Trends in Andrology and Sexual Medicine
Series Editors: Emmanuele A. Jannini, Carlo Foresta, Andrea Lenzi, Mario Maggi

Carlo Foresta
Daniele Gianfrilli *Editors*

Pediatric and Adolescent Andrology

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Trends in Andrology and Sexual Medicine

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Preface

It has been estimated that about one in three men is affected by reproductive and sexual disorders, with variable percentages depending on age and countries. Many of the andrological diseases that occur in adulthood originate before the age of 18 and sometimes even during gestation and neonatal age.

Diagnosing and treating andrological disorders early is crucial, especially because some conditions raised during pediatric age and if they are not promptly recognized, they are managed with greater difficulty by the adult endocrinologist or andrologist. Pediatric and adolescent male subjects could suffer from reproductive and sexual problems, especially cryptorchidism, varicocele, hypogonadism, testicular hypotrophy, congenital anomalies of the genitourinary tract, and sexually transmitted infections. The prevention of this type of disorders should start early, during the childhood and even in pregnancy. It is of extreme importance the identification of early risk factors involved in their development. The male reproductive system is extremely susceptible to external insults during gestational period, after birth and up to puberty. Puberty is a central transitional time between childhood and adulthood, incorporating many physical and psychological changes. The pediatrician and other professionals involved have a pivotal role in the prevention and early diagnosis of male reproductive pathologies with considerable benefits for the community. In fact, it is estimated that prevention and appropriate information to young patients and their parents could lower the incidence of some disorders.

Despite the latest remarkable advances in modern medicine, andrological health is still a poorly addressed issue for many historical and cultural reasons. In some countries, prevention and early diagnosis in andrology have been neglected for far too long and this has contributed to increase the incidence and prevalence of diseases that are otherwise easy to prevent and treat if diagnosed early. Many young people are not correctly followed up by a specialized physicians during their physical, psychosexual, and emotional development. Therefore, diagnoses are often delayed, leading to a worsen patient's prognosis.

This book provides a comprehensive, state-of-the-art overview of the main andrological disorders during childhood and adolescence. The book also addresses the basic knowledge on the genetics and physiology of male reproductive system, the main involved risk factors together with a practical guide for diagnosis and

clinical management. Written by respected and recognized experts in the field, this book is intended to provide a major reference work not only for pediatricians but also for endocrinologists, andrologists, urologists, and adult sexologists who may also treat children and adolescents, as well as for basic and clinical scientists.

Padova, Italy — Carlo Foresta
Roma, Italy — Daniele Gianfrilli

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Csilla Krausz and Viktoria Rosta

1.1 Physiology of Male Sex Determination and Sexual Differentiation

Humans are born with 46 chromosomes, including 22 pairs of autosomes and 1 pair of gonosomes. Sex chromosomes can be X or Y, to specify which sexual determination pathway will be initiated. Generally, most women have 46,XX karyotype and most men 46,XY, but there are a few exceptions, which involve the sex monosomies (45,X0 or 45,Y0), the sex polysomies (i.e., 47,XXX, 47,XYY, or 47,XXY), translocations, rearrangements of chromosomes, or mutations of different genes.

Sex determination is regulated by the temporospatial expression of many different genes with critical dosage effects. Gonadal development is a particularly complex process, involving many genetically regulated, well-synchronized developmental steps, to balance opposing signals and to equilibrate cell proliferation and apoptosis.

Gonadal development starts in the fetal life, around the fourth gestation week, with the formation of the undifferentiated urogenital ridges, containing two different duct systems at the same time, the mesonephric (Wolffian) and the paramesonephric (Müllerian) ducts (Fig. 1.1). They are developed from the intermediate mesoderm, and a week later, they are colonized by primordial germ cells. Genes involved in the formation of bi-potential gonads belong to two groups: homeobox genes and transcriptional factors.

While the expression patterns of genes in the undifferentiated stage of gonadal development are the same in male and female, starting from the 41st gestational day, the gene expression profile will radically change in the two sexes.

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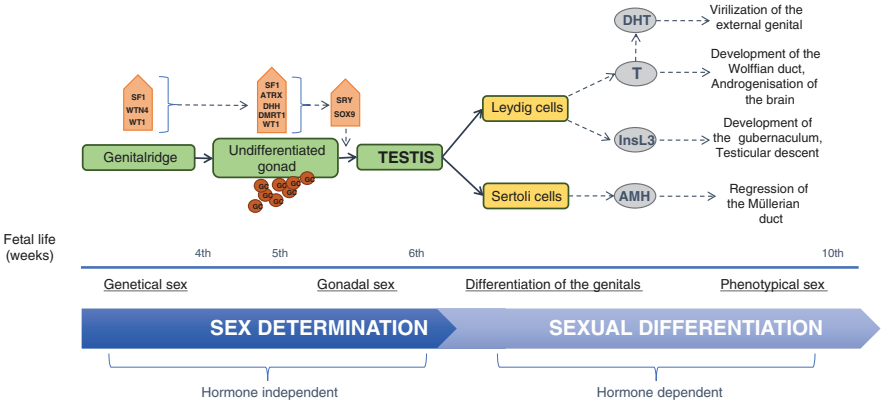

Fig. 1.1 Schematic figure illustrating the processes involved in male sexual development

In fact, the presence/absence of the Y chromosome-linked *Sex Determining gene on Y* (*SRY*) gene will determine the fate of the bi-potential gonadal precursors, i.e., to become either testes or ovaries. *SRY*, which contains 1 single exon, conserves 79 amino acid domains with similarities to a type of DNA-binding domain, the so-called high-mobility group (HMG) box. Apart from *SRY*, the HMG box containing protein family (SOX) involves other 20 transcription factors.

The first step of male gonadal development is linked to the upregulation of SRY expression. The exact mechanism by which the upregulation occurs is still not fully clarified, but a number of factors have been identified as modulators of the *SRY* expression. Among them, some are affecting the *SRY* expression positively, and some negatively. A positive regulator is the Wilms tumor 1 (WT1) protein and specifically its isoform WT1(KTS +), i.e., the protein containing three amino acids, Lys-Thr-Ser (KTS). This gene encodes a transcription factor that takes part in cellular development and cell survival and is also considered as a tumor suppressor. The transcripts of ***WT1*** are expressed in the primordial gonads and subsequently in the Sertoli cells of the testicles or in the granulosa and epithelial cells of the ovary. ***WT1*** is expressed in the same cell lineage, before, during, and after *SRY* expression. This protein has several isoforms, and one of them plays a role in the undifferentiated gonad, whereas the other is essential for testis determination. The two isoforms are the results of alternative splicing at the end of exon 9, and they only differ by the absence (-KTS) or presence (+KTS) of three amino acids. The +KTS isoform upregulates SRY expression through its binding to SRY mRNA, functioning as a post-transcriptional stabilizer. Other positive transcription factors are the *GATA4* with a co-factor, termed as *Friend of GATA2* (*FOG2*), and the *MAP3K4* gene, member of the mitogen-activated protein kinase (MAPK) pathway. *MAP3K4* pathway regulates the phosphorylation of *GATA4* and allows its binding to the *SRY* promoter with subsequent stimulation of *SRY*. *Steroidogenic factor 1* (*SF-1*; alias *NR5A1*) is known as a pivotal gene with multiple functions. It acts as an early modifier of the

expression of several genes required for the initiation and the maintenance of the male determination and differentiation pathways. In addition, it also takes part in the regulation of the hypothalamic-pituitary axis.

In the past, the female sex determination was supposed to be the default pathway in the absence of *SRY*. Several evidences indicate the existence of ovary-specific genes and pathways. These genes (*WNT4*, *RSPO1*, β-*catenin*, *FOXL2*) are also acting as negative modifiers of *SRY* expression.

Following the concerted action of positive modifiers of the *SRY* male-specific gene expression cascade, the testis determination starts. *SRY* signaling in the bipotential gonad acts as an initiator of pre-Sertoli cell differentiation and seminiferous tubule formation through the activation of *SRY-Box 9* (*SOX9*). This gene is a key pro-testis gene for the development of the normal male gonad. *SOX9* is expressed at low levels in both female and male sex, and its upregulation starts immediately with *SRY* expression in Sertoli cells. Sertoli cells are crucial for physiological testis development and for the subsequent Leydig cell differentiation, which are cells producing an essential male hormone, testosterone. After SRY-induced expression of *SOX9*, this protein keeps up its own expression due to autoregulation and positive feedback loops with *fibroblast growth factor 9* (*FGF9*), which activates *FGF receptor 2* (*FGFR2*), and *prostaglandin D2* (*PGD2*), encoded by *PTGDS*. *SOX9* directly inhibits *β-catenin* and indirectly, through *FGF9* which inhibits another female-specific gene, *WNT4*. Additional sex determination gene in human, which activates *SOX9*, *SOX8*, and *PTGSD* and inhibits the ovary-promoting genes, is the *doublesex gene in Drosophila and Mab3-related transcription factor 1* (*DMRT1*). One of the most important negative modifiers of *SOX9* expression is the *nuclear receptor DSSAHC critical region on the X chromosome 1* (*DAX1*, alias *NR0B1*) gene. It is predicted that yet unknown genes and co-factors contribute to the regulation of *SOX9*.

In conclusion, during sex determination, a complex interplay occurs between female and male fates, through a series of opposing feedback loops. Among them, the most crucial antagonism is between the *SRY/SOX9/FGF9* and *WNT4/RSPO1/β--catenin* signaling pathways [1].

Sex determination is followed by sexual differentiation. This process is characterized by sex steroid hormone secretion by the developing gonads, specific to the phenotypical sex, leading to the proper differentiation of the internal and external genitalia.

Differentiated Sertoli cells produce the anti-Müllerian hormone (AMH), which forces the regression of the Müllerian duct, while testosterone produced by Leydig cells induces the differentiation of the Wolffian duct derivatives into the epididymis, the vas deference, and the seminal vesicles. Furthermore, testosterone is converted—due to steroidogenic enzymes—into dihydrotestosterone (DHT), which is crucial for the normal development of the external genitalia and for virilization. The multifunctional *NR5A1* stimulates the expression of insulin-like polypeptide 3 (INSL3) together with maternal hCG and fetal LH. INSL3 is involved in the abdominal phase, whereas testosterone is necessary for the inguinal phase of testes descent.

1.2 Pathophysiology of Male Sex Determination and Sexual Differentiation

According to the Chicago Consensus Statement by Lee et al. (2006, 2016), disorders of sex development (DSD) are defined as congenital conditions within which the development of chromosomal, gonadal, or anatomic sex is abnormal [2, 3]. DSD can derive from defects in pathways related to sex determination or sex differentiation with a broad phenotypic spectrum.

The phenotypic representation of both 46,XY and 46,XX DSD patients depends on the etiology of the disorder and may range from female through male with ambiguous genitalia to male with normal virilization. Depending on the karyotype, disorders of gonadal development may be associated with testicular DSD (T-DSD), ovo-testicular DSD (OT-DSD), and complete/partial gonadal dysgenesis (GD) or be part of rare syndromic forms. Disorders related to androgen biosynthesis and action can be observed in both 46,XX and 46,XY DSD, whereas persistent Müllerian duct syndrome affects 46,XY DSD patients.

1.3 Clinical Manifestations of Mutations in Genes Involved in Sex Determination

1.3.1 46,XX Testicular/Ovo-Testicular DSD

The T/OT-DSD phenotype is largely dependent on the etiology of the disease. The 46,XX male syndrome—first reported by De la Chapelle et al. in 1964 [4]—is a rare, heterogeneous clinical condition with an incidence of about 1:20,000–25,000 male neonates [5]. The majority of 46,XX T-DSD cases are SRY+, with completely differentiated male external and internal genitalia in 85% of cases. Those who are *SRY* negative have ambiguous genitalia and poor virilization and OT-DSD. The incidence of OT-DSD is about 1:100,000 births, and children are usually reared as male due to the size of the phallus. OT-DSD is characterized by the presence of both ovarian and testicular tissues in the same gonad or less commonly in different gonads. Since the ovarian portion of OT is often functionally normal, breast development at puberty can occur due to follicular growth and estradiol production. Additional phenotypical alterations in 46,XX DSD are micropenis, hypospadias, cryptorchidism, and gynecomastia, due to atypical hormonal signaling. Short stature is a common remark, due to the absence of Y chromosome-linked growth control genes (Table 1.2). A constant clinical manifestation in these subjects is azoospermia with high FSH value. It is due to the lack of Y chromosome-linked AZF regions, where essential genes for sperm production are harbored. Depending on the entity of Leydig cell

dysfunction, testosterone level may range from normal to low, but in the majority of cases, LH is high.

Genes Involved in "Male Pathway"

- Sex Determining gene on Y (*SRY*)
  During paternal meiosis, the translocation of *SRY*-containing segments of the Y chromosome onto the X chromosome is the suggested genetic mechanism leading to the large majority (in about 90%) of XX male cases. These 46,XX male, SRY+ patients have usually small stature, gynecomastia, male external/internal genitalia, and decreased testis volume [1].
- SRY-Box genes (*SOX*)
  SRY-negative (SRY–) 46,XX male syndrome can be caused by autosomal gene mutations/over-expression, a gain of function in key testicular pathway genes, which are causing testis differentiation. Ectopic expression, achieved via duplication of ***SOX9***, is the most common mechanism in this group of patients. In addition, rarely, duplications of ***SOX3*** and ***SOX10*** have also been described both in mouse model and in human 46,XX male cases [6]. Both genes present high homology with *SOX9*; hence, the proposed pathogenic mechanism is based on their increased expression which mimics *SOX9* gene's function, leading to the initiation of testis development. These are extremely rare genetic defects with a broad phenotypic spectrum. The affected patients may present with either normal male external/internal or ambiguous genitalia, with T or OT-DSD.
- Fibroblast growth factor 9 (FGF9)
  Large duplication of the ***FGF9*** gene has been identified in a 46,XX *SRY*-negative male patient. This gene takes part of the male sex determination pathways; consequently, its increased dosage explains the observed male phenotype in the absence of *SRY*. The affected patient had male external genitalia with hypospadias and small testes [7].

Genes Involved in the "Female Pathway": *R-Spondin 1* (*RSPO1*) and *Wnt Family Member 4* (*WNT4*)
Loss-of-function mutations in the female pathway are associated with some exceptional forms of 46,XX sex reversal. Mutations in ***RSPO1*** and ***WNT4*** are related with rare autosomal recessive syndromes in human. Besides the abnormal gonadal phenotype, ***RSPO1*** is associated with palmoplantar hyperkeratosis (PPK) and predisposition to squamous cell skin cancer, whereas ***WNT4*** with SERKAL syndrome (SEx Reversal, Kidneys, Adrenal, and Lung dysgenesis) [8]. In addition, ***WNT4*** mutation was identified in a 46,XX DSD female patient, presented with primary amenorrhea, absence of Müllerian structures, unilateral renal agenesis, and clinical signs of androgen excess [1].

Mutations in *NR5A1 Gene,* Involved in Both "Male" and "Female Pathways"

- Nuclear receptor subfamily 5 group A, member 1 (*NR5A1* alias *SF-1*)
  Mutations in this gene represent one of the most frequently found genetic causes of DSD [9]. Since mutations within this gene can disrupt both the testicular and ovarian pathways, they are related to various gonadal development disorders, depending on the karyotype and other phenotypic modifiers (e.g., additional genetic variants). The clinical characteristics of ***NR5A1*** mutation carriers in 46,XY individuals could vary from oligo−/azoospermia to complete sex reversal (OT-DSD). While, at birth, patients show severely undervirilized external genitalia, the majority of them undergo spontaneous virilization during puberty, thanks to conserved Leydig cell function. However, oligozoospermic carriers are at potential risk for late-onset testosterone defect and progressive decrease of sperm production. For these reasons, they should cryopreserve sperm and perform regular endocrine follow-up. In the more severe phenotypes, in about 24% of cases, Müllerian duct derivatives are retained as a consequence of disturbed AMH production in the Sertoli cells. On the other hand, in 46,XX individuals, the phenotypic spectrum includes primary ovarian insufficiency (POI) and severe forms such as T-DSD or OT-DSD. Atypical genitalia are the most frequent presentation ranging from female genitalia to micropenis and penoscrotal hypospadias. In the minority of OT-DSD cases, gonads are not palpable, and the uterus or hemiuterus is present.

1.3.2 46,XY Gonadal Dysgenesis/Ovo-Testicular DSD

Impaired gonadal development in 46,XY karyotype is the consequence of mutations, causing under-expression or over-expression of different sex determination genes. It can result in a heterogenic group of conditions, which are characterized by a varying degree of abnormally configured and differentiated gonads, called as gonadal dysgenesis (GD). In 46,XY complete gonadal dysgenesis (CGD, alias Swyer syndrome or pure gonadal dysgenesis), the disrupted Sertoli cell formation and testis differentiation lead to inadequate levels of testosterone and AMH, hence disrupting the formation of the Wolffian ducts and internal genitalia and driving to Müllerian duct development. Therefore, normal female external genitalia with normal uterus and fallopian tubes can be seen, but the gonadal tissue is replaced with functionless, fibrous tissue, termed as streak gonads. These tissue clumps replace the testicular tissue and might vaguely resemble to ovarian stroma without follicles. In partial gonadal dysgenesis (PGD), the original tissue is retained to some extent. GD may occur unilaterally or bilaterally. Typical clinical findings are the delayed/absent puberty, lack of secondary sex characteristics, and infertility, due to primary amenorrhea (Table 1.1 and Fig. 1.2). The incidence of 46,XY CGD is in approximately 1:80,000 live births and together with 46,XY PGD is 1:20,000 live births. Depending on the underlying gene defect, 46,XY GD may also occur along with other pathologies and syndromes. The etiology is defined only in about 30–40% of cases and it can be due to *SRY*, *MAP3K1*, *SOX9* and other pro-testis gene defects as well as duplication of pro-ovary genes:

Table 1.1 46, XY due to disruption of genes involved in sex determination or steroidogenesis

	Pathology	Pathophysiology	Genetics	External genitalia	Internal genitalia	Possible associated clinical findings
SEX DETERMINATION	**XY-female syndrome (complete form: Swyer sdr)**	Altered sex determination	Mutations/ deletions, which cause under-expression of: *SRY, SOX9, MAP3K1, NR5A1* (alias *SF1*), *CBX2, DHH, WT-1, DMRT1, GATA4, FOG2, FGFR2, ATRX;* Duplications, which cause over-expression of: *WNT4*, *RSPO, DAX1* (alias *NR0B1*); or unknown genetic factors	46,XY: Female external genitalia	46,XY: Normal uterus, fallopian tubes, with (complete/partial) gonadal dysgenesis (non-functional gonads)	Delayed/absent puberty, lack of/sparse secondary sex characteristics, lack of breast development, infertility (primary amenorrhea); osteoporosis/osteopenia; high risk of gonadal germ cell tumors; *WT-1:* Wilm's tumor; *DHH*: polyneuropathy; Syndromes: *SOX9*: Campomelic Dysplasia; *WT-1:* WAGR, Frasier and Denys-Drash sdr; *DMRT1:* Human 9p monosomy sdr; *GATA4* and *FOG2:* congenital heart defects; *FGFR2:* Craniosynostosis sdr; *ATRX*: ATR-X sdr;

(continued)

Table 1.1 (continued)

	Pathology	Pathophysiology	Genetics	External genitalia	Internal genitalia	Possible associated clinical findings
SEXUAL DIFFERENTIATION	**Leydig cell hypoplasia**	Disruption of Leydig cell differentiation	Compound heterozygous or homozygous mutations of *LHCGR*	46,XY: Complete: female external genitalia, +/− mild clitoromegaly or labial fusion; Partial: male external genitalia +/− micropenis, hypospadias	46,XY: male gonads, +/− cryptorchidism	Complete form: absence of secondary sex characteristics, lack of breast development, infertility (primary amenorrhea); Partial form: partial virilization, fertility problems
	7-DHC-R deficiency (Smith-Lemli-Opitz sdr)	Disruption of cholesterol synthesis	Compound heterozygous or homozygous mutations of *DHCR7*	46,XY: ambiguous genitalia, hypoplasic or bifid scrotum; micropenis and hypospadias/ female-like genitalia	46,XY: male gonads, +/− cryptorchidism	Embryonic lethality; congenital malformations (holoprosencephaly, microcephaly, polydactyly, syndactyly) typical facial appearance, cardiovascular and gastrointestinal anomalies, renal agenesis, seizures, hypotonia, mental retardation; Normal puberty with normal hypothalamus, pituitary gland, and adrenal glands (HPA) axis function; in mild cases: fertility unknown

	StAR deficiency	Disruption of cholesterol transfer	Compound heterozygous or homozygous mutations of *STAR*	46,XY: female or ambiguous genitalia with blind vaginal pouch/mild forms: micropenis	46,XY: small testes, +/− cryptorchidism	lipoid CAH, salt wasting, hyponatremia, hypovolemia, hyperkalemia, acidosis, hyperpigmentation, death in infancy; no pubertal development; infertility
	P450scc deficiency	Impaired conversion of cholesterol into pregnenolone	Compound heterozygous or homozygous mutations of *CYP11A1*	46,XY: female or ambiguous genitalia, undervirilization	46,XY: male gonads, +/− cryptorchidism	Prematurity, severe forms: early-onset adrenal insufficiency without CAH, complete hypogonadism, milder forms: late-onset adrenal insufficiency without CAH, mild masculinization
	POR deficiency	Disrupted activity of P450 enzymes	Compound heterozygous or homozygous mutations of *POR*	46,XY: ambiguous genitalia, undermasculinized external genitalia, hypospadias, micropenis	46,XY: male gonads, +/− cryptorchidism	CAH; cortisol deficiency; arterial hypertension; delayed development; hypergonadotropic hypogonadism, PCOS; fertility unknown Antley Bixler Syndrome: skeletal malformations; intellectual disability,

(continued)

Table 1.1 (continued)

	Pathology	Pathophysiology	Genetics	External genitalia	Internal genitalia	Possible associated clinical findings
	3β-HSD2 deficiency	Impaired aldosterone, cortisol and sex hormones production	Compound heterozygous or homozygous mutations of *HSD3B2*	46,XY: ambiguous genitalia: micropenis, perineal hypospadias, bifid scrotum, blind vaginal pouch	46,XY: male gonads	CAH with or without salt wasting; gynecomastia, fertility unknown
	P450c17 deficiency (combined 17α-hydroxylase and 17,20-lyase deficiency)	Disrupted conversion of pregnenolone into 17α-hydroxypregnenolone and DHEA	Compound heterozygous or homozygous mutations of *CYP17A1*	46,XY:female-like or slightly virilized external genitalia, blind vaginal pouch; rarely ambiguous genitalia	46,XY: hypoplastic male gonads; +/− cryptorchidism	Gynecomastia, sparse axillary/pubic hair; infertility (primary amenorrhea) arterial hypertension; hypokalemia
	Isolated 17,20-lyase deficiency	Enzymatic defect in testosterone biosynthesis	Compound heterozygous or homozygous mutations of *CYP17A1*	46,XY: ambiguous genitalia with micropenis, perineal hypospadias; at puberty: poor virilization	46,XY: hypoplastic male gonads, +/− cryptorchidism	Gynecomastia; normal glucocorticoid and mineralocorticoid production
	17β-HSD3 deficiency	Impaired conversion of androstenedione to testosterone	Compound heterozygous or homozygous mutations of *HSD17B3*	46,XY: complete or predominantly female external genitalia, blind vaginal pouch, +/− bifid scrotum; at puberty: virilization	46,XY: male gonads, +/− cryptorchidism	Gynecomastia; social sex change; normal glucocorticoid and mineralocorticoid production, fertility problems

	5α-RD2 deficiency	Impaired conversion of testosterone to DHT	Compound heterozygous or homozygous mutations of *SRD5A2*	46,XY: At birth: almost normal female genitalia/ ambiguous genitalia +/− blind vaginal pouch, bifid scrotum /undervilirized male genitalia +/− hypospadias or isolated micropenis; at puberty: virilization	46,XY: male gonads; +/− prostate hypoplasia; cryptorchidism	Normal testosterone dependent secondary sexual characteristics at puberty (social sex change), fertility problems
	Androgen Insensitivity syndrome (AIS): CAIS—Morris sdr; PAIS—Reifenstein sdr; MAIS—Mild Androgen Insensitivity sdr	Androgen insensitivity	Mutations of AR (X chromosome linked)	46,XY: **CAIS**: female phenotype **PAIS**: undervirilized male genitalia: micropenis, severe hypospadias, bifid scrotum; **MAIS**: normal male genitalia	46,XY: **CAIS**: testes with cryptorchidism; **PAIS:** +/− (bilateral) cryptorchidism; **MAIS:** predominantly normal male gonads	**CAIS**: absent axillary/ pubic hair; infertility (primary amenorrhea); **PAIS**: gynecomastia; infertility (azoospermia); **MAIS:** fertility problems
	Persistent Müllerian Duct Syndrome (PMDS)	Impaired synthesis or action of AMH	Compound heterozygous or homozygous mutations of *AMH* or *AMHR2*	46,XY: normal male appearance	46,XY: Both female (uterus, fallopian tubes, upper vagina) and male internal gonads; +/− cryptorchidism	Fertility problems

Abbreviations: *AMH* Anti-Müllerian hormone, *AMHR2* Anti-Müllerian hormone receptor type 2 gene, *AR* Androgen receptor, *CAH* Congenital adrenal hyperplasia, *CAIS* Complete androgen insensitivity syndrome, *PAIS* Partial androgen insensitivity syndrome, *MAIS* Mild androgen insensitivity syndrome, *PCOS* Polycystic ovary syndrome, *POR* Cytochrome P450 oxidoreductase, *P450scc* Cholesterol side-chain cleavage enzyme, *sdr.* syndrome, *SRY* Sex determining gene on Y chromosome, *StAR* Steroidogenic acute regulatory protein, *3β-HSD2* 3β-hydroxysteroid dehydrogenase type II, *5α-RD2* 5α-reductase type II, *7-DHC-R* 7-Dehydrocholesterol reductase enzyme, *17β-HSD3* 17 β-hydroxysteroid dehydrogenase type III

- Sex Determining gene on Y (*SRY*)
  In approximately 15–20% of affected patients, the cause is the mutation/deletion of the ***SRY*** gene on the Y chromosome. Most of the *SRY* mutations are located in the HMG box and are predominantly de novo mutations. Since the *SRY* gene is the initiator of male sex determination, the malfunction/absence of this gene results in a non-functional gonad with the consequent absence of testicular hormones. The phenotype is a female complete GD.
- Mitogen-Activated Protein Kinase Kinase Kinase 1 gene (MAP3K1)
  The dysregulation of MAPK pathway is implicated in the disrupted development of the gonads, in around 18% of cases. Deleterious mutations of ***MAP3K1*** can result in the under-expression of male pathway genes, since this gene has an important role to balance between *SOX9/FGF9* and *WNT/β-catenin* signaling. Carriers of ***MAP3K1*** mutations present female phenotype with GD.
- SRY-Box genes 9 (*SOX9*)
  As mentioned already, ***SOX9*** is expressed in the undifferentiated gonad of both sexes and is upregulated by the *SRY*; hence, it is evident that the loss-of-function mutations ca`n cause female sex development. This gene is also expressed in other tissues than the testis and involved in other processes, such as chondrogenesis, which can explain the consequent syndromic manifestation of ***SOX9*** mutations. Typically, campomelic dysplasia is associated with this gene mutation. This syndrome is characterized by distinctive facial features, severe skeletal malformations, and 46,XY GD.
- Wilms tumor 1 (*WT1*)
  The +KTS isoform plays a relevant role in testis determination. Diverse syndromes and diseases are associated with ***WT1*** deletions/mutations. The deletions may cause a group of pathologies, termed as WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation). Missense mutations in this gene can cause Denys-Drash syndrome, which is characterized by early diffuse mesangial sclerosis (DMS), Wilms tumor (nephroblastoma), and male pseudohermaphroditism (partial or complete gonadal dysgenesis) with a 46,XY karyotype. On the other hand, the absent production of +KTS due to mutations at the donor site of exon 9 leads to Frasier syndrome. Its typical manifestation is late-onset focal segmental glomerulosclerosis (FSGS) with complete gonadal dysgenesis, but without Wilms tumor.
- Other genes involved in 46, XY GD
  Mutations in other genes with essential function in male gonad development, such as ***DMRT1***, ***DHH***, ***CBX2***, ***NR5A1*** (alias ***SF-1***), ***GATA4***, ***FOG2***, ***FGFR2***, and ***ATRX***, have been rarely reported [10]. Some of them may cause syndromic form with severe anomalies. Mutations in ***DMRT1*** can cause human 9p monosomy syndrome, which is characterized by variable degrees of 46,XY GD, mental retardation, and craniofacial abnormalities. Mutation in ***FGFR2*** gene is associated with 46,XY GD and craniosynostosis. ATR-X syndrome, due to mutations of the ***ATRX*** gene, is associated with severe mental retardation, alpha thalassemia, typical facial appearance, skeletal malformations, and pulmonary, gastrointestinal, and urogenital anomalies.

- On the other hand, duplications in genes involved in the female pathway, such as ***DAX1*** (alias ***NR0B1***), ***WNT4***, and ***RSPO1***, have also been reported in the literature [10].

1.4 Clinical Manifestations of Mutations in Genes Involved in Sexual Differentiation

In contrast to the development of the female external genitalia, which does not require fetal ovarian hormones, the differentiation of male external genitalia requires the action of testosterone and its more potent derivative, DHT. Fetal Leydig cells produce testosterone staring from cholesterol through the sequential actions of various enzymes such as CYP11A1, CYP17A1, HSD3B2, and HSD17B3. In addition, adrenal gland also produces androgens. Noteworthy, some proteins are involved both in sexual determination and also in sexual differentiation, such as the above-mentioned *NR5A1* (alias *SF-1*), while others (e.g., *HSD3B2*, *HSD17B3*, *SDR5A2*) are expressed only during sexual differentiation.

Disorders associated with disrupted androgen production can arise from any step of testosterone synthesis in the testis or in the adrenal glands or from a defect in the conversion of testosterone to DHT in the peripheral tissues (e.g., genital organs and skin, prostate, hair follicles, liver, brain). Disorders of sexual differentiation show a broad spectrum of phenotypes, strongly depending from the underlying etiology. Frequent clinical finding is ambiguous external genitalia at birth; however, in milder forms, only undervirilized genitalia can be seen, which delays the diagnosis of DSD until puberty or even to adulthood (Tables 1.1, 1.2, 1.3 and Fig. 1.2).

1.4.1 Disorders of Androgen Biosynthesis: Reduced Androgen Levels

- 7-dehydrocholesterol reductase deficiency (Smith-Lemli-Opitz syndrome, SLOS) Loss-of-function mutations in the *DHCR7* gene can lead to an inborn error of cholesterol synthesis, due to 7-dehydrocholesterol reductase enzyme deficiency, which converts 7-dehydrocholesterol (7DHC) to cholesterol. The incidence of SLOS is approximately 1:20,000–40,000 per births, and it is most frequent in central and northern Europe. Its clinical manifestation is broad and variable, ranging from early embryonic lethality to different types of malformations and mental retardation postnatally. Typically, these patients may present holoprosencephaly, microcephaly (80% of cases) with a typical facial appearance, postaxial polydactyly of the hands or feet, syndactyly of the second and third toes (95% of cases), and short-proximally placed thumbs, as well as cardiovascular and gastrointestinal anomalies. Concerning genital development ambiguous genitalia, micropenis and hypospadias are observed in the majority of patients (70%) [11].

  Hormonal diagnosis: low plasma cholesterol and elevated plasma 7-dehydrocholesterol levels.

Table 1.2 46,XX DSD due to disruption of genes involved in sex determination or steroidogenesis

	Pathology	Pathophysiology	Genetics	External genitalia	Internal genitalia	Possible associated clinical findings
SEX DETERMINATION	**XX-male syndrome (SRY+)**	Altered sex determination	*SRY* translocation	46,XX: male external genitalia (85%), ambiguous genitalia	46,XX: male gonads	Short stature, infertility (azoospermia)
	XX-male syndrome (SRY-)		Duplications, which cause overexpression of: *SOX9*; *SOX3*; *SOX10*; *FGF9;* Mutations, which cause under-expression of*: RSPO1; WNT4; NR5A1* (alias *SF1*)*; WT-1;*	46,XX: ambiguous genitalia, poor virilization, micropenis; penoscrotal hypospadias	46,XX: testes/ ovotestis cryptorchidism	Short stature, gynecomastia, infertility (azoospermia); *RSPO1:* palmoplantar hyperkeratosis, Predisposition to squamous cell skin cancer; *WT-1:* Wilm's tumour; Syndromes: *WNT4:* SERKAL sdr, *WT-1:* WAGR sdr, Denys-Drash sdr, Frasier sdr
SEXUAL DIFFERENTIATION	**POR deficiency**	Disrupted activity of P450 enzymes	Compound heterozygous or homozygous mutations of *POR*	46,XX: masculinized genitalia/ ambiguous genitalia/ clitoromegaly, labial fusion	46,XX: female gonads	CAH; cortisol deficiency; arterial hypertension; delayed development; PCOS; unknown fertility, Antley Bixler Syndrome: skeletal malformations; intellectual disability,
	21-OH deficiency	Androgen excess	Compound heterozygous or homozygous mutations of *CYP21A2*	46,XX: complete masculinization/ ambiguous genitalia/slight clitoromegaly	46,XX: female gonads	CAH; failure to thrive; hyponatremia; hyperkalemia; acidosis; hypoglycemia; decreased height; precocious puberty; PCOS, excessive hair growth; acne; from normal to reduced fertility;

	11β-OH deficiency	Androgen excess	Compound heterozygous or homozygous mutations of *CYP11B1*	46,XX: complete masculinization/ ambiguous genitalia/slight clitoromegaly	46,XX: female gonads	CAH; low renin hypertension; hypokalemia; decreased height; precocious puberty; excessive hair growth; hirsutism; acne; PCOS, from normal to reduced fertility

Abbreviations: *CAH* Congenital adrenal hyperplasia, *PCOS* Polycystic ovary syndrome, *POR* Cytochrome P450 oxidoreductase, *sdr.* Syndrome; *SRY* Sex determining gene on Y chromosome, *11β-OH* 11β-hydroxylase, *21-OH* 21-hydroxylase

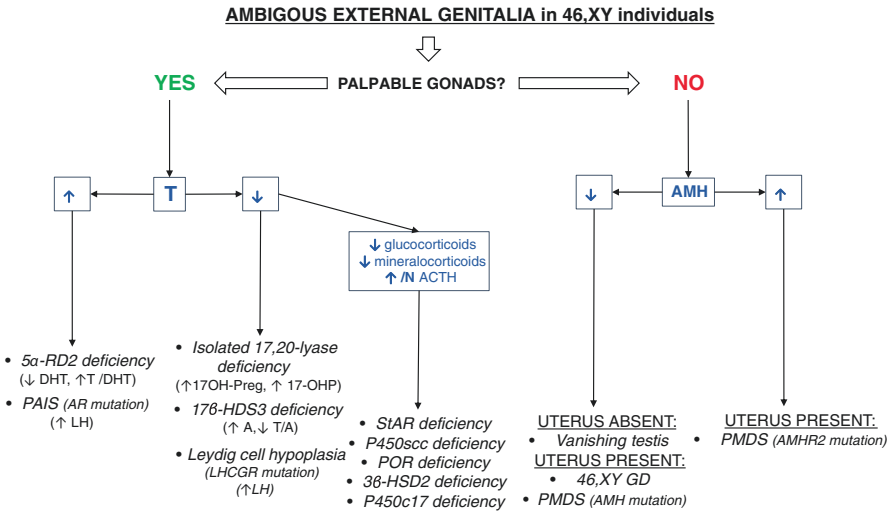

Fig. 1.2 Hormonal features in 46,XY individuals with ambiguous external genitalia

- Congenital lipoid adrenal hyperplasia due to steroidogenic acute regulatory protein (StAR) deficiency
  Cholesterol transfer across the mitochondrial membrane to synthesize pregnenolone is mediated by the steroidogenic acute regulatory (StAR) protein. StAR phosphoprotein can be found in the mitochondria of the adrenal and gonadal cells. Mutations in the gene which encodes StAR result in lipoid congenital adrenal hyperplasia (lipoid CAH), the most severe form of CAH. It is an extremely rare, autosomal recessive condition, with a higher prevalence in Japanese, Korean, and Palestinian populations. The adrenal cortex and the gonads become engorged with cholesterol and cholesterol esters leading to mineralocorticoid, glucocorticoid, and sex steroid deficiency. The most severe form, adrenal insufficiency, manifests in salt wasting, hyponatremia, hypovolemia, hyperkalemia, and acidosis, which consequently lead to a life-threatening condition. Elevated adrenocorticotropic hormone (ACTH) levels due to intrauterine glucocorticoid deficiency can appear in generalized hyperpigmentation at birth. As far as the genital phenotype is concerned, in the case of complete defects, both 46,XY and 46,XX patients have female phenotype. 46,XY patients have testes, with or without cryptorchidism, and no Müllerian derivatives due to the presence of AMH. In the case of partial defect and 46,XY karyotype, genital ambiguity can occur.
  Hormonal diagnosis: elevated ACTH, renin and gonadotropin levels, with low basal values of glucocorticoids, mineralocorticoids, and androgens. In milder forms, partial steroid production can be measured.
- Cholesterol side-chain cleavage enzyme (P450scc) deficiency
  The following step of steroid biosynthesis is the conversion of the intramitochondrial cholesterol into pregnenolone by P450scc. This protein is encoded

by the *CYP11A1* gene and located in the mitochondria of the gonads and the adrenal glands. Compound heterozygous or homozygous mutations of *CYP11A1* gene can cause P450scc deficiency with a similarly severe phenotype like observed in patients with StAR loss-of-function mutations (congenital adrenal insufficiency with 46,XY DSD).

Hormonal diagnosis: high ACTH, renin, and gonadotropin levels, with undetectable or low values of glucocorticoids, mineralocorticoids, and androgens.

- Cytochrome P450 oxidoreductase (POR) deficiency

  POR is required for the activity of all P450 enzymes, such as P450c17, P450c21, and P450aro, which are involved in different steps of steroidogenesis. Homozygous or compound heterozygous mutations in the *POR* gene can lead to a rare form of CAH, due to combined deficiency of P450c17 and P450c21 and accumulation of steroid metabolites. It manifests in syndromic (Antley-Bixler syndrome) or non-syndromic form, with clinical features like cortisol deficiency, altered sex steroid synthesis, DSD, and skeletal malformations. The majority of individuals with POR deficiency have ambiguous genitalia at birth, which can occur in both sexes. 46,XX patients may present with masculinized genitalia, with enlarged clitoris and labial fusion, while 46,XY patients can present with undermasculinized external genitalia, with hypospadias and micropenis. In certain cases, complete sex reversal can occur. The unusual finding that both sexes can present with DSD suggests to an alternative pathway in human androgen synthesis, present only in fetal life, which explains the combination of antenatal androgen excess and postnatal androgen deficiency [12]. Typical congenital craniofacial and skeletal anomalies are, e.g., midface retrusion, craniosynostosis, hand and feet malformations, large joint synostosis, etc. Arterial hypertension may occur due to (mild) mineralocorticoid excess (Tables 1.1 and 1.2).

  Hormonal diagnosis: ACTH plasma concentration is normal or elevated, with normal or low cortisol serum concentration. Pregnenolone, progesterone, 17-hydroxypregnenolone (17OH-Preg), and 17-hydroxyprogesterone values are often elevated at baseline and/or after ACTH stimulation. Androgen serum concentration may be decreased and unresponsive to ACTH or hCG stimulation.

- 3β-hydroxysteroid dehydrogenase type II (3β-HSD2) deficiency

  The 3β-HSD enzyme has two isoforms, which share identity in around 93%. 3β-HSD1 is encoded by the *HSD3B1* gene and expressed mostly in the placenta and peripheral tissues. On the other hand, 3β-HSD2 is encoded by the *HSD3B2* gene and expressed exclusively in the adrenal glands, ovaries, and testes. 3β-HSD2 is not only essential for the synthesis of sex steroids acting on the conversion of dehydroepiandrosterone (DHEA) in androstenedione, but also for the synthesis of mineralocorticoids and glucocorticoids. *HSD3B2* mutations abolishing the enzyme activity lead to CAH with severe salt loss, whereas less severe mutations which reduce 3β-HSD2 enzyme activity result in CAH with mild or no salt wasting. In individuals with 46,XY karyotype, it leads to DSD, due to impaired androgen synthesis. 46,XY DSD patients present ambiguous genitalia,

characterized by micropenis, perineal hypospadias, bifid scrotum, and blind vaginal pouch. During puberty, gynecomastia is a common finding.
Hormonal diagnosis: The gold standard for biochemical diagnosis is measurement of the basal and post-ACTH serum 17OH-Preg and the 17OH-Preg/cortisol ratio. The serum levels of pregnenolone, 17OH-Preg, DHEA, and dehydroepiandrosterone sulfate (DHEAS) are elevated.

- P450c17 (combined 17α-hydroxylase and 17,20-lyase) deficiency
  The conversion of pregnenolone into 17α-hydroxypregnenolone and DHEA is catalyzed by the P450c17 steroidogenic enzyme. It is encoded by the *CYP17* gene, expressed in the endoplasmic reticulum of the adrenal cortex and of the gonads, and has both hydroxylation and lyase functions as well. Defect in the *CYP17* gene has been associated with combined 17α-hydroxylase and 17,20-lyase deficiency. Most of the affected subjects with 46,XY karyotype are phenotypically females with female-like or slightly virilized external genitalia, blind vaginal pouch, and cryptorchidism. Ambiguous genitalia have also been associated with this disease. The Wolffian derivatives are hypoplastic. During puberty, these patients usually present gynecomastia and sparse axillary/pubic hair. Commonly, they are assigned to female social sex and sought treatment due to primary amenorrhea or lack of breast development. High blood pressure and hypokalemia are frequent clinical findings.
  Hormonal diagnosis: typically, massive increase in the 17-deoxysteroid-corticosterone, deoxycorticosterone, and progesterone levels, with decreased aldosterone, cortisol, 17-OH-progesterone, androgen, and estrogen levels. Excessive production of deoxycorticosterone and corticosterone consequently leads to vascular hypertension, decreased levels of renin, and inhibition of aldosterone synthesis.
- Isolated 17,20-lyase deficiency
  Isolated 17,20-lyase deficiency due to mutation in *CYP17* gene may lead to diminished fetal testicular testosterone synthesis with intact cortisol production. 46,XY individuals affected by this disease have ambiguous genitalia with micropenis, perineal hypospadias, and cryptorchidism. During puberty, gynecomastia can occur.
  Hormonal diagnosis: elevated serum 17-OH-progesterone and 17OH-Preg levels, with low levels of androstenedione, DHEA, and testosterone.
- 17β-hydroxysteroid dehydrogenase type III (17β-HSD3) deficiency
  17β-Hydroxysteroid dehydrogenase (17β-HSD) acts on the final step of steroidogenesis. 17β-HSD enzyme has five isoenzymes. Most of them are involved in the estrogen balance, like type I, which is expressed in the ovary and converts estrone into estradiol. Type III (17β-HSD3) is an exception, because it is expressed exclusively in the testes and catalyzes the reduction of androstenedione to testosterone. Homozygous or compound heterozygous mutations in the *HSD17B3* gene cause the defect of 17β-HSD3 enzyme. It is also known as 17β-keto reductase deficiency and is characterized by impaired sex hormone production without impairing glucocorticoid and mineralocorticoid adrenal secretion. It is the most frequent disorder of androgen synthesis. 46,XY

newborns with 17β-HSD3 deficiency have complete or predominantly female external genitalia with a blind vaginal pouch, and the testes are often found in the inguinal canal or in a bifid scrotum. Despite the mainly female-like external genitalia, male internal genitalia, i.e., epididymides, vas deferens, seminal vesicles, and ejaculatory ducts, can be found. Most commonly, the affected males are raised as females. At the time of puberty, virilization occurs, and several patients with 17β-HSD3 deficiency change to male social sex. Testes can be safely maintained, as long as they are positioned inside the scrotum. The phenotype of the affected patients is clinically almost indistinguishable from other causes of 46,XY DSD such as partial androgen insensitivity syndrome (PAIS) or 5α-reductase type II deficiency (both of them will be discussed later), without biochemical assessment.

