelt-Badge 1999). Many transcription factors like SF-1, WT-1, DAX-1, and SOX-9 are also important for gonadal development (Migeon et al. 2002; Mendonca et al. 2002).

Two genes on the SRY region have a major role in male differentiation, SOX9 and FGF9, contributing to testicular cord formation. A novel mutation of the SRY gene has been reported in a XY sex reversal patient (Zhou et al. 2003). A homozygous SOX9 deletion with female external genitalia results in a XY karyotype (Chen et al. 2012). 46, XY sex-reversed patients are associated with mutations in known genes such as SRY, SOX9, WT1, and SF1, but the association is found in less number of patients. This indicates that other unknown genes may influence sex determination (Pannetier et al. 2004). Chromosome X duplications, chromosomes 9 and 10 deletions, and chromosome 17 translocations have been associated with abnormal testicular differentiation, in male-to-female sex reversal in 46, XY individuals (Flejter et al. 1998). SRY protein has been expressed in tubules of streak gonads and rete testis (Salas –Cortes et al. 2000). SRY protein expression is reported in both testicular and ovarian tissues in 46, XX true hermaphrodites. In this case, SRY protein does not inhibit ovary development. It may be suggested that other factors are required for complete testis development, especially downstream of the SRY protein (Salas –Cortes et al. 2000).

6.4.2 Gonadal Sex or Primary Sex Determination

Somatic mesenchyme forms the gonadal stroma. PGCs migrate and colonize in the bipotential gonad. In mosaic gonads, some XX cells may form Sertoli cells, while XY cells may form granulosa cells (Palmer and Burgoyne 1991a). Transgenic mice that express a reporter protein under the control of the SRY promoter shows expression in granulosa cells in an XX gonad, apart from expression in Sertoli cells in XY

gonads (Albrecht and Eicher 2001). Steroid-secreting Leydig cells of testis and theca cells of ovary are probably derived from a single precursor.

6.4.2.1 Precursors for Internal and External Genitalia

The primordia for both male and female internal genital ducts are derived respectively from the Wolffian ducts in males and para-mesonephric or Mullerian duct in females.

The external genitalia in both sexes develop from a single bipotential precursor through the action of androgens. At 6 weeks gestation, failure of conversion of testosterone to DHT or nonaction of DHT leads to incomplete masculinization. There is associated rise in testosterone in response to luteinizing hormone (LH) surge. The rise in testosterone levels remains until the 14th week which promotes phenotypic differentiation during this period. After the 14th week, fetal testosterone levels decrease. But, continued action of testosterone contributes to the growth of the phallus.

In females, urethral folds and genital swellings remain separate forming labia minora and majora, respectively, while genital tubercle forms the clitoris. Androgens in males cause urethral folds to fuse forming urethra. The genital swellings fuse in the midline and form scrotum. The genital tubercle expands and forms glans penis.

6.4.2.2 Genes Involved in Duct Formation

Pax2 is important during development of urogenital structures, and it is expressed in the epithelial derivatives, condensing mesenchyme, Wolffian ducts, and mesonephric tubules (Torres et al. 1995).

Wnt 7a is expressed at the anterior mesonephros and throughout the Mullerian duct (Parr and McMahon 1998).

6.4.3 Phenotypic Sex or Secondary Sex Differentiation

The development of male phenotypic sex is governed by the testicular hormones – AMH and androgens (Jost 1972). The response of urogenital sinus and external genitalia to androgen requires conversion of testosterone into DHT by 5α-reductase enzyme. DHT binds directly to specific high affinity receptor proteins; the hormone-receptor complex induces an increase in transcription of specific mRNA inducing, as final effect, male phenotypic differentiation and in particular virilization of the external genitalia and male urethra. In the ovary, lack of differentiation persists.

6.5 Male Accessory Sex Glands

In males, two epithelial tissues-the epithelial mesodermal origin of the Wolffian duct and the epithelial endodermal origin of the urogenital sinus, are the source of origin of the accessory sex glands. Prostate gland and Cowper's gland are

endodermal in origin. The seminal vesicle has a mesodermal origin. The details of development of genital ducts and male accessory sex glands have been described in the chapter, Genital Ducts and Other Accessory Sex Glands.

Key Questions

1. Describe the types of sexual development.
2. Describe the role of SRY gene in gonadal development.
3. What are the various genes that influence sexual development?
4. Discuss the two phases of testicular descent.

References

Albrecht K, Eicher E. Evidence that Sry is expressed in pre- Sertoli cells and Sertoli and granulosa cells have a common precursor. Dev Biol. 2001;240:92–107.