Hormonal diagnosis: low testosterone/androstenedione (T/A) and estradiol/estrone ratios, due to high serum levels of androstenedione and estrone, and low levels of testosterone. The cut-off value for T/A ratio is 0.8. In addition, hCG test has been proposed as a useful diagnostic tool, showing an increase of Δ4-A more than T [13].

1.4.2 Disorders of Androgen Biosynthesis: Androgen Excess

- 21-Hydroxylase (21-OHD) Deficiency

  The *CYP21A2* gene encodes the 21-hydroxylase enzyme (alias P450c21) which is responsible for the conversion of progesterone to deoxycorticosterone in the aldosterone biosynthesis pathway and 17α-hydroxyprogesterone to 11-deoxycortisol in the cortisol biosynthesis pathway. Severe or complete 21-hydroxylase (21-OHD) deficiency is the most common CAH. There are three forms of 21-OHD: (i) the classic salt wasting form, (ii) the classic simple virilizing form, and (iii) the non-classic form. The classic forms have an overall incidence in the Caucasian population of around 1:20,000–30,000 female newborns. The non-classic form is much more common, with an estimated 1:1000 frequency in the Caucasian population and even greater in other specific ethnic groups, such as Hebrew Ashkenazi (1:27), Hispanic (1:53), or Italians (1:300) [14]. Typical clinical findings in severe forms are hyponatremia, hyperkalemia, hyperreninemia, and hypovolemic shock. These patients can have a phenotype which is corresponding to the karyotype, but it can manifest with ambiguous genitalia or DSD as well. Excessive adrenal androgen production may lead from slight clitoromegaly to complete masculinization of the external genitalia in 46,XX individuals. In most severely virilized females, the social sex is often mistakenly assigned to male sex. Most 46,XY newborns affected by 21-OHD show normal appearance of the external genitalia at birth, and they have no clinical signs which could refer to their disease. Later in life, these patients may experience decreased height, precocious puberty, excessive hair growth, acne, and reduced fertility. Males with the non-classic type may present with early beard growth, enlarged penis, and small testes (Table 1.2).

Hormonal diagnosis: The excess 17-OH-progesterone, which represents the biochemical hallmark of this condition, is converted by the only available accessible pathway into potent androgens, such as testosterone and DHT. In addition, hyperandrogenism is due to the ready conversion of accumulated 17OH-pregnenolone to DHEA by *CYP17A1*.

- 11β-hydroxylase (11β-OHD) Deficiency
  CYP11B1 gene serves for 11β-hydroxylase enzyme production, which is found in the adrenal glands, and converts deoxycorticosterone into corticosterone and 11-deoxycortisol into cortisol. Mutations of *CYP11B1* gene result in 11β-hydroxylase deficiency with CAH. Its estimated frequency is 1:100,000–200,000 newborns, and it is more common among Moroccan Jews living in Israel, affecting approximately 1:5000–7000 newborns. Among all CAH cases, around 5–8% is due to 11β-OHD [14]. The clinical manifestation of 11β-OHD is highly similar to 21-OHD, but with hypertension. The excess production of androgens leads to atypical sexual differentiation, especially in females. In the severe forms, 46,XX newborns can be markedly virilized, with ambiguous genitalia, but with normal internal female gonads. 46,XY fetuses typically show normal sexual development, often with megalopenis and precocious puberty (Table 1.2).
  Hormonal diagnosis: high levels of ACTH, 11-deoxycortisol, 11-deoxycorticosterone, DHEA, androstenedione, and testosterone.

1.4.3 Disorders of Testosterone Metabolism

- 5α-reductase type II (5α-RD2) Deficiency
  The conversion of testosterone to DHT is catalyzed by the 5α-reductase enzyme, which has two isoenzymes. Isoenzyme 1 is encoded by the SRD5A1 gene and expressed at low levels in embryonic tissues, whereas after birth and during later life, it is expressed in the brain, liver, and non-genital skin. Its role is not well defined yet. Isoenzyme 2, which is encoded by the SRD5A2 gene, is predominantly expressed in external genital tissues and in the prostate. DHT is specially needed for the physiological development of the prostate, penis, and scrotum. Homozygous or compound heterozygous loss-of-function mutations of the SRD5A2 gene lead to 5α-reductase type II (5α-RD2) deficiency, which is a rare autosomal recessive disorder, associated with 46,XY DSD. Patients with 5α-RD2 can have ambiguous genitalia, but also apparently normal female or undervirilized male genital phenotype at birth. In the case of female-like external genitalia, normal male internal reproductive structures are observed, often with prostate hypoplasia and undescended testicles. In subjects with predominantly male external genitalia, hypospadias or isolated micropenis has also been described. During puberty, patients exhibit virilization without breast development and change gender identity from female to male. It is pivotal to make the proper diagnosis in infancy by biochemical and molecular studies before gender assignment or any surgical intervention because these patients should be considered as males.

Hormonal diagnosis: in childhood low DHT serum levels, with elevated testosterone/DHT ratio after hCG stimulation test [13]. At puberty or in young adult men, the basal hormonal evaluation demonstrates normal/elevated serum testosterone levels with low DHT levels and normal/elevated testosterone/DHT ratio.

1.4.4 Disorders of Androgen Action

- Androgen Insensitivity syndrome (AIS): Morris Syndrome and Reifenstein Syndrome
  Mutations of the X chromosome-linked *androgen receptor* gene (*AR*) are associated with variable form of androgen insensitivity. Obviously, male phenotype could be seen only in milder forms of this condition, ranging from undervirilization to normal male genitals with diminished sperm production. The prevalence of AIS is depending whether the androgen insensitivity is complete (CAIS), partial (PAIS), or mild (MAIS). The prevalence of CAIS is 2–5/100,000 in subjects with 46,XY karyotype. CAIS (alias Morris syndrome) is associated with a female phenotype. Estrogen-dependent secondary sexual characteristics may develop in these subjects as a result of excess aromatization of androgens, but without pubic and axillary hair growth. The uterus, cervix, and proximal vagina are absent because of the action of AMH, produced by Sertoli cells during fetal life. PAIS is thought to be at least as frequent as CAIS. In PAIS (alias Reifenstein syndrome), the typical phenotype is micropenis with severe hypospadias, bifid scrotum, and cryptorchidism. In MAIS, the habitual clinical sign is infertility without associated genital anomalies.
  Hormonal diagnosis: Typical signs of hormone resistance are observed, i.e., serum testosterone values are either within or above the normal range for young or adult men, with inappropriately high LH levels. FSH and inhibin B levels are usually in the normal range. High androgen sensitivity index can be found (calculated as the product of serum testosterone levels multiplied by serum LH levels; e.g., high LH levels in the presence of relatively high testosterone levels might suggest mild resistance).

1.4.5 Disruption of Leydig Cell Differentiation

- Leydig cell hypoplasia
  Leydig cell differentiation from mesenchymal cells is essential for later testosterone synthesis in this cell type. Several genes are involved in the development of fetal Leydig cells, among them the *Desert Hedgehog* (*DHH*) and *NR5A1* (alias *SF-1*) genes. *DHH* is produced by the Sertoli cells and specifies the fetal Leydig cell (FLC) lineage in the primordial gonad through a paracrine signaling mechanism. *NR5A1* (alias *SF-1*) is expressed in Leydig cells, and based on mouse models, a combinatorial expression of *DHH* and *NR5A1* is needed for physiological

Leydig cell development and for the replacement of FLCs by adult Leydig cells. *NR5A1* is an essential protein for hormone biosynthesis. Leydig cells are stimulated by both hCG during fetal life and LH after birth. Both hormones act through the binding and activation of a common receptor (LHCGR), located in the cell membrane. Several different mutations in the *LHCGR* gene are also associated with 46,XY DSD due to Leydig cell hypoplasia.

It is a rare disorder with unknown prevalence. A typical clinical finding in patients with Leydig cell hypoplasia is the lack of intrauterine and pubertal virilization, due to the lack or to diminished testosterone secretion. In the case of a complete form, the typical phenotype is normal female external genitalia, leading to female sex assignment, despite the 46,XY karyotype. On the other hand, partial forms manifest with a wide spectrum of phenotypes. Most frequently, male external genitalia can be found with micropenis and/or hypospadias. Additionally, cryptorchidism may occur. During puberty, these subjects undergo partial virilization with mostly normal testis volume.

Hormonal diagnosis: The determination of serum testosterone level can be assessed by hCG stimulation test. These patients have subnormal testosterone response to hCG test prior puberty, whereas during and after puberty, the hCG test shows absent/impaired increase of all testicular androgens. Hormonal assessment shows elevated serum gonadotropin levels, with a clear predominance of LH over FSH levels.

1.4.6 Persistent Müllerian Duct Syndrome (PMDS)

Mutations in the gene encoding for AMH or for its receptor (AMHR-II) cause decreased levels or impaired action of AMH leading to the failure of Müllerian duct regression. Persistent Müllerian duct syndrome (PMDS) is an extremely rare condition with around 150 documented cases globally. It is also called as hernia uterine inguinale, because it is often diagnosed accidentally during hernia repair or during orchidopexy (type of surgical intervention, which brings the undescended testis into the scrotum). 46,XY carriers bear both male external genitalia and gonads (often undescended), with female internal genitalia (uterus, fallopian tubes, and upper vagina). PMDS is a complex and anatomically variable disease classified in three main phenotypic categories, depending on the position of the testes and the uterus. In the "female" type (60–70% of patients), the testes and epididymes are connected to the fallopian tubes in the abdomen and are in analogue positions to the ovaries. In the "hernia uterine inguinale" type (20–30% of patients), one of the testes may be found in the hernia sac or in the scrotum, while the other is located in the abdomen. Transverse testicular ectopia (TTE) refers to the condition, when the two testes are in the same hernia sac along with the uterus and uterine tubes. It occurs in about 10% of cases [15].

Hormonal diagnosis: show a corresponding picture of testicular failure, with high FSH and low testosterone levels. Mutated *AMH* leads to decreased AMH level. On the other hand, in the case of AMH receptor mutation, the AMH level is high. There is a testosterone response to hCG stimulation test (Table 1.3).

Table 1.3 Hormone parameters in DSD according to the etiology

FSH	LH	T	Additional relevant biochemical/hormonal features		Diagnosis
↑	↑	↓		↓ AMH	**XY- gonadal dysgenesis/ ovo-testicular DSD (impaired sex determination)**
↑	↑/N	↓/N		–	**XX- testicular/ ovo-testicular DSD (impaired sex determination)**
N	N/↑	↓	↑ ACTH ↓ glucocorticoids	↓ cholesterol, ↑ 7-dehydrocholesterol, ↓mineralocorticoids	**7-DHC-R deficiency[a] (Smith-Lemli-Opitz sdr)**
↑	↑	↓		↓mineralocorticoids, ↑ renin	**StAR deficiency[a]**
↑	↑	↓		↓mineralocorticoids ↑ renin	**P450scc deficiency[a]**
↑	↑	↓		↑ pregnenolone, ↑ progesterone, ↑ 17OH-Preg, ↑17-OH-Progesterone	**POR deficiency[a]**
↑	↑	↓/N		↑ pregnenolone, ↑ 17OH-Preg, ↑DHEA, ↑ DHEAS	**3β-HSD2 deficiency[a]**
↑	↑	↓		↑ corticosterone, ↑17-deoxysteroid-corticosterone, ↑ progesterone, ↓ 17-OH-Progesterone, ↓ renin	**P450c17 deficiency[a] (combined 17α-hydroxylase and 17,20-lyase deficiency)**
↑/N	↓	↑		↑ 17OH-Preg, ↑ 17OH-Progesterone, ↓mineralocorticoids, ↑ DHEA	**21-OH deficiency[a]**
↑/N	↓	↑		↑ 11 deoxycortisol and 11 deoxycortisosterone, ↑ DHEA	**11β-OH deficiency[a]**
↑	↑	↓		↑ 17OH-Preg, ↑ 17-OH-Progesterone, ↓ androstenedione, ↓ DHEA	**Isolated 17,20-lyase deficiency**
↑	↑	↓		↑ androstenedione, ↑ estrone; T/Androstenedione ↓	**17β-HSD3 deficiency**
N	↓/N	↑/N		↓ DHT; T/DHT↓	**5α-RD2 deficiency**
↑/N	↑	↑/N		–	**Androgen Insensitivity syndrome**
↑	↑↑	↓/N		–	**Leydig cell hypoplasia**
↑	↑	↓		*AMH* mutation: ↓ AMH, *AMHR2* mutation: ↑AMH	**Persistent Müllerian Duct Syndrome (PMDS)**

Abbreviations: *ACTH* Adrenocorticotropic hormone, *AMH* Anti-Mullerian hormone, *AMHR2* *Anti-Mullerian hormone receptor 2* gene, *DHEA* Dehydroepiandrosterone, *DHT* Dihydrotestosterone, *FHS* Follicle stimulating hormone, *LH* Luteinizing hormone, *N* Normal, *POR* Cytochrome P450 oxidoreductase, *P450scc* Cholesterol side-chain cleavage enzyme, *StAR* Steroidogenic acute regulatory protein, *T* Testosterone, *3β-HSD2* 3β-hydroxysteroid dehydrogenase type II, *5α-RD2* 5α-reductase type II, *7-DHC-R* 7-Dehydrocholesterol reductase enzyme, *11β-OH* 11β-hydroxylase, *17β-HSD3* 17 β-hydroxysteroid dehydrogenase type III, *17OH-Preg* 17-hydroxypregnenolone, *17-OH-Progesterone* 17-hydroxyprogesterone, *21-OH* 21-hydroxylase

[a]Depending on the severity of the enzymatic defect ACTH, glucocorticoids and mineralocorticoids maybe normal

1.5 Diagnosis and Clinical Management of DSD Patients

DSD is a rare endocrine disease with low number of affected individuals for each etiological category. This peculiarity together with the highly heterogeneous phenotypes implies that the diagnosis is complex and requests special expertise. The diagnostic work-up of this condition includes extensive **physical examination**, with **assessment of the genitals**, **biochemical** and **genetic analyses**, **imaging studies**, and in some cases **surgical exploration** (e.g., laparoscopy, gonadal biopsy) for the confirmation. Biochemical assessment is highly informative about the underlying pathology, and it is essential in order to plan appropriate hormonal treatments. If it is possible, chromatographic and mass spectrometric methods are recommended for exact steroid hormone measurements, although these methods are not yet widely available and standardization between laboratories is an issue. The ideal diagnostic evaluation should include genetic analyses since the identification of the genetic defect implies the possibility of individualized patient's care. The genetic diagnosis is achieved in the majority of 46,XX males, whereas in 46,XY DSD patients, the underlying genetic cause remains unknown in about 70% of cases. Regular follow-up is advised to these patients due to the frequent occurrence of comorbidities and increased incidence of malignancies.

The results of the diagnostic tests need to be discussed within a multidisciplinary team in order to provide a holistic approach to the affected individuals. The management is based on the strict collaboration of endocrinologists, urologists, gynecologists, andrologists, clinical geneticists, psychologists, nurses, and social workers.

Key Points in the General Clinical Approach *Medical treatment options:* Gonadal function is compromised in the large majority of cases. Therefore, **sex hormone therapy** is necessary for pubertal induction and for hormone replacement therapy (HRT) during life. HRT serves to enhance gender identity and to induce or reinforce secondary sex characteristics, as well as for general well-being and to avoid cardiovascular and bone comorbidities (osteopenia/osteoporosis) later in life. It is important to state that both gender identity and sexual orientation seem to be determined during fetal development, under the influence of sex hormone production by the developing gonad. This is especially relevant in pathologies such as 5α-RD2 and 17β-HSD3 deficiency, where virilization occurs at puberty (if the gonads were not removed in childhood) with the consequent female-to-male social sex change. These patients, during their fetal life, are exposed to normal testosterone levels which leads to the virilization of the brain. Hence, if female sex is assigned, it will likely lead to distress related to gender dysphoria [16, 17]. It is therefore extremely important to diagnose this genetic defect as early as possible in order to avoid the removal of the testes and raise these individuals as boys [18].

HRT is based on estrogen or testosterone substitution, depending on the patients' social sex. Pubertal induction should be performed usually at the age of 10–12 in girls and 11–13 in boys, and the standard therapeutic regimen should simulate physiological puberty. In the case of patients with female gender identity, with or

without uterus, the hormonal substitution starts with low-dose estrogen (e.g., 0.07–0.15 mg/day orally), and this dose should be gradually increased every 6 months over 2–3 years. The maintenance dose is, for example, 0.625 mg/day of conjugate estrogen, with additional progesterone substitution (e.g., 50 mg/day, from the first to the 12th day of the month) to induce menstruation. Progesterone therapy is not required in patients without uterus. Low-dose transdermal estrogen treatment is also a viable option for HRT. Anti-androgens are used as adjuvants to estrogen, especially in the reduction of male sexual characteristics and the suppression of testosterone to female levels [10].

For patients with male gender identity, available options are intramuscular testosterone ester depot injections, oral testosterone undecanoate, and transdermal preparations. The initial dose of depot injections is 25–50 mg/month, while the maintenance dose in adults is 150–250 mg every 2–4 weeks or 1000 mg every 3 months [10]. The medical treatment for micropenis is high-dose testosterone therapy (e.g., 500 mg of testosterone cypionate injection/week). Maximum increase of penile length can be obtained after 6 months of therapy. Hormonal therapy is used for pubertal suppression in DSDs associated with precocious puberty.

Patients with CAH require lifelong **glucocorticoid** and often additional **mineralocorticoid replacement therapy**. In the case of SLOS, the primary therapeutic approach focuses on **cholesterol supplementation**, to enhance cholesterol production and/or accretion, and to decrease the accumulation of potentially toxic cholesterol precursors. The most widely accepted forms of dietary cholesterol supplementation are egg yolks and/or crystalline or powder form cholesterol suspension.

Gender assignment: Gender assignment is a difficult and critical decision in the management of DSDs. This process should be managed in reference centers by experienced teams. They should take into account, among others, cultural and religious factors and the impact of the decision on the adult life of the affected individuals (e.g., gender dysphoria, drastic surgical interventions, dissatisfaction with the appearance of genitalia). The current criteria for gender assignment are based on (i) the psychosexual outcomes in adults, (ii) the potential for fertility, and (iii) the available hormonal and surgical options [17].

Surgical treatment options: The aim is to repair sexual ambiguity, with the removal of the internal structures, which are inadequate for the patient's social sex and to construct a normal-looking, appropriate external genitalia. There is a growing tendency to postpone surgery, until the patient will be able to take part in the decision-making. If surgery is performed at early ages, mutilating and irreversible interventions should be avoided. Depending on the patients' desired gender, the surgical procedures consist of procedures like phalloplasty, scrotoplasty, resection of the vaginal pouch, proximal/distal urethroplasty, orchidopexy, vaginoplasty, excision of Müllerian duct remnants, and breast surgery. Apart from AIS patients, all ectopic male gonads are at increased risk for malignancies and need to be mobilized to an extra-abdominal, inguinal, or preferably scrotal position to allow monitoring by self-examination and imaging. If this relocation fails, surgical removal of the gonad should be considered. In the case

of 46,XY GD, gonadal streaks even more often become cancerous; therefore, they are usually removed surgically as the diagnosis occurs [19]. The timing of gonadectomy is controversial but will mainly depend on the anticipated malignancy risk of the gonad. As the risk is very low at pre-pubertal age, the final decision on gonadectomy usually can be deferred, if the gonad can be monitorized safely. In the case of undesired masculinizing or feminizing effects of sex steroids, which is in discordance with the social gender identity, prior intervention may be carried out. Correction of hypospadias is advised early, in the first 2 years of life.

Fertility: Rarely, DSD individuals with (partially) functional testicles may undergo **assisted reproductive techniques** (ART). For instance, **microdissection testicular sperm extraction** (micro-TESE) may offer possibility to find sperm in the testis. The harvested spermatozoa may be cryopreserved and used for later **intracytoplasmic sperm injection** (ICSI). If the atypical gonad does not contain gametes, ART with donor sperm can be taken into consideration. In the case of 46,XY DSD with female phenotype and with uterus, successful pregnancies following egg donation have been described. Adoption, as a final option for the couple, should be discussed with the patient and his/her partner.

1.6 Conclusions

The understanding of the genetic aspects and of the molecular mechanisms behind human sex development and DSD has been greatly improving over the past decades. It became clear that mutations in the same gene could be associated with a spectrum of phenotypes suggesting a role for modifier genes and oligogenic inheritance. In cases where clinical and biochemical exams are clearly indicating a distinct gene defect (e.g., 21-OHD, AIS), targeted gene sequencing is the usual approach. Following the diffusion of novel sequencing technologies (massive parallel sequencing), genetic testing based on gene panel is becoming the first-level test allowing the diagnosis of both monogenic and oligogenic cases. However, despite technological advances and progresses in our knowledge about the biology of DSD, still many new genes need to be discovered. The identification of the genetic basis for each DSD case is pivotal for the clinical management. Many gaps in our knowledge concerning this heterogeneous group of rare pathologies are currently addressed through large-scale networks. Thanks to the worldwide interaction between researchers and clinicians, major advancements in the management of social and clinical aspects are expected in the near future.

References

1. Knarston I, Ayers K, Sinclair A. Molecular mechanisms associated with 46,XX disorders of sex development. Clin Sci. 2016;130(6):421–32.
2. Lee PA, Houk CP, Ahmed SF, Hughes IA. International consensus conference on intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. International consensus conference on intersex. Pediatrics. 2006;118(2):e488–500.

3. Lee PA, Nordenström A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al. Global disorders of sex development update since 2006: perceptions, approach and care. HRP. 2016;85(3):158–80.
4. de la Chapelle A, Hortling H, Niemi M, Wennström J. XX sex chromosomes in a human male. Acta Med Scand. 1964;175(s412):25–38.
5. Vorona E, Zitzmann M, Gromoll J, Schüring AN, Nieschlag E. Clinical, endocrinological, and epigenetic features of the 46,XX male syndrome, compared with 47,XXY Klinefelter patients. J Clin Endocrinol Metab. 2007;92(9):3458–65.
6. Baetens D, Verdin H, De Baere E, Cools M. Update on the genetics of differences of sex development (DSD). Best Pract Res Clin Endocrinol Metab. 2019;33(3):101271.
7. Chiang H-S, Wu Y-N, Wu C-C, Hwang J-L. Cytogenic and molecular analyses of 46,XX male syndrome with clinical comparison to other groups with testicular azoospermia of genetic origin. J Formos Med Assoc. 2013;112(2):72–8.
8. Baxter RM, Vilain E. Translational genetics for diagnosis of human disorders of sex development. Annu Rev Genomics Hum Genet. 2013;14:371–92.
9. Domenice S, Machado AZ, Ferreira FM, Ferraz-de-Souza B, Lerario AM, Lin L, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. Birth Defects Res C Embryo Today. 2016;108(4):309–20.
10. Domenice S, Arnhold IJP, Costa EMF, Mendonca BB. 46,XY disorders of sexual development. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al., editors. Endotext [Internet]. South Dartmouth, MA: MDText.com, Inc.; 2000. http://www.ncbi.nlm.nih.gov/books/NBK279170/.
11. Mendonca BB, Costa EMF, Belgorosky A, Rivarola MA, Domenice S. 46,XY DSD due to impaired androgen production. Best Pract Res Clin Endocrinol Metab. 2010;24(2):243–62.
12. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet. 2004;363(9427):2128–35.
13. Bertelloni S, Russo G, Baroncelli GI. Human chorionic gonadotropin test: old uncertainties, new perspectives, and value in 46,XY disorders of sex development. Sex Dev. 2018;12(1–3):41–9.
14. Baronio F, Ortolano R, Menabò S, Cassio A, Baldazzi L, Di Natale V, et al. 46,XX DSD due to androgen excess in monogenic disorders of steroidogenesis: genetic, biochemical, and clinical features. Int J Mol Sci. 2019;20(18)
15. Ren X, Wu D, Gong C. Persistent Müllerian duct syndrome: a case report and review. Exp Ther Med. 2017;14(6):5779–84.
16. Fisher AD, Ristori J, Fanni E, Castellini G, Forti G, Maggi M. Gender identity, gender assignment and reassignment in individuals with disorders of sex development: a major of dilemma. J Endocrinol Invest. 2016;39(11):1207–24.
17. Guerrero-Fernández J, Azcona San Julián C, Barreiro Conde J, de la Vega JA B, Carcavilla Urquí A, Castaño González LA, et al. Management guidelines for disorders/different sex development (DSD). Anales de Pediatría (English Edition). 2018;89(5):315.e1–315.e19.
18. Bertelloni S, Scaramuzzo RT, Parrini D, Baldinotti F, Tumini S, Ghirri P. Early diagnosis of 5alpha-reductase deficiency in newborns. Sex Dev. 2007;1(3):147–51.
19. Wolffenbuttel KP, Hersmus R, Stoop H, Biermann K, Hoebeke P, Cools M, et al. Gonadal dysgenesis in disorders of sex development: diagnosis and surgical management. J Pediatr Urol. 2016;12(6):411–6.

Impact of Endocrine Disruptors on Male Sexual Development

2

Alberto Ferlin, Andrea Di Nisio, Luca De Toni, and Carlo Foresta

2.1 Introduction

Reproductive health has emerged as an important healthcare need involving many clinical and public health issues, including sexually transmitted infections (STIs), declining fertility, and rising rates of testicular cancer [1–4]. Importantly, it is now recognized that many causes and risk factors for testicular dysfunction and infertility indeed act early during life [5]. Many andrological pathologies that we see in adults actually arose in younger age, due to the strong susceptibility and vulnerability of male gonads to external insults, starting from gestational age and during all growth phases.

Of particular scientific and public interest is the possible contribution of endocrine disruptors to increased incidence of male sexual and reproductive problems, such as infertility, hypogonadism, cryptorchidism, hypospadias, and testicular cancer. An endocrine-disrupting chemical (EDC) is defined as "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action" [6]. Contamination from EDCs is almost inevitable, when such chemicals are used in occupational activities or are widely dispersed across the environment. The daily used products like pesticides, plastic items containing bisphenol A and phthalates, flame retardants, personal care products containing antimicrobials, heavy metals, and perfluoroalkyls are regularly being manufactured in industries. These are some of the most potential candidates as testicular disruptors among EDCs (Table 2.1).

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Table 2.1 Main Endocrine Disrupting Chemicals (EDCs) and related mechanisms and effects during the three main phases of male sexual development

	Pre-natal period		Neo-natal period/Infancy		Childhood/Adolescence	
EDCs	Mechanisms	Effects	Mechanisms	Effects	Mechanisms	Effects
BPA	Foetal Leydig cell dysfunction; germ cell toxicity	Reduced T and/or INSL3; impaired germ cells maturation; impaired HPG maturation	GnRH interference; impaired germ cells maturation	Impaired HPG maturation; congenital malformations (crtyptorchidism, hypospadias)	HPG disruption; reduced steroidogenesis; testicular toxicity on germ cells and Leydig cells	Delayed puberty; reduced spermatogenesis; developmental disorders; delayed sexual maturation; reduced testicular volume
Phthalates	Histological alterations; Leydig cell dysfunction	Reduced T and/or INSL3; impaired germ cells maturation	Impaired germ cells maturation	Congenital malformations (crtyptorchidism, hypospadias)	Reduced steroidogenesis; testicular toxicity	Impaired spermatogenesis
PFAS	Impairment of foetal Leydig cells, germ cells and Sertoli cells	Reduced T and/or INSL3; congenital malformations; impaired germ cells maturation; impaired HPG maturation	GnRH interference; impaired germ cells maturation	Impaired HPG maturation; congenital malformations (crtyptorchidism, hypospadias); reduced AGD	HPG dysruption; reduced steroidogenesis	Delayed puberty; reduced spermatogenesis; developmental disorders; delayed sexual maturation; reduced testicular volume

Abbreviations: *BPA* Bisphenol A, *PFAS* Perlfuoroalkyl substances, *T* Testosterone, *INSL3* Insulin-like 3 hormone, *HPG* Hypothalamic–pituitary gland, *GnRH* Gonadotropin-releasing hormone, *AGD* Anogenital distance

Although the biological effects of many EDCs are well known at the molecular and cellular levels in in vitro studies, their mechanism of action is not readily and easily assessed in vivo, as their effects can appear after prolonged and/or continuous exposure to a low dose. Importantly, the effects can be transgenerational, and therefore two or three generations are necessary to highlight some modest effects, making epidemiological studies in humans very challenging. Furthermore, these effects are often the result of the simultaneous interaction of several substances (mixture effect) at low doses. On the contrary, in vitro and animal studies often use single compounds at a high dose. Human EDC-related diseases are more likely to be the results of long-term exposure to low concentrations of EDC mixtures, rather than of acute exposure to single compound at high concentration. Anyway, many EDCs that have been linked to impaired male sexual and reproductive development and function seem to act as antiandrogenic and/or estrogenic compounds, after binding or mimicking the actions of either the androgen receptor (AR) or the estrogen receptor (ER). EDCs are highly heterogeneous and can be classified according to their origins in (i) natural and artificial hormones (e.g., phytoestrogens, omega-3 fatty acids, contraceptive pills, and thyroid medicines), (ii) drugs with hormonal side effects (e.g., naproxen, metoprolol, and clofibrate), (iii) industrial and household chemicals (e.g., phthalates, alkylphenoletoxilate detergents, plasticizers, solvents), and (iv) side products of industrial and household processes (e.g., polycyclic aromatic hydrocarbons, dioxins, pentachlorobenzene). As a consequence, many pathways might be disrupted, depending on the period of life when they act (ranging from impairment of sexual differentiation, organogenesis, spermatogenesis, steroidogenesis) and the cocktail of contaminants involved.

In general, three main phases of a man life are particularly susceptible for subsequent normal testis development and function (Fig. 2.1): the intrauterine phase, the neonatal phase comprising the so-called minipuberty in the first months of life, and puberty. However, even during infancy, when the testes are apparently "sleeping," damaging causes with permanent effects on testicular function can occur. This is,

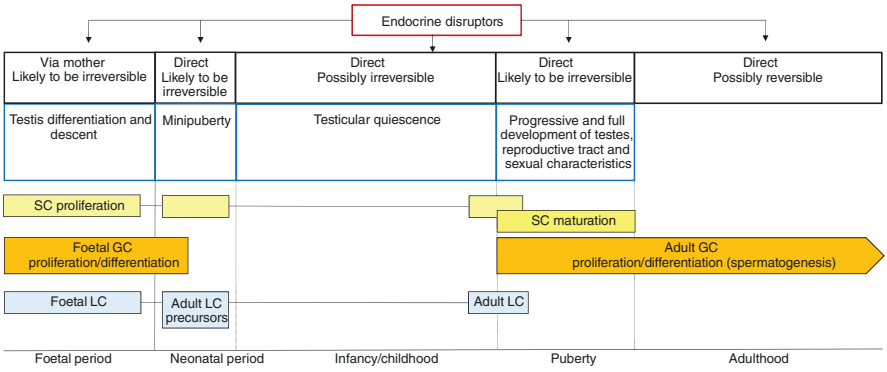

Fig. 2.1 Windows of susceptibility for testicular development and function from the fetal period to adulthood and effects of EDCs. *SC* Sertoli cells, *GC* Germ cells, *LC* Leydig cells

for example, the case of the iatrogenic, devastating effect of chemotherapy in this period of life. Risk factors acting via the mother during pregnancy might compromise definitively testicular function later in life, by disrupting fetal germ cell proliferation and differentiation and Sertoli cell proliferation and establishing the Leydig cell population (Fig. 2.1). Similarly, risk factors acting directly on minipuberty might compromise germ, Sertoli, and Leydig cell differentiation and proliferation. Iatrogenic, environmental, and lifestyle risk factors during childhood might interfere above all with the germ cell compartment, and those acting during puberty might disrupt Sertoli cell maturation, the establishment of adult Leydig cell population, and spermatogenesis (Fig. 2.1) [5, 7]. These fundamental phases of vulnerability are also important when dealing with EDCs, even if the intrauterine, transplacental phase seems to be the most important for future and transgenerational effects.

Also adolescence is a vulnerable window for the development and maturation of the genitourinary tract [8]. Risk factors, lifestyles, and EDC effects in adolescence may negatively affect adult health as well as that of future generations, through epigenetics.

A large body of evidence has been published dealing with various molecular and cellular aspects of the action of EDCs and their association with urogenital diseases. However, most studies focused on a single or single class of EDCs. Evidence from epidemiological and clinical studies is less robust, for the intrinsic difficulties highlighted above. Indeed, a systematic review and meta-analysis [9] of epidemiological studies reporting association between male reproductive disorders and exposures (documented by biochemical analyses of biospecimens) to chemicals that have been included in the European Commission's list of Category I EDCs showed that there is evidence for a small increased risk following prenatal and postnatal exposure to some persistent environmental chemicals, but the evidence is low, with an overall odds ratio across all exposures and outcomes of 1.11 (95% CI 0.91–1.35).

Most studies focused on bisphenol A and phthalates [10] and more recently on perfluoroalkyl compounds (PFC) [11].

2.2 Bisphenol A

Bisphenols, and in particular the phenol compound 2,2-bis(4-hydroxyphenyl)propane, universally known as bisphenol A (BPA), are widely used as additives for the production of plastic materials, such as polycarbonate, phenol and epoxy resins, polyesters and polyacrylates, as well as antioxidant in foodstuffs and cosmetics [12, 13]. Specifically, nearly the 75% of the industrial production of BPA is intended for the manufacture of polycarbonate-based products, which find wide application in food industry such as containers for food and beverages, in plastic dishes, in kitchen utensils, in containers for microwave cooking, and, until 2011, in bottles [14]. Of note, BPA is also used in epoxy resin films used as binary patina: the internal coatings in the cans for canned food [15].

As a result, there is a significant risk of human exposure to BPA through ingestion, skin contact, or inhalation [16, 17]. Epidemiological data from the United

States have reported detectable levels of BPA in urine samples from more than 90% of the general population, resulting in a major problem of exposure to chemical substance [18].

Concerns about BPA issues on the human health date back to the 1930s, when severe impact on male sexual development had been suggested. From a mechanistic point of view, the most relevant risks associated with the exposure to BPA are mainly due to its action as an EDC. Available reports in the late 1990s firstly documented a stimulating activity of BPA on ERα [19, 20] and were confirmed later [21–23]. In addition, unconjugated BPA showed a binding activity to other two receptors: the G protein-coupled estrogen receptor 30 (GPR30), also known as membrane estrogen receptor alpha (mERα) [24, 25], and the orphan nuclear estrogen-related receptor gamma (ERR-gamma) [26, 27]. Finally, experimental animal studies demonstrated that BPA binds also to AR, the peroxisome proliferator-activated receptor gamma (PPAR-gamma), and the thyroid hormone receptor [22].

A wide amount of data from animal studies shows a clear effect of BPA on male reproductive system, even at very low doses. In rodent models, BPA exposure has been associated with reduced sperm count and significant reductions of the absolute weights of the testes and seminal vesicles [28–35]. Furthermore, the exposure to BPA has been associated with the alteration of other non-conventional markers of sperm quality such as the index of DNA fragmentation, suggesting a possible role as mutagen [31, 36–45]. Also, acrosomal integrity, an overall marker of the fertilization potential, was significantly reduced by PBA exposure in murine models [29].

Several studies have been performed to disclose the possible disruption of the hypothalamus–pituitary–testis (HPT) axis associated with BPA exposure in animal models, with the result of a fairly complex picture that invariably leads to the impaired production of testosterone [30, 46], by both direct effects on steroidogenesis of the Leydig cells [42, 47, 48] and indirect effects on HPT. This latter is mediated by indirect suppression of the pituitary LH release through the massive aromatase upregulation in the testes [49]. Importantly, because of its high lipid solubility, BPA undergoes to transplacental transfer in animal models with a consequent detection in cord blood, an evidence reported also in humans [50–52]. Accordingly, BPA exposure during the prenatal period was associated with the impairment of both fetal development and the endocrine function of the testis, with reduced Leydig cell proliferation and fetal testosterone production [53–55]. Maternal exposure to BPA was associated with reduced sperm count and motility in male offspring and, in turn, with post-implantation loss and decreased litter size [56]. Of note, very recent studies disclosed some transgenerational effects associated with BPA exposure [57].

Despite the large availability of data in animal models, fewer studies assessed the possible relationship between BPA exposure and semen quality in humans. A negative association between urinary BPA and sperm concentration [58], motility, morphology, and sperm DNA damage has been described [59]. However, two independent studies on male partners from infertile couples attending infertility clinics were not able to retrieve any significant association between BPA urinary concentration and altered semen parameters [60, 61].

Another field of investigation pursued was the possible correlation between exposure to BPA and alteration of the endocrine pattern, but widely varying scenarios can be observed. Lower serum levels of follicle-stimulating hormone (FSH) in exposed workers compared to those non-exposed were found [62], but also a positive and significantly association with serum testosterone levels was observed [63]. Another study found increased serum testosterone, free testosterone, LH, and estradiol in subjects pertaining to higher urinary BPA concentrations quartile, compared with the lowest quartile. Subjects in the highest urinary BPA quartile also showed reduced progressive sperm motility compared with the lowest quartile [64]. On the contrary, urinary BPA concentrations were found positively associated with serum SHBG levels and inversely correlated with free androgen index (FAI) [61].

Finally, few studies aimed to assess the possible impact of BPA exposure on the overall fertility potential in males through the overall evaluation of the relationship between BPA levels and the reproductive outcome in the setting of assisted reproduction facilities. Minimal association between paternal urinary propyl paraben levels and reduced live birth rate in a correlation model corrected by possible confounders has been reported [65]. However, no significant association emerged between paternal urinary BPA and reproductive outcomes after fertility treatments. On the other hand, urinary BPA concentration in either males or females was not associated with increased time to pregnancy [66].

Overall, available data are supportive of detrimental role of BPA on semen parameters, but this is not accompanied by clear data on sex hormones and on fertility outcomes. As suggested by other authors [67], within the limits of the availability of data in humans, a possible reconciling explanation could rely on a greater direct toxicity of BPA on germ line cells, rather than in an albeit important endocrine disruption of the HPT axis.

In conclusion, BPA represents one of the most controversial chemical pollutants, with the typical features of an EDC. Early toxicological evidence on BPA date back to nearly 30 years ago, when major interference with estrogen signaling pathway was claimed. Since that time, a wide range of cell mechanisms of both endocrine and metabolic disruption have been claimed by the use of experimental models. In particular, major impairment of the HPT axis has been recognized as associated with the exposure to BPA during both the fetal and the adult life, resulting in altered testis development, impaired endocrine function, and infertility. To this regard, direct disruption of sperm characteristics such as reduced motility performances and development of genetic abnormalities have been identified. On the other hand, data obtained in humans are actually limited and poorly conclusive to identify a strict causal role of BPA in reduced male fertility potential.

Methodological differences and different study populations are factors that can explain some discrepancies. Moreover, available clinical outcomes, such as semen parameters and time to pregnancy, are likely susceptible of variation related to many different confounding factors. It should be noted that, as for most of chemical pollutants, the identification of a reliable marker of exposure remains a major issue. Specifically, for BPA, urinary concentrations are surely reliable data from an analytical point of view but may not be representative of the real exposure to BPA due

to its short half-life. To this regard, Vitku et al. reported that BPA levels in blood plasma were positively correlated with BPA levels in semen, but only seminal BPA was negatively associated with seminal quality [68]. Finally, the cross-sectional design of the available studies surely provides proof of association but limited evidence of causality.

One of the main problems associated with exposure to endocrine disruptors, in general, and to BPA, in particular, is represented by the potential activity at low concentrations. This represents a critical issue during the development phases, such as embryo/fetal life, newborn, or peri-pubertal age, since the effects in these time windows may be irreversible and are generally detected only at adulthood. Accordingly, populations at higher risk include pregnant women, infants, and adolescents (Fig. 2.1). On these bases, the current European law restricted the use of BPA in the production of packaging and materials in direct contact with food by limiting migration rate to 0.05 mg/kg of food and prescribing the total absence in products for newborns, from food to food containers and clothes [69]. In addition, based on new toxicological data and methodologies, the European authorities adjusted the tolerable daily intake from 50 to 4 μg/kg body weight/day with an overall lowering rate of 12 times, highlighting the increasing level of attention for these health concerns.

2.3 Phthalates

Phthalates are employed in virtually all industrial applications and consumer products as additives and used as plasticizers in a broad range of industrial and commercial products [70, 71]. The most commonly used phthalates are di-(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and benzyl butyl phthalate (BzBP). More than 75% of DEHP produced worldwide is used in plastic products. The other phthalates are largely used in personal care products like foams, shampoos, dyes, lubricants, and food packaging materials [72]. Since these compounds are not covalently bound polymers, their exposure to heat over time has the potential to favor their migration into food [73]. Indeed, plasticizers such as phthalate esters, because of their antiandrogen and estrogen-like activity, are indicated as major EDCs. Both in vitro and in vivo toxicology studies have demonstrated their endocrine-disrupting potential in model organisms, with endpoints such as antiandrogen effects, reproductive abnormalities, testicular lesions, and reduced sperm production [74]. However, as for other EDCs, dose ranges used for traditional reproductive toxicological studies were much higher than those observed in human epidemiological studies. Therefore, it is not surprising that these studies do not entirely align with the human studies. Nevertheless, in vitro and in vivo toxicology studies with low exposures to phthalates were linked to decreased semen quality and male infertility in animals, as well as to decreased androgen production and steroidogenesis [66, 75–83]. Phthalates have mostly shown the antiandrogen effect on testicular function during steroid formation [84–86]. Furthermore, phthalates as well as their metabolites (e.g., DEHP/MEHP, DBP/MBP) have stimulatory

effects at low doses through inducing the production of progesterone, testosterone, steroidogenesis-related proteins, and gene expression [77, 78, 80–83]. The adverse effects of phthalates on sperm quality were confirmed by ex vivo studies, where spermatozoa were exposed to high concentrations of phthalates, showing that sperm motility was affected and that cytotoxicity was caused at long-term exposures (>3 days) to the metabolite DEHP [87]. In parallel, DHEP has been shown to inhibit testosterone production, when cultured in vitro with explants derived from human testes [88].

Epidemiological studies reported an association between phthalate exposure and altered seminal parameters [89]. It is important to note that exposure of infants to phthalates is due to both maternal exposure and breastfeeding. In fact, breast milk levels of the phthalate metabolites are positively associated with maternal diet and water consumption.

Studies in humans corroborated the in vitro findings and suggested that exposure to phthalate metabolites is correlated with lower motility of spermatozoa in men from subfertile couples [90]. The DNA damage induced in spermatozoa and the motility and morphology of the spermatozoa were weakly associated with the exposure to phthalates [91–94], whereas an inverse association between MEHP exposure and testosterone and estradiol levels was reported [95].

Apart from infertility, data available on the effect of phthalates on male reproductive health are limited [96]. Phthalates are rapidly metabolized and excreted in urine and feces, and therefore the assessment of exposure to phthalates in humans relies on the measurement of urinary concentrations of phthalate metabolites. However, little or even no attention is given to the possible accumulation of unmetabolized phthalates in different tissues [97]. This evidence raises some concerns about the appropriateness of parameters employed as index of exposure to contaminants, in particular for those substances like phthalates that, showing specific tissue accumulation, may exert risk associated with long-term exposures [85]. To this regard, quantification of both parent compound and corresponding metabolites in specific body fluids may represent an informative parameter with better correlation with clinical parameters [86].

2.4 Perfluoroalkyl Compounds

Perfluoroalkyl compounds (PFCs) or substances (PFAS) are a class of organic molecules characterized by fluorinated hydrocarbon chains extensively used in industry and consumer products including oil and water repellents, coatings for cookware, carpets, and textiles. PFCs possess unique physical chemical properties due to their amphiphilic structures and their strong carbon-fluorine bonds. Therefore, long-chain PFCs are non-biodegradable and bioaccumulate in the environment [98, 99]. PFCs have been found in humans and in the global environment, and their toxicity, environmental fate, and sources of human exposure have been a major subject of research. Currently, 23 PFCs are distinguished, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), which are the predominant forms in

human and environmental samples. Both in vitro and animal studies on PFC toxicity have shown a detrimental effect of PFOA and PFOS on testicular function, through alteration of steroidogenic machinery and subsequent defect of spermatogenesis [100–104]. Among the endocrine effects of PFOS in particular, it should be emphasized that this compound can affect the HPT axis activity [105, 106]. It is also able to exert its toxicity at testicular level [107], as reported in rats [105, 108] and in testis models [109]. According to a recent study on male rats [110], high doses of PFOS orally administered for 28 days seem to modify the relative gene and protein receptor expressions of several hormones of the reproductive axis (GnRH, LH, FSH, and testosterone). Recently, exposure to PFOA was associated with reduction in sperm motility through alteration of sperm membrane fluidity [111].

Various PFC compounds have been found in human serum [112], seminal fluid [113], breast milk [114], and even umbilical cord [115], suggesting a lifelong exposure to PFCs in humans, from fetal stages until the adult life. Indeed, PFCs act as endocrine disruptors on the fetus and newborns, leading to developmental defects [116]. This has led to strict regulation of PFOA and PFOS use in industrial processes, as the compounds were added to the Annex B of the Stockholm Convention on Persistent Organic Pollutants. In addition to the health concerns related to fetal development, epidemiological studies have focused also on the relationship between PFCs and human fertility. In utero exposure to PFOA was associated later in adult life with lower sperm concentration and total sperm count and with higher levels of LH and FSH [117].

Besides the impact of PFCs on the professionally exposed populations, recent evidence of pollution from chemical industries producing PFCs have emerged also in the general population from at least four different areas worldwide: Mid-Ohio valley in the United States, Dordrecht area in Netherlands, Shandong district in China, and Veneto region in Italy [118]. Despite strong evidence pointing toward a negative role of PFCs on male reproductive function, to date, few evidence is available on the actual effect of these substances on seminal parameters in men, with conflicting results [113, 119, 120]. Two cross-sectional studies reported negative associations of PFOS, or high PFOA and PFOS combined, with the proportion of morphologically normal spermatozoa in adult men [119, 121]. Furthermore, in a study of men attending an in vitro fertilization clinic, Raymer et al. [113] reported that LH and free testosterone significantly and positively correlated with plasma levels of PFOA, although PFOA was not associated with semen quality. Conflicting results are reported also for the association between PFCs and sperm DNA quality, although a significant trend is evident for increased DNA fragmentation in exposed men [120, 122, 123]. In infertile males, PFOS levels were higher than fertile counterparts, together with a higher gene expression of ERα, ERβ, and AR [124, 125], suggesting that PFC activity might be linked also to the genetic expression of sex hormones' nuclear receptors. With respect to AR, PFOS and PFOA induce a decrease of the protein expression of this receptor in the hypothalamus and pituitary gland as well as in the testis [126]. These findings clearly suggest an antiandrogenic potential of PFCs. More recently, in a cross-sectional study on 212 exposed males from the Veneto region in Italy, and 171 non-exposed controls, increased levels of

PFCs in plasma and seminal fluid positively correlated with circulating testosterone and with a reduction of semen quality, testicular volume, penile length, and anogenital distance [127]. Furthermore, the antiandrogenic property of PFOA was related to antagonism on the binding of testosterone to AR [127].

In conclusion, in men, there is little evidence for an association between PFC exposure and semen quality or levels of reproductive hormones. As is the case for many epidemiological studies, causality cannot be definitively established in these studies, largely because of their cross-sectional design. However, the consistency of findings in pre-clinical studies strongly suggests a causal relationship for some endpoints.

2.5 Conclusions

EDCs can potentially cause harmful effects to the male reproductive system. In addition to the classical action of EDCs that includes the agonism and/or antagonism with hormone and nuclear receptors, the last decade of scientific research has given significant advances in the field of molecular biology that identified several compounds as endocrine disruptors, by interfering with the cell cycle, the apoptotic machinery, and the epigenetic regulation of the target cells [128]. However, action mechanisms should not be generally extrapolated since each chemical has different routes to interfere with endocrine activity. Among the tens of known EDCs, BPA, phthalates, and PFCs are particularly intriguing for male sexual and reproductive consequences given the strong experimental evidence of effects on hormone nuclear receptors (AR and/or ER), HPT axis, and direct action on spermatogenesis and steroidogenesis. However, epidemiological studies in humans have shown controversial and inconsistent results. This discrepancy can be attributed to several factors that could affect the outcome of the studies, notably to the complexity of the clinical protocols used, the degree of occupational or environmental exposure, the selection of the target group under investigation, the determination of the variables measured, and the sample size of the subjects examined. Despite the lack of consistency in the results of the human studies, the overall conclusion points toward a positive association between exposure to EDCs and alteration of the reproductive system.

References

1. Khabbaz RF, Moseley RR, Steiner RJ, Levitt AM, Bell BP. Challenges of infectious diseases in the USA. Lancet. 2014;384:53–63.
2. Slater C, Robinson AJ. Sexual health in adolescents. Clin Dermatol. 2014;32:189–95.
3. Stephen EH, Chandra A, King RB. Supply of and demand for assisted reproductive technologies in the United States: clinic- and population-based data, 1995-2010. Fertil Steril. 2016;105:451–8.
4. Nigam M, Aschebrook-Kilfoy B, Shikanov S, Eggener S. Increasing incidence of testicular cancer in the United States and Europe between 1992 and 2009. World J Urol. 2015;33:623–31.

5. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson AM, Eisenberg ML, Jensen TK, Jørgensen N, Swan SH, Sapra KJ, Ziebe S, Priskorn L, Juul A. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev. 2016;96:55–97.
6. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. EDC-2: the endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev. 2015;36(6):E1–E150.
7. Sharpe RM. Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc Lond Ser B Biol Sci. 2010;365:1697–712.
8. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4:254–64.
9. Bonde JP, Flachs EM, Rimborg S, Glazer CH, Giwercman A, Ramlau-Hansen CH, Hougaard KS, Høyer BB, Hærvig KK, Petersen SB, Rylander L, Specht IO, Toft G, Bräuner EV. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. Hum Reprod Update. 2016;23(1):104–25.
10. Pallotti F, Pelloni M, Gianfrilli D, Lenzi A, Lombardo F, Paoli D. Mechanisms of testicular disruption from exposure to bisphenol A and phthalates. J Clin Med. 2020;9(2):pii: E471.
11. Di Nisio A, Foresta C. Water and soil pollution as determinant of water and food quality/contamination and its impact on male fertility. Reprod Biol Endocrinol. 2019;17(1):4.
12. Murata M, Kang JH. Bisphenol A and all cell signaling pathways. Biotechnol Adv. 2017;36:311–27.
13. Lyons G. Bisphenol Q: a known endocrine disruptor. A WWF European toxics programme report. WWF European toxics programme: Godalming, Surrey. Registered Charity No 201707; 2000. http://www.google.it/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwjQ4uXOnoDoAhWttIsKHeNUAywQFjAAegQIBxAB&url=http%3A%2F%2Fassets.panda.org%2Fdownloads%2Fbisphenol.pdf&usg=AOvVaw1KMvI7KSfb4MEwV1Ce3KvD
14. Ehrlich S, Calafat AM, Humblet O, Smith T, Hauser R. Handling of thermal receipts as a source of exposure to bisphenol a. JAMA. 2014;311:859–60.
15. Pivnenko K, Pedersen GA, Eriksson E, Astrup TF. Bisphenol A and its structural analogues in household waste paper. Waste Manag. 2015;44:39–47.
16. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24:139–77.
17. Vandenberg LN, Hunt PA, Myers JP, vom Saal FS. Human exposures to bisphenol A: mismatches between data and assumptions. Rev Environ Health. 2013;28:37–58.
18. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ Health Perspect. 2008;116:39–44.
19. Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP, Gaido KW. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. Mol Cell Endocrinol. 1998;142:203–14.
20. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998;139:4252–63.
21. Li L, Wang Q, Zhang Y, Niu Y, Yao X, Liu H. The molecular mechanism of bisphenol A (BPA) as an endocrine disruptor by interacting with nuclear receptors: insights from molecular dynamics (MD) simulations. PLoS One. 2015;10:e0120330.
22. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol. 2007;24:199–224.
23. Viñas R, Jeng YJ, Watson CS. Non-genomic effects of xenoestrogen mixtures. Int J Environ Res Public Health. 2012;9:2694–714.

24. Dong S, Terasaka S, Kiyama R. Bisphenol A induces a rapid activation of Erk1/2 through GPR30 in human breast cancer cells. Environ Pollut. 2011;159:212–8.
25. Wozniak AL, Bulayeva NN, Watson CS. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca2+ fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ Health Perspect. 2005;113:431–9.
26. Matsushima A, Kakuta Y, Teramoto T, Koshiba T, Liu X, Okada H, Tokunaga T, Kawabata SI, Kimura M, Shimohigashi Y. Structural evidence for endocrine disruptor bisphenol A binding to human nuclear receptor ERR gamma. J Biochem. 2007;142:517–24.
27. Okada H, Tokunaga T, Liu X, Takayanagi S, Matsushima A, Shimohigashi Y. Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-gamma. Environ Health Perspect. 2008;116:32–8.
28. Al-Hiyasat AS, Darmani H, Elbetieha AM. Effects of bisphenol A on adult male mouse fertility. Eur J Oral Sci. 2002;110:163–7.
29. Wisniewski P, Romano RM, Kizys MML, Oliveira KC, Kasamatsu T, Giannocco G, Chiamolera MI, Dias-da-Silva MR, Romano MA. Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic–pituitary–testicular axis. Toxicology. 2015;329:1–9.
30. Gurmeet K, Rosnah I, Normadiah MK, Das S, Mustafa AM. Detrimental effects of bisphenol A on development and functions of the male reproductive system in experimental rats. EXCLI J. 2014;13:151–60.
31. Dobrzynska MM, Radzikowska J. Genotoxicity and reproductive toxicity of bisphenol A and X-ray/bisphenol A combination in male mice. Drug Chemical Toxicol. 2013;36:19–26.
32. Tainaka H, Takahashi H, Umezawa M, Tanaka H, Nishimune Y, Oshio S, Takeda K. Evaluation of the testicular toxicity of prenatal exposure to bisphenol A based on microarray analysis combined with MeSH annotation. J Toxicol Sci. 2012;37:539–48.
33. Tiwari D, Vanage G. Mutagenic effect of bisphenol A on adult rat male germ cells and their fertility. Reprod Toxicol (Elmsford, NY). 2013;40:60–8.
34. Salian S, Doshi T, Vanage G. Neonatal exposure of male rats to bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. Toxicology. 2009;265:56–67.
35. Qiu LL, Wang X, Zhang XH, Zhang Z, Gu J, Liu L, Wang Y, Wang X, Wang SL. Decreased androgen receptor expression may contribute to spermatogenesis failure in rats exposed to low concentration of bisphenol A. Toxicol Lett. 2013;219:116–24.
36. Tiwari D, Vanage G. Mutagenic effect of bisphenol A on adult rat male germ cells and their fertility. Reprod Toxicol. 2013;40:60–8.
37. Minamiyama Y, Ichikawa H, Takemura S, Kusunoki H, Naito Y, Yoshikawa T. Generation of reactive oxygen species in sperms of rats as an earlier marker for evaluating the toxicity of endocrine-disrupting chemicals. Free Radic Res. 2010;44:1398–406.
38. Chitra KC, Latchoumycandane C, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology. 2003;185:119–27.
39. Liu C, Duan W, Li R, Xu S, Zhang L, Chen C, He M, Lu Y, Wu H, Pi H, Luo X, Zhang Y, Zhong M, Yu Z, Zhou Z. Exposure to bisphenol A disrupts meiotic progression during spermatogenesis in adult rats through estrogen-like activity. Cell Death Dis. 2013;4:e676.
40. Rashid H, Ahmad F, Rahman S, Ansari RA, Bhatia K, Kaur M, Islam F, Raisuddin S. Iron deficiency augments bisphenol A-induced oxidative stress in rats. Toxicology. 2009;256:7–12.
41. Wu HJ, Liu C, Duan WX, Xu SC, He MD, Chen CH, Wang Y, Zhou Z, Yu ZP, Zhang L, Chen Y. Melatonin ameliorates bisphenol A-induced DNA damage in the germ cells of adult male rats. Mutat Res. 2013;752:57–67.
42. D'Cruz SC, Jubendradass R, Jayakanthan M, Rani SJ, Mathur PP. Bisphenol A impairs insulin signaling and glucose homeostasis and decreases steroidogenesis in rat testis: an in vivo and in silico study. Food Chem Toxicol. 2012;50:1124–33.
43. Kabuto H, Hasuike S, Minagawa N, Shishibori T. Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. Environ Res. 2003;93:31–5.

44. Anjum S, Rahman S, Kaur M, Ahmad F, Rashid H, Ansari RA, Raisuddin S. Melatonin ameliorates bisphenol A-induced biochemical toxicity in testicular mitochondria of mouse. Food Chem Toxicol. 2011;49:2849–54.
45. Fang Y, Zhou Y, Zhong Y, Gao X, Tan T. Effect of vitamin E on reproductive functions and anti-oxidant activity of adolescent male mice exposed to bisphenol A. Wei Sheng Yan Jiu. 2013;42:18–22.
46. El-Beshbishy HA, Aly HA, El-Shafey M. Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. Toxicol Ind Health. 2013;29:875–87.
47. Lan HC, Wu KY, Lin IW, Yang ZJ, Chang AA, Hu MC. Bisphenol A disrupts steroidogenesis and induces a sex hormone imbalance through c-Jun phosphorylation in Leydig cells. Chemosphere. 2017;185:237–46.
48. Gonçalves GD, Semprebon SC, Biazi BI, Mantovani MS, Fernandes GSA. Bisphenol A reduces testosterone production in TM3 Leydig cells independently of its effects on cell death and mitochondrial membrane potential. Reprod Toxicol. 2018;76:26–34.
49. Xi W, Lee CK, Yeung WS, Giesy JP, Wong MH, Zhang X, Hecker M, Wong CKC. Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypothalamus-pituitary-gonadal axis of CD-1 mice. Reprod Toxicol. 2011;31:409–17.
50. Zhang T, Sun H, Kannan K. Blood and urinary bisphenol A concentrations in children, adults, and pregnant women from China: partitioning between blood and urine and maternal and fetal cord blood. Environ Sci Technol. 2013;47:4686–94.
51. Wan Y, Choi K, Kim S, Ji K, Chang H, Wiseman S, Jones PD, Khim JS, Park S, Park J, Lam MHW, Giesy JP. Hydroxylated polybrominated diphenyl ethers and bisphenol A in pregnant women and their matching fetuses: placental transfer and potential risks. Environ Sci Technol. 2010;44:5233–9.
52. Balakrishnan B, Henare K, Thorstensen EB, Ponnampalam AP, Mitchell MD. Transfer of bisphenol A across the human placenta. Am J Obstet Gynecol. 2010;202:393e1.
53. Ben Maamar M, Lesné L, Desdoits-Lethimonier C, Coiffec I, Lassurguère J, Lavoué V, Deceuninck Y, Antignac JP, Le Bizec B, Perdu E, Zalko D, Pineau C, Chevrier C, Dejucq-Rainsford N, Mazaud-Guittot S, Jégou B. An investigation of the endocrine-disruptive effects of bisphenol a in human and rat fetal testes. PLoS One. 2015;10:e0117226.
54. Lv Y, Li L, Fang Y, Chen P, Wu S, Chen X, Ni C, Zhu Q, Huang T, Lian Q, Ge RS. In utero exposure to bisphenol A disrupts fetal testis development in rats. Environ Pollut. 2019;246:217–24.
55. Hong J, Chen F, Wang X, Bai Y, Zhou R, Li Y, Chen L. Exposure of preimplantation embryos to low-dose bisphenol A impairs testes development and suppresses histone acetylation of StAR promoter to reduce production of testosterone in mice. Mol Cell Endocrinol. 2016;427:101–11.
56. Salian S, Doshi T, Vanage G. Perinatal exposure of rats to bisphenol A affects the fertility of male offspring. Life Sci. 2009;85:742–52.
57. Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One. 2013;8:e55387.
58. Li DK, Zhou Z, Miao M, He Y, Wang J, Ferber J, Herrinton LJ, Gao E, Yuan W. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil Steril. 2011;95:625–30.
59. Meeker JD, Ehrlich S, Toth TL, Wright DL, Calafat AM, Trisini AT, Ye X, Hauser R. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reprod Toxicol. 2010;30:532–9.
60. Goldstone AE, Chen Z, Perry MJ, Kannan K, Louis GM. Urinary bisphenol A and semen quality, the LIFE study. Reprod Toxicol. 2015;51:7–13.
61. Mendiola J, Jorgensen N, Andersson AM, Calafat AM, Ye X, Redmon JB, Drobnis EZ, Wang C, Sparks A, Thurston SW, Liu F, Swan SH. Are environmental levels of bisphenol a associated with reproductive function in fertile men? Environ Health Perspect. 2010;118:1286–91.

62. Hanaoka T, Kawamura N, Hara K, Tsugane S. Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. Occup Environ Med. 2002;59:625–8.
63. Galloway T, Cipelli R, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Money C, McCormak P, Merlzer D. Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environ Health Perspect. 2010;118:1603–8.
64. Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, Skakkebaek NE, Juul A, Jørgensen N, Andersson AM. Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. Environ Health Perspect. 2014;122:478–84.
65. Dodge LE, Williams PL, Williams MA, Missmer SA, Toth TL, Calafat AM, Hauser R. Paternal urinary concentrations of parabens and other phenols in relation to reproductive outcomes among couples from a fertility clinic. Environ Health Perspect. 2015;123:665–71.
66. Buck Louis GM, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K. Urinary bisphenol A, phthalates, and couple fecundity: the longitudinal investigation of fertility and the environment (LIFE) study. Fertil Steril. 2014;101:1359–66.
67. Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, Padmanabhan V, Taylor HS, Swan SH, VandeVoort CA, Flaws JA. Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013. Environ Health Perspect. 2014;122:775–86.
68. Vitku J, Heracek J, Sosvorova L, Hampl R, Chlupacova T, Hill M, Sobotka V, Bicikova M, Starka L. Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. Environ Int. 2016;89–90:66–173.
69. EFSA COMMISSION REGULATION (EU) 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use WHO—endocrine disrupting chemicals; 2012.
70. Barr DB, Silva MJ, Kato K, Reidy JA, Malek NA, Hurtz D, et al. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Environ Health Perspect. 2003;111:1148–51.
71. Guo Y, Wu Q, Kannan K. Phthalate metabolites in urine from China, and implications for human exposures. Environ Int. 2011;37:893–8.
72. Guo Y, Weck J, Sundaram R, Goldstone AE, Louis GB, Kannan K. Urinary concentrations of phthalates in couples planning pregnancy and its association with 8-hydroxy-2′-deoxyguanosine, a biomarker of oxidative stress: longitudinal investigation of fertility and the environment study. Environ Sci Technol. 2014;48:9804–11.
73. Skinner MK. Endocrine disruptors in 2015: epigenetic transgenerational inheritance. Nat Rev Endocrinol. 2015;12:68–70.
74. Meeker JD, Ferguson KK. Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011-2012. J Clin Endocrinol Metab. 2014;99:4346–52.
75. Bao A-M, Man X-M, Guo X-J, Dong H-B, Wang F-Q, Sun H, et al. Effects of di-n-butyl phthalate on male rat reproduction following pubertal exposure. Asian J Androl. 2011;13:702–9.
76. Bloom MS, Whitcomb BW, Chen Z, Ye A, Kannan K, Buck Louis GM. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum Reprod. 2015;30:2645–57.
77. Fan J, Traore K, Li W, Amri H, Huang H, Wu C, et al. Molecular mechanisms mediating the effect of mono-(2-Ethylhexyl) phthalate on hormone-stimulated steroidogenesis in MA-10 mouse tumor Leydig cells. Endocrinology. 2010;151:3348–62.
78. Gunnarsson D, Leffler P, Ekwurtzel E, Martinsson G, Liu K, Selstam G. Mono-(2-ethylhexyl) phthalate stimulates basal steroidogenesis by a cAMP-independent mechanism in mouse gonadal cells of both sexes. Reproduction. 2008;135:693–703.

79. Han X, Cui Z, Zhou N, Ma M, Li L, Li Y, et al. Urinary phthalate metabolites and male reproductive function parameters in Chongqing general population, China. Int J Hyg Environ Health. 2014;217:271–8.
80. Hu Y, Dong C, Chen M, Lu J, Han X, Qiu L, et al. Low-dose monobutyl phthalate stimulates steroidogenesis through steroidogenic acute regulatory protein regulated by SF-1, GATA-4 and C/EBP-beta in mouse Leydig tumor cells. Reprod Biol Endocrinol. 2013;11:72.
81. Li Y, Hu Y, Dong C, Lu H, Zhang C, Hu Q, et al. Vimentin-Mediated Steroidogenesis Induced by Phthalate Esters: Involvement of DNA Demethylation and Nuclear Factor κB. Delmas D, editor. PLoS One. 2016;11:e0146138.
82. Savchuk I, Söder O, Svechnikov K. Mono-2-Ethylhexyl phthalate stimulates androgen production but suppresses mitochondrial function in mouse Leydig cells with different steroidogenic potential. Toxicol Sci. 2015;145:149–56.
83. Chen X, Liu YN, Zhou QH, Leng L, Chang Y, Tang NJ. Effects of low concentrations of Di-(2-ethylhexyl) and mono-(2-ethylhexyl) phthalate on steroidogenesis pathways and apoptosis in the murine Leydig tumor cell line MLTC-1. Biomed Environ Sci. 2013;26:986–9.
84. Dees JH, Gazouli M, Papadopoulos V. Effect of mono-ethylhexyl phthalate on MA-10 Leydig tumor cells. Reprod Toxicol. 2001;15:171–87.
85. Fiandanese N, Borromeo V, Berrini A, Fischer B, Schaedlich K, Schmidt J-S, et al. Maternal exposure to a mixture of di(2-ethylhexyl) phthalate (DEHP) and polychlorinated biphenyls (PCBs) causes reproductive dysfunction in adult male mouse offspring. Reprod Toxicol. 2016;65:123–32.
86. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect. 2008;116:1092–7.
87. Pant N, Pant A, Shukla M, Mathur N, Gupta Y, Saxena D. Environmental and experimental exposure of phthalate esters: the toxicological consequence on human sperm. Hum Exp Toxicol. 2011;30:507–14.
88. Desdoits-Lethimonier C, Albert O, Le Bizec B, Perdu E, Zalko D, Courant F, et al. Human testis steroidogenesis is inhibited by phthalates. Hum Reprod. 2012;27:1451–9.
89. Hauser R, Sokol R. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult male. Fertil Steril. 2008;89:e59–65.
90. Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, et al. Phthalate exposure and human semen parameters. Epidemiology. 2003;14:269–77.
91. Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, et al. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. Hum Reprod. 2007;22:688–95.
92. Duty SM, Calafat AM, Silva MJ, Brock JW, Ryan L, Chen Z, et al. The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. J Androl. 2004;25:293–302.
93. Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology. 2006;17:682–91.
94. Liu L, Bao H, Liu F, Zhang J, Shen H. Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality, a primary study. Environ Int. 2012;42:78–83.
95. Meeker JD, Calafat AM, Hauser R. Urinary metabolites of Di(2-ethylhexyl) phthalate are associated with decreased. J Androl. 2009;30:287–97.
96. Kay VR, Bloom MS, Foster WG. Reproductive and developmental effects of phthalate diesters in males. Crit Rev Toxicol. 2014;44:467–98.
97. Rusyn I, Peters JM, Cunningham ML. Modes of action and species-specific effects of di-(2-ethylhexyl)phthalate in the liver. Crit Rev Toxicol. 2006;36:459–79.
98. Conder JM, Hoke RA, De Wolf W, Russell MH, Buck RC. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ Sci Technol. 2008;42:995–1003.
99. Steenland K, Zhao L, Winquist A. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup Environ Med. 2015;72:373–80.

100. Biegel LB, Liu RCM, Hurtt ME, Cook JC. Effects of ammonium Perfluorooctanoate on Leydig-cell function: in vitro, in vivo, and ex vivo studies. Toxicol Appl Pharmacol. 1995;134:18–25.
101. Shi Z, Zhang H, Liu Y, Xu M, Dai J. Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to Perfluorododecanoic acid. Toxicol Sci. 2007;98:206–15.
102. Wan HT, Zhao YG, Wong MH, Lee KF, Yeung WSB, Giesy JP, et al. Testicular signaling is the potential target of Perfluorooctanesulfonate-mediated subfertility in male Mice1. Biol Reprod. 2011;84:1016–23.
103. Zhang H, Lu Y, Luo B, Yan S, Guo X, Dai J. Proteomic analysis of mouse testis reveals perfluorooctanoic acid-induced reproductive dysfunction via direct disturbance of testicular steroidogenic machinery. J Proteome Res. 2014;13:3370–85.
104. Kang JS, Choi JS, Park JW. Transcriptional changes in steroidogenesis by perfluoroalkyl acids (PFOA and PFOS) regulate the synthesis of sex hormones in H295R cells. Chemosphere. 2016;155:436–43.
105. López-Doval S, Salgado R, Pereiro N, Moyano R, Lafuente A. Perfluorooctane sulfonate effects on the reproductive axis in adult male rats. Environ Res. 2014;134:158–68.
106. Pereiro N, Moyano R, Blanco A, Lafuente A. Regulation of corticosterone secretion is modified by PFOS exposure at different levels of the hypothalamic–pituitary–adrenal axis in adult male rats. Toxicol Lett. 2014;230:252–62.
107. Qiu L, Zhang X, Zhang X, Zhang Y, Gu J, Chen M, et al. Sertoli cell is a potential target for perfluorooctane sulfonate-induced reproductive dysfunction in male mice. Toxicol Sci. 2013;135:229–40.
108. Jensen AA, Leffers H. Emerging endocrine disrupters: perfluoroalkylated substances. Int J Androl. 2008;31:161–9.
109. Zhang Y, Beesoon S, Zhu L, Martin JW. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ Sci Technol. 2013;47:10,619–27.
110. López-Doval S, Salgado R, Lafuente A. The expression of several reproductive hormone receptors can be modified by perfluorooctane sulfonate (PFOS) in adult male rats. Chemosphere. 2016;155:488–97.
111. Šabović I, Cosci I, De Toni L, Ferramosca A, Stornaiuolo M, Di Nisio A, Dall'Acqua S, Garolla A, Foresta C. Perfluoro-octanoic acid impairs sperm motility through the alteration of plasma membrane. J Endocrinol Investig. 2020;43(5):641–52.
112. Olsen GW, Lange CC, Ellefson ME, Mair DC, Church TR, Goldberg CL, et al. Temporal trends of Perfluoroalkyl concentrations in American red cross adult blood donors, 2000–2010. Environ Sci Technol. 2012;46:6330–8.
113. Raymer JH, Michael LC, Studabaker WB, Olsen GW, Sloan CS, Wilcosky T, et al. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. Reprod Toxicol. 2012;33:419–27.
114. Kubwabo C, Kosarac I, Lalonde K. Determination of selected perfluorinated compounds and polyfluoroalkyl phosphate surfactants in human milk. Chemosphere. 2013;91:771–7.
115. Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, et al. Trans-placental transfer of thirteen Perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol. 2011;45:7465–72.
116. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16:972–8.
117. Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Kristensen SL, Halldorsson TI, et al. Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect. 2013;121:453–8.
118. Ingelido AM, Abballe A, Gemma S, Dellatte E, Iacovella N, De Angelis G, et al. Biomonitoring of perfluorinated compounds in adults exposed to contaminated drinking water in the Veneto region, Italy. Environ Int. 2018;110:149–59.
119. Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebæk NE, Jørgensen N. Do Perfluoroalkyl compounds impair human semen quality? Environ Health Perspect. 2009;117:923–7.

120. Louis GMB, Chen Z, Schisterman EF, Kim S, Sweeney AM, Sundaram R, et al. Perfluorochemicals and human semen quality: the LIFE study. Environ Health Perspect. 2015;123:57–63.
121. Toft G, Jönsson BAG, Lindh CH, Giwercman A, Spano M, Heederik D, et al. Exposure to perfluorinated compounds and human semen quality in arctic and European populations. Hum Reprod. 2012;27:2532–40.
122. Specht IO, Hougaard KS, Spanò M, Bizzaro D, Manicardi GC, Lindh CH, et al. Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances—a study of spouses of pregnant women in three geographical regions. Reprod Toxicol. 2012;33(4):577–83.
123. Governini L, Guerranti C, De Leo V, Boschi L, Luddi A, Gori M, et al. Chromosomal aneuploidies and DNA fragmentation of human spermatozoa from patients exposed to perfluorinated compounds. Andrologia. 2015;47:1012–9.
124. La Rocca C, Alessi E, Bergamasco B, Caserta D, Ciardo F, Fanello E, et al. Exposure and effective dose biomarkers for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in infertile subjects: preliminary results of the PREVIENI project. Int J Hyg Environ Health. 2012;215:206–11.
125. La Rocca C, Tait S, Guerranti C, Busani L, Ciardo F, Bergamasco B, et al. Exposure to endocrine disruptors and nuclear receptors gene expression in infertile and fertile men from Italian areas with different environmental features. Int J Environ Res Public Health. 2015;12:12426–45.
126. Foresta C, Tescari S, Di Nisio A. Impact of perfluorochemicals on human health and reproduction: a male's perspective. J Endocrinol Investig. 2018;41(6):639–45.
127. Di Nisio A, Sabovic I, Valente U, Tescari S, Rocca MS, Guidolin D, Dall'Acqua S, Acquasaliente L, Pozzi N, Plebani M, Garolla A, Foresta C. Endocrine disruption of androgenic activity by perfluoroalkyl substances: clinical and experimental evidence. J Clin Endocrinol Metab. 2019;104(4):1259–71.
128. Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. Environ Toxicol Pharmacol. 2017;51:56–70.

From Genetics to Epigenetics: New Insights into Male Reproduction

3

Marica Franzago and Liborio Stuppia

3.1 Introduction (Genetic Versus Epigenetic Cause of Male Infertility)

Male infertility represents a growing concern worldwide, since 9–15% of males in reproductive age are infertile in different geographic areas [1–4]. Despite the large number of tools available for the study of this condition, so far in about 30–40% of infertile males, a specific cause of the disease cannot be identified, and these patients are defined as affected by "idiopathic male infertility" [5]. Great interest has been devoted in the last decades to the investigations of possible genetic causes of male infertility. Nevertheless, so far the only genetic conditions affecting male infertility in a substantial number of cases are 47,XXY karyotype and Yq microdeletions in non-obstructive azoospermia or severe oligozoospermia [6, 7] and *CFTR* gene mutations in obstructive azoospermia. Due to the availability of high-throughput techniques for gene analysis, several variants in a number of additional genes have been detected [8, 9]. Nevertheless, the overall prevalence of gene mutations detectable in infertile males still appears to account for no more than 25% cases. In this picture, it is essential to look to different directions. A very promising topic of

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research in this field is represented by epigenetics, that is, the effect of environmental factors able to affect gene expression. Technically speaking, epigenetics, a term first coined by Conrad Waddington in the 1940s [10], can be defined as the study of meiotic and/or mitotic inherited changes in the function of specific genes not related to modification in the DNA sequence [11]. The relevance of epigenetic effects on sperm gene expression can be synthetized as follows: (i) several environmental factors, including psychological stress, are known to affect male infertility and all of them are suspected to perform their activity by means of epigenetic mechanisms; (ii) sperm is one of the very few human tissues in which epigenetic modifications can be directly analyzed in the cells involved by the disease; (iii) epigenetic modifications can be transmitted, raising the question about the possible effects on the risk of congenital or late-onset disorders in the offspring; and (iv) epigenetic modifications are reversible, thus representing a very good candidate for personalized treatment, in particular in male partners enrolled in assisted reproduction technique (ART) protocols.

In this section, we will describe the most recent discoveries in the field of epigenetic of male fertility by stressing the possible risks derived from the transgenerational transmission of epigenetic modifications and the therapeutic perspectives for infertile males.

3.2 Molecular Basis of Epigenetics

DNA methylation, histone modifications, and small non-coding RNAs represent the main epigenetics marks [12]. These mechanisms play an important role in a number of biological processes involving chromatin structure and are tightly linked to the field of reproductive biology.

The major regulator of gene expression is DNA methylation, a dynamic process that adds a methyl group on the fifth carbon of the cytosines within a CpG site, thereby forming 5-methylcytosine. These dinucleotides are clustered in the differently methylated regions (DMRs) often placed near gene regulatory regions, such as the promoter [13]. In most instances, hypermethylation suppresses gene expression, preventing the transcription factors' and DNA polymerases' recruitment. DNA methylation is under the control of DNA methyltransferases (DNMTs) and enzymes of the demethylation pathway such as the ten-eleven translocation (TET), the thymine-DNA-glycosylase (TDG), and the DNA base excision repair (BER) [14]. There are two types of DNMTs, namely, DNMT1, which mainly plays a role in the maintenance of methylation of the newly synthesized strand after each DNA replication cycle, and DNMT3a–DNMT3b playing a major role in "de novo" methylation, occurring in specific chromosomal regions during early embryogenesis. Cytosine methylation can occur also in non-CpG sites (CpA, CpT, CpC). Although the significance of these variants is still unknown, it is restricted to specific cell types, such as pluripotent stem cells, oocytes, neurons, and glial cells [15]. The second epigenetic mark is represented by post-translational modifications of the amino terminal tails of the core histones (H2A, H2B, H3, H4), which include

methylation, acetylation, sumoylation, and phosphorylation. They can be easily induced and removed by many different enzymes increasing or decreasing access to DNA by the transcriptional machinery such as enhancers or repressors [16]. Many distinct histone modifications, on one or more tails, act sequentially or in combination, and this system of gene regulation is termed the "histone code" [17]. Generally, the methylation, which frequently occurs on histones H3 and H4 on specific lysine (K) and arginine (A) residues, is a key regulator for both activation and inhibition of transcription. For instance, methylation of H3K4 and H3K36 is usually considered as an activation mark, while when occurring at H3K9, H3K27, and H4K20 as a repressive mark. Acetylation and deacetylation at lysine residues are also important histone changes, modulated by the combined action of two enzymes known as histone acetyltransferase (HAT) and histone deacetylase (HDAC) that work to modify chromatin conformation. Acetylation correlates with the condensation of the chromatin and leads to transcription. Conversely, deacetylation of the lysine residues leads to chromatin condensation, making genes transcriptionally inactive. Then, phosphorylation of serines is associated with transcriptionally active chromatin because it precedes acetylation at lysine residues [18].

Finally, different RNAs, including short-chain non-coding RNAs, such as small interfering (siRNAs, 19–24 pb), microRNA (miRNAs, 19–24 pb), piwi interacting RNA (piRNAs, 26–31 pb), and long non-coding RNA (lncRNAs, generally >200 pb), are a class of relatively newly identified gene expression regulators [19, 20]. These non-coding RNAs do not encode functional proteins, being anyway able to regulate gene expression of encoding genes to control cell differentiation. A specific feature of epigenetic control is represented by genomic imprinting, involving about 150 genes, in which the gene expression is limited to one of the two parental alleles. Genomic imprinting determines the transcription rate of genes through a fine balance between the two parental alleles' expression that is established during gametogenesis and maintained throughout life. Each paternal and maternal allele contains different DMRs located near imprinted genes that affect gene expression. Several disorders related to alterations of this process are known, such as Prader-Willi syndrome (PWS), Angelman syndrome (AS), Beckwith-Wiedemann syndrome, and Silver-Russell syndrome [21].

3.3 Epigenetics and Human Spermatogenesis

Spermatogenesis is a highly complex and well-organized biological process in which spermatozoa are produced from spermatogonial stem cells. The formation of a mature sperm into the testicular tubule lumen requires three different transitions: (i) from mitotic spermatogonia to spermatocytes; (ii) from meiotic spermatocytes to spermatids; and (iii) from spermatids to mature spermatozoa (spermiogenesis). The spermatogenic process requires approximately 74 days, and the estimated daily sperm production ranges from 150 to 275 million spermatozoa in normal men [22].

During the different steps of spermatogenesis, a strict and precise regulation of gene expression at transcriptional, post-transcriptional, and epigenetic level

occurs. During spermiogenesis, the chromatin undergoes further condensation, due to the replacement of 90–95% histones with protamines, small molecules rich in arginine, inducing sperm DNA compaction. The protamination of sperm chromatin plays an important role in conferring extreme stability to the core of the sperm nucleus, improving sperm motility, protecting the sperm genome from oxidation and harmful molecules all along the female reproductive system, and blocking the transcriptional activity of the sperm DNA [23]. This process can be considered as a first example of epigenetic mechanism of gene expression control in sperms. In the first step of histone-protamine transition, histone hyperacetylation induces a loose chromatin structure; as a result, the histones are replaced with transition proteins (TP1 and TP2) [24]. The second step occurs in elongating spermatids in which the transition proteins are replaced with protamines [25]. Mature spermatid nuclei present two types of protamines, and the ratio of P1/P2 protamines is equal to one in fertile men. P1/P2 ratio appears to be critical for male fertility; in fact, the variations in sperm protamine expression and derangement of ratio have been correlated with the inadequate chromatin condensation, the increase in the DNA fragmentation, and important alterations in sperm parameters, such as motility and counts [26–28]. In addition, during the entire process of spermatogenesis, sperm cells undergo important modifications in DNA methylation. In fact, the erasure of DNA methylation is followed by de novo methylation that takes place before meiosis. In the process of de novo DNA methylation, the DNMT3A/B is recruited also through the cofactor DNMT3L activity, after birth at the stage of pachytene spermatocyte. Then, DNMT1 is responsible from maintenance of methylation profile. In addition, dynamics of histone modifications are critical for the spermatogenesis and early embryogenesis [29], modifying DNA accessibility to transcription factors. Generally, the methylation of H3K4 in spermatogonial stem cells is required to begin differentiation toward spermatocytes, whereas it diminishes during meiosis, promoting DNA silencing. The methylation level of H3K9 and H3K27 increases during meiosis, whereas histone H3-K9 methylation is removed to onset of spermiogenesis, promoting gene activation [30]. Specific enzymes such as histone methyltransferase (HMT) and histone demethylase (HDM) regulate these methylation patterns. In addition, the processes of acetylation and deacetylation of H3 and H4 lysine residues occur by means of HAT and HDAC activity. In particular, H3 and H4 lysine residues' acetylation is high in male stem cells but is removed during meiosis [30]. Moreover, the reacetylation of H4K plays a crucial role for correct histone to protamine transition in elongating spermatids [24].

The spermatozoon contains many specific RNAs, mRNAs, miRNAs, piRNAs, and tRNA fragments (tRNAs) which are markers of male infertility, particularly concerning spermiogenesis [30]. The study of biogenesis of the RNA payload of mature sperm is an area of intensive ongoing investigation because RNAs contribute to embryo development, but to date, this process is not completely understood [31]. Recently, it has been demonstrated that sperms carry RNAs previously synthesized in epididymal somatic cells, suggesting that soma-germline RNA transfer occurs in male mammals via vesicular transport [31, 32]. An abnormal epigenetic

reprogramming that may occur during each stage of spermatogenesis adversely not only affects male fertility and the outcome of in vitro fertilization (IVF) but also has consequences on the embryo and on the offspring's health [33, 34].

3.4 Factors Affecting Epigenetic Modifications in Sperm

A crucial question in the context of the study of epigenetic modifications during spermatogenesis is the one related to the external factors able to induce these changes (Fig. 3.1). In the last decade, a growing interest has been devoted to the study of two main factors invoked as possible causes of epigenetic alterations: (i) paternal lifestyle and (ii) exposure to environmental factors.

3.4.1 Lifestyle Factors (Diet, Exercise, Obesity, Smoke)

It has been demonstrated from a long time that couples with overweight, obese, or heavy smoker male partners have increased risk of infertility [35–38]. At present, it is definitively clear that exposure to periconceptional adverse lifestyle factors (unhealthy diet, physical inactivity, tobacco smoking, and alcohol abuse) can result not only in defect in spermatogenesis process but also in recurrent implantation failure, miscarriage, prematurity, and congenital malformations [39]. In this view, it has been strongly suggested that these exogenous agents can have adverse impact

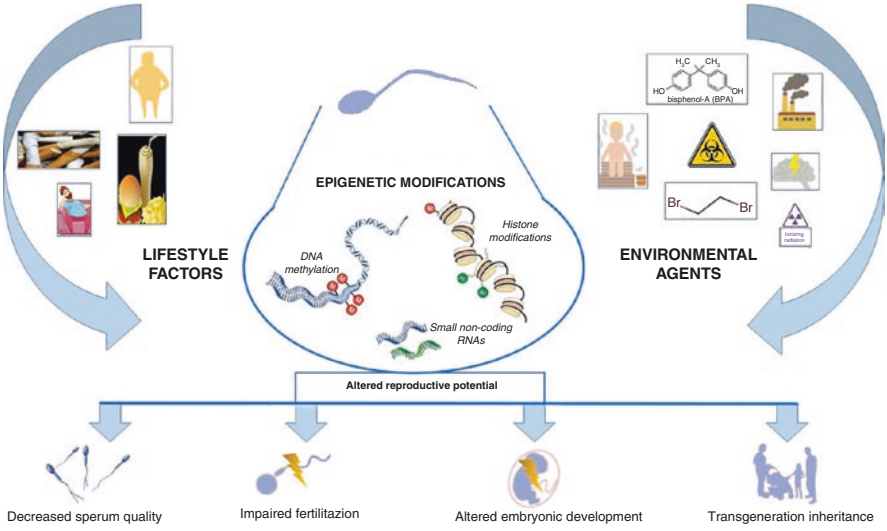

Fig. 3.1 Epigenetic modifications induced by environmental agents and lifestyle factors can lead to spermatogenesis failure, altered embryonic development and the offspring phenotype during lifetime

upon male individual health status, including reproduction, through an aberrant epigenetic regulation [40, 41].