Birk OS, Casiano DE, Wassif CA, Cogliati T, Zhao L, Zhao Y, et al. The LIM homeobox gene Lhx9 is essential for mouse gonad formation. Nature. 2000;403:909–13.

Capel B, Albrecht KH, Washburn LL, Eicher EM. Migration of mesonephric cells into the mammalian gonad depends on Sry. Mech Dev. 1999;84:127–31.

Chen SY, Lin SJ, Tsai LP, Chou YY. Sex-reversed acampomelic campomelic dysplasia with a homozygous deletion mutation in SOX9 gene. Urology. 2012;79:908–11.

El-Khairi R, Achermann JC. Steroidogenic factor-1 and human disease. Semin Reprod Med. 2012;30:374–81.

Flejter WL, Fergestad J, Gorski J, Varvill T, Chandrasekharappa S. A gene involved in XY sex reversal is located on chromosome 9, distal to marker D9S1779. Am J Hum Genet. 1998;63:794–802.

Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, Ziegler U, et al. Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell. 2001;106:319–29.

Hanley NA, Ball SG, Clement-Jones M, Hagan DM, Strachan T, Lindsay S, et al. Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. Mech Dev. 1999;87:175–80.

Hilscher B, Hilscher W, Bulthoff-Ohnolz B, Kramer U, Birke A, Pelzer H, et al. Kinetics of gametogenesis. I. Comparative histological and autoradiographic studies of oocytes and transitional prospermatogonia during oogenesis and prespermatogenesis. Cell Tissue Res. 1974;154:443–70.

Hunter J. Observations on the state of the testis in the foetus and on the hernia congenita. In: Hunter W, editor. William Hunter's medical commentaries. London: Hamilton; 1762. p. 75–89.

Hutson JM, Li R, Southwell BR, Newgreen D, Cousinery M. Regulation of testicular descent. Pediatr Surg Int. 2015;31:317–25.

Jost A. A new look at the mechanism controlling sex differentiation in mammals. Johns Hopkins Med J. 1972;130(1):38–53.

Jost A. Basic sexual trends in the development of vertebrates. Sex, hormones and behavior. Amsterdam: Elsevier; 1979. CIBA Foundation Symposium no. 62.

Mendonca BB, Domenice S, Costa EMF, Arnhold IJP. Male pseudohermaphroditism. In: New M, editor. Paediatric Endocrinology. www.Endotext.org. 2002.

Migeon CJ, Wisniewski AB, Brown TR. Androgen insensitivity syndrome. In: Chrousos G, editor. Hormone resistance and hypersensitivity States. Baltimore/Lippincott: Williams and Wilkins; 2002. p. 409–39.

Miyamoto N, Yoshida M, Kuratani S, Matsuo I, Aizawa S. Defects of urogenital development in mice lacking Emx2. Development. 1997;124:1653–64.
Nightingale SS, Western P, Hutson JM. The migrating gubernaculum grows like a "limb bud". J Pediatr Surg. 2008;43(2):387–90.
Palmer SJ, Burgoyne PS. In situ analysis of fetal, prepuberal and adult XX_XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. Development. 1991a;112:265–8.
Palmer SJ, Burgoyne PS. XY follicle cells in the ovaries of XO/XY and XO/XY/XXY mosaic mice. Development. 1991b;111:1017–9.
Pannetier M, Mandon-Pépin B, Copelli S, Fellous M. Molecular aspects of female and male gonadal development in mammals. Paediatr Endocrinol Rev. 2004;1:274–87.
Parr BA, McMahon AP. Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. Nature. 1998;395:707–10.
Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, et al. The candidate Wilms' tumour gene is involved in genitourinary development. Nature. 1990;346:194–7.
Salas-Cortés L, Jaubert F, Nihoul-Feketé C, Brauner R, Rosemblatt M, Fellous M. SRY protein is expressed in ovotestis and streak gonads from human sex-reversal. Cytogenet Cell Genet. 2000;91:212–6.
Siitera PK, Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. J Clin Endocrinol Metab. 1974;38(1):113–25.
Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. Best Pract Res Clin Endocrinol Metab. 2015;29:607–19.
Swain A, Lovell-Badge R. Mammalian sex determination: a molecular drama. Genes Dev. 1999;13:735–67.
Torres M, Gómez-Pardo E, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. Development. 1995;121:4057–65.
Wilhelm D, Palmer S, Koopman P. Sex determination and Gonadal development in mammals. Physiol Rev. 2007;87:1–28.
Zhou C, Li LY, Fu JJ, Mo YQ, Zhong CG, Lu GX. 46, XY female sex reversal patient with a novel point mutation in the coding sequence of the SRY gene. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2003;20(5):369–72.