The sperm epigenome is dynamically reactive to environmental and lifestyle stressors, but to date, the degree to which exogenous factors influence the fidelity of epigenetic reprogramming is unknown [42]. Several animal studies have demonstrated that the sperm epigenome may be responsive to dietary factors, and few recent studies appear to confirm this correlation in humans as well. In fact, obese men, already known to be more likely affected by oligo-azoospermia as compared to normal weight men [43, 44], have been also identified as carriers of a distinct epigenetic marks in sperm, in particular at genes that control brain development and function [45]. Moreover, the weight loss induced after gastric bypass (GBP) led to a progressive modification of the sperm epigenome, with about 1500 genes, some of which implicated in the central control of appetite, showing modifications in their epigenetic status 1 week after the surgery, and about 3000 genes 1 year after GBP [45]. In addition, the association between male obesity and DNA methylation alterations in spermatozoa at the regulatory regions of imprinted genes, which are growth effectors, plays an important role in early embryonic and fetal growth [42].

Alcohol consumption is also associated with deterioration of semen parameters [46, 47], and it has been demonstrated the alcohol's effect in lowering methyltransferase activity in sperm resulting in demethylation of normally hypermethylated imprinted regions in sperm DNA [48].

In addition, a 3-month supplementation of folate and anti-oxidants, such as vitamin C, vitamin E, lycopene, zinc, and selenium, improved the sperm quality and increased global sperm DNA methylation in infertile men [49]. These results in humans underline the need of further research to understand if some harmful consequences of unhealthy diet could be normalized through specific personalized nutrition [50].

The negative impact of smoking on sperm count, motility, and morphology has been well characterized [51] and is probably due to alterations in sperm DNA methylation patterns [52] as well as in the expression of sperm miRNAs involved in the mediation of the pathways of cell death and apoptosis [53].

Emerging evidence in humans indicates that also physical exercising influences epigenetic pattern in sperm cells; in fact, a 3-month endurance training intervention modified the methylation of genes associated with a wide range of diseases, such as schizophrenia, Parkinson's disease, cervical cancer, and leukemia [54]. Similarly, alterations of DNA methylation occurred in spermatozoa collected before and after a 6-week endurance exercise intervention, as well as after 3 months without exercise training [55]. Taken together, these observations suggest that the modifiable lifestyle factors could offer a therapeutic opportunity for the subfertile male. In particular, the plasticity of epigenetic modifications could represent a target for possible future interventions in improving the quality of human sperm. In fact, since the spermatogenesis process is completed in a few weeks, a restricted period of the personal lifestyle care could restore a physiological epigenetic mark of the sperm [56].

3.4.2 Environmental Factors (Endocrine Disruptors, Psychological Stress, Pollution, Heat, Toxins, Ionizing Radiation)

Also environmental factors, such as heat, psychological stress, pollution, toxins, and ionizing radiation, have been shown to affect the epigenetic state in sperm.

It has been evidenced in animal models that the heat can alter the DNA methylation programming in the paternal genome [57, 58]. In particular, perturbations of H3K27me3 in sperm chromatin induced by heat higher than 65 °C may decrease embryo implantation rate [57]. In this view, it has been proposed that varicocele, a condition inducing increased temperature in the testis, could induce alteration of DNA methylation in spermatozoa, thus explaining its potential negative effect on spermatogenesis [59, 60].

It has been documented that prolonged exposure to sauna sessions in normozoospermic subjects induces a significant but reversible impairment of spermatogenesis including alterations of sperm parameters, mitochondrial function, and sperm DNA packaging. Moreover, continuous sauna exposure leads to a complex gene response, including expression of genes involved in heat stress and hypoxia [61].

Another environmental factor able to induce epigenetic modifications in germ cells is represented by psychological stress, which is a well-known factor of reduced male fertility [62]. In this view, animal and human data suggest that the exposure of early life stress, such as abusive and/or dysfunctional family behavior, alters expression of specific sperm miRNA family [63].

A further increase in our knowledge about the relationship between stress and sperm function has been provided by the recently developed field of epididymosomes. These structures, considered as mediators of epididymal soma-spermatozoa intercellular communication, are represented by small membrane-bound vesicles released by the soma of epididymis that encapsulates an impressive cargo of miRNAs, the majority of which are represented in the miRNA signature of sperm. These vesicles are now considered as candidate vectors to facilitate the epigenetic information transfer to sperm [32]. Epididymosome miRNA cargo may be vulnerable to perturbation following paternal exposure to various forms of stress [64–66]. Further research about how epididymal soma responds to environmental cues to alter the molecular cargo of epididymosomes is warranted.

The role of radiation exposure as a very strong risk factor for male infertility is very well known from a long time. For example, men treated with radiation for childhood cancers show an elevated sperm DNA fragmentation index and an increased risk of negative consequences in terms of fertility [67]. On the other hand, limited data are available as concerning the association between occupational radiation exposure and risk of fertility and reproductive outcomes [68, 69]. In this view, it is important to stress that ionizing radiations have been invoked as a risk factor also for epigenetic modifications [70].

Other exposures, such as environmental chemicals, including metals (cadmium, arsenic, nickel, chromium, methylmercury), peroxisome proliferators (trichloroethylene, dichloroacetic acid, trichloroacetic acid), air pollutants (particulate matter,

black carbon, benzene), and endocrine-disrupting/reproductive toxicants (bisphenol A, diethylstilbestrol, persistent organic pollutants, dioxin), have been proved to modify epigenetic pathways in both experimental and epidemiological studies [71]. The harmful effects of endocrine-disrupting factors or their metabolites in disturbing spermatogenesis both at the level of the hypothalamic-pituitary axis and in the testis are well known [41, 72–75]. Endocrine-disrupting chemicals can potentially interfere with male and female reproductive systems either through direct interaction with hormone receptors or via epigenetic and cell cycle regulatory modes of action [76]. For example, the exposure to bisphenol A (BPA), which is present in many manufacture polycarbonate plastics and epoxy resins, including food and beverage containers and dental composites, has been correlated to anti-androgenic/anti-estrogenic effects and poor semen parameters [77]. The exposure to this environmental chemical could impair gene expression via affecting DNA hydroxymethylation in spermatogenesis, thus reducing human sperm quality [78]. Other investigations evidenced that chronical exposure to ethylene dibromide, used as a fumigant and in gasoline, was associated with a reduced sperm count and altered morphology and motility [79, 80]. This effect is probably due to the ethylene dibromide binding to histones and disturb of DNA packing, as evidenced in animal models [81].

Air pollution is correlated with increased air content of carbon monoxide (CO), nitrous dioxide (NO2), sulfur dioxide (SO2), ozone, lead (Pb), and particulate matter (PM), which is of particular interest, because it can carry a group of compounds that includes several endocrine disruptors. It has been observed that exposure to specific air pollutants alter sperm DNA integrity [82–84]. It is worth to mention that damage to spermiogenesis induced by air pollution exposure and in particular its genotoxic effects and epigenetic alterations require further investigation [71, 85]. Mice exposed to steel plant air showed germline mutations, DNA damage, and global hypermethylation compared to controls, and interestingly, this epigenetic mark persisted following removal from the environmental exposure [86]. Future studies in humans should strive to elucidate the underlying molecular mechanisms by which toxicant compound exposure may affect the sperm DNA damage and epigenetic modifications leading to affect fertility. Moreover, further research could especially determine whether DNA methylation changes determined by air pollutants are transmitted transgenerationally.

3.5 Transgenerational Inheritance

One of the most surprising features of epigenetic modifications of sperm DNA is the evidence that some of these changes can be retained after fertilization by the zygote and can escape the epigenetic reprograming occurring during embryo development. As a matter of fact, until a few years ago, the only example of transmission of epigenetic modifications to the offspring was related to the effects of maternal environmental exposure during pregnancy. Nevertheless, more recently, it has been evidenced that also paternal exposures to toxins, stress, nutrition, and other factors could play a crucial role in transgenerational epigenetic inheritance [87]. In other

words, it has been demonstrated that life experiences of fathers may be transmitted though the germline to offspring and perhaps subsequent generations in the form of epigenetic modification of sperm DNA. This leads some authors to suggest the novel term of "paternal exposome" as origin of health and disease in the offspring derived from paternal influences ("Paternal Origins of Health and Disease," POHaD) [50].

3.6 Assisted Reproductive Technique (ART)

Epigenetic modifications in the offspring conceived by ART have been largely documented both in animal models and in human [88]. One of the most frequently invoked causes of this effect is represented by technical stress induced by ART in terms of ovulation induction, manipulation of eggs, and embryo culture [89–93].

In particular, a crucial role of in vitro culture conditions has been suggested in the case of "large offspring syndrome" (LOS) evidenced in sheep and cattle after ART [94–96]. However, also epigenetic defects in the gametes used in ART have been invoked as a possible cause of epigenetic alterations in the embryo, due to the presence of DNA methylation changes in the sperm of men with defects of spermatogenesis [97, 98].

More recently, a large number of studies have confirmed the presence of alterations potentially related to epigenetic mechanisms in the offspring generated by ART [99–101]. Significant changes in both cardiac systolic and diastolic function in the ART population during childhood have been evidenced by Liu et al., who suggested an increased risk of early-onset myocardial alterations in children generated by ART [99]. The risk of cardiovascular dysfunction has been confirmed by Guo et al., who evidenced a statistically significant increase in blood pressure in 1–22-year-old children conceived by IVF [100]. An increased risk for cardiometabolic diseases has been suggested also by Kosteria et al. by the analysis of the proteomic profile of children born after intracytoplasmic sperm injection (ICSI) [101]. On the other hand, an increased risk for neurodevelopmental disorders, including autism spectrum disorder (ASD), in ART children has not been clearly demonstrated [102].

These data raise the question about long-term health implications of ART, mainly as concerning the risk of developing metabolic syndrome, type 2 diabetes, cardiovascular diseases (CVD), and infertility. Large-scale epidemiological studies are required to clarify if ART-conceived children are actually at increased risk of lifelong diseases and if epigenetic mechanisms are at the base of this process [103, 104].

3.7 Conclusions

The very impressive increase of the studies in the field of the role played by epigenetic modifications in male reproduction is disclosing an unexpected number of possible consequences of such alterations during the entire lifetime of the individual and this offspring.

The relationship between sperm epigenetic signatures and clinical outcomes in ART represents one of the most intriguing topics in this field. In fact, males are better candidate than women both for the identification of epigenetic markers in their gametes and for the development of novel therapeutic strategies aimed to induce a reversibility of epigenetic alterations [45, 49, 56]. In particular, since spermatogenesis process is completed in a few weeks, and it has been demonstrated in obese species that epigenetic pattern of sperm DNA can be restored in a short time, it is possible to suggest that a few months period of care of the personal lifestyle could provide a benefit for the quality of male gametes. The efficiency of this approach could be easily assessed by a simple analysis of sperm samples before and after treatment. Thus, personalized nutrition and specific attention to male lifestyle could represent a very powerful and costless tool in the prevention and restoration of epigenetic alterations induced by unhealthy lifestyle or exposure to environmental agents [105].

References

1. Geelhoed DW, Nayembil D, Asare K, Schagen van Leeuwen JH, van Roosmalen J. Infertility in rural Ghana. Int J Gynaecol Obstet. 2002;79(2):137–42. https://doi.org/10.1016/S0020-7292(02)00237-0.
2. Klemetti R, Raitanen J, Sihvo S, Saarni S, Koponen P. Infertility, mental disorders and well-being—a nationwide survey. Acta Obstet Gynecol Scand. 2010;89(5):677–82. https://doi.org/10.3109/00016341003623746.
3. Louis JF, Thoma ME, Sørensen DN, McLain AC, King RB, Sundaram R, Keiding N, Buck Louis GM. The prevalence of couple infertility in the United States from a male perspective: evidence from a nationally representative sample. Andrology. 2013;1:741–8. https://doi.org/10.1111/j.2047-2927.2013.00110.x.
4. Datta J, Palmer MJ, Tanton C, Gibson LJ, Jones KG, Macdowall W, Glasier A, Sonnenberg P, Field N, Mercer CH, et al. Prevalence of infertility and help seeking among 15000 women and men. Hum Reprod. 2016;31:2108–18. https://doi.org/10.1093/humrep/dew123.
5. Katz DJ, Teloken P, Shoshany O. Male infertility - The other side of the equation. Aust Fam Physician. 2017;46(9):641–6.
6. Stuppia L, Gatta V, Antonucci I, Giuliani R, Scioletti AP, Palka G. Genetic testing in couples undergoing assisted reproduction technique protocols. Expert Opin Med Diagn. 2009;3(5):571–83. https://doi.org/10.1517/17530050902970986. Epub 2009 Jul 29.
7. Thirumavalavan N, Gabrielsen JS, Lamb DJ. Where are we going with gene screening for male infertility? Fertil Steril. 2019;111(5):842–50. https://doi.org/10.1016/j.fertnstert.2019.03.036. Review.
8. Fenz Araujo T, Friedrich C, Paiva Grangeiro CH, Martelli LR, Emich J, Wyrwoll MJ, Kliesch S, Simões AL, Tüttelmann F. Sequence analysis of 37 candidate genes for male infertility: challenges in variant assessment and validating genes. Andrology. 2019; https://doi.org/10.1111/andr.12704.
9. Tüttelmann F, Ruckert C, Röpke A. Disorders of spermatogenesis: perspectives for novel genetic diagnostics after 20 years of unchanged routine. Med Genet. 2018;30(1):12–20. https://doi.org/10.1007/s11825-018-0181-7. Epub 2018 Feb 26.
10. Waddington CH. The epigenotype. Endeavour. 1942;1:18–20. https://doi.org/10.1093/ije/dyr184. Epub 2011 Dec 20.
11. Riggs AD, Matienssen RA, Russo VEA. Introduction. In: Epigenetic mechanisms of gene regulation. Cold Spring Harbour, NY: Cold Spring Harbour Laboratory Press; 1996. p. 1–4.
12. Holliday R. Epigenetics: a historical overview. Epigenetics. 2006;1:76–80. https://doi.org/10.4161/epi.1.2.2762.

13. Uysal F, Akkoyunlu G, Ozturk S. DNA methyltransferases exhibit dynamic expression during spermatogenesis. Reprod Biomed Online. 2016;33:690–702. https://doi.org/10.1016/j.rbmo.2016.08.022.
14. Kohli RM, Zhang Y. TET enzymes, TDG and the dynamics of DNA demethylation. Nature. 2013;502(7472):472–9. https://doi.org/10.1038/nature12750.
15. Patil V, Ward RL, Hesson LB. The evidence for functional non-CpG methylation in mammalian cells. Epigenetics. 2014;9(6):823–8. https://doi.org/10.4161/epi.28741. Epub 2014 Apr 9.
16. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693–705. https://doi.org/10.1016/j.cell.2007.02.005.
17. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;403(6765):41–5. https://doi.org/10.1038/47412.
18. Jenuwein T, Allis CD. Translating the histone code. Science. 2001;293:1074–80.
19. Dadoune JP. Spermatozoal RNAs: what about their functions? Microsc Res Tech. 2009;72:536–51. https://doi.org/10.1002/jemt.20697.
20. Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (review). Oncol Rep. 2017;37(1):3–9. https://doi.org/10.3892/or.2016.5236. Epub 2016 Nov 8.
21. Butler MG. Genomic imprinting disorders in humans: a mini-review. J Assist Reprod Genet. 2009;26:477–86. https://doi.org/10.1007/s10815-009-9353-3.
22. Neto FT, Bach PV, Najari BB, Li PS, Goldstein M. Spermatogenesis in humans and its affecting factors. Semin Cell Dev Biol. 2016;59:10–26. https://doi.org/10.1016/j.semcdb.2016.04.009. Epub 2016 Apr 30.
23. Rathke C, Baarends WM, Awe S, Renkawitz-Pohl R. Chromatin dynamics during spermiogenesis. Biochim Biophys Acta. 1839;2014:155–68. https://doi.org/10.1016/j.bbagrm.2013.08.004.
24. Sonnack V, Failing K, Bergmann M, Steger K. Expression of hyperacetylated histone H4 during normal and impaired human spermatogenesis. Andrologia. 2002;34:384–90. https://doi.org/10.1046/j.1439-0272.2002.00524.x.
25. Meistrich ML, Mohapatra B, Shirley CR, Zhao M. Roles of transition nuclear proteins in spermiogenesis. Chromosoma. 2003;111:483–8.
26. Carrell DT, Liu L. Altered protamine 2 expression is uncommon in donors of known fertility, but common among men with poor fertilizing capacity, and may reflect other abnormalities of spermiogenesis. J Androl. 2001;22:604–10. https://doi.org/10.1002/j.1939-4640.2001.tb02220.x.
27. de Mateo S, Gázquez C, Guimerà M, Balasch J, Meistrich ML, Ballescà JL, Oliva R. Protamine 2 precursors (Pre–P2), protamine 1 to protamine 2 ratio (P1/P2), and assisted reproduction outcome. Fertil Steril. 2009;91:715–22. https://doi.org/10.1016/j.fertnstert.2007.12.047.
28. Carrell DT. Epigenetics of the male gamete. Fertil Steril. 2012;97:267–74. https://doi.org/10.1016/j.fertnstert.2011.12.036.
29. Ge SQ, Li SL, Zhao ZH, Sun QY. Epigenetic dynamics and interplay during spermatogenesis and embryogenesis: implications for male fertility and offspring health. Oncotarget. 2017;8(32):53,804–18. https://doi.org/10.18632/oncotarget.17479.
30. Boissonnas CC, Jouannet P, Jammes H. Epigenetic disorders and male subfertility. Fertil Steril. 2013;99:624–31. https://doi.org/10.1016/j.fertnstert.2013.01.124.
31. Sharma U, Sun F, Conine CC, Reichholf B, Kukreja S, Herzog VA, Ameres SL, Rando OJ. Small RNAs are trafficked from the epididymis to developing mammalian sperm. Dev Cell. 2018;46(4):481–494.e6. https://doi.org/10.1016/j.devcel.2018.06.023.
32. Reilly JN, McLaughlin EA, Stanger SJ, Anderson AL, Hutcheon K, Church K, Mihalas BP, Tyagi S, Holt JE, Eamens AL, Nixon B. Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome. Sci Rep. 2016;6:31794. https://doi.org/10.1038/srep31794.
33. Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460:473–8. https://doi.org/10.1038/nature08162.

34. Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. Clin Epigenetics. 2015;7:120. https://doi.org/10.1186/s13148-015-0155-4.
35. Sallmén M, Sandler DP, Hoppin JA, Blair A, Baird DD. Reduced fertility among overweight and obese men. Epidemiology. 2006;17(5):520–3. https://doi.org/10.1097/01.ede.0000229953.76862.e5.
36. Nguyen RH, Wilcox AJ, Skjærven R, Baird DD. Men's body mass index and infertility. Hum Reprod. 2007;22(9):2488–93. https://doi.org/10.1093/humrep/dem139.
37. Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. Hum Reprod. 2007;22(1):188–96. https://doi.org/10.1093/humrep/del364.
38. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sørensen TIA, Olsen J. Subfecundity in overweight and obese couples. Hum Reprod. 2007;22(6):1634–7. https://doi.org/10.1093/humrep/dem035.
39. Steegers-Theunissen RPM, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. Hum Reprod Update. 2013;19:640–55. https://doi.org/10.1093/humupd/dmt041.
40. Alegría-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. Epigenomics. 2011;3:267–77. https://doi.org/10.2217/epi.11.22.
41. Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013;11:66. https://doi.org/10.1186/1477-7827-11-66.
42. Soubry A, Guo L, Huang Z, Hoyo C, Romanus S, Price T, Murphy SK. Obesity-related DNA methylation at imprinted genes in human sperm: results from the TIEGER study. Clin Epigenetics. 2016;8:51. https://doi.org/10.1186/s13148-016-0217-2.
43. Sermondade N, Faure C, Fezeu L, Lévy R, Czernichow S. Obesity and increased risk for oligozoospermia and azoospermia. Arch Intern Med. 2012;172(5):440–2. https://doi.org/10.1001/archinternmed.2011.1382.
44. Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. Hum Reprod Update. 2013;19(3):221–31. https://doi.org/10.1093/humupd/dms050.
45. Donkin I, Versteyhe S, Ingerslev LR, Qian K, Mechta M, Nordkap L, Mortensen B, et al. Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. Cell Metab. 2016;23(2):369–78. https://doi.org/10.1016/j.cmet.2015.11.004.
46. Gaur DS, Talekar MS, Pathak VP. Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. Indian J Pathol Microbiol. 2010;53(1):35. https://doi.org/10.4103/0377-4929.59180.
47. Jensen TK, Swan S, Jørgensen N, Toppari J, Redmon B, et al. Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. Hum Reprod. 2014;29:1801–9. https://doi.org/10.1093/humrep/deu118.
48. Ouko LA, Shantikumar K, Knezovich J, Haycock P, Schnugh DJ, Ramsay M. Effect of alcohol consumption on CpG methylation in the differentially methylated regions of H19 and IG-DMR in male gametes: implications for fetal alcohol spectrum disorders. Alcohol Clin Exp Res. 2009;33:1615–27. https://doi.org/10.1111/j.1530-0277.2009.00993.x.
49. Tunc O, Tremellen K. Oxidative DNA damage impairs global sperm DNA 21 methylation in infertile men. J Assist Reprod Genet. 2009;26:537–44. https://doi.org/10.1007/s10815-009-9346-2.
50. Soubry A. POHaD: why we should study future fathers. Environ Epigenet. 2018;4(2):dvy007. https://doi.org/10.1093/eep/dvy007. eCollection 2018 Apr.
51. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. Eur Urol. 2016;70(4):635–45. https://doi.org/10.1016/j.eururo.2016.04.010.

52. Jenkins TG, James ER, Alonso DF, Hoidal JR, Murphy PJ, Hotaling JM, Cairns BR, Carrell DT, Aston KI. Cigarette smoking significantly alters sperm DNA methylation patterns. Andrology. 2017;5(6):1089–99. https://doi.org/10.1111/andr.12416. Epub 2017 Sep 26.
53. Marczylo EL, Amoako AA, Konje JC, Gant TW, Marczylo TH. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? Epigenetics. 2012;7:432–9. https://doi.org/10.4161/epi.19794.
54. Denham J, O'Brien BJ, Harvey JT, Charchar FJ. Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. Epigenomics. 2015;7(5):717–31. https://doi.org/10.2217/epi.15.29. Epub 2015 Apr 13.
55. Ingerslev LR, Donkin I, Fabre O, Versteyhe S, Mechta M, et al. Endurance training remodels sperm-borne small RNA expression and methylation at neurological gene hotspots. Clin Epigenetics. 2018;10:12. https://doi.org/10.1186/s13148-018-0446-7.
56. Franzago M, La Rovere M, Guanciali Franchi P, Vitacolonna E, Stuppia L. Epigenetics and human reproduction: the primary prevention of the non-communicable diseases. Epigenomics. 2019; https://doi.org/10.2217/epi-2019-0163.
57. Chao SB, Chen L, Li JC, Ou XH, Huang XJ, Wen S, Sun QY, Gao GL. Defective histone H3K27 trimethylation modification in embryos derived from heated mouse sperm. Microsc microanal. 2012;18:476–82. https://doi.org/10.1017/S1431927612000396.
58. Rahman MB, Kamal MM, Rijsselaere T, Vandaele L, Shamsuddin M, Van Soom A. Altered chromatin condensation of heat-stressed spermatozoa perturbs the dynamics of DNA methylation reprogramming in the paternal genome after in vitro fertilisation in cattle. Reprod Fertil Dev. 2014;26(8):1107–16. https://doi.org/10.1071/RD13218.
59. Tavalaee M, Bahreinian M, Barekat F, Abbasi H, Nasr-Esfahani MH. Effect of varicocelectomy on sperm functional characteristics and DNA methylation. Andrologia. 2015;47(8):904–9. https://doi.org/10.1111/and.12345.
60. Garolla A, Torino M, Miola P, Caretta N, Pizzol D, Menegazzo M, Bertoldo A, Foresta C. Twenty-four hour monitoring of scrotal temperature in obese men and men with a varicocele as a mirror of spermatogenic function. Hum Reprod. 2015;30(5):1006–13. https://doi.org/10.1093/humrep/dev057. Epub 2015 Mar 15.
61. Garolla A, Torino M, Sartini B, Cosci I, Patassini C, Carraro U, Foresta C. Seminal and molecular evidence that sauna exposure affects human spermatogenesis. Hum Reprod. 2013;28(4):877–85. https://doi.org/10.1093/humrep/det020. Epub 2013 Feb 14.
62. Bhongade MB, Prasad S, Jiloha RC, Ray PC, Mohapatra S, Koner BC. Effect of psychological stress on fertility hormones and seminal quality in male partners of infertile couples. Andrologia. 2015;47(3):336–42. https://doi.org/10.1111/and.12268. Epub 2014 Mar 26.
63. Dickson DA, Paulus JK, Mensah V, Lem J, Saavedra-Rodriguez L, Gentry A, Pagidas K, Feig LA. Reduced levels of miRNAs 449 and 34 in sperm of mice and men exposed to early life stress. Transl Psychiatry. 2018;8(1):101. https://doi.org/10.1038/s41398-018-0146-2.
64. Pang TYC, Short AK, Bredy TW, Hannan AJ. Transgenerational paternal transmission of acquired traits: stress-induced modification of the sperm regulatory transcriptome and offspring phenotypes. Curr Opin Behav Sci. 2017;14:140–7. https://doi.org/10.1016/j.cobeha.2017.02.007. Epub 2017 Mar 8.
65. Hur SSJ, Cropley JE, Suter CM. Paternal epigenetic programming: evolving metabolic disease risk. J Mol Endocrinol. 2017;58(3):R159–68. https://doi.org/10.1530/JME-16-0236. Epub 2017 Jan 18.
66. Rowold ED, Schulze L, Van der Auwera S, Grabe HJ. Paternal transmission of early life traumatization through epigenetics: do fathers play a role? Med Hypotheses. 2017;109:59–64. https://doi.org/10.1016/j.mehy.2017.09.011. Epub 2017 Sep 18.
67. Romerius P, Ståhl O, Moëll C, Relander T, Cavallin-Ståhl E, Gustafsson H, Löfvander Thapper K, Jepson K, Spanò M, Wiebe T, Lundberg Giwercman Y, Giwercman A. Sperm DNA integrity in men treated for childhood cancer. Clin Cancer Res. 2010;16(15):3843–50. https://doi.org/10.1158/1078-0432.CCR-10-0140.
68. Fischbein A, Zabludovsky N, Eltes F, Grischenko V, Bartoov B. Ultramorphological sperm characteristics in the risk assessment of health effects after radiation exposure among salvage

workers in Chernobyl. Environ Health Perspect. 1997;105:1445–9. https://doi.org/10.1289/ehp.97105s61445.
69. Zhou DD, Hao JL, Guo KM, Lu CW, Liu XD. Sperm quality and DNA damage in men from Jilin Province, China, who are occupationally exposed to ionizing radiation. Genet Mol Res. 2016 Mar;22:15(1). https://doi.org/10.4238/gmr.15018078.
70. Kumar D, Salian SR, Kalthur G, Uppangala S, Kumari S, Challapalli S, Chandraguthi SG, Krishnamurthy H, Jain N, Kumar P, Adiga SK. Semen abnormalities, sperm DNA damage and global hypermethylation in health workers occupationally exposed to ionizing radiation. PLoS One. 2013;8(7):e69927. https://doi.org/10.1371/journal.pone.0069927.
71. Baccarelli A, Bollati V. Epigenetics and environmental chemicals. Curr Opin Pediatr. 2009;21(2):243–51.
72. Jeng HA, Yu L. Alteration of sperm quality and hormone levels by polycyclic aromatic hydrocarbons on airborne particulate particles. J Environ Sci Health A. 2008;43(7):675–81.
73. Hammoud A, Carrell DT, Gibson M, Sanderson M, Parker-Jones K, Peterson CM. Decreased sperm motility is associated with air pollution in salt Lake City. Fertil Steril. 2010;93:1875–9.
74. Hammoud SS, Purwar J, Pflueger C, Cairns BR, Carrell DT. Alterations in sperm DNA methylation patterns at imprinted loci in two classes of infertility. Fertil Steril. 2010;94:1728–33.
75. Anawalt BD. The silent spermatozoon: are man-made endocrine disruptors killing male fertility? Asian J Androl. 2013;15:165–8. https://doi.org/10.1038/aja.2012.148.
76. Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. Environ Toxicol Pharmacol. 2017;51:56–70. https://doi.org/10.1016/j.etap.2017.02.024. Epub 2017 Mar 6.
77. Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, et al. Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. Environ Health Perspect. 2014;122:478–84. https://doi.org/10.1289/ehp.1307309.
78. Zheng H, Zhou X, Li DK, Yang F, Pan H, Li T, Miao M, Li R, Yuan W. Genome-wide alteration in DNA hydroxymethylation in the sperm from bisphenol A-exposed men. PLoS One. 2017;12:e0178535. https://doi.org/10.1371/journal.pone.0178535.
79. Schrader SM, Turner TW, Ratcliffe JM. The effects of ethylene dibromide on semen quality: a comparison of short-term and chronic exposure. Reprod Toxicol (Elmsford, NY). 1988;2:191–8.
80. Bretveld R, Brouwers M, Ebisch I, Roeleveld N. Influence of pesticides on male fertility. Scand J Work Environ Health. 2007;33(1):13–28.
81. Amir D. The spermicidal effect of ethylene dibromide in bulls and rams. Mol Reprod Dev. 1991;28:99–109. https://doi.org/10.1002/mrd.1080280116.
82. Selevan SG, Borkovec L, Slott VL, Zudova Z, Rubes J, Evenson DP, Perreault SD. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect. 2010;108:887–94. https://doi.org/10.1289/ehp.00108887.
83. Rubes J, Rybar R, Prinosilova P, Veznik Z, Chvatalova I, Solansky I, Sram RJ. Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res. 2010;683:9–15. https://doi.org/10.1016/j.mrfmmm.2009.09.010.
84. Radwan M, Jurewicz J, Polańska K, Sobala W, Radwan P, Bochenek M, Hanke W. Exposure to ambient air pollution—does it affect semen quality and the level of reproductive hormones? Ann Hum Biol. 2016;43:50–6.
85. Vecoli C, Montano L, Andreassi MG. Environmental pollutants: genetic damage and epigenetic changes in male germ cells. Environ Sci Pollut Res Int. 2016;23(23):23,339–48. Epub 2016 Sep 26. https://doi.org/10.1007/s11356-016-7728-4.
86. Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ, Berndt ML, Pogribny IP, Koturbash I, Williams A, et al. Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. Proc Natl Acad Sci U S A. 2008;105:605–10.

87. Tollefsbol TO. Generational epigenetic inheritance. In: Tollefsbol TO, editor. Transgenerational epigenetics. Cambridge, MA: Academic Press; 2019. p. 1–10.
88. Canovas S, Ross PJ, Kelsey G. Coy PDNA methylation in embryo development: epigenetic impact of ART (assisted reproductive technologies). BioEssays. 2017;39 https://doi.org/10.1002/bies.201700106.
89. Bowman P, McLaren A. Viability and growth of mouse embryos after in vitro culture and fusion. J Embryol Exp Morphol. 1970;23:693–704.
90. Roemer I, Reik W, Dean W, Klose J. Epigenetic inheritance in the mouse. Curr Biol. 1997;7:277–80.
91. Dean W, Bowden L, Aitchison A, Klose J, Moore T, Meneses JJ, Reik W, Feil R. Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes. Development. 1998;125:2273–82.
92. Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biol Reprod. 2000;62:1526–35.
93. Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol Reprod. 2001;64:918–26.
94. Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nat Genet. 2001;27:153–4.
95. Young LE, Schnieke AE, McCreath KJ, Wieckowski S, Konfortova G, Fernandes K, Ptak G, Kind AJ, Wilmut I, Loi P, et al. Conservation of IGF2-H19 and IGF2R imprinting in sheep: effects of somatic cell nuclear transfer. Mech Dev. 2003;120:1433–42.
96. Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. Rev Reprod. 1998;3:155–63.
97. Filipponi D, Feil R. Perturbation of genomic imprinting in oligozoospermia. Epigenetics. 2009;4:27–30.
98. Kobayashi H, Hiura H, John RM, Sato A, Otsu E, Kobayashi N, Suzuki R, et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. Eur J Hum Genet. 2009;17:1582–91. https://doi.org/10.1038/ejhg.2009.68.
99. Liu H, Zhang Y, Gu HT, Feng QL, Liu JY, Zhou J, Yan F. Association between assisted reproductive technology and cardiac alteration at age 5 years. JAMA Pediatr. 2015;169:603–5. https://doi.org/10.1001/jamapediatrics.2015.0214.
100. Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, Huang HF. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. Fertil Steril. 2017;107:622–31. https://doi.org/10.1016/j.fertnstert.2016.12.007.
101. Kosteria I, Tsangaris GT, Gkourogianni A, Anagnostopoulos A, Papadopoulou A, Papassotiriou I, Loutradis D, Chrousos GP, Kanaka-Gantenbein C. Proteomics of children born after intracytoplasmic sperm injection reveal indices of an adverse cardiometabolic profile. J Endocr Soc. 2017;1:288–301. https://doi.org/10.1210/js.2016-1052.
102. La Rovere M, Franzago M, Stuppia L. Epigenetics and Neurological Disorders in ART. Int J Mol Sci. 2019; 20(17). pii: E4169. https://doi.org/10.3390/ijms20174169. Review.
103. Whitelaw N, Bhattacharya S, Hoad G, Horgan GW, Hamilton M, Haggarty P. Epigenetic status in the offspring of spontaneous and assisted conception. Hum Reprod. 2014;29:1452–8.
104. Chen M, Heilbronn LK. The health outcomes of human offspring conceived by assisted reproductive technologies (ART). J Dev Orig Health Dis. 2017;8:388–402. https://doi.org/10.1017/S2040174417000228.
105. Szarc vel Szic K, Declerck K, Vidaković M, Vanden Berghe W. From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition? Clin Epigenetics. 2015;7:33. https://doi.org/10.1186/s13148-015-0068-2. eCollection 2015.

4 Medical and Surgical Treatment of Congenital Anomalies of Male Genital Tract

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4.1 Introduction

Normal sexual development is a multistep process which requires three different main phases (Jost model, [1–2]). The establishment of chromosomal sex allows the differentiation of the indifferent gonadal ridge into either testes or ovaries. In particular, in males, the expression of the product of the gene called SRY (sex determination region of Y chromosome), which occurs around the sixth week of gestation, guides the differentiation of both Sertoli and Leydig cells leading to the production of specific hormones allowing for the development of phenotypically male internal and external genital anatomy ([1–2]; see also Fig. 4.1). Testosterone (T) production usually begins between the eighth and the ninth weeks of gestation and, along with the contribution of the testis release of INSL-3 (insulin-like factor-3), regulates testis descent from the abdomen to the scrotum (see Fig. 4.1). In addition, whereas T mainly contributes to the development of internal genitalia from Wolff's ducts, its

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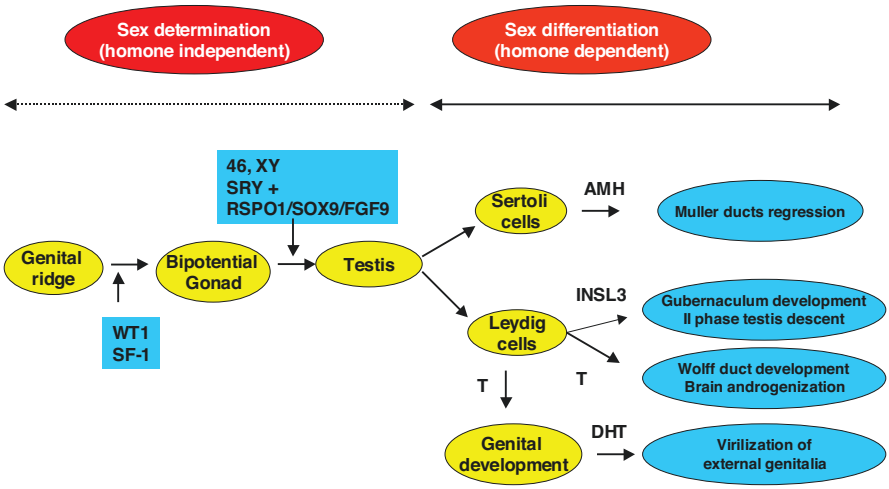

Fig. 4.1 Pathways from primordial gonad to sex determination and to sex differentiation: key genes and testicular hormone actions are summarized. *AMH* Anti-müllerian hormone, *T* Testosterone, *INSL-3* Insulin like 3, *DHT* Dihydrotestosterone

conversion to the more potent androgen dihydrotestosterone (DHT) is mainly involved in the regulation of prostate and external male genitalia differentiation ([1–2]; see also Fig. 4.1). At the same time, the Sertoli cell production of anti-Müllerian hormone (AMH) allows Müllerian duct regression. However, Müllerian duct remnants can persist in the adult male, located in the appendix testis, or in the prostatic utricle, an expansion of the prostatic urethra ([1–2]; see also Fig. 4.1).

It is important to recognize that luteinizing hormone (LH) receptor expression on Leydig cells occurs around the 12th week of gestation. Hence, for at least 3 weeks of gestation, T production appears to be independent of human chorionic gonadotropin of LH regulation [1, 2]. Accordingly, the rare condition of so-called Leydig cell hypoplasia syndrome (estimated incidence of 1:1000,000 newborns) due to loss-of-function mutations on the LH receptor (LHR), or LH/hCG receptor (LHCGR), is characterized by female external genitalia and bilateral retained abdominal testes with the presence of T-dependent Wolff-derived structures, such as epididymides and vasa deferentia [3].

Milder forms of the latter syndrome, due to milder LHCGR function, have also been described. The latter forms are characterized by a broader array of phenotypic expression with micropenis, severe hypospadias, and disorder of sex differentiation and virilization [3].

Hence, according to the Jost model, three different steps are crucial for normal sexual development (sex chromosomal establishment, differentiation of the testis or ovary, and hormonal production). The perturbation of each of these phases can be the cause of an impaired male genital tract development. Several conditions are known to be involved. However, in the vast majority of cases, the real etiology of male genitalia tract abnormalities remains unknown.

In this chapter, the most frequent and most important male genitalia tract abnormalities will be summarized. In addition, possible medical and surgical approaches will be discussed.

4.2 Disorders of Male Sexual Development (DSD)

About 1:4500 infants is born with abnormalities of the external genitalia [4]. DSD include widely different clinical conditions in which chromosomal, gonadal, or anatomical sex development is atypical [4, 5]. In 2008, Hughes et al. [5] introduced a new classification of DSD conditions, starting from a karyotype analysis. In particular, the previously used term "intersex" was abolished since it is considered discriminating and offensive [5]. According the new classification, three main categories are recognized: sexual chromosome DSD, 46,XX DSD, and 46,XY DSD (see Table 4.1). Overall, DSD is clinically characterized by the presence of ambiguous external genitalia at birth, leading to possible difficulties in gender definition. However, it is important to recognize that even some conditions in which there are no "genital ambiguities" and in which the "social" sex of the person is not in doubt (e.g., Klinefelter's, Turner's, Rokitansky's, androgenic complete insensitivity), but one (or more than one) chromosomal/molecular mechanism of sex development is "atypical," were also included (Table 4.1). The specific pathognomonic characteristics of each condition included in the umbrella of DSD are beyond the aim of the present chapter and have been reviewed elsewhere [4, 5]. Some more details will be provided for those defects related to androgen biosynthesis problems or actions.

4.2.1 DSD Due to Androgen Biosynthesis Problems or Actions

T and its related sex-derived steroids are all derived from cholesterol. Hence, several genes involved in steroidogenesis can underlay DSD defects (see Table 4.1).

1. *2.1.1 Congenital lipoid adrenal hyperplasia* represents the most severe genetic disorder of steroidogenesis. It results as a consequence of steroidogenic acute regulatory protein (StAR) gene mutations, involved in the first step of the complex enzymatic steroidogenic process [6]. In this condition, Leydig cells are lost early on during gestation, and the affected 46,XY fetuses are characterized by female external genitalia and blind vaginal pouch at birth, without the development of Müllerian duct derivatives [6]. In addition, due to associated adrenal insufficiency, this condition represents a life-threading condition at birth. A milder phenotype can be present with the retention of about 20–25% of normal StAR activity [6].
2. 2.1.2 A similar phenotype is observed in the presence of *gene mutations in the 20,22-desmolase enzyme*, which is involved in the conversion of cholesterol to pregnenolone ([6]; Fig. 4.2).
3. *3β-Hydroxysteroid dehydrogenase (3β-HSD)* catalyzes the conversion from hydroxyl to keto group during steroidogenesis (Fig. 4.2). The 3β-HSD deficiency is a rare condition in which 46,XY newborns are able to synthetize only small amounts of androgens by peripheral conversion of adrenal and testicular DHEA resulting in minor defects including small phallus and hypospadias and cryptorchidism [6].

Table 4.1 Proposed classification of causes of disorders of sex development (DSDs)

Sex chromosome DSD	46 XY DSD	46,XX DSD
A: 47,XXY (Klinefelter syndrome and variants) **B: 45,X (turner syndrome and variants)** **C: 45,X/46,XY (mixed gonadal dysgenesis)**	**A: Disorders of gonadal (testicular) development** *1. Complete or partial gonadal dysgenesis* (e.g. SRY,SOX9,SFI, WT1, DHH etc) *2. Ovotesticular DSD* *3. Testis regression*	**A: Disorders of gonadal (ovarian) development** *1. Gonadal dysgenesis* *2. Ovotesticular DSD* *3. Testicular DSD*(e.g.*SRY+, dup SOX9, RSP01)*
D: 46,XX/46,XY (chimerism)	**B: Disorders in androgen synthesis or action** *1. Disorders of androgen synthesis* a. LH receptor mutations b. Smith-Lemli-Opitz syndrome c. Steroidogenic acute regulatory protein mutations d. Cholesterolside-chain cleavage (CYP11A1) e. 3 β-hydroxysteroid dehydrogenase 2(HSD3B2) f. 17 β-hydroxysteroid dehydrogenase (HSD17B3) g. 5 α-reductase 2(SRD5A2) *2. Disorders of androgen action* a. Androgen insensitivity syndrome b. Drugs and environmental modulators	**B: Androgen excess** *1. Fetal* a. 3 β-hydroxysteroid dehydrogenase 2(HSD3B2) b. 21-hydroxylase(CYP21A2) c. P450 oxidoreductase(POR) d. 11 β -hydroxylase (CYP11B1) e. Glucocorticoid receptor mutations *2. Feto-placental* a. Aromatase deficiency(CYP19) b. Oxidoreductase deficiency(POR) *3. Maternal* a. Maternal virilizing tumours (e.g. luteomas) b. Androgenic drugs
	C: Other *1. Syndromic associations of male genital development* (e.g. cloacal anomalies, Robinow, Aarskog, hand-foot-genital, popliteal pterygium) *2. Persistent Mullerian duct syndrome* *3. Vanishing testis syndrome* *4. Isolated hypospadias(CXorf 6)* *5. Congenital hypogonadotropichypogonadism* *6. Cryptorchidism (INSL3,GREAT)* *7. Environmental influence*	**C: Other** *1. Syndromic associations (e.g. cloacal anomalies)* *2. Mullerian agenesis/hypoplasia (e.g. MURCS)* *3. Uterine abnormalities (e.g. MODY5)* *4. Vaginal atresis (e.g. KcKusick-Kaufman)* *5. Labial adhesions*

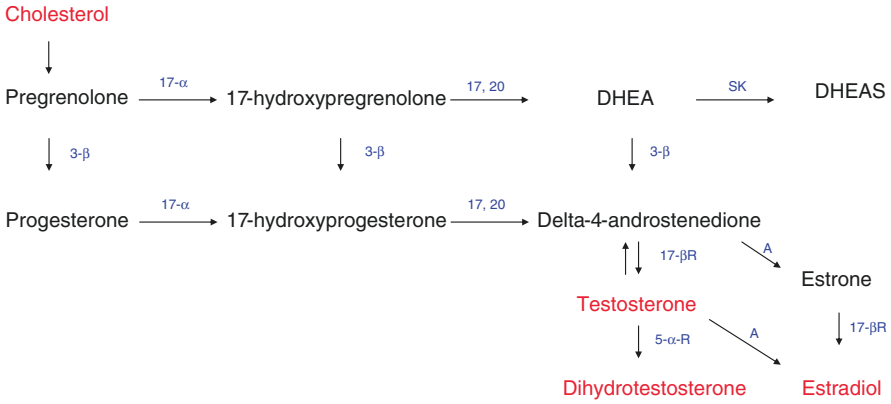

Fig. 4.2 Schematic representation of sex steroid synthesis. *17α* 17α-hydroxylase (CYP17, P450c17), *17,20* 17,20 lyase (also mediated by CYP17), *3β* 3β-hydroxysteroid dehydrogenase, *17βR* 17β-reductase, *5αR* 5α-reductase, *DHEA* Dehydroepiandrosterone, *DHEAS* DHEA sulfate, *A* Aromatase (CYP19)

4. 17α-Hydroxylase/17,20-Lyase

   Both the 17α-hydroxylase and the 17,20-lyase are catalyzed by the same gene (P450c17) as the 17,20-lyase is mainly important for sex steroid production (Fig. 4.2, [6]). Mutations on P450c17 gene cause adrenal insufficiency in males and absent or incomplete development of external genitalia [6].
5. 2.1.5 17β-Hydroxysteroid Dehydrogenase (17β-HSD)

   Three isoenzymes have so far been discovered [6]. The 17β-HSD type 3 (17β-HSD3) is involved in the conversion of androstenedione to T (Fig. 4.2). Male children with 17β-HSD3 deficiency display DSD, with hypogonadism, hypospadia, micropenis, and inguinal or intra-abdominal testes [6].
6. 2.1.6 Morris Syndrome

   Morris syndrome or complete androgen insensitivity syndrome (AIS) is a rare condition (1:20000–1:100.000) caused by inactivating mutations of the X-linked androgen receptor gene in a 46,XY male [7]. The lack of androgen action leads to the development of normal female external genitalia with a short blind vagina, absence of Müllerian-derived internal genitalia (uterus and fallopian tubes), and bilateral retained testis [7]. At puberty, usually, normal breast development occurs with diminished or absent secondary terminal hairs and primary amenorrhea. Milder forms of AIS have also been described with a large spectrum of clinical phenotypes from DSD to milder virilization [7].
7. 2.1.7 Steroid 5a-Reductase 2 Deficiency

   It is a rare autosomal recessive DSD, described for the first time in 1961 by Nowakowski and Lenz [8]. Two different 5a-reductase isoenzymes involved in the conversion of T to DHT have been isolated. Whereas the role of isoenzyme 1 has not yet been well defined, isoenzyme 2 is expressed in genital tissues and in the prostate, and it is crucial for the normal development of these structures [8]. Several mutations in the gene coding for isoenzyme 2 have been described.

Depending on the level of enzyme activity, the clinical phenotype ranges from an almost female external genitalia to an undervirilized male genitalia [8]. Usually, during adolescence, a normal muscle mass occurs with male gender identity orientation in the vast majority of cases [8].

4.2.2 Diagnosis of DSD

Overall, the clinical phenotype depends on the underlying defects and ranges from a complete female phenotype such as in the case of Morris syndrome (or complete androgen insensitivity) to milder defects of male genitalia tract development or isolated abnormalities such as cryptorchidism or hypospadias [4, 5]. Correct and early clinical diagnosis represents a crucial step in the management of DSD. Besides chromosomal evaluation, a steroid profile is essential in differentiating the various enzymatic defects potentially causing congenital adrenal hyperplasia (CAH) which represent the most frequent cases of DSD in women. In addition, measurement of AMH should also be performed. Radiological and/or ultrasound testing is useful in determining the presence of a uterus and the location of gonads. Furthermore, an hCG stimulation test can be performed to evaluate for abnormalities in testosterone. Despite this approach, it is important to recognize that molecular diagnosis is possible in no more than 20% of cases and no more than 50% of patients with 46,XY karyotype and ambiguous genitalia arrive at a definitive diagnosis [4, 5, 9].

4.2.3 Treatment

Gender identity throughout a lifespan is quite variable across DSD syndromes. Hence, the correct sex assignment at birth, as well as the timing for surgical correction of genitalia abnormalities, still represents a challenge for all physicians involved in the treatment of DSD. The absence of specific outcome-based guidelines requires the presence of an experienced interdisciplinary team of specialists including pediatric endocrinologists, pediatric urologists/surgeons, geneticists, and psychologists who should be all involved along with the child's family in the final decision. In addition, the parents and family need psychosocial support and education prior to making surgical management decisions.

Once gender is assigned, the child should be raised in this gender, although flexibility should be considered. In many cases, the newborn period after the diagnosis establishment and gender assignment represents the best period for the first surgical approach. However, in many cases, especially in 46,XY children with androgen biosynthesis defects, the gender identity stability is quite complex, so much so that some centers recommend postponing surgical intervention until the patient can participate in the decision-making [10]. During childhood, the pediatric endocrinologist plays the most important role in the management of a child with a DSD. The main focus in this period is growth optimization along with the treatment of

disease-specific issues [10]. Finally, adolescence represents a challenging period due to sexual maturation. Clinicians involved in the management of DSD should allow and favor the development of sexual characteristics in line with patient gender identity. During this period, it is important to recognize the possibility of reconsidering gender assignment in subjects with 46,XY DSD and intact gonads or, if not already done, reinforce gender identity with gonadectomy due to a mismatch between the assigned sex and gender identity or to reduce cancer risk [10]. In addition, a review of prior surgeries, identification of surgical complications, as well as the determination of the possible need for future surgery represent other crucial issues in this period.

4.2.3.1 Medical Therapy

Hypogonadism in DSD people is quite frequent, and it can be the result of the dysgenetic gonads with impaired sex steroid production or a consequence of specific defects in sex steroid biosynthesis or action. In addition, previous gonadectomy during the prepubertal years represents a further additional cause of hypogonadism [11, 12]. Substitutive sex steroid hormones should be administered in subjects with 46,XY DSD and hypogonadism with the aim to induce normal sexual characteristic development and normal bone maturation. Doses of T should be individualized and suitable for the patient's age, and therapy should be initiated only after obtaining the fully informed consent of both the patient and the parents [7, 11, 12].

Puberty Induction in Phenotypic Males with 46,XY Disorders of Sex Development

Several T preparations for oral, transdermal, and intramuscular administration with different pharmacokinetics are available [13–16]. A long-lasting ester (i.e., T enanthate) at 50–75 mg/month is used initially and gradually increased to 100–150 mg/month before finally reaching 250 mg dosage every 2–3 weeks. The most used non-injectable forms of T replacement therapy (TRT) include transdermal gel preparations (1% T strength, 50–100 mg of T in 5–10 g of gel daily, or the metered-dose gel formulation of 2% T strength, 60–80 mg of T in 3–4 g of gel applied daily, with an absorption rate of about 10%). If T gel is used, the starting dose is 2.5 mg/daily with a gradual increase. The long-acting injectable T undecanoate preparation (1000 mg ampoules for i.m. injection) is also rather expensive and is usually administered every 12 weeks and could be preferably used for maintenance therapy [17]. Alternatively, the oral route can be used, but sustained blood levels of T are difficult to achieve, because it first passes through the liver. Moreover, the more active 17α-alkylated derivatives of T have a potential risk of hepatotoxicity. The only safe oral T treatment is T undecanoate capsules (40 mg each), which must be initially administered once a day with a meal and gradually titrated upward to two and then three times/day [13–16].

In prepubertal children with deficiency of 5alpha-reductase type 2, the use of DHT gel has been successfully used to increase penile length [18]; however, its use to improve virilization at puberty in this condition has yet to be better elucidated [11, 18].

Finally, some clinical case reports have suggested a possible benefit from supraphysiological doses of T in subjects with AIS to improve phenotypic virilization [19, 20]; however, it is important to recognize that prospective studies in these patients are lacking [7].

Puberty Induction in Phenotypic Females with 46,XY Disorders of Sex Development

In patients raised as females, administration of estrogens is crucial for sexual development. As in the case of TRT, low dosages of estrogens should be used at the beginning, and therapy should be individualized and increased based on the clinical response [11]. No consensus exists among pediatric endocrinologists regarding the best estrogen formulation to be used for pubertal induction [11]. However, some evidence indicates that the growth hormone-insulin-like growth factor-I pathway is more influenced by oral when compared to transdermal estrogen administration, suggesting a clinical advantage of the latter route of administration [11]. Accordingly, beneficial effects of the transdermal route of administration over oral preparations have been reported for other outcomes including bone tissue, insulin sensitivity, blood pressure, as well as inflammatory markers [11]. Unfortunately, oral estrogens still represent the most frequently used preparation to induce puberty by pediatric endocrinologists in Europe as well as in the USA [11].

4.2.3.2 Surgical Therapy

Feminizing genitoplasty can be applicable to all forms of DSD where reconstruction to achieve feminized genitalia is desired. The cosmetic results are now excellent, and the complications, in particular the risks of vaginal stenosis, are limited. More complicated is the phallic reconstruction surgery. Phenotypically, male external genitalia vary greatly, in patients with a DSD, and there are no guidelines or standards to be used. Hence, a case-by-case decision should be followed taking many variables into account.

Phallic Reconstruction

Several reconstructive surgical procedures are available in order to make the most feminized genitalia appear and function like a typical male [9].

- *Chordee Without Hypospadias:* Chordee is a condition characterized by a downward or upward penile curvature at the junction of the head and shaft of the penis. The problem is usually caused by tethering of the ventral foreskin or secondary to corporal disproportion. In the first condition, the dorsal foreskin is swung around to the ventral side of the penis to correct the ventral defect. Conversely, in the latter condition, the correction deals with either lengthening the shorter ventral corpora cavernosa, or shortening the longer dorsal corpora cavernosa, or doing both [9].
- *Distal Hypospadias:* Several surgical approaches are available, but the most commonly used technique is the tubularized incised plate (TIP) urethroplasty [21]. Usually in distal hypospadias, the ventral urethral plate distal to the hypospadiac meatus is often intact although frequently too narrow. Hence, the TIP

consists in rolling it back into a tube and connecting it to the hypospadiac meatus. In addition, an incision is made right in the midline of the plate vertically allowing the plate to widen [21].

- *Proximal Hypospadias* represents a more challenging procedure with a higher frequency of complication and reoperation rate [21]. Usually, a two-staged approach is the most frequent technique used [21]. During the first operation, the chordee and foreskin flaps are corrected, whereas the urethroplasty is performed in the second stage [21].
- *Penoscrotal Transposition and Bifid Scrotum:* This approach should be reserved for the less virilized forms of male DSD where the scrotum appears like the labia and the hypospadiac meatus is usually located in the perineum. The hypospadias and chordee can be corrected in a two-staged approach as previously mentioned in the case of proximal, whereas scrotal reconstruction needs a specific approach [9]. In the case of penoscrotal transposition, the penis is below the scrotum instead of above it, requiring the use of scrotal skin flaps to be pulled down the scrotum [9].

Feminizing Genitoplasty

Although tremendous improvement in feminizing surgery has been made, the surgery timing and the best technique to be used remain controversial issues [9].

Clitoroplasty: The most important target of this surgical approach is to preserve and to avoid injury of the neurovascular bundles. The initial incision is made at the junction of the inner and outer surface of the preputial skin. This approach allows for the preservation of the inner surface of the skin to create a clitoral hood. The tunics are left intact, whereas the corporal incisions are made longitudinally along the ventrum of each corporal body away from the neurovascular bundles, which are located at the 11:00 and 1:00 on the dorsum of the clitoris. The erectile tissue can be removed from near the glans to the bifurcation of the corpora. Finally, the tunics are folded back allowing the glans to be secured to the corpora at their bifurcation [9].

- *Vaginoplasty* Several techniques have been described in order to exteriorize the vagina from the urogenital (UG) sinus. The most commonly used technique deals with the use of an omega-shaped perineal "flap" to place into the posteriorly opened vagina. The main advantages consist of avoiding complete separation of the vagina from the urogenital sinus, thereby lessening the risk of vaginal stenosis and injury to the continence mechanism [9]. The "pull-through" procedure is used for the high confluence situation. The vagina is completely separated from the UG sinus which becomes the urethra. The separated vagina is then "pulled through" to the perineum [9]. Although sometimes necessary, this technique is associated with a higher risk of side effects including urethrovaginal fistula, vaginal stenosis, and injury to the continence mechanism [9].
- Total Urogenital Mobilization (TUM) is required in the most complicated cases [22]. With this approach, the entire urogenital system (UG sinus, urethra, vagina, and bladder) is circumferentially dissected and moved toward the perineum [9, 22].
- *Labioplasty* This technique allows the labia minora and a clitoral hood to be created from the available clitoral or scrotum skin [9].

4.3 Isolated Cryptorchidism

Cryptorchidism is a quite frequent clinical condition characterized by a unilateral or bilateral retained testis at birth. A large nationwide observational study from longitudinal register data of all Swedish-born boys 0–18 years of age, diagnosed with cryptorchidism from 2001 to 2014, reported a cumulative childhood prevalence of 1.8% (95% CI, 1.5–2.0), with a higher rate observed in boys born prematurely, small for gestational age, or with low birth weight [23, 24]. Accordingly, it has been reported that 80% of cryptorchid testes descend by the first year of life (the majority within 3 months), making the true incidence of cryptorchidism around 1% overall [24].

4.3.1 Pathophysiology

Much evidence has documented that a combination of genetic favorable background and environmental factors can simultaneously play an important role in the pathogenetic issues related to the development of cryptorchidism [23–25]. A large case-control study performed in 600 infants with cryptorchidism compared to 300 non-cryptorchid male children aged 1–4 years documented a statistically significant association between bilateral and persistent cryptorchidism and genetic alterations, including Klinefelter's syndrome and receptor of insulin-like 3 (INSL-3) receptor gene mutations [25]. INSL-3 is highly expressed in both fetal and adult Leydig cells, and deletion of its gene or its receptor gene in mice causes cryptorchidism due to a failure of gubernaculum development [24]. In addition, interaction analysis studies have documented that genetic polymorphisms in genes involved in environmental endocrine disruptor metabolism are associated with a risk of cryptorchidism [26]. Accordingly, an association between endocrine disruptors, chemicals that can interfere with endocrine (or hormonal) systems, and cryptorchidism has been documented [24]. Similarly, all conditions associated with an impairment of hypothalamus-pituitary-testis axis are associated with an increased risk of cryptorchidism [27].

4.3.2 Surgical Treatment

The main risks related to cryptorchidism are infertility and testis cancer. Substantial data have suggested that early surgery, as soon as the age of probable spontaneous descent has passed (9 months of age), would have a beneficial effect on germ cell development. A previous randomized controlled trial (RCT) documented that testicular volumes and the number of germ and Sertoli cells at surgery were significantly greater in children who had orchiopexy and at 9 months than at 3 years of age [28]. As expected, intra-abdominal testes were associated with the largest degree of germ cell depletion [28]. Similar results were recently reported in an updated meta-analysis including 15 studies and comparing orchidopexy at less than 1 year of age

with orchidopexy at 1 year or more of age [29]. The association between histopathology at biopsy at orchiopexy and hormone levels or semen analysis in adulthood are conflicting. However, some evidence documented that a more severe phenotype at histopathology might identify patients with lower sperm counts, lower sperm density, and higher FSH levels in adulthood [24].

4.3.3 Medical Treatment

Whereas surgical treatment represents the choice in the case of cryptorchidism, medical treatment is still a conflicting approach although some evidence suggests a possible role in retractile or ascending testes. A previous meta-analysis including 13 RCTs showed that hCG (usually hCG IM 500 IU 2×/wk. for 5 wk.; 250 IU for age <2 years and 1000 IU for age >6 years) or GnRH (using a specific device) were associated with a limited success rate of 24% and 19%, respectively. In addition, the effect was significant in subjects with bilateral cryptorchidism, but not in those with a unilateral problem. All side effects were transitory and not severe, but if they have long-term risks was not clear [30]. Previous studies have documented that despite surgical success, a total of 47.5% of unilateral and 78% of bilateral cryptorchid males had their sperm concentration in the infertility range according to WHO standards [31]. In order to improve long-term surgery outcomes, adjuvant GnRH therapy has been advocated. A previous meta-analysis with a literature search up to September 2013 and including ten eligible studies concluded that GnRH have a significant overall increased mean effect estimate and increased relative chance of having normal germ cell values per tubule at the time of surgery [32]. However, it should be recognized that each study used varying hormonal treatments, making comparisons between studies difficult [32]. Other authors proposed post-surgical use of GnRH, suggesting positive outcomes in adulthood [33]. Again, the evidence is still conflicting.

4.4 Isolated Hypospadias

Hypospadias represents one of the most common congenital anomalies in men. Typically, it is characterized by proximal displacement of the urethral opening, penile curvature, and a ventrally deficient hooded foreskin. In the vast majority of cases (about 70%), the urethral meatus is located distally on the penile shaft, whereas in a minor number of subjects (about 30%), the defect is more proximal and frequently associated with other urogenital deformities [34]. The prevalence seems to be stable and is highest in North America (34.2:10,000 births) and lowest in Asia (0.6:10,000 births) [21]. In the vast majority of cases, the real etiology of hypospadias remains unknown although a multifactorial origin has been advocated [34]. Familiar predisposition has been reported in 7% of cases although in only 30% of hypospadias (syndromic forms), a clear genetic cause has been found [35]. Accordingly, in the presence of hypospadias, a specific endocrinological

evaluation is required in order to exclude androgen production/action defects [34]. Much evidence indicates that environmental factors can play a crucial role in the pathogenesis of hypospadias. In particular, endocrine disruptors, chemicals derived from environmental pollution with anti-androgen or estrogen-like actions, can interfere with normal sexual development favoring the appearance of hypospadias [34]. In line with the aforementioned hypotheses, the term *testicular dysgenesis syndrome* has been introduced to describe a possible association among some male reproductive disorders (cryptorchidism, hypospadias, male subfertility, and testicular cancer) which can be interlinked and originate from a disturbed testicular development [36].

4.4.1 Treatment

Medical treatment is indicated only when hypospadias is a sign of a more complex disorder which includes a defect of the hypothalamus–pituitary–testis axis (see above). In the presence of penile length below the third percentile (microphallus), T therapy has been proposed in order to increase anatomical proportions, before surgery procedures [38]. The current evidence of this approach is, however, limited and of poor quality [34, 38].

Surgical treatment represents the gold standard in the presence of hypospadias. The goal of hypospadias repair is to achieve cosmetic and functional normality. According to current guidelines, surgery is recommended between 6 and 18 months of age based on the severity and the need for multiple procedures [37]. The specific surgical approach and its outcome depend on the type and severity of the defect (see DSD section). Available data indicate that overall, cosmetic outcome and sexual function are considered satisfactory in more than 70% and 80%, respectively, of all patients after hypospadias repair [38]. As expected, worse results are observed in patients treated for proximal and complex hypospadias [39].

4.5 Anorchia

Bilateral congenital anorchia has been reported in around 1:20,000 males, whereas the unilateral form is four times more frequent [40]. Possible congenital causes of anorchia include mutation in steroidogenic factor 1 (SF1) gene or deletion of SRY gene. The former condition is usually called “vanishing testis syndrome,” and it is associated with micropenis [41]. On the other hand, the most frequent acquired anorchia seems to be the consequence of intrauterine torsion [40].

The clinical phenotype of men with bilateral anorchia depends on the time in which the disease acts, ranging from milder defects to more severe forms of DSD [40]. Conversely, in unilateral anorchia, the intact testis is able to produce sufficient amounts of androgens, and disorders of sexual differentiation do not occur [40].

4.6 Congenital Penile Curvature

Congenital penile curvature (CPC) is a quite frequent condition, characterized by angulation of the erect penis, most commonly in the ventral and/or lateral site. The reported prevalence ranges from 0.5 to 10%, but these data are probably underestimated, since many mild cases go unrecognized due to the absence of functional limitations [42]. Rarely CPC is associated with urethral plate malformation such as hypospadias. In the case of isolated CPC, the potential etiologies include asymmetric corporal length (corporal disproportion), fibrosis of Dartos or Buck's fascia, and even a congenitally shortened urethra [42].

Surgical correction represents the gold standard therapy for CPC. Three main procedure have been described: (1) *plication techniques* (including excisional corporoplasty, incisional corporoplasty, or plication-only), (2) *grafting techniques* (including total or partial tunica/plaque excision, or tunica/plaque incision, followed by grafting of the tunica albuginea defect), and (3) *correction of curvature with simultaneous penile prosthesis implantation (PPI)* which is usually reserved to patients with erectile dysfunction refractory to medical treatment [42]. A recent review of the literature including 34 studies and 2155 patients with CPC documented that excisional corporoplasty and incisionless plication were the preferred surgical methods [42]. The same study showed that overall outcome is excellent with minimal side effects [42].

References

1. Bertelloni S, Dati E, Baroncelli GI. Disorders of sex development: hormonal management in adolescence. Gynecol Endocrinol. 2008;24:339–46.
2. Hughes IA. Disorders of sex development: a new definition and classification. Best Pract Res Clin Endocrinol Metab. 2008;22:119–34.
3. Huhtaniemi I, Alevizaki M. Gonadotrophin resistance. Best Pract Res Clin Endocrinol Metab. 2006;20:561–76.
4. Pasterski V, Prentice P, Hughes IA. Impact of the consensus statement and the new DSD classification system. Best Pract Res Clin Endocrinol Metab. 2010;24:187–95.
5. Hughes IA, Houk C, Ahmed SF, Lee PA, Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology Consensus Group. Consensus statement on management of intersex disorders. J Pediatr Urol. 2006;2:148–62.
6. Bose HS, Sugawara T, Strauss JF 3rd, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med. 1996;335:1870–8.
7. Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, MacDougall J. Androgen insensitivity syndrome. Lancet. 2012;380:1419–28.
8. Nowakowski H, Lenz W. Genetic aspects in male hypogonadism. Recent Prog Horm Res. 1961;17:53–95.
9. DiSandro M, Merke DP, Rink RC. Review of current surgical techniques and medical management considerations in the treatment of pediatric patients with disorders of sex development. Horm Metab Res. 2015;47:321–8.
10. Diamond M, Beh HG. Changes in the management of children with intersex conditions. Nat Clin Pract Endocrinol Metab. 2008;4:4–5.
11. Bertelloni S, Dati E, Baroncelli GI. Disorders of sex development: hormonal management in adolescence. Gynecol Endocrinol. 2008;24:339–46.

12. Fisher AD, Ristori J, Fanni E, Castellini G, Forti G, Maggi M. Gender identity, gender assignment and reassignment in individuals with disorders of sex development: a major of dilemma. J Endocrinol Investig. 2016;39:1207–24.
13. Maggi M, Buvat J. Standard operating procedures: pubertas tarda/delayed puberty—male. J Sex Med. 2013;10:285–93.
14. Salonia A, Rastrelli G, Hackett G, Seminara SB, Huhtaniemi IT, Rey RA, Hellstrom WJG, Palmert MR, Corona G, Dohle GR, Khera M, Chan YM, Maggi M. Paediatric and adult-onset male hypogonadism. Nat Rev Dis Primers. 2019;5:38.
15. Corona G, Sforza A, Maggi M. Testosterone replacement therapy: long-term safety and efficacy. World J Mens Health. 2017;35:65–76.
16. Corona G, Rastrelli G, Reisman Y, Sforza A, Maggi M. The safety of available treatments of male hypogonadism in organic and functional hypogonadism. Expert Opin Drug Saf. 2018;17:277–92.
17. Giagulli VA, Triggiani V, Carbone MD, Corona G, Tafaro E, Licchelli B, Guastamacchia E. The role of long-acting parenteral testosterone undecanoate compound in the induction of secondary sexual characteristics in males with hypogonadotropic hypogonadism. J Sex Med. 2011;8:3471–8.
18. Odame I, Donaldson MD, Wallace AM, Cochran W, Smith PJ. Early diagnosis and management of 5a-reductase deficiency. Arch Dis Child. 1992;67:720–3.
19. Grino PB, Isidro-Gutierrez RF, Griffin JE, Wilson JD. Androgen resistance associated with a qualitative abnormality of the androgen receptor and responsive to high dose androgen therapy. J Clin Endocrinol Metab. 1989;68:578–84.
20. Weidemann W, Peters B, Romalo G, Spindler KD, Schweikert HU. Response to androgen treatment in a patient with partial androgen insensitivity and a mutation in the deoxyribonucleic acid-binding domain of the androgen receptor. J Clin Endocrinol Metab. 1998;83:1173–6.
21. Springer A, Krois W, Horcher E. Trends in hypospadias surgery: results of a worldwide survey. Eur Urol. 2011;60:1184–9.
22. Pena A. Total urogenital mobilization: an easier way to repair cloacas. J Pediatr Surg. 1997;32:267–8.
23. Bergbrant S, Omling E, Björk J, Hagander L. Cryptorchidism in Sweden: a nationwide study of prevalence, operative management, and complications. J Pediatr. 2018;194:197–203.e6.
24. Lee PA, Houk CP. Cryptorchidism. Curr Opin Endocrinol Diabetes Obes. 2013;20:210–6.
25. Ferlin A, Zuccarello D, Zuccarello B, Chirico MR, Zanon GF, Foresta C. Genetic alterations associated with cryptorchidism. JAMA. 2008;300:2271–6.
26. Qin XY, Kojima Y, Mizuno K, Ueoka K, Massart F, Spinelli C, Zaha H, Okura M, Yoshinaga J, Yonemoto J, Kohri K, Hayashi Y, Ogata T, Sone H. Association of variants in genes involved in environmental chemical metabolism and risk of cryptorchidism and hypospadias. J Hum Genet. 2012;57:434–41.
27. Cortes D, Holt R, de Knegt VE. Hormonal aspects of the pathogenesis and treatment of cryptorchidism. Eur J Pediatr Surg. 2016;26:409–17.
28. Kollin C, Stukenborg JB, Nurmio M, Sundqvist E, Gustafsson T, Söder O, Toppari J, Nordenskjöld A, Ritzén EM. Boys with undescended testes: endocrine, volumetric and morphometric studies on testicular function before and after orchidopexy at nine months or three years of age. J Clin Endocrinol Metab. 2012;97:4588–95.
29. Allin BSR, Dumann E, Fawkner-Corbett D, Kwok C, Skerritt C, Paediatric Surgery Trainees Research Network. Systematic review and meta-analysis comparing outcomes following orchidopexy for cryptorchidism before or after 1 year of age. BJS Open. 2018;2:1–12.
30. Bu Q, Pan Z, Jiang S, Wang A, Cheng H. The effectiveness of hCG and LHRH in boys with cryptorchidism: a meta-analysis of randomized controlled trials. Horm Metab Res. 2016;48:318–24.
31. Hadziselimovic F. Is hormonal treatment of congenital undescended testes justified? A debate. Sex Dev. 2019;13:3–10.

32. Chua ME, Mendoza JS, Gaston MJV, Luna SL Jr, Morales ML Jr. Hormonal therapy using gonadotropin releasing hormone for improvement of fertility index among children with cryptorchidism: a meta-analysis and systematic review. J Pediatr Surg. 2014;49:1659–67.
33. Hadziselimovic F. Successful treatment of unilateral cryptorchid boys risking infertility with LH-RH analogue. Int Braz J Urol. 2008;34:319–26.
34. van der Horst HJ, de Wall LL. Hypospadias, all there is to know. Eur J Pediatr. 2017;176:435–41.
35. Sagodi L, Kiss A, Kiss-Toth E, Barkai L. Prevalence an possible causes of hypospadias. Orv Hetil. 2014;155:978–85.
36. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16:972–8.
37. Riedmiller H, Androulakakis P, Beurton D, Kocvara R, Gerharz E, European Association of Urology. EAU guidelines on paediatric urology. Eur Urol. 2001;40:589–99.
38. Wright I, Cole E, Farrokhyar F, Pemberton J, Lorenzo AJ, Braga LH. Effect of preoperative hormonal stimulation on postoperative complication rates after proximal hypospadias repair: a systematic review. J Urol. 2013;190:652–9.
39. Rynja SP, de Jong TP, Bosch JL, de Kort LM. Functional, cosmetic and psychosexual results in adult men who underwent hypospadias correction in childhood. J Pediatr Urol. 2011;7:504–15.
40. Pirgon O, Dundar BN. Vanishing testes: a literature review. J Clin Res Pediatr Endocrinol. 2012;4:116–20.
41. Philibert P, Zenaty D, Lin L, Soskin S, Audran F, Léger J, Achermann JC, Sultan C. Mutational analysis of steroidogenic factor 1 (NR5a1) in 24 boys with bilateral anorchia: a French collaborative study. Hum Reprod. 2007;22:3255–61.
42. Sokolakis I, Hatzichristodoulou G. Current trends in the surgical treatment of congenital penile curvature. Int J Impot Res. 2019; https://doi.org/10.1038/s41443-019-0177-0. [Epub ahead of print].

Disorders of Pubertal Development: From Hypogonadotropic Hypogonadism to Constitutional Delay of Puberty

5

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5.1 Background

Puberty is of paramount importance in the life of a young person. Puberty is the phase whereby secondary sexual characteristics develop, growth progresses towards final adult stature and reproductive ability is achieved. Rapid and complex changes involve physical, mental and social adjustments. Pubertal progression consists of specific sequential events. Disordered development can have adverse consequences, both physically and psychosocially [1]. Understanding the sequence of normal pubertal events is important in order to distinguish between normal, normal variants and pathological syndromes [2].

5.2 Normal Puberty

In order to have an understanding of disorders of pubertal development, it is first necessary to describe and comprehend normal puberty. Here, we focus exclusively on male development.

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5.2.1 Physiology

The hypothalamic-pituitary-gonadal (HPG) axis is active during early development in utero until the neonatal period. This neonatal phase is termed 'mini-puberty' and lasts up to age 6 months. The pubertal axis then becomes dormant after infancy and remains so during childhood, with very low levels of gonadotrophins and testosterone. The first stage in puberty is disinhibition of this axis, triggering reactivation. Gonadotrophin-releasing hormone (GnRH) is secreted in a pulsatile manner by neurosecretory cells of the hypothalamus into the hypothalamic-hypophyseal portal circulation [3]. This portal system serves as a direct vascular link between the hypothalamus and the anterior pituitary. GnRH stimulates gonadotropic cells in the anterior pituitary to synthesise and release luteinising hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates testosterone synthesis in the testicular Leydig cells. FSH, in conjunction with testosterone, stimulates germ cell maturation, inducing spermatogenesis.

Adrenal androgens, secreted from the zona reticularis (ZR), also make an important contribution to the formation of secondary sexual characteristics, particularly pubic and axillary hair (pubarche). In normal puberty, adrenal and HPG axis maturations coincide; however, they are independent processes. Therefore, a child with signs of adrenarche may still experience delayed puberty. Pubarche is usually a result of adrenarche (maturation of the ZR of the adrenal gland), associated with skin changes and development of adult-type body odour.

5.2.2 External Pubertal Development

External pubertal development can be objectively classified using the Tanner staging system. This system has some limitations, most notably that it may not apply to all populations, having been designed on a small population of English children. However, it is still widely used internationally. In boys, the first external change is an increase in testicular volume (gonadarche) to 4 mL [4]. Orchidometers are used to help gauge size (Fig. 5.1) [5]. After this, the penis lengthens, followed by the development of sperm (spermarche) and then by achievement of peak height velocity [6, 7]. Adrenal features of puberty may begin anywhere along this progression but usually begin between initial testicular enlargement and the development of sperm.

5.2.3 Onset of Puberty

Although pubertal development follows a specific sequence, with relatively predictable onset and progression, judgement of which adolescents require further investigation can be challenging [8]. The onset of puberty can vary greatly, influenced by both intrinsic and extrinsic factors. Sex, race, ethnicity, nutrition, environmental exposure and genetic factors all contribute to this variability. Generally, it is advised to consider investigation when abnormalities fall 2–2.5 standard

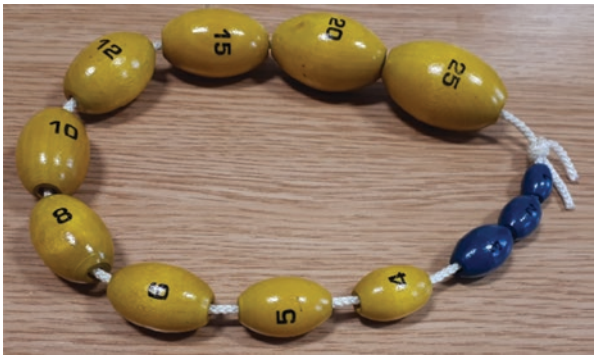

Fig. 5.1 A photo of an orchidometer

deviations (SDs) outside the population mean. Normal puberty in boys is expected to start from age 9 years for the 'early developers' and up to age 14 years for the 'late developers'.

5.3 Normal Variants of Puberty

Any deviation from normal puberty is likely to induce stress and anxiety in both the patient and his family. However, normal variants of early or delayed puberty must be differentiated from true pathological disorders.

Early puberty discussions are beyond the scope of this chapter and will be covered in another part of this book.

5.3.1 Delayed Puberty

If there are no signs of puberty in boys by age 14 years, then this is regarded as delayed puberty. Delayed puberty is seven times more frequent in boys than girls. Girls are more likely than boys to have a pathological cause. Since pubertal onset is not perfectly normally distributed, population studies suggest that up to 5% of children may be affected by delayed puberty.

The most common normal variant of delayed puberty is constitutional delay of growth and puberty (CDGP), accounting for up to 60% of cases of delayed puberty in boys. This is a diagnosis of exclusion. In CDGP the onset of puberty occurs more than 2 standard deviations (SDs) later than the population mean age of onset. Eventually, normal sequential puberty does occur. In CDGP the HPG axis is intact but remains dormant beyond the expected age for puberty. It may be more accurate to describe CDGP as 'a delay in puberty and growth' as it is the delay in puberty which causes the delay in growth. CDGP can produce significant anxiety in boys, particularly because of short stature in comparison with their peers and the apparent lack of pubertal development. Typically, reassurance is often all that is needed, but in some children, medical intervention may be indicated, discussed later.

5.4 Disorders of Puberty

We must now consider the disorders that can affect pubertal development.

5.4.1 Incomplete/Absent Puberty

Puberty is deemed absent when there is lack of development of secondary sexual characteristics by age of 14 years. This syndrome of impaired gonadal function is termed 'hypogonadism'. Features of adrenarche do not indicate pubertal development. Hypogonadism can be classified as hypergonadotropic (primary) or hypogonadotropic (secondary).

Aspects relating to hypergonadotropic hypogonadism are covered elsewhere in this book.

Hypogonadotropic (secondary) hypogonadism involves failure of the HPG axis, typically, a pituitary or hypothalamic problem (some texts will refer to hypothalamic hypogonadism as 'tertiary'). It is termed 'hypogonadotropic hypogonadism', due to the failure of hypothalamic production of GnRH or failure of pituitary production of LH and FSH.

Hypogonadotropic hypogonadism can be divided into functional, congenital or acquired types.

5.5 Functional Hypogonadotropic Hypogonadism

The term functional hypogonadotropic hypogonadism is particularly used to describe pubertal delay or arrest that is induced by chronic disease or stress. Psychosocial deprivation, intense exercise, anorexia and malnutrition are all linked to hypogonadotropic hypogonadism. This is thought to be an adaptive mechanism to prevent reproduction in suboptimal circumstances.

5.5.1 Nutritional Hypogonadotropic Hypogonadism

Puberty is an energy-demanding period of development, requiring sufficient calorie intake. During puberty, there is significant weight gain. Approximately 50% of adult body weight is gained during adolescence (average 9 kg/year), with peak weight velocity in males occurring at about the same time as peak height velocity. Under the influence of testosterone and growth hormone there are associated changes in the body composition and relative proportions of water, fat and bone [9].

The metabolic control of puberty is determined by the action of different central neurotransmitters and peripheral hormones. These sense the metabolic state of the individual and interact with the hypothalamic GnRH neurons. Studies have

confirmed the importance of leptin (an adipose hormone) in a permissive regulatory role in puberty. Leptin is secreted from adipocytes proportionally to the body fat content. Leptin levels fall dramatically in conditions of starvation. There are also interactions with kisspeptin (from the Kiss 1 Gene). Kisspeptin is an upstream regulator of GnRH [10]. Increases in kisspeptin have been shown to increase GnRH, which subsequently leads to increased LH pulsatility, thus triggering puberty [11, 12]. Figure 5.2 illustrates some of the hormonal interplay between puberty and nutrition [13]. There is compelling evidence from animal models that there are changes in kisspeptin expression in response to a negative energy balance (calorie restriction). In most studies, there was a reduction in KISS1 expression in response to calorie restriction/starvation [14]. This translates clinically to delayed or arrested puberty, as seen in malnutrition (undernutrition), anorexia nervosa, chronic illness with malabsorption or excessive exercise without matched calorie intake.

Calorie deficit due to poverty-related malnutrition is relatively uncommon in the developed world. The United Kingdom (UK) government does not routinely collect data on food poverty, and this has been largely left to the charitable sectors. According to recent international data, more children in the United Kingdom (10%) live in severe food insecurity compared with other countries in Europe, where the

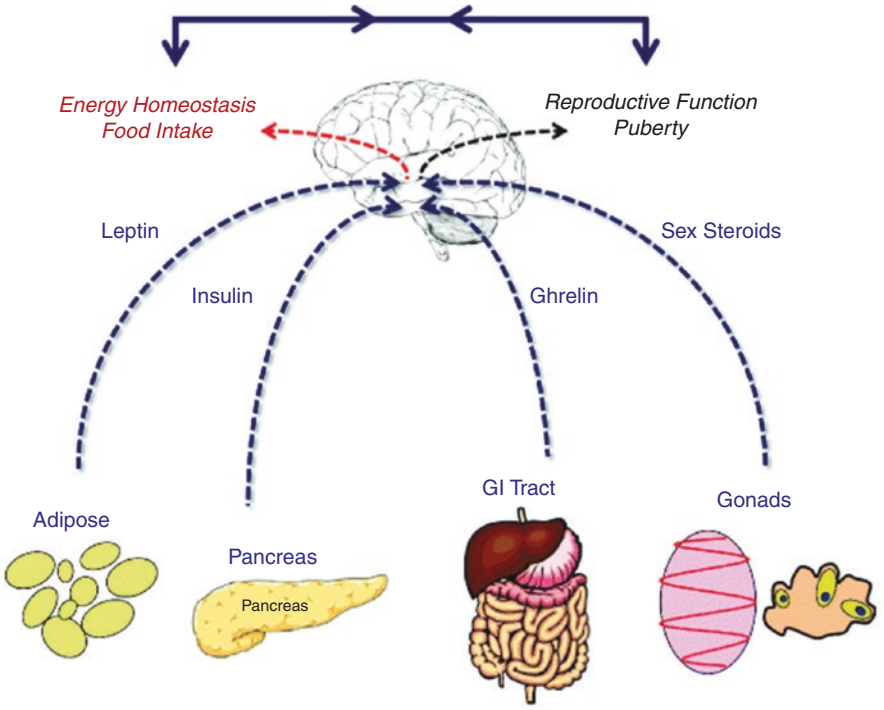

Fig. 5.2 Hormonal interplay between puberty and nutrition (Kisspeptin Signaling in Reproductive Biology (2013) Springer publications)

average is 4% [15]. It is unlikely that many endocrinologists will routinely manage children with hypogonadotropic hypogonadism due to malnutrition secondary to poverty.

Many chronic illnesses are associated with malnutrition, through reduced food absorption or an increased catabolic state. Examples include Crohn's disease, cystic fibrosis, cardiac failure, and coeliac disease - which is often under diagnosed and under-treated. The negative energy balance will result in a delayed pathophysiology similar to malnutrition.

Eating disorders (EDs) are historically considered to predominantly affect females; however, epidemiological studies indicate that males are also at risk of developing EDs. A recent report from 2018 quoted ED rates of 1.2% and 2.2% in Canadian and Australian adolescent males, respectively, and 1.2% in young adult Dutch men [16]. These values are likely to be underestimates as most boys and men will under-report symptoms of EDs. EDs which result in calorie deficit, e.g. anorexia nervosa and bulimia nervosa, typically cause functional hypogonadotropic hypogonadism. Puberty has frequently been identified as a time of risk for developing EDs. Sex steroids, in particular, appear to have a role in unmasking genetic risks for developing EDs, although this phenomenon is more apparent in girls than boys [17, 18].

Excessive exercise can have a role in both the development and maintenance of a number of EDs and is most frequently seen in people with anorexia nervosa as a way of purging calories. It is also often used as a way to manage mood or affect in people with EDs, either to help produce a positive mood state or to help avoid a negative mood state related to not exercising. Reports show that excessive exercise is frequently one of the most persistent symptoms of EDs, often interfering with recovery [19]. It is important to note that excessive exercise can also occur in a non-pathological state, e.g. long-distance or marathon runners, scholastic wrestling, dancers and gymnasts. These sports emphasise strict weight control and high-energy output and predispose to functional hypogonadotropic hypogonadism [9].

Contrastingly, it is interesting to note that energy excess such as that seen in obesity or the metabolic syndrome is also associated with functional hypogonadotropic hypogonadism [20, 21]. In obese adolescent boys, serum testosterone concentrations were reported to be up to 40–50% lower than matched normal BMI peers [22].

5.5.2 Psychosocial Factors

Stress and/or depression are known to cause functional hypogonadotropic hypogonadism, and this is likely to be hypothalamic in origin. Research has shown that high levels of corticotrophin-releasing hormone (CRH) have a direct inhibitory action on the kisspeptin system. Other studies have shown that at the time of puberty there is a reduction in CRH activity and blockade of CRH actions in the brain; with anti-CRF drugs stimultating earlier puberty. This would account for why puberty is delayed in people who are chronically stressed. More recent research is focusing on

the role of the amygdala -which is already known to regulate emotion, enhance the stress response, and control anxiety [23].

5.5.3 Clinical Impact of Functional Hypogonadotropic Hypogonadism

As described above, functional hypogonadotropic hypogonadism can cause an overall delay in growth and puberty. In addition, the underlying cause of the calorie deficit, e.g. malnutrition or malabsorption, may also result in other disorders, for example, anaemia, osteopenia and/or deficiencies of minerals, vitamins, essential fatty acids and amino acids and trace elements.

Delayed puberty/growth can lead to emotional and psychological dysfunction and reduced educational attainments. Bullying and victimisation can result in increased depression and anxiety [24]. Reports also show that boys with a history of delayed puberty have a greater risk for metabolic and cardiovascular disorders [25].

5.5.4 Assessment of Suspected Functional Hypogonadotropic Hypogonadism

Investigation will be guided by the history and examination findings, but a basic structure to follow is outlined in Table 5.1.

5.5.5 Management of Functional Hypogonadotropic Hypogonadism

Efforts should be made to address the underlying cause of the functional hypogonadotropic hypogonadism. In most cases, these causes are reversible, and puberty should commence/continue once the underlying issue is resolved.

It is also important to ensure that clear explanations of the conditions and risks are given to both the patient and the family. Clinicians should ensure the child has adequate support to meet their psychological needs. These may extend to concerns

Table 5.1 Assessment and investigation of suspected functional hypogonadotropic hypogonadism

History	Examination	Investigations
Presenting complaint Diet Exercise Social circumstances Past medical history Medication history Family history of illnesses/puberty	Height Weight BMI General system examination Appropriate endocrine assessment, e.g. thyroid Pubertal assessment and staging	Bone age assessment LH FSH Testosterone Second line as needed: Renal, liver, bone profile Thyroid function, prolactin, Full blood count, ESR, CRP, coeliac screen

Table 5.2 Causes of congenital and acquired hypogonadotropic hypogonadism

Congenital
Isolated GnRH deficiency
Kallmann syndrome (with anosmia)
Without anosmia
GnRH deficiency associated with obesity and/or mental retardation
Prader-Willi syndrome
Laurence-Moon-Biedl syndrome
Idiopathic hypogonadotropic hypogonadism
Acquired
Tumours
Craniopharyngiomas
Pituitary adenomas and cysts
Germinomas, gliomas, meningiomas, astrocytomas
Pituitary apoplexy
Infiltrative disease
Granulomatous disease
Histiocytosis
Haemochromatosis
Drugs
Anabolic steroids
Opiates
Marijuana
Iatrogenic
Post-surgery
Post-radiotherapy

relating to personal identity, psychosexual development and future fertility. In some cases, testosterone therapy may be required, as discussed in the management section. Quite often this group of patients may need to have an ongoing input beyond the paediatric age cut-off (usually 16 years in most institutions). These young and emerging adults should be seen in an endocrine transition clinic, ideally with input from both paediatric and adult endocrine teams [24].

We will now consider causes of congenital and acquired hypogonadotropic hypogonadism. These have been summarised in Table 5.2.

5.6 Congenital Hypogonadotropic Hypogonadism

Congenital pituitary abnormalities, causing gland hypoplasia or aplasia, often affect multiple hormones, which may have presented earlier in life. There are also 'migration disorders' resulting from abnormal migration of GnRH neurons during embryonic development. In normal development, GnRH neurons are derived from the olfactory placode. Several genes may be implicated in disrupting this migration and subsequent adhesion. Therefore, congenital hypogonadotropic hypogonadism may be linked to anosmia (lack of sense of smell). In this case, it is defined as Kallmann syndrome [26]. Associated features of Kallmann syndrome include cleft lip/palate, sensorineural deafness and cerebellar ataxia. As there are several implicated genes,

there are several modes of inheritance: X-linked recessive, autosomal dominant and autosomal recessive.

Other congenital syndromes may also affect the HPG axis, resulting in hypogonadotropic hypogonadism. Prader-Willi syndrome is a complex genetic condition affecting hypothalamic function, muscle tone and cognitive development. Bardet Biedl syndrome is another multifaceted genetic condition linked to HPG axis dysfunction, as well as retinal, renal, limb and cognitive abnormalities. Rarely, there are specific hypothalamic receptor abnormalities that lead to failure of GnRH secretion. Other genetic causes of isolated GnRH deficiency occur with a wide spectrum of clinical presentation ranging from microphallus and cryptorchidism in the neonatal period to delayed or arrested puberty in adolescence [27]. The diagnosis is confirmed biochemically followed by imaging to confirm normal appearance of the hypothalamus and pituitary on MRI. The main differential diagnosis is with CDGP and can be very challenging to differentiate. Unless there are clear associated features such as anosmia or prior genetic testing, the diagnosis can be difficult to determine until the individual is at least 18 years.

5.7 Acquired Hypogonadotropic Hypogonadism

Acquired hypogonadotropic hypogonadism is often secondary to structural central nervous system lesions such as pituitary adenoma, craniopharyngioma or autoimmune hypophysitis. Radiotherapy to the head is an iatrogenic cause of secondary hypogonadism.

5.7.1 Pituitary Tumours

Pituitary tumours can be classified as intrasellar and suprasellar, with the former being largely made up of (>90%) pituitary adenomas. The latter is mostly represented by disorders in embryogenesis such as craniopharyngioma, germinoma and dermoid and epidermoid cysts. Neoplastic and infiltrative processes can also occur including gliomas, meningiomas, germ cell tumours arising from the pituitary stalk, granulomatous disease including sarcoidosis and histiocytosis (see Table 5.1) and the iron deposition disorder, haemochromatosis. Craniopharyngiomas are the most common cause of hypopituitarism in childhood. Adenomas are the most common cause of the pituitary lesions to present in childhood and adolescence.

Craniopharyngiomas arise from squamous rest cells in the remnant of Rathke's pouch between the adenohypophysis and neurohypophysis. They are rare with an annual incidence of 0.5–2 per million; however 30–50% of cases are present in childhood and adolescence and account for 1–4% childhood intracranial tumours [28, 29]. Although they are histologically benign, their papillae or cysts may invade and compress local structures.

Craniopharyngiomas commonly present with the neurological symptoms of headache and visual field defects coupled with manifestations of endocrine

deficiency such as stunted growth and delayed puberty. At diagnosis GH is the most common axis deficiency (75%), followed by FSH and LH (40%) and then ACTH and TSH deficiency (25%). Posterior pituitary deficit in the form of diabetes insipidus is less common (17%) [30]. In a child presenting with delayed puberty and gonadotrophin deficiency combined with short stature, headache and visual disturbance, craniopharyngioma would be high on the list of differentials. Diagnosis is made with gadolinium-enhanced MRI, although CT may also be used and is specifically useful for identifying calcification in association with craniopharyngiomas. Surgery is the mainstay of treatment and is increasingly a balanced approach between aiming for total resection and also achieving optimal functional outcome. Radiotherapy is also used where significant residual remains post-operatively and risk of recurrence deemed high. Recent identification of the BRAFV600E mutation in papillary craniopharyngiomas has led to trials of combination therapy with BRAF and MEK inhibitors and subsequent therapeutic response reported [31, 32], giving potential for other adjuvant therapy options in the future.

Pituitary adenomas are a relatively rare cause of hypogonadotropic hypogonadism in childhood, with an estimated average annual incidence of 0.1/million children [33]. Prolactinoma is the most frequent adenoma cell type in children, followed by corticotrophs and somatotrophs [34]. Non-functioning adenomas, TSH- and gonadotrophin-secreting adenomas are very rare, accounting for only 3–6% of all pituitary tumours in children. Diagnosis of functioning (hormone-secreting) pituitary adenomas is usually clinical with confirmation of the lesion on contrast MRI and identification of co-existing pituitary dysfunction with biochemistry. Prolactinomas tend to present in the peripubertal age group with deficiency of the pituitary-gonadal axis. This manifests as menstrual irregularities in girls and gynaecomastia and delayed puberty in boys. Large adenomas show a predominance of neurological symptoms.

Prolactinomas may cause hypogonadotropic hypogonadism due to compression of the pituitary gonadotrophs; however they also lead to secondary hypogonadism due to the suppressive effect of hyperprolactinaemia. Both normalisation of prolactin levels and reduction in size of the prolactinoma usually occur on treatment with dopamine agonist therapy. Other pituitary adenomas are likely to be large, causing structural sequelae in the form of headache and visual field disturbance as well as the hypopituitarism. First-line treatment for other functioning pituitary adenomas and non-functioning adenomas is trans-sphenoidal adenomectomy. Adjuvant somatostatin analogue therapy and radiotherapy are options when surgery is non-curative.

5.7.2 Pubertal Effects of Treatment for Childhood Malignancies

The 5-year survival in childhood and adolescent cancers is now in excess of 80%. It is estimated that 1 in 1000 young adults in the United Kingdom is a childhood cancer survivor [35]. This impressive statistic has brought with it the long-term endocrine effects of the treatment for childhood cancer.

Cranial radiotherapy involving the pituitary and hypothalamus commonly results in long-term dysfunction in gonadotrophin secretion—hypogonadotropic hypogonadism. The effect of radiotherapy on any organ is dictated by the dose of radiotherapy, fraction size, number of fractions, modality of the radiotherapy and the time since exposure [36]. As a result, the radiotherapy dose is generally divided into small pulses given successively over time to reduce the damage to healthy tissues adjacent to the abnormal lesion. The endocrine consequences of radiotherapy take time to develop and increase with time from exposure.

Proton beam radiotherapy has been used increasingly frequently over the past decade. This modality focuses the radiotherapy onto a smaller area with less scatter to neighbouring tissues, aiming to reduce damage to healthy tissues. The long-term outcomes of proton beam versus conventional radiotherapy will become clearer with time. These factors, along with the age of the individual, may lead to absent pubertal development, pubertal arrest or later disruption in gonadal and sexual function. The smaller the radiation dose, the later these effects of cranial radiotherapy are generally seen. Conversely, radiotherapy to the hypothalamus may initially be associated with precocious pubertal development as the hypothalamic 'break' is removed, allowing inappropriate activation of the hypothalamic-pituitary pathway and initiating puberty at a younger age than normal [37].

Testicular radiotherapy and chemotherapy treatments can result in hypergonadotropic hypogonadism, due to generalised gonadal damage. This area is discussed elsewhere in this book.

5.8 Management of Hypogonadotropic Hypogonadism

In constitutional delay, treatment is not generally necessary, and often reassurance, understanding and regular review are sufficient. However, due to the distress, treatment may be initiated to induce puberty [38]. Careful consideration is required, as if started at too young an age, treatment may affect final height achieved. Treatment may be initiated after 14 years of age.

For those with permanent hypogonadotropic hypogonadism, treatment needs to continue into adult life. The main aim is start at a low dose with slow increments over a 2–3 year period to mimic testosterone levels during natural pubertal development [39]. This is slowly increased over 2–3 years to an adult dose.

In pubertal arrest, an appropriate dose of intramuscular testosterone can be initiated depending on the stage of puberty completed prior to the pubertal arrest.

5.9 Pubertal Induction

5.9.1 Testosterone Esters

In general, puberty is induced with testosterone esters which are given by deep IM (intramuscular) injection.

1. For constitutional delay of puberty:
   (a) Start testosterone 50 mg every 4 weeks by IM injection for 6 months.
   (b) At the end of this period, if testicular volume has increased, this suggests that spontaneous puberty has begun and treatment can be stopped.
   (c) If testicular volume is <8 mL, then growth may slow on stopping testosterone. If height velocity rather than pubertal progression is the major issue, then consider a further 6-month treatment of 50 mg testosterone every 4 weeks.
   (d) If no progression in testicular volume, consider continuing 50 mg of the testosterone every 4 weeks for further 6 months. Cases should be discussed individually in the post-clinic meeting.
2. For induction of puberty (not constitutional delay):
   (a) Testosterone should be commenced at a low dose of 50 mg every 4 weeks. (Consider starting earlier at 12 years and using lower doses/slower progression in boys with bilateral anorchia.)
   (b) The dose is then gradually increased over 2–3 years to maintain a normal pace of pubertal development until the full adult dose is reached. After 6 months, consider increasing to 100 mg testosterone every 4 weeks, for further 6 months, then increase to 150 mg for 6 months and then 200 mg for further 6 months and then increase to the adult dose of 250 mg given every 4 weeks. A lower dose may be needed in smaller individuals.

5.9.2 Other Options

Intramuscular testosterone remains the most popular method of pubertal induction in the United Kingdom, but recent studies have indicated a role for other preparations of testosterone such as transdermal gels or oral preparations [40].

1. Oral testosterone undecanoate has been used to initiate puberty and is licensed but not commonly used due to variable absorption and hepatic first pass metabolism leading to variable drug levels. The fluctuating levels between doses lead to symptomatic variations and prevent true physiological simulation, making it less reliable for pubertal induction. The usual starting dose is 40 mg on alternate days, increasing to 40 mg a day after 6–8 months (or according to response), then to 80 mg once daily for further 6–8 months and finally up to a maximum of 120 mg daily. Oral testosterone has a short half-life and must be taken with food for satisfactory absorption and has a tendency to be 5α-reduced to dihydrotestosterone (DHT) in the gut.
2. Transdermal testosterone can also be considered. Transdermal gels are currently unlicensed in the United Kingdom for pubertal induction, and dosing regimens have been extrapolated from adult data. Topical gel can be applied to the skin and is available in a metered-dose pump. A usual starting dose is one press of the canister piston which delivers 0.5 g of gel containing 10 mg testosterone. This

can be increased by one press every 6 months. The dose can be applied to the abdomen (entire dose over an area of at least 10 by 30 cm) or to *both* inner thighs (one half of the dose over an area of at least 10 by 15 cm for each inner thigh). The gel should be applied to clean, dry, intact skin. It should be rubbed in gently with one finger until dry, and then the application site should be covered, preferably with loose clothing. Hands should then be washed with soap and water. Daily rotation between the abdomen and inner thighs is recommended to minimise application site reactions. Transfer of the gel to the skin of children and women should be avoided.

3. Implanted testosterone is available for adult androgen deficiency but is not used for induction of puberty.
4. Lifelong testosterone substitution can be via the IM route or transdermal. Once patients have been stabilised on the adult dose of testosterone for a while then, consider changing to 3 weekly, and then they can be converted to long-acting intramuscular testosterone undecanoate, 1 g IM given every 12 weeks. Prior to the second dose, pre-dose bloods can be requested to include FBC (full blood count) with haematocrit, testosterone and prostatic-specific antigen (PSA). If the transdermal route is preferred, change to testosterone sachets 5 mL sachet daily (=50 mg) or testosterone gel 6 metered doses daily (=60 mg).
5. HCG/FSH. Although physiologically potent, HCG and FSH are not routinely used in induction of puberty because they are time-consuming and expensive and require multiple injections. They will likely be ineffective in the case of gonadal damage. Where used, they are initiated after commencing testosterone treatment. FSH is initiated first at 150 i.u. 3 times a week by subcutaneous injection. Two to three months later, HCG is started at 1500 I.U. twice a week by subcutaneous injection, and the testosterone supplementation then stopped. The dose of HCG is then titrated against physiological testosterone production to maintain normal testosterone levels. Generally these are limited to use in clinical research protocols and in adult fertility clinics for stimulating spermatogenesis in males with hypogonadotropic hypogonadism.

5.9.3 Side Effects

Occasionally boys commencing on testosterone may become moody and slightly aggressive. Injected testosterone may result in fluctuating mood, energy level and libido caused by testosterone levels that rise rapidly upon injection and then fall too low before the next dose. Too rapid an increase in dose may result in premature fusion of the epiphyses.

In adult life, testosterone replacement is sufficient to maintain normal sexual function, but if fertility is desired, gonadotrophins or pulsatile GnRH is used. Further description of fertility induction is outside the scope of this chapter.

5.10 Conclusion

Puberty is an important transitional period in a young person's life. We have summarised the normal physiology of puberty and when puberty is delayed this can be constitutional, functional or pathological. We have discussed the investigations and management strategies above, and therapeutic protocols will depend on the specific cause.

References

1. Mobbs EJ. The psychological outcome of constitutional delay of growth and puberty. Horm Res. 2005;63(Suppl 1):1–66.
2. Traggiai C, Stanhope R. Disorders of pubertal development. Best Pract Res Clin Obstet Gynaecol. 2003;17(1):41–56.
3. Wu FC, et al. Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive immunofluorometric assay. J Clin Endocrinol Metab. 1996;81(5):1798–805.
4. Zachmann M, et al. Testicular volume during adolescence. Cross-sectional and longitudinal studies. Helv Paediatr Acta. 1974;29(1):61–72.
5. Prader A. Testicular size: assessment and clinical importance. Triangle. 1966;7(6):240–3.
6. Largo RH, Prader A. Pubertal development in Swiss boys. Helv Paediatr Acta. 1983;38(3):211–28.
7. Nielsen CT, et al. Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. J Clin Endocrinol Metab. 1986;62(3):532–5.
8. Bozzola M, et al. Delayed puberty versus hypogonadism: a challenge for the pediatrician. Ann Pediatr Endocrinol Metab. 2018;23(2):57–61.
9. Rogol AD, Clark PA, Roemmich JN. Growth and pubertal development in children and adolescents: effects of diet and physical activity. Am J Clin Nutr. 2000;72(2 Suppl):521S–8S.
10. Sanchez-Garrido MA, Tena-Sempere M. Metabolic control of puberty: roles of leptin and kisspeptins. Horm Behav. 2013;64(2):187–94.
11. Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014;20(4):485–500.
12. Harter CJL, Kavanagh GS, Smith JT. The role of kisspeptin neurons in reproduction and metabolism. J Endocrinol. 2018;238(3):R173–83.
13. Castellano JM, Tena-Sempere M. Metabolic regulation of kisspeptin. Adv Exp Med Biol. 2013;784:363–83.
14. Wolfe A, Hussain MA. The emerging role(s) for Kisspeptin in metabolism in mammals. Front Endocrinol (Lausanne). 2018;9:184.
15. The-Food-Foundation. UK and global malnutrition: the new normal. International Learning Series/1 2017 06/10/2019]. https://foodfoundation.org.uk/wp-content/uploads/2017/07/1-Briefing-Malnutrition_v4.pdf.
16. Limbers CA, Cohen LA, Gray BA. Eating disorders in adolescent and young adult males: prevalence, diagnosis, and treatment strategies. Adolesc Health Med Ther. 2018;9:111–6.
17. Klump KL. Puberty as a critical risk period for eating disorders: a review of human and animal studies. Horm Behav. 2013;64(2):399–410.
18. Timko CA, DeFilipp L, Dakanalis A. Sex differences in adolescent anorexia and bulimia nervosa: beyond the signs and symptoms. Curr Psychiatry Rep. 2019;21(1):1.
19. Mirror-Mirror. Excessive exercise and eating disorders. 07/10/2019]. https://www.mirror-mirror.org/excessive-exercise.htm.

20. Dandona P, Dhindsa S. Update: hypogonadotropic hypogonadism in type 2 diabetes and obesity. J Clin Endocrinol Metab. 2011;96(9):2643–51.
21. Dhindsa S, et al. Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. J Clin Endocrinol Metab. 2004;89(11):5462–8.
22. Mogri M, et al. Testosterone concentrations in young pubertal and post-pubertal obese males. Clin Endocrinol. 2013;78(4):593–9.
23. O'Byrne, K. Stress and timing of puberty: is the amygdala the key? https://gtr.ukri.org/project/666E67E5-D529-4FBE-B880-B25F45CE0E26. Accessed 10 Oct 2019.
24. Dwyer AA, et al. Transition in endocrinology: hypogonadism in adolescence. Eur J Endocrinol. 2015;173(1):R15–24.
25. Zhu J, Chan YM. Adult consequences of self-limited delayed puberty. Pediatrics. 2017;139(6):e20163177.
26. Kim SH. Congenital hypogonadotropic hypogonadism and Kallmann syndrome: past, present, and future. Endocrinol Metab (Seoul). 2015;30(4):456–66.
27. Boehm U, et al. Expert consensus document: European consensus statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11(9):547–64.
28. Karavitaki N, et al. Craniopharyngiomas. Endocr Rev. 2006;27(4):371–97.
29. Müller HL. Childhood craniopharyngioma. Pituitary. 2013;16(1):56–67.
30. Müller HL. Craniopharyngioma. Endocr Rev. 2014;35(3):513–43.
31. Aylwin SJ, Bodi I, Beaney R. Pronounced response of papillary craniopharyngioma to treatment with vemurafenib, a BRAF inhibitor. Pituitary. 2016;19(5):544–6.
32. Rostami E, et al. Recurrent papillary craniopharyngioma with BRAFV600E mutation treated with neoadjuvant-targeted therapy. Acta Neurochir. 2017;159(11):2217–21.
33. Gold EB. Epidemiology of pituitary adenomas. Epidemiol Rev. 1981;3:163–83.
34. Colao A, Loche S. Prolactinomas in children and adolescents. Endocr Dev. 2010;17:146–59.
35. Robison LL, Hudson MM. Survivors of childhood and adolescent cancer: life-long risks and responsibilities. Nat Rev Cancer. 2014;14(1):61–70.
36. Laughton SJ, et al. Endocrine outcomes for children with embryonal brain tumors after risk-adapted craniospinal and conformal primary-site irradiation and high-dose chemotherapy with stem-cell rescue on the SJMB-96 trial. J Clin Oncol. 2008;26(7):1112–8.
37. Chemaitilly W, et al. Central precocious puberty following the diagnosis and treatment of paediatric cancer and central nervous system tumours: presentation and long-term outcomes. Clin Endocrinol. 2016;84(3):361–71.
38. Richmond EJ, Rogol AD. Male pubertal development and the role of androgen therapy. Nat Clin Pract Endocrinol Metab. 2007;3(4):338–44.
39. Dunkel L, Quinton R. Transition in endocrinology: induction of puberty. Eur J Endocrinol. 2014;170(6):R229–39.
40. Wei C, Crowne EC. Recent advances in the understanding and management of delayed puberty. Arch Dis Child. 2016;101(5):481–8.

Disorders of Pubertal Development: Precocious Puberty

6

Marco Cappa and Laura Chioma

6.1 Introduction

Puberty is a complex physical and psychological process that culminates with a complete sexual maturation, comprising the reproductive capacity. Pubertal onset requires activation of hypothalamic neurons to increase pulsatile GnRH secretion, with the gene network involved in its activation gradually coming to light. The synthesis of GnRH starts early in fetal life, with this system being active during approximately the first 6–9 months of life in boys (known as 'minipuberty'), and then the gonadotrope axis becomes quiescent. The timing of puberty is highly heritable, and the reactivation of hypothalamic GnRH secretion is determined by genetic, ethnic, nutritional, and environmental influences [1].

The transition from childhood to puberty is determined by the reactivation of the hypothalamus-pituitary-gonad axis [2] and controlled by neuroendocrine and metabolic factors [3, 4]. The secretion of GnRH is controlled by kisspeptin and its receptor KISS1R and is modulated by the increased stimulatory effect of neurokinin B and its receptor and the reduced inhibitory effect of dynorphin and its receptor, resulting in increased GnRH secretion with pulsatile pattern (Fig. 6.1).

Furthermore, the GnRH pulsatility is under excitatory and inhibitory control, so at the onset of puberty, the excitatory signal increases, while the inhibitory one decreases [5]. The major neurotransmitter responsible for the inhibition of GnRH secretion during childhood is gamma-aminobutyric acid (GABA), while glutamate, neuropeptide Y, endorphins, opioids, and melatonin are responsible for activating the GnRH pulse generator and consequently setting up the timing of puberty.

In conclusion, the increased frequency and range of GnRH secretion, along with the increase in excitatory input of kisspeptin through KNDy neurons and glutamate

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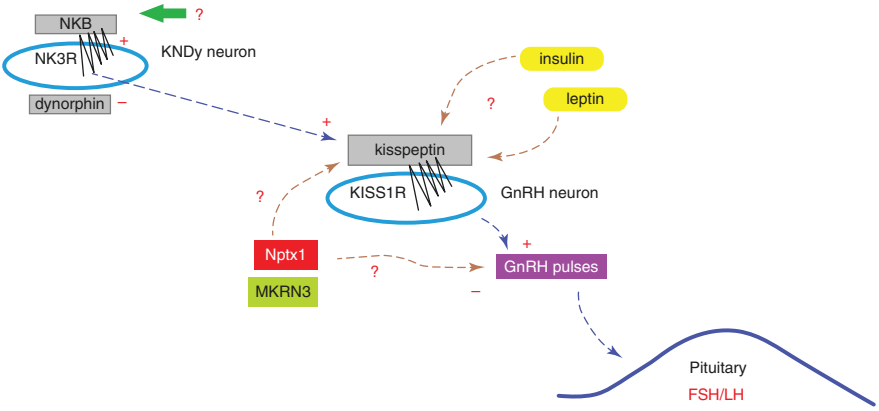

Fig. 6.1 Hypothalamus-pituitary-gonad axis activation in puberty. The GnRH pulse is induced by kisspeptin and its receptor KISS1R and is modulated by neurokinin B (NKB) and its receptor NK3R on KNDy neurons, likely through increased kisspeptin levels. Nptx levels increase during puberty, and its levels are inversely associated with MKRN3, responsible for repressing pubertal initiation, although the exact role is still unknown. Gain-of-function mutations of kisspeptin and KISS1R gene and loss-of-function mutations of MKRN3 have been associated with CPP (modified by Aguirre RS et al.) [32]

and the decrease in inhibitory signal from GABA neurons, mark the beginning of puberty [6].

Metabolic control is another important factor which influences the onset of puberty, particularly in girls; in fact, important information about the nutritional status and energy reserves are indirectly sent to the GnRH neurons by insulin and leptin signal pathway through mostly unidentified intermediary inputs (Fig. 6.1) [7, 8]. During the peripubertal period, there is a change in body composition and sensitivity to insulin, in fact a higher body fat content leads to an earlier pubertal maturation, and early puberty is in turn associated with a higher risk of obesity later in life [9].

In the last two decades, different studies have shown that the onset of puberty was advanced by 12–18 months [10] and some of the hypothesized causes include the role of nutritional status and growth but also the influence of extrinsic factors such as the exposure to the endocrine-disrupting chemicals (EDCs) [11]. EDCs cause hypomethylation and potentially should be able to modify the pubertal process [12–14]. This class of chemicals is capable of interfering with steroid hormone activity, particularly estrogens and antiandrogens as demonstrated in animal models [15], and seems also to be linked to the shift in puberty timing [16].

Variations in the timing of pubertal development are inheritable. This has been demonstrated in the studies on homozygous twins compared to dizygotic twins [17, 18]. The knowledge of the underlying mechanisms, including genes that explain variance, is still unclear. Recently, some rare genetic causes of early puberty have been reported, and three genes have been identified in the pathogenesis of central precocious puberty: KISS1 [19] encoding kisspeptin, its KISS1R receptor [20], and MKRN3, a gene deemed to act as a hypothalamic repressor on the gonadal axis (Fig. 6.1).

Secondary sexual development should be classified according to Tanner stage, evaluating pubic hair (P) and genital (G) development in boys (Fig. 6.2). The first external sign of puberty in boys is change from G1 to G2 stage, including enlargement of the testes with testicular volume greater than 4 mL or testicular length greater than 25 mm [21], with a normal pubertal development at the age of 9–14 years with an average age of 11.5 years.

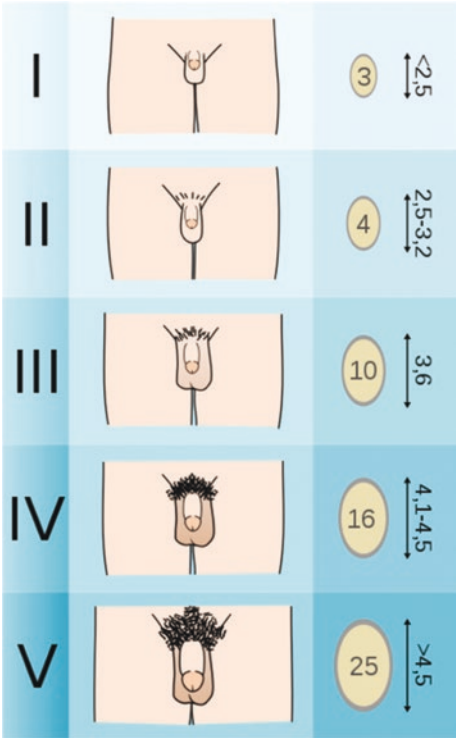

Fig. 6.2 Tanner stages of pubertal development

Pubic hair development (P):

- Stage 1: prepubertal, with no pubic hair
- Stage 2: sparse, straight pubic hair along the base of the penis
- Stage 3: hair is darker, coarser, and curlier, extending over the mid-pubis
- Stage 4: hair is adult-like in appearance but does not extend to thighs
- Stage 5: hair is adult in appearance, extending from thigh to thigh

External genitalia development (G):

- Stage 1: prepubertal
- Stage 2: enlargement of testes and scrotum, scrotal skin reddens, and changes in texture
- Stage 3: enlargement of penis, further growth of testes
- Stage 4: increased size of penis with growth in breadth and development of glans, testes and scrotum larger, scrotal skin darker
- Stage 5: adult genitalia

Precocious puberty is defined as the onset of sexual characteristics before 9 years in boys, at a chronological age 2–2.5 SD before the mean age of pubertal onset for Caucasian population [21].

Based on the definition of precocious puberty, the prevalence rate is expected to be around 2%, or 2 every 100 children. However, population studies have demonstrated different rates depending on the population studied. Nine-year Danish national registries provide a very low incidence for boys (<1 per 10,000) and a prevalence fivefold lower than girls (<5 per 10,000 vs 20–23 per 10,000 in girls) [22].

Precocious puberty can be classified based upon the underlying pathologic process as:

- Central precocious puberty (CPP, also known as gonadotropin-dependent precocious puberty) is due to early maturation of the hypothalamic-pituitary-gonadal axis. CPP is characterized by maturation of testicular and penile enlargement and pubic hair in boys.
- Peripheral precocious puberty (PPP, also known as gonadotropin-independent precocious puberty) is caused by excess secretion of sex hormones from the gonads or adrenal glands, exogenous sources of sex steroids, or ectopic production of gonadotropin from a germ cell tumor.
- Benign or nonprogressive pubertal variants, including isolated androgen-mediated sexual characteristics (such as pubic and/or axillary hair, acne, and apocrine odor) in boys that result from early activation of the hypothalamic–pituitary–adrenal axis (premature adrenarche). Both of these disorders can be a variant of normal puberty.

In at least 50% of cases of precocious pubertal development, pubertal manifestations will regress or stop progressing, and no treatment is necessary [23]. Although the mechanism underlying these cases of nonprogressive precocious puberty is unknown, the gonadotropic axis is not activated.

6.2 Central Precocious Puberty

Central precocious puberty (CPP, also known as gonadotropin-dependent precocious puberty) is due to early maturation of the hypothalamic-pituitary-gonadal axis, and it is the most common mechanism of precocious puberty. Although the onset is early, the pattern and timing of pubertal events are usually normal.

6.2.1 Causes

Several cerebral malformations and acquired insults have been associated with CPP (Table 6.1), although idiopathic disease is described in 25–60% of boys, as reported by some authors [24]. The most frequently detected brain abnormalities associated

Table 6.1 Causes of CPP

CNS lesions—congenital malformations
• Hypothalamic hamartoma
• Suprasellar arachnoid cysts
• Hydrocephalus
• Glioma or neurofibromatosis type 1
• Tuberous sclerosis
• Septo-optic dysplasia
• Chiari II malformations and myelomeningocele
CNS lesions—acquired insults
• Tumors: astrocytoma, ependymoma, pinealoma, hypothalamic or optic glioma, craniopharyngioma, dysgerminoma (non-hCG secreting), meningioma
• Post-insults (perinatal, infection trauma, radiotherapy)
• Granulomatous disease
• Cerebral palsy
No CNS lesions
• Idiopathic
• Endocrine disruptors
• No CNS lesions—congenital causes
• Genetic changes: gain-of-function mutations in the genes encoding kisspeptin (KISS1) and kisspeptin receptor (KISS1R [formerly called GPR54]), loss-of-function mutation in makorin ring finger 3 (MKRN3)
• Chromosomal abnormalities
No CNS lesions—acquired conditions
• International adoption
• Early exposure to sex steroids (secondary central precocious puberty)

with CPP include hypothalamic hamartomas, encephalitis, hydrocephalus, neurofibromatosis type 1, meningomyelocele, and neonatal encephalopathy [25]. The hypothalamic hamartoma, known as hamartoma of the tuber cinereum, represents the most common cause of organic cause of CPP, usually in children before the age of 4 years. It is a benign congenital tumor composed of GnRH neurons or transforming growth factor (TGF)α-producing astroglial cells that could cause premature activation of pulsatile GnRH release [26]. The disease phenotype caused by hamartomas can be associated with neurological abnormalities, such as gelastic (laughing or crying), focal, or generalized tonic-clonic seizures, and cognitive impairment [27].

Other CNS tumors associated with CPP include astrocytomas, ependymomas, pinealomas, hypothalamic or optic gliomas, craniopharyngiomas, dysgerminomas (non-hCG secreting), and meningiomas. In patients with neurofibromatosis, CPP is usually, but not always, associated with an optic glioma [28].

Cranial irradiation, particularly at high dose used for CNS malignancies, may cause CPP in boys with consequent rapid progress of bone age maturation and risk of short stature [29, 30].

CPP has been associated also with congenital or acquired lesions, such as hydrocephalus, cysts, trauma, inflammatory disease, tuberous sclerosis, or septo-optic dysplasia.

Specific genetic mutations have been associated with CPP, although they represent a minority of cases. Studies have described [19, 20, 31] the activation of the genes KISS1, which encodes kisspeptin, and KISS1R (formerly called GPR54), which encodes the kisspeptin receptor, and the inactivation of the MKRN3 gene in the premature reactivation of GnRH secretion, which was previously deemed idiopathic (Fig. 6.1) [32].

MKRN3, an imprinted gene located on the Prader-Willi syndrome critical region (15q11-q13), encodes makorin ring finger protein 3 and represents one of the main factors involved in repressing pubertal initiation. Thus, loss-of-function mutations in this gene would lead to diminished inhibition and early onset of puberty. The MKRN3 protein is derived only from RNA transcribed from the paternally inherited copy of the gene, because of maternal imprinting [31]. Segregation analysis of families with central precocious puberty caused by MKRN3 mutations clearly shows an autosomal dominant inheritance with complete penetrance. MKRN3 mutations have been demonstrated to be associated with up to 46 percent of familial cases of CPP [33] as well as identified in children with apparently sporadic disease [34]. Because of the imprinting pattern (maternal silencing) of MKRN3, the disease phenotype can be inherited from an asymptomatic father who carries an MKRN3 mutation. Indeed, genotype analysis of patients who have MKRN3 mutations and no family history of premature sexual development showed that paternal inheritance was present in all studied cases [34]. Findings from these initial studies suggested that the familial nature of this disorder is probably under-recognized, because of the difficulty of obtaining a precise family history from the father and the likelihood of underdiagnosis of early testicular enlargement. A growing list of loss-of-function mutations of MKRN3 has been identified in several affected families from different ethnic groups [31–40]. Remarkably, patients with MKRN3 mutations had typical clinical and hormonal features of premature activation of the reproductive axis, including early pubertal signs such as testis and pubic hair development, accelerated linear growth, advanced bone age, and raised basal or GnRH-stimulated LH concentrations.

Distinct chromosomal abnormalities have been associated with complex syndromic phenotypes that could include premature sexual development caused by activation of the hypothalamic-pituitary-gonadal axis. Among these syndromes are the 1p36 deletion, 7q11.23 microdeletion (Williams-Beuren syndrome) [41], 9p deletion [42], maternal uniparental disomy of chromosomes 7 (Silver-Russell syndrome) and 14 (Temple syndrome) [43], inversion duplication of chromosome 15 [44], de novo interstitial deletion and maternal uniparental disomy of chromosome 15 (Prader-Willi syndrome) [45], and a de novo deletion in cyclin-dependent kinase-like 5 gene (CDKL5, located in the Xp22 region) [46], with a phenotype reminiscent of Rett syndrome.

Internationally adopted children have 10–20 times increased risk of developing CPP, particularly when adopted after 2 years of age as reported by Danish national registries, while the risk of developing CPP was only marginally increased in children immigrating with their family [47]. The reason for this finding is unclear, but it has been hypothesized that nutritional deprivation in early life followed by

increased adiposity after adoption and stressful psychosocial factors trigger the pubertal maturation. Additionally, environmental effects including early life exposure to endocrine-disrupting factors, such as estrogenic and antiandrogenic chemicals, can affect pubertal onset. Indeed, most of adopted children coming from malaria-endemic countries had been exposed to the insecticide dichlorodiphenyltrichloroethane (DDT) during prenatal life and infancy [48]. DDT has prominent estrogenic properties, and its derivative dichlorodiphenyldichloroethylene (DDE) is regarded as antiandrogenic [49]. These findings initially suggested the potential involvement of DDT in the early pathogenesis of sexual precocity in exposed children, although additional animal and human studies are needed to establish the role of these chemicals in pubertal disorders.

Long-term exposure to high serum levels of sex steroids, as occur in sex steroid-producing tumors, testotoxicosis, McCune-Albright syndrome, and poorly controlled congenital adrenal hyperplasia, leads to an increased growth rate, to an accelerated bone age, and acts as trigger of the maturation of hypothalamic centers that are important for the initiation of puberty. The decrease in sex steroids following the treatment of the primary underlying disorder causes an activation of the precociously matured hypothalamic GnRH pulse generator via feedback mechanisms, resulting in secondary CPP [50].

6.2.2 Evaluation

The evaluation is warranted in boys presenting with signs of secondary sexual development before the age of 9 years, beginning with a medical history and physical examination. In most cases, radiographic measurement of bone age is performed to determine whether there is a corresponding increase in epiphyseal maturation.

6.2.3 Medical History and Physical Examination

The first step in evaluating a child suspected for CPP is to obtain a complete family history (age at onset of puberty in parents and siblings) and personal history, including when the initial pubertal changes were first noted and progression of pubertal manifestations. Usually children with CPP present normal sequence of pubertal development but, at an earlier age, with a rapid rate of linear growth and skeletal maturation due to high concentration of sex steroids. In addition, other questions are directed toward any evidence of possible CNS dysfunction, such as headaches, changes of behavior or vision, seizures, or previous history of CNS disease or trauma. Physical examination includes height, weight, and height velocity (HV, measured as cm/year). Children with CPP display an early growth acceleration with HV over 95th percentile for age. Secondary sexual development should be assessed to determine the sexual maturity rating and classified according to Tanner stage, which means evaluating pubic hair (P) and genital (G) development in boys (Fig. 6.2). The genital evaluation is made with

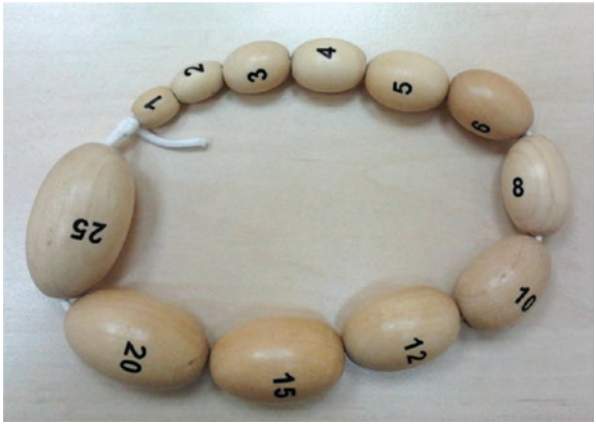

Fig. 6.3 Prader orchidometer

measurements of the testicular volume, rather than with penile size because penile growth is not an early event in puberty and accurate measurement is difficult and awkward for the adolescent boy. CPP should be suspected when Tanner's genital stage changes from G1 to G2 including enlargement of the testes with testicular volume greater than 4 mL or testicular length greater than 25 mm usually associated with Tanner's pubic hair initial development [21]. On the contrary, CPP is ruled out when Tanner's pubic hair stage changes from P1 to P2 without enlargement of testicular volume. For all these reasons, the measurement of testicular volume is critical on physical evaluation and is typically measured using the Prader orchidometer (Fig. 6.3).

6.2.4 Bone Age

The evaluation of epiphyseal maturation by radiographic assessment of bone age (Greulich and Pyle or TW2/TW3 20 bones atlas) of patients with CPP can help in differential diagnosis of precocious puberty. In fact, the bone age of patients with CPP is generally advanced by at least 1 year or by more than 2 standard deviations (SDs), in relation to chronological age [51]. However, the absence of advanced bone age is not a reason to discontinue follow-up assessment when increased growth velocity and other clinical symptoms of progressive puberty are present. Bone age is also used to predict adult height, although this prediction tends to overestimate adult height and is not very reliable [52].

6.2.5 Laboratory evaluation

In boys, testosterone is an excellent marker for sexual precocity, because most patients have morning plasma testosterone values in the pubertal range [51]. Tandem mass spectroscopy methods are more sensible than immunoassay methods to distinguish between prepubertal and early pubertal testosterone concentrations. The gold

standard biochemical diagnosis of CPP is based on the assessment of gonadotropins, mainly LH, at 30 or 45 min after stimulation with exogenous GnRH or GnRH-releasing hormone agonists [51]. Cutoff concentrations for an LH peak higher than 5 IU/L, assessed by ultrasensitive immunoassays, are usually indicative of an activated gonadotropic axis [51, 53]. Children with CPP tend to have a more prominent LH increase after stimulation with a peak LH-to-FSH ratio of 0.6–1.0 suggesting for diagnosis. However, its sensitivity and specificity are not greater than the GnRH-stimulated peak LH alone [54]. With the development of laboratory methods that make use of monoclonal antibodies such as immunofluorometric, immunochemiluminometric, and electrochemiluminometric assays, which have higher sensitivity and specificity than radioimmunoassay methods with a lower limit of detection of ≤0.1 mUI/mL, it has been suggested to use morning baseline LH to assess the activation of the gonadotropic axis, avoiding the need for GnRH agonist test [54]. However, unless levels of LH are clearly elevated, it is advisable to confirm the diagnosis of progressive CPP with a stimulation test.

Gonadotropins evaluations are not reliable in boys younger than 6 or 12 months, because baseline gonadotropin concentrations are usually high due to "minipuberty" period.

6.2.6 Imaging

Magnetic resonance imaging (MRI) of the brain should be performed in boys with confirmed CPP to rule out any hypothalamic or CNS lesion (Table 6.1). The prevalence of such lesions is higher in boys (40–90%) than in girls and more frequent in younger patients with rapid progression of pubertal development [24].

6.2.7 Treatment

Long-acting GnRH agonists are the gold standard treatment for CPP. They provide a continuous stimulation to the pituitary gonadotrophs, instead of physiologic pulsatile secretion of hypothalamic GnRH, leading to desensitization of the gonadotroph cells and decreases in release of LH and FSH [55]. This treatment can be used for patients with idiopathic or neurogenic CPP, such as for secondary CPP. However, not all patients require treatment, and the decision depends upon the rate of pubertal progression, height velocity, and the estimated adult height. In fact, boys with CPP who present before 9 years of age with a rapid progression of maturation benefit the most from therapy because they have early epiphyseal fusion and reduced adult height if they are not treated [56]. In contrast, boys who have a very slowly progressive variant of CPP usually do not require any therapy because their adult height without treatment is concordant with their mid-parental height range [57]. Based upon the data reported above, the primary goal of treatment for CPP is to allow a child to grow to a normal adult height and secondarily to relieve psychosocial stress.

GnRH agonist administration results in an initial transient stimulation of gonadotropin secretion from the pituitary, followed by a complete, but reversible, suppression of the pituitary-gonadal axis. Several GnRH agonists are available in various long-acting depot forms as intramuscular injections for monthly, 3 or 6 monthly dosing, or 12 monthly subcutaneous implant. Their approval for use and recommended doses in precocious puberty vary in different countries (Table 6.2). The most widely used drugs are triptorelin and leuprorelin monthly or 3 monthly intramuscular depot [56]. A recent meta-analysis confirms that the quarterly formulation is similarly effective in suppressing the pituitary-gonadal axis to the monthly formulation with the advantage of less injections required, although few data are available in male population [58].

Short-acting GnRH agonist formulations, including daily subcutaneous injections and multiple daily dosing intranasal sprays, are also available, though the depot preparations are preferable for their greater effectiveness in pubertal suppression in addition to an improved compliance.

The use of GnRH agonist results in the regression or stabilization of pubertal symptoms, lowering of growth velocity to normal prepubertal values, and decrease of bone-age advancement [56]. Progression of testicular development usually indicates poor compliance, treatment failure, or incorrect diagnosis, demanding further assessment.

Table 6.2 Long-acting GnRH agonists for the treatment of CPP

GnRH agonist	Availability	Dose, frequency, and method of administration
Histrelin acetate subcutaneous implant	US	50 mg implant surgically inserted every 12 months
Leuprolide acetate (leuprorelin)	US, CAN, EU, AU, SA, elsewhere	Intramuscular depot injection, given every 28 days: • US: PW ≤25 kg, 7.5 mg; PW >25 kg, 11.25 mg • EU: 3.75 mg Intramuscular depot injection, given every 12 weeks • US: 11.25, 22.5, or 30 mg (criteria for selection of the dosage have not been established) • EU: 11.25 mg
Goserelin acetate	US, UK, EU, CAN, SA, elsewhere	Subcutaneous implant injected into the anterior abdominal wall: 3.6 mg every 28 days or 10.8 mg every 12 weeks
Triptorelin pamoate	UK, EU, SA, elsewhere	Intramuscular depot injection every 28 days: PW ≤20 kg: 1.875 mg PW 20–30 kg: 2.5 mg PW >30 kg: 3.75 mg An 11.25/22.5 mg intramuscular depot injection every 12/24 weeks, respectively, is available in some countries

US United States, *CAN* Canada, *EU* European Union, *AU* Australia, *SA* South America, *UK* United Kingdom, *PW* Patient weight

The duration of GnRH agonist therapy should be long enough to optimize final adult height yet still allow progression of pubertal characteristics at an age that is concurrent with the individual's peers, although the optimum time to discontinue treatment has not been formally established due to lack of data available about GnRH agonist treatment in boys. As described for girl, commonly the treatment withdrawal is recommended between 12 and 12.5 years of bone age in boys or when a marked deceleration of growth occurs to avoid the loss of physiologic pubertal growth spurt [59]. When monthly GnRH agonist therapy is stopped, normal puberty returns within few months.

Treatment may be associated with headaches and menopausal symptoms (e.g., hot flushes); these adverse effects are generally transient and resolve spontaneously or with symptomatic treatment. Local complications, including sterile abscesses at injection sites, occur in 3–13% of patients [60]. Fat mass tends to increase with treatment, whereas lean mass and bone density tend to decrease, although longitudinal studies indicate that the prevalence of obesity does not increase during or after treatment and that bone density is normal after the cessation of treatment [61, 62].

6.3 Peripheral Precocious Puberty

Peripheral precocious puberty (PPP, also known as gonadotropin-independent precocious puberty) is due to secretion of sex hormones not depending to an early maturation of the hypothalamic-pituitary-gonadal axis. Indeed FSH and LH levels are usually suppressed in PPP, and they do not increase after GnRH stimulation. Further characterization is based upon whether the sexual characteristics are appropriate for the child's gender (isosexual) or inappropriate, with feminization of boys (controsexual).

6.3.1 Causes

PPP is caused by excess secretion of sex hormones derived either from the gonads or adrenal glands or from exogenous sources.

Leydig cell tumor is a testosterone-secreting tumor responsible of PPP in boys. Usually, it should be considered in any boy with asymmetric testicular enlargement. Even if a distinct mass cannot be palpated and none is evident on ultrasonography, the larger testis should be biopsied if it enlarges during follow-up. These tumors are almost always benign and are readily cured by surgical removal with radical orchiectomy [63].

Human chorionic gonadotropin-secreting germ cell tumors secrete hCG, which in boys activates LH receptors on the Leydig cell resulting in increased testosterone production. The increase in testicular size is less than expected for the serum testosterone levels and degree of pubertal development because most of the testis enlargement is made up of tubular elements FSH- depending for maturation. These tumors occur in the gonads, brain (often in the pineal region), liver, retroperitoneum, and

anterior mediastinum, reflecting sites of embryonic germ cells before their coalescence in the gonadal ridge. The histology of hCG-secreting tumors ranges from dysgerminoma, which responds readily to therapy, to the more malignant embryonal cell carcinoma and choriocarcinoma [64]. Familial male-limited precocious puberty, also known as testotoxicosis, is caused by an activating mutation in the LH receptor gene, which results in premature Leydig cell maturation and testosterone secretion. Affected boys typically present between 1 and 4 years of age [65]. Although this rare disorder is inherited as an autosomal dominant character, only boys are affected because activation of both LH and FSH receptors is required for estrogen biosynthesis in girls.

Children with severe, long-standing primary hypothyroidism occasionally present PPP with premature testicular enlargement, known as the "overlap" or Van Wyk-Grumbach syndrome. The proposed mechanism is cross-reactivity and stimulation of the FSH receptor by high serum thyrotropin (TSH) concentrations, because both TSH and FSH share a common alpha subunit [66]. The signs of pubertal development regress after thyroxine therapy onset.

Exogenous estrogen or testosterone exposure can cause PPP in boys with contra- and isosexual development, respectively. Feminization, with gynecomastia in boys, has been attributed to excess estrogen exposure from creams, ointments, and sprays (e.g., oral contraceptive pills, estrogen-based cream for menopausal symptoms) [67]. Other possible sources of estrogen exposure include contamination of food with hormones, phytoestrogens that share a chemical structure with estrogen (e.g., soy), and folk remedies with estrogenic activity such lavender oil and tea tree oil [68].

Multiple case reports of transdermal testosterone products causing virilization in children have also been reported [69].

Adrenal causes of excess androgen production include androgen-secreting tumors (i.e., Cushing's syndrome, adrenal neoplasia) and enzymatic defects in adrenal steroid biosynthesis as nonclassic (or late-onset) congenital adrenal hyperplasia (NCCAH). Boys with an adrenal cause of PPP may present premature pubarche without testicular enlargement (testes have testicular volume less than 4 mL or diameter less than 2.5 cm). Rarely, adrenal tumors can lead to feminization due to both androgen and estrogen production, the latter because of intra-adrenal aromatization of androgen or for peripheral aromatization [70].

McCune-Albright syndrome (MAS) is a rare disorder defined as the triad of PPP, irregular café-au-lait skin pigmentation, and fibrous dysplasia of the bone. Patients with MAS have a somatic (postzygotic) mutation of the alpha subunit of the Gs protein that activates adenylyl cyclase [71]. This mutation leads to continued stimulation of endocrine function (e.g., precocious puberty, thyrotoxicosis, gigantism or acromegaly, Cushing's syndrome, prolactin-secreting adenoma, and hypophosphatemic rickets) with a markedly various clinical phenotype, depending on which tissues are affected by the mutation. PPP is the most commonly reported manifestation of MAS, although is less common in boys than girls. There is a high prevalence of ultrasound-detected testicular pathology, including hyper- and hypoechoic lesions (most likely representing areas of Leydig cell hyperplasia), microlithiasis, and focal calcifications [72]. Mutations can be also found in other non-endocrine organs (such

as the liver and heart) resulting in cholestasis and/or hepatitis, intestinal polyps, and cardiac arrhythmias, respectively. A heightened risk of malignancy has also been reported [73].

6.4 Evaluation

6.4.1 Medical History and Physical Examination

As described for CPP, the first step in evaluating a child suspected for PPP is to obtain a complete family history to identify familiar causes and personal history, including when the initial pubertal changes were first noted, progression of pubertal manifestations, and to rule out an estrogen or androgen exogenous exposition. Usually children with PPP have a peripheral source of gonadal hormones and are more likely to display deviations from the normal sequence of pubertal development with a rapid rate of linear growth and skeletal maturation. The physical examination could permit to identify the possible cause of PPP. In fact, patients with mild or asymmetric testicular enlargement could be suspected for testicular causes, cutaneous café-au-lait spots could evocate MAS, while premature pubarche without testicular enlargement is suggestive for adrenal origin of sexual hormones. Finally, children with isolated contrasexual development such as gynecomastia may have been exposed to exogenous estrogen.

6.4.2 Bone Age

As for CPP, the bone age of patients with PPP is generally advanced by at least 1 year or by more than 2 standard deviations (SDs), in relation to chronological age [51], except for boys with hypothyroidism who present a delayed bone age.

6.4.3 Laboratory Evaluation

The peripheral causes of PP are commonly characterized to have elevated morning plasma testosterone values as in the pubertal range, with suppressed gonadotropins (FSH and LH) [51]. In boys with premature pubarche without testicular enlargement, measurement of adrenal steroids may help to distinguish between PPP and benign premature adrenarche. Indeed, elevated level of early morning 17-hydroxyprogesterone (17-OHP) over 1000 ng/dL is diagnostic for Non Classical Congenital Adrenal Hyperplasia (NCCAH), while moderately elevated level between 200 and 1000 ng/dL is suggestive for NCCAH, and a high-dose (250 mcg) ACTH stimulation test is recommended to confirm the diagnosis [74]. Elevated level of DHEAS concentration may be due to a rare case of adrenal tumor or to a rare ACTH-dependent Cushing's disease when associated with elevated 24-h urinary free cortisol (UFC), late-night salivary cortisol, and early morning ACTH serum values.

Furthermore, the hCG dosage is recommendable to evaluate for the possibility of an hCG-secreting tumor, as well as the thyroid evaluation with TSH concentration to rule out a suspected chronic primary hypothyroidism.

6.4.4 Imaging

Ultrasound examination of the testes could be performed in boys with PPP and mild or asymmetric testicular enlargement to evaluate for the possibility of a Leydig cell tumor, testotoxicosis, or MAS. Finally, abdominal ultrasound and/or computerized tomography (CT) scan should be performed if adrenal tumor is suspected.

6.4.4.1 Treatment

The treatment of PPP is directed to removing or blocking the production of the excess sex hormones, depending on the cause. Testicular and adrenal tumors are treated by surgery, while the identification and removal of exogenous sexual steroids permit the regression of pubertal changes.

Glucocorticoid replacement therapy is only recommended in boys with NCCAH associated with an advanced bone age coupled with a poor height prediction compared to family target height [75].

Testotoxicosis and MAS in boys are treated with a combination of an antiandrogen (androgen receptor antagonist such as spironolactone or bicalutamide) and an aromatase inhibitor, such as anastrozole or Letrozole, which inhibit the conversion of testosterone to estradiol, thereby retarding further bone maturation. Although the data provided by this combined therapy appear promising, long-term studies are needed to further define the safety and efficacy of these pharmacological agents. In the past, ketoconazole was included in treatment regimen as inhibitor of steroid synthesis, but its use is limited due to its potential hepatotoxicity and adrenal insufficiency side effect [76, 77]. Testicular Leydig cell hyperplasia is common in MAS, but surgical treatment is avoided to preserve fertility.

The decrease of sex steroid levels may lead to CPP, often when associated with an advanced bone age; therefore GnRH agonist treatment is required.

6.5 Benign or Nonprogressive Pubertal Variants

Premature adrenarche is a very mild form of hyperandrogenism, characterized by the appearance of pubic and/or axillary hair prior to the age of 9 years in boys associated with a mild elevation in serum dehydroepiandrosterone sulfate (DHEAS) for age (typically 40–115 mcg/dL or 1.1–3.1 mcmol/L) [78]. Children with premature adrenarche tend to be taller than average and to have a bone age and linear growth rate that are above average but still within the normal range, with normal predicted adult height for the family target height. It is commonly assumed that the process is caused by premature development of the adrenal zona reticularis as a variant of normal development, although several studies described that premature adrenarche

could be associated with obesity and with low birth weight probably due to insulin resistance [78–80].

Some cases of premature pubarche, known as "idiopathic premature pubarche," occur in children with normal androgen levels and likely reflect increased sensitivity of the pilosebaceous unit to age-appropriate levels of androgen [81].

Nonprogressive or intermittently progressive precocious puberty represents a form of CPP that appears clinically stable or with very slow progression in their pubertal signs. The bone age is typically not as advanced compared with boys with true CPP, and serum LH concentrations are within the pre- or early pubertal range, indicating that the hypothalamic–pituitary–gonadal axis is not fully activated. In these cases, treatment with a GnRH agonist is not needed because their adult height is not affected [57].

All of these forms described above require no specific endocrine treatment but only clinical monitoring for evidence of pubertal progression to distinguish them from true CPP or PPP cases.

References

1. Palmert MR, Boepple PA. Variation in the timing of puberty: clinical spectrum and genetic investigation. J Clin Endocrinol Metab. 2001;86(6):2364–8.
2. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4:254–64.
3. Walvoord EC. The timing of puberty: is it changing? Does it matter? J Adolesc Health. 2010;47:433–9.
4. Tony M. Plant: neuroendocrine control of the onset of puberty. Front Neuroendocrinol. 2015;38:73–88.
5. Uenoyama Y, Tsukamura H, Maeda KI. KNDy neuron as a gatekeeper of 574 puberty onset. J Obstet Gynaecol Res. 2014;4:1518–26.
6. Sultan C, Gaspari S, Maimoun L, Kalfa N, Paris F. Disorders of puberty. Best Pract Res Clin Obstet Gynaecol. 2018;48:62–89.
7. Castellano JM, Tena-Sempere M. Metabolic control of female puberty: potential therapeutic targets. Expert Opin Ther Targets. 2016;20:1181–93.
8. Manfredi-Lozano M, Roa J, Ruiz-Pino F, Piet R, Garcia-Galiano D, Pineda R, et al. Defining a novel leptin-melanocortin-kisspeptin pathway involved in the metabolic control of puberty. Mol Metab. 2016;5:844–57.
9. Kaplowitz PB. Link between body fat and the timing of puberty. Pediatrics. 2008;121(Suppl 3):S208–17.
10. Sørensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal 564 timing in healthy Danish boys: associations with body mass index. J Clin Endocrinol Metab. 2010;95:263–70.
11. Aksglaede L, Sørensen K, Petersen JH, Skakkebaek NE, Juul A. Recent 567 decline in age at breast development: the Copenhagen Puberty Study. Pediatrics. 2009;123:e932–9.
12. Euling SY, Selevan SG, Pescovitz OH, Pescovitz OH, Skakkebaek NE. Role of environmental factors in the timing of puberty. Pediatrics. 2008;121(Suppl 3):S167171.
13. Kempinas Wde G. Environmental factors in dysregulation of puberty timing and progression. Reprod Toxicol. 2014;44:v–vi.
14. Harley KG, Rauch SA, Chevrier J, Kogut K, Parra KL, Trujillo C, et al. Association of prenatal and childhood PBDE exposure with timing of puberty in boys and girls. Environ Int. 2017;100:132–8.
15. Goldman JM, Laws SC, Balchak SK, Cooper RL, Kavlock RJ. Endocrine disrupting chemicals: prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat—a focus on the EDSTAC recommendations. Crit Rev Toxicol. 2000;30:135–96.

16. Wang RY, Needham LL, Barr DB. Effects of environmental agents on the attainment of puberty: considerations when assessing exposure to environmental chemicals in the National Children's Study. Environ Health Perspect. 2005;113:1100–7.
17. Kaprio J, Rimpela A, Winter T, Viken RJ, Rimpela M, Rose RJ. Common genetic influences on BMI and age at menarche. Hum Biol. 1995;67:739–53.
18. Silventoinen K, Haukka J, Dunkel L, Tynelius P, Rasmussen F. Genetics of pubertal timing and its associations with relative weight in childhood and adult height: the Swedish Young Male Twins Study. Pediatrics. 2008;121:e885–91.
19. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, et al. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab. 2010;95:2276–80.
20. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med. 2008;358:709–15.
21. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45:13–24.
22. Teilmann G, Pedersen CB, Jensen TK, Skakkebaek NE, Juul A. Prevalence and incidence of precocious pubertal development in Denmark: an epidemiologic study based on national registries. Pediatrics. 2005;116:1323–8.
23. Kaplowitz P. Clinical characteristics of 104 children referred for evaluation of precocious puberty. J Clin Endocrinol Metab. 2004;89:3644–50.
24. De Sanctis V, Corrias A, Rizzo V, Bertelloni S, Urso L, Galluzzi F, et al. Etiology of central precocious puberty in males: the results of the Italian Study Group for Physiopathology of Puberty. J Pediatr Endocrinol Metab. 2000;13(Suppl 1):687–93.
25. Stephen MD, Zage PE, Waguespack SG. Gonadotropin-dependent precocious puberty: neoplastic causes and endocrine considerations. Int J Pediatr Endocrinol. 2011;2011:184502.
26. Jung H, Ojeda SR. Pathogenesis of precocious puberty in hypothalamic hamartoma. Horm Res. 2002;57(suppl 2):31–4.
27. Cukier P, Castro LH, Banaskiwitz N, Teles LR, Ferreira LR, Adda CC, et al. The benign spectrum of hypothalamic hamartomas: infrequent epilepsy and normal cognition in patients presenting with central precocious puberty. Seizure. 2013;22:28–32.
28. Listernick R, Charrow J, Gutmann DH. Intracranial gliomas in neurofibromatosis type 1. Am J Med Genet. 1999;89(1):38–44.
29. Armstrong GT, Chow EJ, Sklar CA. Alterations in pubertal timing following therapy for childhood malignancies. Endocr Dev. 2009;15:25–39.
30. Ogilvy-Stuart AL, Clayton PE, Shalet SM. Cranial irradiation and early puberty. J Clin Endocrinol Metab. 1994;78(6):1282–6.
31. Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, et al. Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl J Med. 2013;368:2467–75.
32. Aguirre RS, Eugster EA. Central precocious puberty: From genetics to treatment. Best Pract Res Clin Endocrinol Metab. 2018;32(4):343–54.
33. Simon D, Ba I, Mekhail N, Ecosse E, Paulsen A, Zenaty D, et al. Mutations in the maternally imprinted gene MKRN3 are common in familial central precocious puberty. Eur J Endocrinol. 2016;174(1):1–8.
34. Macedo DB, Abreu AP, Reis AC, Montenegro LR, Dauber A, Beneduzzi D, et al. Central precocious puberty that appears to be sporadic caused by paternally inherited mutations in the imprinted gene makorin ring finger 3. J Clin Endocrinol Metab. 2014;99:E1097–103.
35. Settas N, Dacou-Voutetakis C, Karantza M, Kanaka-Gantenbein C, Chrousos GP, Voutetakis A. Central precocious puberty in a girl and early puberty in her brother caused by a novel mutation in the MKRN3 gene. J Clin Endocrinol Metab. 2014;99:E647–51.
36. Schreiner F, Gohlke B, Hamm M, Korsch E, Woelfle J. MKRN3 mutations in familial central precocious puberty. Horm Res Paediatr. 2014;82:122–6.
37. de Vries L, Gat-Yablonski G, Dror N, Singer A, Phillip M. A novel MKRN3 missense mutation causing familial precocious puberty. Hum Reprod. 2014;29:2838–43.
38. Bulcao Macedo D, Nahime Brito V, Latronico AC. New causes of central precocious puberty: the role of genetic factors. Neuroendocrinology. 2014;100:1–8.

39. Neocleous V, Shammas C, Phelan MM, Nicolaou S, Phylactou LA, Skordis N. In silico analysis of a novel MKRN3 missense mutation in familial central precocious puberty. Clin Endocrinol (Oxf). 2016;84(1):80–4.
40. Lee HS, Jin HS, Shim YS, Jeong HR, Kwon E, Choi V, et al. Low frequency of MKRN3 mutations in central precocious puberty among Korean girls. Horm Metab Res. 2016;48(2):118–22.
41. Partsch CJ, Japing I, Siebert R, Gosch A, Wessel A, Sippell WG, et al. Central precocious puberty in girls with Williams syndrome. J Pediatr. 2002;141:441–4.
42. Cisternino M, Della Mina E, Losa L, Madè A, Rossetti G, Bassi LA, et al. Idiopathic central precocious puberty associated with 11 mb de novo distal deletion of the chromosome 9 short arm. Case Rep Genet. 2013;2013:978087.
43. Hoffmann K, Heller R. Uniparental disomies 7 and 14. Best Pract Res Clin Endocrinol Metab. 2011;25:77–100.
44. Grosso S, Balestri P, Anichini C, Bartalini G, Pucci L, Morgese G, et al. Pubertal disorders in inv dup(15) syndrome. Gynecol Endocrinol. 2001;15:165–9.
45. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader–Willi syndrome. Genet Med. 2012;14:10–26.
46. Saletti V, Canafoglia L, Cambiaso P, Russo S, Marchi M, Riva D. A CDKL5 mutated child with precocious puberty. Am J Med Genet A. 2009;149A:1046–51.
47. Teilmann G, Pedersen CB, Skakkebaek NE, Jensen TK. Increased risk of precocious puberty in internationally adopted children in Denmark. Pediatrics. 2006;118(2):e391–9.
48. Parent AS, Franssen D, Fudvoye J, Gérard A, Bourguignon JP. Developmental variations in environmental influences including endocrine disruptors on pubertal timing and neuroendocrine control: revision of human observations and mechanistic insight from rodents. Front Neuroendocrinol. 2015;38:12–36.
49. Rasier G, Parent AS, Gérard A, Denooz R, Lebrethon MC, Charlier C, et al. Mechanisms of interaction of endocrine-disrupting chemicals with glutamate-evoked secretion of gonadotropin-releasing hormone. Toxicol Sci. 2008;102:33–41.
50. Partsch CJ, Heger S, Sippell WG. Management and outcome of central precocious puberty. Clin Endocrinol (Oxf). 2002;56(2):129–48.
51. Carel JC, Léger J. Precocious puberty. N Engl J Med. 2008;358:2366–77.
52. Carel JC, Lahlou N, Roger M, Chaussain JL. Precocious puberty and statural growth. Hum Reprod Update. 2004;10:135–47.
53. Neely EK, Hintz RL, Wilson DM, Lee PA, Gautier T, Argente J, et al. Normal ranges for immunochemiluminometric gonadotropin assays. J Pediatr. 1995;127:40–6.
54. Brito VN, Batista MC, Borges MF, Latronico AC, Kohek MB, Thirone AC, et al. Diagnostic value of fluorometric assays in the evaluation of precocious puberty. J Clin Endocrinol Metab. 1999;84:3539–44.
55. Lahlou N, Carel JC, Chaussain JL, Roger M. Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics. J Pediatr Endocrinol Metab. 2000;13(suppl 1):723–37.
56. Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR, et al; ESPE-LWPES GnRH Analogs Consensus Conference Group. Consensus statement on the use of gonadotropin-releasing hormone analogs in children. Pediatrics 2009;123(4):e752-e762
57. Lazar L, Pertzelan A, Weintrob N, Phillip M, Kauli R. Sexual precocity in boys: accelerated versus slowly progressive puberty gonadotropin-suppressive therapy and final height. J Clin Endocrinol Metab. 2001;86(9):4127–32.
58. Bertelloni S, Mucaria C, Baroncelli GI, Peroni D. Triptorelin depot for the treatment of children 2 years and older with central precocious puberty. Expert Rev Clin Pharmacol. 2018;11(7):659–67.
59. Carel JC, Roger M, Ispas S, Tondu F, Lahlou N, Blumberg J, et al. Final height after long-term treatment with triptorelin slow release for central precocious puberty: importance of statural growth after interruption of treatment. French study group of Decapeptyl in Precocious Puberty. J Clin Endocrinol Metab. 1999;84:1973–8.

60. Carel JC, Lahlou N, Jaramillo O, et al. Treatment of central precocious puberty by subcutaneous injections of leuprorelin 3-month depot (11.25 mg). J Clin Endocrinol Metab. 2002;87:4111–6.
61. Palmert MR, Mansfield MJ, Crowley WF Jr, Crigler JF Jr, Crawford JD, Boepple PA. Is obesity an outcome of gonadotropin-releasing hormone agonist administration? Analysis of growth and body composition in 110 patients with central precocious puberty. J Clin Endocrinol Metab. 1999;84:4480–8.
62. Bertelloni S, Baroncelli GI, Sorrentino MC, Perri G, Saggese G. Effect of central precocious puberty and gonadotropin releasing hormone analogue treatment on peak bone mass and final height in females. Eur J Pediatr. 1998;157:363–7.
63. Urban MD, Lee PA, Plotnick LP, Migeon CJ. The diagnosis of Leydig cell tumors in childhood. Am J Dis Child. 1978;132(5):494–7.
64. Englund AT, Geffner ME, Nagel RA, Lippe BM, Braunstein GD. Pediatric germ cell and human chorionic gonadotropin-producing tumors. Clinical and laboratory features. Am J Dis Child. 1991;145(11):1294–7.
65. Shenker A, Laue L, Kosugi S, Merendino JJ Jr, Minegishi T, Cutler GB Jr. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature. 1993;365(6447):652–4.
66. Cabrera SM, DiMeglio LA, Eugster EA. Incidence and characteristics of pseudoprecocious puberty because of severe primary hypothyroidism. J Pediatr. 2013;162(3):637–9.
67. Franklin SL. Effects of unintentional exposure of children to compounded transdermal sex hormone therapy. Pediatr Endocrinol Rev. 2011;8(3):208–12.
68. Henley DV, Lipson N, Korach KS, Bloch CA. Prepubertal gynecomastia linked to lavender and tea tree oils. N Engl J Med. 2007;356(5):479–85.
69. Martinez-Pajares JD, Diaz-Morales O, Ramos-Diaz JC, Gomez-Fernandez E. Peripheral precocious puberty due to inadvertent exposure to testosterone: case report and review of the literature. J Pediatr Endocrinol Metab. 2012;25(9–10):1007–12.
70. Moreno S, Guillermo M, Decoulx M, Dewailly D, Bresson R, Proye C. Feminizing adrenocortical carcinomas in male adults. A dire prognosis. Three cases in a series of 801 adrenalectomies and review of the literature. Ann Endocrinol (Paris). 2006;67(1):32–8.
71. Lumbroso S, Paris F, Sultan C, European Collaborative Study. Activating Gsalpha mutations: analysis of 113 patients with signs of McCune-Albright syndrome—a European Collaborative Study. J Clin Endocrinol Metab. 2004;89(5):2107–13.
72. Boyce AM, Chong WH, Shawker TH, Pinto PA, Linehan WM, Bhattacharryya N, et al. Characterization and management of testicular pathology in McCune-Albright syndrome. J Clin Endocrinol Metab. 2012;97(9):E1782–90.
73. Chanson P, Salenave S, Orcel P. McCune-Albright syndrome in adulthood. Pediatr Endocrinol Rev. 2007;4(Suppl 4):453–62.
74. Armengaud JB, Charkaluk ML, Trivin C, Tardy V, Bréart G, Brauner R, et al. Precocious pubarche: distinguishing late-onset congenital adrenal hyperplasia from premature adrenarche. J Clin Endocrinol Metab. 2009;94(8):2835–40.
75. Joint LWPES/ESPE CAH Working Group. Consensus statement on 21-hydroxylase deficiency from the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. J Clin Endocrinol Metab. 2002;87(9):4048–53.
76. Leschek EW, Jones J, Barnes KM, Hill SC, Cutler GB. Six-year results of spironolactone and testolactone treatment of familial male-limited precocious puberty with addition of deslorelin after central puberty onset. J Clin Endocrinol Metab. 1999;84:175–8.
77. Haddad N, Eugster E. An update on the treatment of precocious puberty in McCune-Albright syndrome and testotoxicosis. J Pediatr Endocrinol Metab. 2007;20(6):653–61.
78. Mäntyselkä A, Jääskeläinen J, Lindi V, Viitasalo A, Tompuri T, Voutilainen R, et al. The presentation of adrenarche is sexually dimorphic and modified by body adiposity. J Clin Endocrinol Metab. 2014;99(10):3889–94.
79. Utriainen P, Jääskeläinen J, Romppanen J, Voutilainen R. Childhood metabolic syndrome and its components in premature adrenarche. J Clin Endocrinol Metab. 2007;92(11):4282–5.

80. Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, et al. Avon Longitudinal Study of Parents and Children Study Team. Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. J Clin Endocrinol Metab. 2004;89(6):2647–51.
81. Lappalainen S, Utriainen P, Kuulasmaa T, Voutilainen R, Jääskeläinen J. Androgen receptor gene CAG repeat polymorphism and X-chromosome inactivation in children with premature adrenarche. J Clin Endocrinol Metab. 2008;93(4):1304–9. https://doi.org/10.1210/jc.2007-2707.

Clinical Management and Treatment of Varicocele in the Adolescence

7

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Abbreviations

ASRM	American Society for Reproductive Medicine
AUA	American Urological Association
EAU	European Association of Urology
ESPU	European Society for Pediatric Urology
FSH	Follicle-stimulating hormone
HSP	Heat shock proteins
LH	Luteinizing hormone
NcP	Nutcracker phenomenon
OR	Oxidative stress
PRF	Peak retrograde flow
ROS	Reactive oxygen species

7.1 Introduction

Varicocele is defined as the tortuosity and abnormal dilation of the pampiniform plexus draining the testis. According to evidences, varicocele is reported in 19–41% of patients with primary infertility and in up to 80% of the patients with secondary infertility [1].

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Table 7.1 Prevalence of varicocele in childhood and adolescence [3]

Age (years)	Prevalence (%)
2–6	0.88
7–10	1
11–14	7.8
15–19	14.1

The overall prevalence is 15% in adulthood, and this data is also confirmed during adolescence [1]. Accordingly, a prevalence of 15.7% has been found among a cohort of 7000 young men with a median age of 19 years in a European study [2]. A Turkish investigation involving 4052 children and adolescents reported an increasing prevalence after the onset of puberty [3] (Table 7.1).

Current evidence, mostly arising from studies on adult patients, indicates that varicocele negatively affects the testicular function. In adults, varicocele has a negative role on testicular function. Hence, as a matter of fact, poorer semen quality, pregnancy outcomes [4–6], and testosterone levels (as meta-analytic data indicate [7]) have been reported in patients with varicocele compared to healthy controls.

There is a lack of knowledge concerning the impact of varicocele during adolescence. Indeed, despite consensus has been reached in the last decade on the management and treatment of adulthood varicocele [8], these topics are still unclear and debated during adolescence.

During pubertal development, the rapid increase of testicular volumes and consequent hormone changes are responsible of the huge heterogeneity observed in adolescent population. Therefore, a standard approach is difficult and may be planned according to the stage of pubertal development. Currently, the challenge is to know which patient should be treated and when and what type of treatment should be proposed [9].

The aim of this review is to discuss the impact of adolescent varicocele on the testicular function from an endocrinological perspective. The current positions on diagnosis, management, and treatment of the European Society for Pediatric Urology (ESPU), the European Association of Urology (EAU), the American Urological Association (AUA), and the American Society for Reproductive Medicine (ASRM) will be examined.

7.2 Pathogenesis of Testicular Damage

Varicocele is potentially able to affect conventional semen parameters in the youth. In this regard, according to a recent meta-analysis comparing 357 varicocele patients aged 15–24 with 427 age-matched controls, the varicocele group had a significant decrease in sperm concentration (24 million/mL), motility (7.5%), and morphology (1.7%) [10].

These findings have been confirmed also elsewhere, and a role for varicocele-induced testicular hypotrophy in the determination of sperm abnormalities has been proposed. Accordingly, Tanner stage V adolescent patients (14–20 years old) with testicular volume asymmetry greater that 10% showed lower sperm concentration

and total motile sperm count compared with those having a lower testicular asymmetry. An even higher decrease was found in those with values >20% (medians of total motile sperm counts were of 64, 32, and 10 million in patients with 10%, 15%, and 20% testicular asymmetry, respectively) [11].

Several theories have been suggested to explain the mechanisms by which varicocele may impact on testicular function.

Varicocele, by causing scrotal hyperthermia, could affect spermatogenesis. A decreased expression of the heat shock proteins (HSPs) can promote heat stress, being the latter associated with the markers of oxidative stress (OS) and apoptosis. Testis weight can be affected by transient exposure to high temperature by interfering with spermatogenesis. Also, leucocyte trapping, which is induced by blood stasis in varicose veins, causes reactive oxygen species (ROS) release and promotes testicular hypoxia.

Individual susceptibility to OS and thermogenic damage may be conferred by constitutive low antiapoptotic and increased proapoptotic gene expression (*HSP*, *metallothionein-1*, *BCL-2*, *BAX*, *PHUDA1*, *PRM2*, *CCIN*), thus providing reasons why some patients are more susceptible to high-degree varicocele-induced testicular damage than others [12].

The childhood testis is mainly made of immature and actively proliferating Sertoli cells, secreting anti-Müllerian hormone (AMH). Testicular volume reflects the degree of Sertoli cell proliferation in this phase of life. At the onset of puberty, Sertoli cells secrete lower amounts of AMH and lose the ability to proliferate, leaving the immature state and acquiring maturity. Since each Sertoli cell can support a defined number of germ cells, the final number of Sertoli cells reached at puberty will determine the spermatogenetic potential [13].

Any event interfering with Sertoli cell proliferation and maturation in childhood may potentially impair testicular volume and the spermatogenetic potential in adolescence and adulthood. Noteworthy, since high temperature can impair Sertoli cell proliferation [14], varicocele may hypothetically lower the final Sertoli cell number in childhood (at least in some cases), thus causing a damage that cannot be reverted later in life. This evidence highlights the importance of proper management and treatment of childhood and adolescent varicocele.

7.3 Diagnostic Evaluation

Adolescent varicocele is asymptomatic in most of cases, although symptoms such as chronic fullness or scrotal or inguinal swelling may occasionally occur [9]. It is left-sided in the 90% and bilateral only in the 3% of cases. This is due to the testicular venous drainage entering the left renal vein with a 90° angle. By contrast, testicular right-side venous flux drains directly into the inferior vena cava forming an obtuse angle [9, 15].

Adolescent varicocele is mostly diagnosed during routine medical examination for school or sports or by testicular auto-palpation. The physical examination is the first step to make the diagnosis; it is required for genital inspection and testis,

epididymis, and deferent duct palpation, in supine position. The testicular volume is estimated by Prader orchidometer. Varicocele is appreciated in standing position. It usually presents as a plexus of veins having a consistency of a "bag of warms," and the Valsalva maneuver may be evoked to ascertain the presence of blood reflux. According to the Dubin and Amelar clinical staging, varicocele may be classified into four grades: grade 0, which defines subclinical varicocele (not clinically, detectable only by ultrasound); grade I, indicating a palpable varicocele only during the Valsalva maneuver; grade II, when varicocele is appreciable without the need to evoke the Valsalva maneuver; and grade III, referring to a varicocele that is already visible at inspection [16].

Doppler ultrasound is needed to assess the varicocele grade, evaluating the maximum vein diameter and the peak retrograde flow (PRF). By Doppler examination, following the Sarteschi's scale, varicocele can be scored in five different degrees, based on the time when reflux is detected and the extension of varicosity [17] (Table 7.2).

The nutcracker phenomenon (NcP) is due to the compression of the left renal vein between the aorta and the mesentery artery. This causes renal venous hypertension and dilatation of collateral veins, thus predisposing to varicocele. In the case of abdominal pain, hematuria, proteinuria, left-sided flank/lower abdominal pain, varicose veins, urinary frequency, and left-sided varicocele with a vein lumen diameter >3 mm NcP may be suspected [18]. In such cases, Doppler ultrasound of the renal vessels is useful. Ultrasound criteria suggestive for NcP have been proposed [18], and they are summarized in Table 7.3.

Very few data are reported about the prevalence of NcP in young patients: some authors have suggested that it is frequent in adolescents, since it has been diagnosed in 77 patients with higher velocity ratios than those without in a cohort of 182

Table 7.2 Ultrasound varicocele degree classifications

Scale	Degree	Description
Sarteschi	I	Reflux detected only during Valsalva maneuver, in the absence of evident scrotal varicosity during US study
	II	Small posterior varicosity that extends to the superior pole of the testis. Their diameter increase and the reflux became detectable in the supratesticular region only during Valsalva maneuver
	III	Vessels appear enlarged in the superior pole only in standing position. No enlargement can be detected in supine position. Reflux is observed only during Valsalva maneuver
	IV	Vessels appear enlarged in supine position. Dilatation is more marker during Valsalva maneuver
	V	Venous ectasia is detected in prone and supine position. Reflux occurs at rest, and it does not increase during Valsalva maneuver
Dubin	0	Moderate and transient venous reflux during Valsalva maneuver
	I	Persistent venous reflux that ends before that Valsalva maneuver is completed
	II	Persistent venous reflux through the entire Valsalva maneuver
	III	Venous reflux is basally detected and does not change during Valsalva maneuver

Table 7.3 Symptoms, signs, and ultrasound criteria suggestive for nutcracker phenomenon [18]

	Description
Signs and symptoms	Hematuria
	Proteinuria
	Varicose veins
	Urinary frequency
	Left-sided flank/lower abdominal pain
Ultrasound criteria	Reduced aortic/superior mesenteric artery angle (normal values: 38–65°)
	Left renal vein compression at the origin of aorta and superior mesenteric artery
	Increase flow velocity at the left renal vein
	Left-sided varicocele with a vein lumen diameter >3 mm

adolescents with clinical varicocele. According to these data, no influence on testicular symmetry, initial surgery, or re-operative surgery was observed [19].

Since the Prader orchidometer overestimates the true testicular volume, scrotal ultrasound should be requested, since a more precise estimation is important to decide for varicocele repair. Ultrasound testicular volume is usually calculated using the ellipsoid formula (length × width × thickness × 0.52) [20].

Elastosonography is a noninvasive technique which evaluates testis elasticity, already adopted in undescended pediatric testis or adult varicocele. It may play a prognostic role in the management of varicocele since a significant change in testicular elasticity has been found in patients with varicocele only in the case of volume asymmetry >20% [21]. However, this technique is not currently used in the clinical practice.

Sperm analysis is needed for a proper management of adolescent varicocele. It has to be requested at least 1.5 years after puberty onset [22]. Unfortunately this parameter is not frequently requested by pediatricians. Indeed, only the 13% of American pediatric urologists routinely request sperm analysis in adolescent patients with varicocele. Up to a half of them admit discomfort in requesting this exam and discussing semen collection with patients and parents [23]. This is worrying considering the negative impact of varicocele on sperm parameters during adolescence. In agreement, adolescent with varicocele may have lower sperm count [24] and sperm motility, vitality, and morphology compared to age-matched controls [25], being the sperm motility even more affected as maximal blood flow velocity, basal blood flow velocity, and the pampiniform vein diameter increase [25]. Furthermore, varicocele has been shown to affect sperm concentration and total motile sperm count in Tanner V patients, especially in the case of testicular asymmetry [11].

Despite the lack of consensus, the hormone profile may be useful for the workup of adolescent varicocele. Increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and lower inhibin B serum levels have been found in patients with varicocele [2, 26]. AMH and inhibin B may be particularly useful when puberty is not already started and sperm analysis cannot be performed, especially when gonadotropins and testosterone levels are still not suggesting of puberty start. Indeed, despite testes have been considered silent in childhood for a long time, they

secrete AMH and inhibin B in this phase. Particularly, serum AMH and inhibin B levels have been suggested as markers of testicular function in prepubertal age [13]. Accordingly, impaired AMH and inhibin B levels have been reported in prepubertal and pubertal boys with varicocele [27, 28].

7.4 Management

Since no clear consensus has been still reached, management of adolescent varicocele is partly controversial. Varicocele repair is not always needed due to the spontaneous catch-up growth and sperm recovery that have been observed in some cases. Therefore, conservative management (monitoring and follow-up) can be suggested in selected cases. The current challenge is to identify those markers which may reliably predict which patient would benefit from varicocele repair.

Tanner V patients with no painful varicocele and normal testicular volume may be candidate to conservative management. A retrospective analysis of 216 patients with these features showed a decreased total motile sperm count (<20 million) in 45% of cases at baseline. A half of these patients with poor sperm parameters had a spontaneous recovery, suggesting that poor sperm parameters persist in 22.5% of cases with no painful varicocele and normal testicular volume. No additional marker was used to further typify these patients [29].

Testicular asymmetry has been already addressed as a prognostic marker and may be used in the decisional flowchart. Since catch-up growth has been found to occur with no intervention in 85% of adolescents with a >15% testicular asymmetry [30], different testicular volume measurements at distinct follow-up times should be reasonably performed prior to decide for varicocele repair [30]. The latter could be suggested in case of failure of testicular growth.

Similarly, the PRF has been proposed as a predictor of persistent or worsening testicular asymmetry in adolescent varicocele. According to a recent study, adolescents with testicular asymmetry ≥20% which persists even after 13.2 months of follow-up and PRF ≥38 cm/s should be considered for varicocelectomy. By contrast, those having a PRF <30 cm/s should be monitored [31]. A pilot study has combined testicular volume asymmetry with PRF values in the attempt to increase the accuracy of such markers. Persistent testicular asymmetry was associated with ≥20% asymmetry and PRF >38 cm/s (the so-called 20/38 harbinger). Indeed, 94% of patients with the 20/38 harbinger did not have catch-up growth after a 15.5-month surveillance, thus suggesting that intervention may be required in these patients [32, 33]. When borderline asymmetry or PRF is present, intervention may be indicated in case of abnormality of sperm parameters [34].

To summarize, "at-risk" patients deserving consideration for intervention are those presenting the following signs and symptoms [15]:

(a) Persistent abnormal semen parameters with no evidence of recovery at the follow-up
(b) Pain

(c) Persistent altered testicular volume with asymmetry >15–20% and no evidence of catch-up growth at follow-up
(d) PRF >38 cm/s
(e) Failure of testicular development
(f) 20/38 harbinger (which can be extended to 15% asymmetry)

In addition, decreased AMH levels in children with varicocele may need careful surveillance due to the likely occurrence of Sertoli cell dysfunction [13]. However, longitudinal studies are needed to confirm this hypothesis.

7.5 Treatment Options

Varicocele repair can be made by radiological or surgical intervention. Sixty-four consecutive young patients (age range 13–19 years) have been recently assessed for testicular volume and sperm outcome before and after percutaneous scleroembolization. Compared to the not treated control group, spermatozoa release per unit of testis volume improved significantly. Therefore, early scleroembolization may improve the sperm output in the adolescent phase of testicular growth [35]. Similarly, the efficacy of surgical intervention has clearly been demonstrated by meta-analytic data on 1475 patients. Indeed, the average proportion of catch-up growth following treatment was 76.4%, being the testicular volume asymmetry significantly reduced in the groups with ≥10% and ≥20% asymmetry. This highlights the advantages of surgical intervention when the discrepancy is ≥10% in adolescent varicocele [36].

The impact of varicocele repair on the paternity rate is controversial. A study on 661 patients with varicocele (372, aged 15.3 years, treated with scleroembolization and 289, aged 17.1 years, conservatively followed) reported the achievement of paternity in 85% of those followed-up and in 78% of treated patients, indicating no effect of scleroembolization on the paternity rate later in life [37]. By contrast, microsurgical or surgical varicocele repair during adolescence increased both sperm parameters and the paternity rate (OR 3.63) compared to conservative option [38].

Meta-analytic data of the EAS/ESPU [39] and the ASRM [40] confirm the efficacy of both radiological and surgical approaches, with no demonstration of superiority of any technique. More in detail, the ASRM performed a meta-analysis aimed at understanding whether youth (15–24 years) varicocele may impact on sperm parameters and if its correction improves sperm outcome. Data collected from 357 patients with varicocele and 427 controls showed a significantly decrease in sperm concentration, motility, and morphology. Studies in which varicocele repair was performed using the "Palomo" (open or laparoscopic) technique ($n = 5$), scleroembolization ($n = 1$), and inguinal or subinguinal intervention with magnification ($n = 4$) were included to analyze the second outcome. Overall, varicocele repair improved sperm concentration and motility. Each technique was effective in ameliorating the sperm outcome, despite scleroembolization was used in only one single study [10]. More recently, a meta-analysis on a total of 16,130 patients (7–21 years) developed by the EAS/ESPU societies included testicular volume into the

outcomes. Varicocele repair resulted in testicular volume (+1.52 mL) and sperm concentration (+25.54 million/mL) improvement compared to the observation group. Surgical and radiological interventions were analyzed, finding similar results. Lower frequency of hydrocele formation was observed in lymphatic versus non-lymphatic sparing surgery [39].

Finally, both radiological and surgical approaches may be suggested for varicocele repair. Alternative strategies (e.g., anastomosis of the proximal part of the spermatic vein with the inferior epigastric vein) have to be considered in NcP [40].

7.6 Established Guidelines

No guideline specifically focuses on the management and the treatment of adolescent varicocele. Current knowledge comes from guidelines dealing with the management of male infertility [41]. The ASRM/SMRU/AUA practice committee [41] suggests varicocele evaluation using the Dubin and Amelar clinical classification and to perform Doppler ultrasound only in case it is inconclusive. Treatment is advisable when reduced testicular volume or sperm abnormalities are present, while it is contraindicated in subclinical varicocele. However, a proper testicular volume cutoff to suggest varicocele repair is not indicated, and no specific treatment is recommended. Follow-up is suggested at least annually.

The EAU guidelines on male infertility [42] similarly suggest to use the Dubin and Amelar clinical grading classification and scrotal ultrasound to confirm the clinical findings. However, indications for treatment of adolescent varicocele are not mentioned, and no clear benefit of varicocele repair is mentioned. Subinguinal microscopic approach is depicted to have lower recurrence rate and complications.

By contrast, the benefit of childhood and adolescent varicocele repair has been clearly shown by the recent EAS/ESPU and ASRM meta-analysis [39, 40].

7.7 Conclusions and Authors' Recommendations

The evaluation of varicocele should be clinically performed using the Dubin and Amelar scale at first. Scrotal ultrasound with echo color Doppler should be requested to precisely estimate the testicular volume and asymmetry as well as PRF. Hormone assessment (including AMH and inhibin B in childhood and LH, FSH, and total testosterone in adolescence) should be performed for a more comprehensive evaluation of the testicular function. Importantly, sperm analysis has to be requested at least 1.5 years after puberty onset. Doppler ultrasound of renal vessels should be requested in selected cases (Table 7.3).

The negative impact of childhood and adolescent varicocele on testicular growth and sperm output has been clearly shown. Spontaneous testicular catch-up growth can be observed in some cases. Some markers may be used to identify those patients who will likely benefit from varicocele repair. These mainly include testicular volume asymmetry and PRF. Conservative management may be recommended in

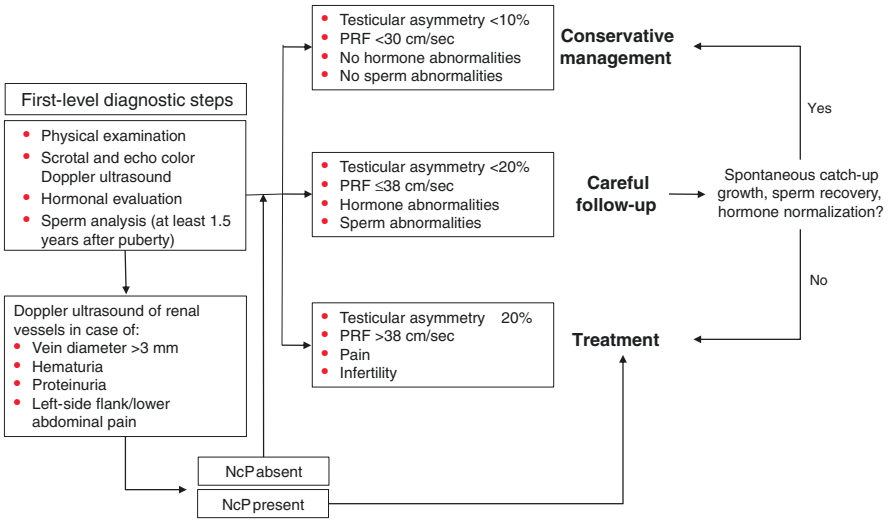

Fig. 7.1 Management of childhood and adolescent varicocele

patients with PRF <30 cm/s, testicular asymmetry <10%, and no evidence of sperm and hormonal abnormalities. In patients with 10–20% testicular volume asymmetry, 30 < PRF ≤ 38 cm/s, and/or sperm abnormalities, careful follow-up is advisable. In case of absent catch-up growth or sperm recovery, varicocele repair could be suggested. Finally, treatment can be indicated at the initial presentation in painful varicocele, testicular volume asymmetry ≥20%, PRF >38 cm/s, infertility, failure of testicular development, and severe sperm abnormalities. Based on the current evidence, both radiological and surgical intervention could be proposed. A practical flowchart of management of adolescent varicocele is proposed in Fig. 7.1.

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References

1. Agarwal A, Deepinder F, Cocuzza M, Agarwal R, Short RA, Sabanegh E, Marmar JL. Efficacy of varicocelectomy in improving semen parameters: new meta-analytical approach. Urology. 2007;70(3):532–8.
2. Damsgaard J, Joensen UN, Carlsen E, Erenpreiss J, Blomberg Jensen M, Matulevicius V, Zilaitiene B, Olesen IA, Perheentupa A, Punab M, Salzbrunn A, Toppari J, Virtanen HE, Juul A, Skakkebæk NE, Jørgensen N. Varicocele is associated with impaired semen quality and reproductive hormone levels: a study of 7035 healthy young men from six European countries. Eur Urol. 2016;70(6):1019–29. https://doi.org/10.1016/j.eururo.2016.06.044. Epub 2016 Jul 14.

3. Akbay E, Cayan S, Doruk E, Duce MN, Bozlu M. The prevalence of varicocele and varicocele-related testicular atrophy in Turkish children and adolescents. BJU Int. 2000;86(4):490–3.
4. Mongioì LM, Mammino L, Compagnone M, Condorelli RA, Basile A, Alamo A, La Vignera S, Morgia G, Russo GI, Calogero AE. Effects of varicocele treatment on sperm conventional parameters: surgical varicocelectomy versus sclerotherapy. Cardiovasc Intervent Radiol. 2019;42(3):396–404.
5. Kirby EW, Wiener LE, Rajanahally S, Crowell K, Coward RM. Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review and meta-analysis. Fertil Steril. 2016;106(6):1338–43. https://doi.org/10.1016/j.fertnstert.2016.07.1093. Epub 2016 Aug 12.
6. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Effects of varicocelectomy on sperm DNA fragmentation, mitochondrial function, chromatin condensation, and apoptosis. J Androl. 2012;33(3):389–96.
7. Li F, Yue H, Yamaguchi K, Okada K, Matsushita K, Ando M, Chiba K, Fujisawa M. Effect of surgical repair on testosterone production in infertile men with varicocele: a meta-analysis. Int J Urol. 2012;19(2):149–54. https://doi.org/10.1111/j.1442-2042.2011.02890.x. Epub 2011 Nov 8.
8. Cho CL, Esteves SC, Agarwal A. Indications and outcomes of varicocele repair. Panminerva Med. 2019;61(2):152–63.
9. Chung JM, Lee SD. Current issues in adolescent varicocele: pediatric urological perspectives. World J Mens Health. 2018;36(2):123–31. https://doi.org/10.5534/wjmh.170053. Epub 2018 Mar 22.
10. Nork JJ, Berger JH, Crain DS, Christman MS. Youth varicocele and varicocele treatment: a meta-analysis of semen outcomes. Fertil Steril. 2014;102(2):381–387.e6.
11. Diamond DA, Zurakowski D, Bauer SB, Borer JG, Peters CA, Cilento BG Jr, Paltiel HJ, Rosoklija I, Retik AB. Relationship of varicocele grade and testicular hypotrophy to semen parameters in adolescents. J Urol. 2007;178(4 Pt 2):1584–8.
12. Hassanin AM, Ahmed HH, Kaddah AN. A global view of the pathophysiology of varicocele. Andrology. 2018;6(5):654–61. https://doi.org/10.1111/andr.12511. Epub 2018 Jul 6.
13. Condorelli RA, Cannarella R, Calogero AE, La Vignera S. Evaluation of testicular function in prepubertal children. Endocrine. 2018;62(2):274–80.
14. Hu JT, Shao CH, Wang PT, Liu Y, Hao WY, Feng YL, Liu SH, Wang XS. High temperature reduces the proliferation of and occludin expression in rat Sertoli cells in vitro. Zhonghua Nan Ke Xue. 2012;18(10):920–4.
15. Macey MR, Owen RC, Ross SS, Coward RM. Best practice in the diagnosis and treatment of varicocele in children and adolescents. Ther Adv Urol. 2018;10(9):273–82. https://doi.org/10.1177/1756287218783900. eCollection 2018 Sep.
16. Dubin L, Amelar RD. Varicocele size and results of varicocelectomy in selected subfertile men with varicocele. Fertil Steril. 1970;21(8):606–9.
17. Pauroso S, Di Leo N, Fulle I, Di Segni M, Alessi S, Maggini E. Varicocele: Ultrasonographic assessment in daily clinical practice. J Ultrasound. 2011;14(4):199–204.
18. Englund KM, Rayment M. Nutcracker syndrome: a proposed ultrasound protocol. Austr J Ultrasound Med Banner. 2018;21(2):75–8.
19. Hannick JH, Blais AS, Kim JK, Traubici J, Shiff M, Book R, Lorenzo AJ. Prevalence, Doppler ultrasound findings, and clinical implications of the nutcracker phenomenon in pediatric varicoceles. Urology. 2019;128:78–83.
20. Condorelli RA, Calogero AE, Vicari E, Mongioi' L, Burgio G, Cannarella R, Giacone F, Iacoviello L, Morgia G, Favilla V, Cimino S, La Vignera S. Reduced seminal concentration of CD45pos cells after follicle-stimulating hormone treatment in selected patients with idiopathic oligoasthenoteratozoospermia. Int J Endocrinol. 2014;2014:372060.
21. Jedrzejewski G, Osemlak P, Wieczorek AP, Nachulewicz P. Prognostic values of shear wave elastography in adolescent boys with varicocele. J Pediatr Urol. 2019;15(3):223.e1–5.

22. Dabaja AA, Wosnitzer MS, Bolyakov A, Schlegel PN, Paduch DA. When to ask male adolescents to provide semen sample for fertility preservation? Transl Androl Urol. 2014;3(1):2–8.
23. Fine RG, Gitlin J, Reda EF, Palmer LS. Barriers to use of semen analysis in the adolescent with a varicocele: survey of patient, parental, and practitioner attitudes. J Pediatr Urol. 2016;12(1):41.e1–6.
24. Haans LC, Laven JS, Mali WP, te Velde ER, Wensing CJ. Testis volumes, semen quality, and hormonal patterns in adolescents with and without a varicocele. Fertil Steril. 1991;56(4):731–6.
25. Paduch DA, Niedzielski J. Semen analysis in young men with varicocele: preliminary study. J Urol. 1996;156(2 Pt 2):788–90.
26. Romeo C, Arrigo T, Impellizzeri P, Manganaro A, Antonuccio P, Di Pasquale G, Messina MF, Marseglia L, Formica I, Zuccarello B. Altered serum inhibin b levels in adolescents with varicocele. J Pediatr Surg. 2007;42(2):390–4.
27. Niu XB, Tang J, Wang HB, Yan L, Zhang CY, Wang GC, Liang J, Dou XY, Fu GB. Inhibin B level helps evaluate the testicular function of prepubertal patients with varicocele. Zhonghua Nan Ke Xue. 2018;24(7):618–21.
28. Trigo RV, Bergadá I, Rey R, Ballerini MG, Bedecarrás P, Bergadá C, Gottlieb S, Campo S. Altered serum profile of inhibin B, pro-alphaC and anti-Müllerian hormone in prepubertal and pubertal boys with varicocele. Clin Endocrinol. 2004;60(6):758–64.
29. Chu DI, Zderic SA, Shukla AR, Srinivasan AK, Tasian GE, Weiss DA, Long CJ, Canning DA, Kolon TF. The natural history of semen parameters in untreated asymptomatic adolescent varicocele patients: A retrospective cohort study. J Pediatr Urol. 2017;13(1):77.e1–5.
30. Kolon TF, Clement MR, Cartwright L, Bellah R, Carr MC, Canning DA, Snyder HM. Transient asynchronous testicular growth in adolescent males with a varicocele. J Urol. 2008;180(3):1111–4; discussion 1114-5.
31. Kozakowski KA, Gjertson CK, Decastro GJ, Poon S, Gasalberti A, Glassberg KI. Peak retrograde flow: a novel predictor of persistent, progressive and new onset asymmetry in adolescent varicocele. J Urol. 2009;181(6):2717–22; discussion 2723.
32. Van Batavia JP, Badalato G, Fast A, Glassberg KI. Adolescent varicocele-is the 20/38 harbinger a durable predictor of testicular asymmetry? J Urol. 2013;189(5):1897–901.
33. Cimador M, Castagnetti M, Gattuccio I, Pensabene M, Sergio M, De Grazia E. The hemodynamic approach to evaluating adolescent varicocele. Nat Rev Urol. 2012;9(5):247–57.
34. Glassberg KI. My indications for treatment of the adolescent varicocele (and why?). Transl Androl Urol. 2014;3(4):402–12.
35. Mancini M, Carrafiello G, Melchiorre F, Pelliccione F, Andreassi A, Mantellassi G, Ahmed Said Z, Pecori Giraldi F, Banderali G, Folli F. Early varicocelectomy by percutaneous sclero-embolization improves seminiferous tubules spermatozoa release in the adolescent phase of testicular growth. Andrologia. 2019;51(7):e13286.
36. Li F, Chiba K, Yamaguchi K, Okada K, Matsushita K, Ando M, Yue H, Fujisawa M. Effect of varicocelectomy on testicular volume in children and adolescents: a meta-analysis. Urology. 2012;79(6):1340–5.
37. Bogaert G, Orye C, De Win G. Pubertal screening and treatment for varicocele do not improve chance of paternity as adult. J Urol. 2013;189(6):2298–303.
38. Çayan S, Şahin S, Akbay E. Paternity rates and time to conception in adolescents with varicocele undergoing microsurgical varicocele repair vs observation only: a single institution experience with 408 patients. J Urol. 2017;198(1):195–201.
39. Silay MS, Hoen L, Quadackaers J, Undre S, Bogaert G, Dogan HS, Kocvara R, Nijman RJM, Radmayr C, Tekgul S, Stein R. Treatment of varicocele in children and adolescents: a systematic review and meta-analysis from the European Association of Urology/European Society for Paediatric Urology Guidelines Panel. Eur Urol. 2019;75(3):448–61.
40. Dong W, Yao Y, Huang H, Han J, Zhao X, Huang J. Surgical management of nutcracker phenomenon presenting as left varicocele in adolescents: a novel approach. J Pediatr Urol. 2014;10(3):424–9.

41. Practice Committee of the American Society for Reproductive Medicine, Society for Male Reproduction and Urology. Report on varicocele and infertility: a committee opinion. Fertil Steril. 2014;102(6):1556–60.
42. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, Krausz C, European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on male infertility: the 2012 update. Eur Urol. 2012;62(2):324–32.

8 Congenital Causes of Hypergonadotropic Hypogonadism: Anorchia and Klinefelter Syndrome

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8.1 Anorchia

8.1.1 Etiology and Diagnosis

Anorchia can be defined as the absence of functional testicular tissue in a normally virilized boy with a normal karyotype. The incidence is approximately 1 in 20,000 live births and in 1 in 177 cases of bilateral cryptorchidism [1]. The condition can be either congenital or acquired. The etiology of congenital anorchia is unclear, but genetic factors together with fetal growth restriction and/or low birth weight, as well as the intrauterine environment, have been suggested. Testicular tissue must have been present and functioning in utero up to at least the 16th week of gestation, whereafter gonads are lost ("vanishing or regressed testis syndrome") after completion of the sexual differentiation until the neonatal period or in early childhood. This is consistent with the association with a blind-ending spermatic cord, as the presence of the spermatic cord structure supports the testis having been present during early intrauterine life [2]. In boys born with non-palpable testes, verification of intra-abdominal functional testicular tissue is important due to increased risk of germ cell cancer later in life. Anorchid patients on the other hand have no functional

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testicular tissue, although atrophic testicular remnants consisting of fibrous tissue may persist. The reports of some familial cases suggest a genetic origin. In fact mutational analysis of *NR5A1* in 24 individuals with bilateral anorchia and micropenis from the French Collaborative Anorchia study found one individual who had a *NR5A1* mutation [3], whereas sequencing of target genes involved in gonadal development and testicular descent (*SRY*, *NR5A1*, *INSL3*, *MAMLD1*) in another study of 26 anorchid patients revealed no mutations [4]. This may suggest that other genes contribute to the phenotype.

8.1.2 Clinical and Biochemical Presentation

Male infants with anorchia typically present with absent testes but normal penile size. However, micropenis has been reported in 5–50% of cases [5].

In boys with non-palpable testes at birth, diagnostic evaluation includes karyotype and reproductive hormones and some forms of abdominal imaging (ultrasound or magnetic resonance imaging (MRI)). Frequently, a laparoscopy is performed as gold standard. Pilot studies have suggested that serum concentrations of anti-Müllerian hormone (AMH) [6], inhibin B, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) may differentiate between boys with bilateral cryptorchidism and anorchia [4]. Moreover, a human chorionic gonadotropin (hCG) stimulation may signify the existence of functional testis tissue [7], whereas ultrasonography, CT, or MRI failed to detect the presence or absence of intra-abdominal testes [8]. Finally, exploratory laparoscopy may be considered, although this remains controversial.

8.2 Klinefelter Syndrome

8.2.1 Etiology and Diagnosis

Klinefelter syndrome (KS) is characterized by the presence of one extra X chromosome, 47,XXY in 80–90% of cases [9], and higher-grade aneuploidies (e.g., 48,XXXY, 49,XXXXY) or mosaicism (47,XXY/46,XY) in the remaining [10, 11]. Males with mosaic KS often have few if any symptoms, whereas the presence of increasing numbers of extra X chromosomes is associated with an increasingly severe phenotype [12]. One in 660 newborn males carries an extra X chromosome [10, 13]. KS is the most frequent sex chromosome disorder in the male and the most frequent genetic cause of infertility found in 3% of infertile and 11% of azoospermic men [14].

The classical description of the adult male with KS includes primary testicular failure with small hyalinized testes, hypergonadotropic hypogonadism, infertility, tall stature, eunuchoidism, and an increased risk for comorbidities (e.g., osteoporosis, metabolic syndrome, and psychosocial problems). However, the phenotypic spectrum is very wide, and the main clinical features may not become evident until after the onset of puberty.

KS may be diagnosed prenatally or in general during three main stages of life: (1) in childhood because of developmental delay, behavioral disturbances, or excessive growth, (2) in peripuberty because of delayed or poor pubertal development, gynecomastia, or small testes or as in earlier stages of life due to behavioral or growth disturbances, or (3) in adulthood during diagnostic workup for infertility, or because of hypogonadism or gynecomastia. Except from small testes, no consistent clinical features or specific abnormalities irrespective of age have been identified, and a definitive diagnosis can only be made by genetic testing [15].

Because of the phenotypic variability and relatively nonspecific and subtle symptoms in childhood and most probably also because of lack of awareness among health professionals, KS is a highly underdiagnosed condition. Approximately 75% of males with KS remain undiagnosed [10]. In addition, in individuals who are diagnosed, less than 10% are diagnosed before puberty [10]. These statistics may be changing as some countries adopt routine genetic screening prenatally [16].

8.2.2 Genetics

KS is characterized by the presence of one or more extra X chromosomes. The extra X chromosome occurs as a consequence of either a paternal nondisjunction in the first meiotic division (approximately 50% of cases) or a maternal nondisjunction in the first or second meiotic division (approximately 50% of cases) or during a postzygotic division [17].

The natural history of the Klinefelter phenotype and the reason for the variable phenotype are not completely elucidated despite extensive research during recent years. The possible influence of gene dosage, skewed X-inactivation, CAG repeat length in exon 1 of the androgen receptor (*AR*) gene, and parental origin of the supernumerary X chromosome has been investigated and seems related to at least some of the phenotypic characteristics of KS, although data are sparse and inconsistent [18]. The CAG repeat length in *AR* may be related to at least some of the phenotypic characteristics of KS, although no clear relation has been identified [12, 19–25]. In addition, no clear phenotypic differences have been identified in studies on the possible influences of parental origin of the X chromosome [19, 26, 27], although one group demonstrated a higher incidence of developmental problems in speech/language (88% versus 59%) and motor impairment (77% versus 46%) in paternally inherited cases [28]. Furthermore, the increased copy number of the *SHOX* gene has been shown to be associated with tall stature [29].

By the use of new technologies and more detailed analysis of the methylome and transcriptome in KS, more recent studies have identified genome-wide alterations and have pointed out new candidate genes including noncoding genes that may be involved in the KS phenotype and comorbidities associated with KS [30–35]. Single-cell sequencing of blood cells from one KS male showed changes in the transcriptome of both autosomes and the X chromosome [32], whereas epigenetic and transcriptional analyses of single germ cells showed a normal DNA methylation profile of selected germ cell-specific markers compared with spermatogonia and

sperm from men with normal spermatogenesis [36]. In the latter study, variations in DNA methylation of imprinted regions were found [36].

8.2.3 Clinical and Biochemical Presentation

8.2.3.1 Infancy

Infants with KS are phenotypically male with normal birth length, weight, and head circumference [37]. The prevalence of cryptorchidism [38] is increased, and small testis volume and reduced penile length have been reported [27, 37], whereas micropenis seems to be rare. Additionally, subtle dysmorphic signs such as fifth finger clinodactyly, high-arched palate, hypertelorism, and hypotonia may be observed [27, 37]. Other congenital anomalies, such as structural heart disease, renal anomalies, cleft lip or palate, or clubfoot, are rarely reported in infants with KS.

Infants with KS present with a hormone surge that resembles but may differ from the so-called minipuberty [37, 39–42]. Most studies of the "minipuberty" in KS are challenged by poor testosterone assay metrics and low numbers of boys. However, one single study based on liquid chromatography/tandem mass spectrometry (LC/MS) measurements reported testosterone (T) concentrations within the normal range albeit significantly lower than controls. Twenty percent of the boys aged 2–180 days had T concentrations below the fifth percentile of the normal range, and only 5 of 38 infants aged 17–152 days presented with a T concentration above the median of the controls [40, 43]. Interestingly, insulin-like peptide-3 (INSL3) concentrations are within the normal range during "minipuberty" [40]. Despite a potential degree of Leydig cell impairment, LH concentrations are normal and correlate with T and INSL3 at this age [37, 39–42].

Although existing studies are quite small, infants with KS have normal inhibin B and AMH but elevated FSH indicating an impaired Sertoli cell function [39–42].

8.2.3.2 Prepubertal Boys

During childhood, growth tends to accelerate, and from the age of 5–6 years, boys with KS are significantly taller than controls and than expected for their genetic target. In addition, they may develop eunuchoid body proportions [27, 29, 44–47]. Importantly, these boys may have normal body proportions and may be of normal or low stature. Testicular volume and penile length are generally smaller than average [27, 48]. In one study, 17% of prepubertal boys met the criteria for micropenis [48].

Hormone assessments in prepubertal boys with KS have similar challenges as mentioned above. In addition, the very low expected serum concentrations of gonadotropins and testosterone are often below the limit of detection for the assays during this developmental stage. Until recently, prepubertal T, INSL3, inhibin B, AMH, LH, and FSH concentrations have been perceived as normal [15, 42, 45, 49–54]. However, newer studies have shed light on the fact that these boys may already be androgen deficient before puberty [27, 48]. Zeger et al. found T concentrations in the lower quartile of normal in 75% of the boys [27], and Davis et al. have

reported T concentrations measured by LC/MS below the lower limit of normal (≤3 ng/dL) in 49% of the prepubertal boys with KS [48]. Interestingly, despite low T, LH concentrations were normal [27] or significantly lower [48] as compared with healthy controls. FSH was increased in 20% of the boys, but no correlation was found between FSH and inhibin B or AMH, which tended to be low and high, respectively, in a subset of the patients [48] (Fig. 8.1).

8.2.3.3 Puberty

In general, boys with KS enter puberty at the expected age, with testicular enlargement and advancing genital development, although penile length may be reduced [15, 27, 49, 52, 55]. The increase in testicular volume peaks around mid-puberty, whereafter a decrease to prepubertal size may be observed [55–57].

Gynecomastia is a physiological phenomenon seen in 20–70% of otherwise healthy pubertal boys [58] and has been reported with an equal frequency (18–59%) in pubertal KS boys [27, 47, 48, 59, 60]. However, gynecomastia may be more likely to persist in KS, particularly if hypogonadism is present and untreated.

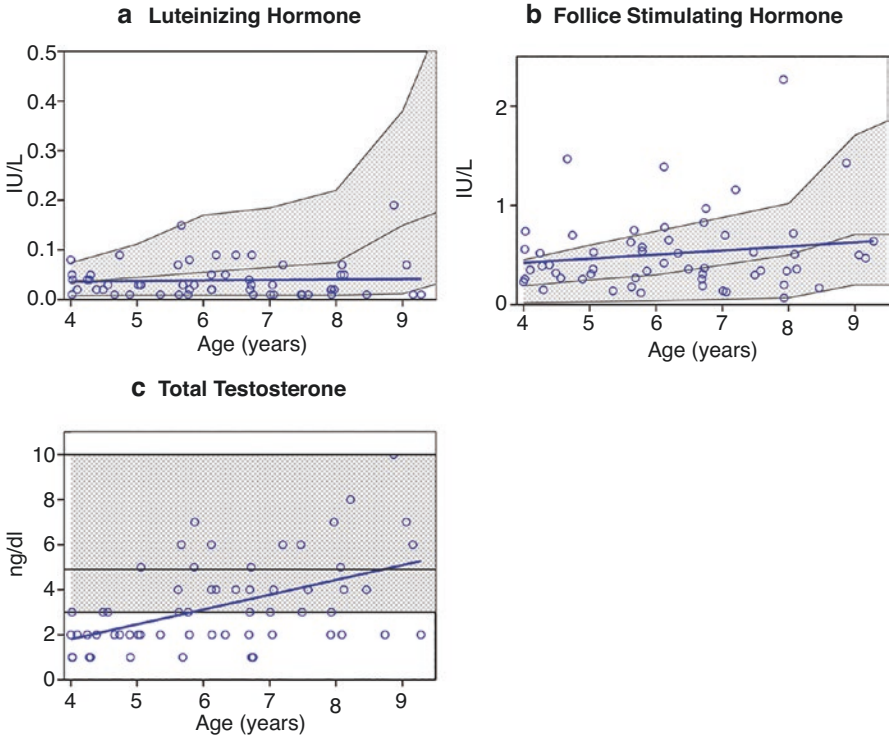

Fig. 8.1 Serum hormone concentrations in 60 prepubertal boys with KS 4–9.5 years of age [48]. Luteinizing hormone (**a**) and total testosterone (**c**) were significantly lower in boys with KS, while follicle-stimulating hormone (**b**) was significantly higher. Individual values are in open circles with a solid regression line; gray-shaded areas are the laboratory-provided normal ranges for age

Hypergonadotropic hypogonadism becomes evident in most boys with KS at the time of puberty. Initially, T and INSL3 concentrations increase in response to increasing concentrations of LH. However, around mid-puberty, LH and FSH increase to very high concentrations followed by a levelling off in T and INSL3 that remain in the lower half of the normal range in the majority, whereas inhibin B declines to an undetectable level [27, 42, 49, 53, 54]. AMH declines in puberty, but the decline is postponed as compared with healthy boys, likely reflecting lower intratesticular testosterone concentrations [42, 53, 57] (Fig. 8.2).

8.2.3.4 Adulthood

According to the first description of patients with KS by Harry F. Klinefelter (1942), the adult patient with KS presents with tall stature, eunuchoid body proportions, gynecomastia, and small testes [61]. However, usually not all, but only some, of these characteristics are present. T concentrations are usually sufficient for normal

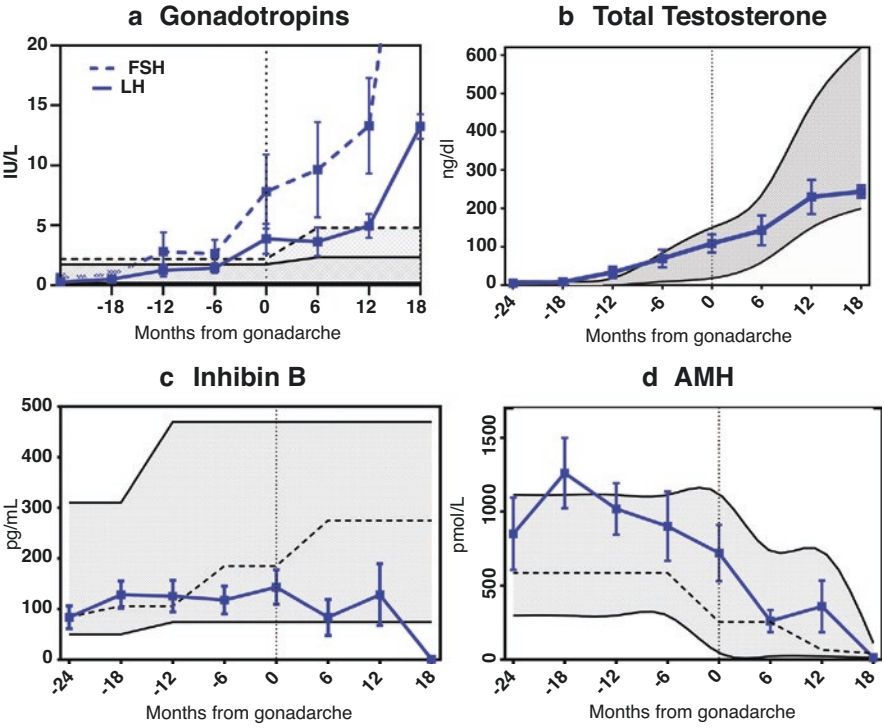

Fig. 8.2 Peripubertal hormone concentrations in 12 boys with KS in the months before and after gonadarche (testicular volume 4 mL). Gonadotropins (**a**) are already elevated at the time of gonadarche and continue to rise. Testosterone (**b**) and inhibin B (**c**) are within the normal range but do not increase as robustly as expected after gonadarche. Anti-Müllerian hormone (**d**) is higher than expected prior to gonadarche, falling to very low concentrations by ~18 months after gonadarche. Data represent the mean and standard error of the mean, with laboratory-provided normal ranges for age within the gray-shaded area

virilization, but some patients have signs of decreased virilization (e.g., lack of voice deepening, sparse face and body hair, lower muscle mass, reduced penile length) [62].

Adult males with KS present with hypergonadotropic hypogonadism with highly elevated LH and FSH and low to low-normal T. AMH and INSL3 concentrations are reduced as compared with healthy males, and inhibin B is typically unmeasurable [42, 54, 63].

8.2.4 Testicular Histology

The histology of the adult KS testes is well described and characterized by extensive fibrosis and hyalinized tubules with patchy areas including Sertoli cell-only tubules, tubules with Sertoli cells and few spermatogonia, and in some cases foci with single tubules with preserved complete spermatogenesis. The presence of two types of Sertoli cell-only tubules (type A and B) with either differentiated Sertoli cells or immature sex-chromatin-positive Sertoli cells, respectively, is pathognomonic for the histology in KS in adulthood [64]. Additionally, massive Leydig cell hyperplasia and the presence of large Leydig cell nodules are characteristic [65–67] (Fig. 8.3).

It has been the general perception that the testicular degeneration and depletion of the germ cells accelerate at the onset of puberty [57], whereas data from Klinefelter fetuses and prepubertal boys have been sparse and contradictory [68]. More recent studies have shown germ cell loss in KS fetuses as evidenced by an absent increase in MAGE-A4-positive cells [34], whereas Van Saen et al. found a high variation in the number of germ cells within samples but no difference in the number of MAGE-A4-positive cells in fetuses with KS as compared to controls [67]. In the latter study, the number of MAGE-A4-positive cells was reduced in pre- and peripubertal KS boys aged 4–16 years as compared to controls [67], and fibrosis was first observed in peripubertal boys.

8.2.5 Fertility

The majority of patients with KS have nonobstructive azoospermia and have historically been regarded as sterile, although motile spermatozoa may be found in the ejaculate in a small subset of patients [12, 62, 69, 70]. For more than two decades, however, fertility preservation has been possible using testicular sperm extraction (TESE) or micro-TESE (mTESE) combined with intracytoplasmic sperm injection (ICSI). The sperm retrieval rates are 43% and 45% using TESE and mTESE, respectively [71]. Birth rates are not reported in the majority of studies of TESE outcome, but a recent study found that only 10.1% of KS couples pursuing fertility preservation treatment had their biologically own child(ren) [72].

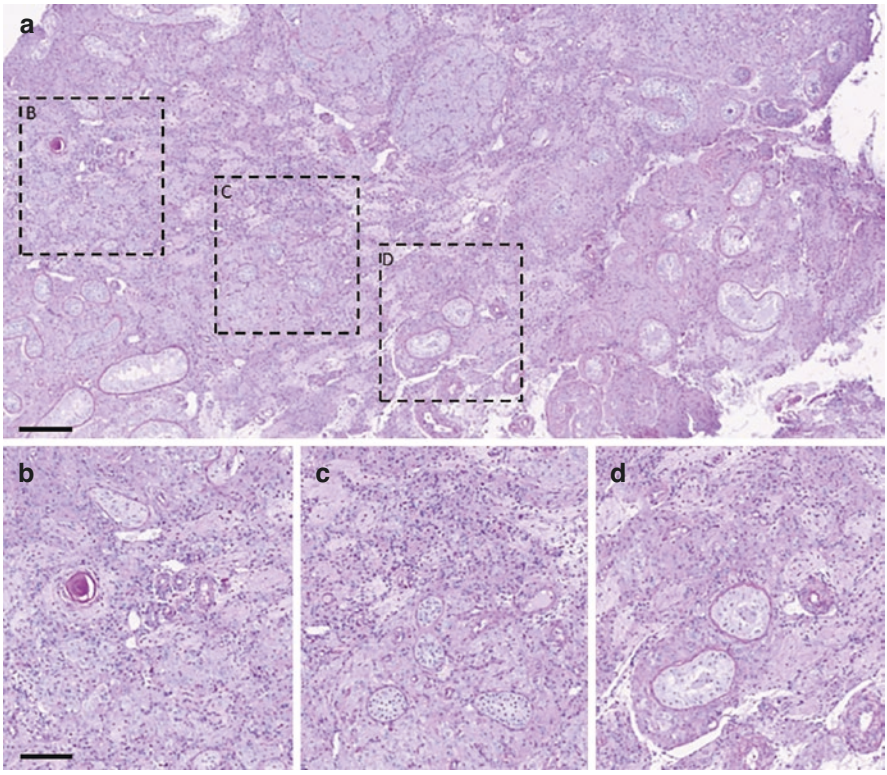

Fig. 8.3 Testicular histology in a 22-year-old patient with 47, XXY. (**A**) Heterogeneous adult pattern showing large Leydig cell nodules, "ghost tubules," type A and B Sertoli cells, and a microlith. (**B**) Microlith. (**C**) Incompletely differentiated type B Sertoli cells. (**D**) Differentiated type A Sertoli cells. The bar in A denotes 250 μm, the bar in B denotes 100 μm, and the same magnification is used in **C** and **D**. All stained with periodic acid-Schiff

No predictors of successful TESE/mTESE have been identified although some studies have shown higher sperm retrieval rates with younger age [73–83]. Based on histological studies suggesting an accelerated germ cell depletion at the onset of puberty, TESE/mTESE has been offered to prepubertal and adolescent KS boys for cryopreservation of spermatozoa and, in research protocols, spermatogonial stem cells or testicular tissue [67, 80, 84–87]. However, a recent meta-analysis failed to document an effect of age [71], and indeed, sperm retrieval rates seem to be lower in adolescents younger than 16 years [86].

Given that exogenous testosterone suppresses the hypothalamic-pituitary-gonadal axis, there is at least hypothetical concern that testosterone treatment may reduce sperm retrieval rates. History of testosterone treatment did not alter the success of TESE in one study [80], whereas others have reported high success rates in 14–22-year-old KS adolescents even with current treatment with topical testosterone and aromatase inhibitor [88]. However, in the meta-analysis by Corona et al., it

was concluded that data on retrieval rates and effects of testosterone treatment were insufficient for conclusions [71].

A recent 24-question survey on current practices regarding fertility and andrology was submitted to members of the Society for the Study of Male Reproduction, the Pediatric Endocrine Society, and the Endocrine Society. Fertility preservation practices in adolescents with KS varied between the 232 experts who responded. All specialties encouraged sperm banking in late puberty but disagreed with the practice in early puberty [89].

8.2.6 Neurodevelopmental and Psychosocial Presentation

Boys with KS have a higher prevalence of hypotonia, which may contribute to motor delays. Language delays are also more common in boys with KS, and in prospective birth cohort studies, 75% of boys received speech therapy in early childhood [90]. Average cognitive abilities (IQ) are in the normal range but 5–10 points lower than sibling controls, with relative strengths in nonverbal domains and weaknesses in verbal domains. Learning disabilities (particularly in reading) affect over half of boys with KS [91]. Executive function deficits and attention-deficit disorder are also appreciated in KS and can manifest as behavioral difficulties [92–94]. Social deficits can be present, and around 10% of boys meet criteria for autism spectrum disorder [95, 96]. Psychiatric conditions can also manifest in later childhood and adolescence, with anxiety and depression being the most common and often exacerbated by social, behavioral, or learning difficulties.

8.2.7 Gender Identity, Sexuality, and Sexual Function

Most men with KS experience normal sexual function [97, 98], have the same number of sexual partners and same intercourse frequency, but may have later sexual debut as compared with controls [99]. However, a recent study assessing sexual function using International Index of Erectile Function Questionnaires (IIEF-15) found significantly more erectile dysfunction (ED), decreased orgasmic function, and lower intercourse satisfaction, as well as delayed ejaculation in KS patients (n = 132) as compared with controls (n = 313) from the general population (matched on age, zip code, and educational level) [99]. Lower sexual desire in KS may be associated with testosterone deficiency and was shown to improve after testosterone therapy [100]. In contrast, ED was associated with neurocognitive, psychosexological, and relational problems, and no effect of testosterone therapy was observed [100].

Significantly more men with KS than controls have reported being homosexual (3.4% versus 0.4%) or bisexual (6.7% versus 1.1%) in one study [99], and gender dysphoria and hypersexuality have also been reported [101–104].

8.2.8 Comorbidities

Sparse data report skeletal abnormalities (scoliosis, kyphosis, pes planus, elbow dysplasia, pectus excavatum, and pectus carinatum), high-arched palate, fifth finger clinodactyly, and joint laxity [27, 47]. In adult men with KS, bone mineral density is decreased, and there are a higher morbidity and mortality from hip and vertebral fractures; however bone mineral density in children with KS was reported as normal in one study [105]. Hand tremor may be observed during childhood, present in 4 out of 16 boys in one study [47], and can worsen with age, particularly in males with supernumerary sex chromosomes [106, 107]. Seizure disorders may be more common than the general population but affect a minority of males with KS [108].

Adult men with KS have a high prevalence of type 2 diabetes and cardiovascular disease, leading to a high morbidity and mortality [11, 109–113]. Type 2 diabetes affects approximately 20% of men with KS, and as many as 50% have metabolic syndrome. Precursors of this unhealthy metabolic profile may be appreciated at a young age. Boys with KS tend to have normal body mass index (BMI) but increased waist-to-hip ratio and body fat percentage suggestive of central adiposity [48, 53, 105]. Prepubertal boys have also been shown to have dyslipidemia with high triglycerides and low HDL cholesterol, characteristic of insulin resistance and potentially a precursor to the metabolic dysfunction appreciated in men with KS [46, 48].

The risk of autoimmune diseases (lupus, rheumatoid arthritis, Sjögren syndrome, Addison's disease, hypothyroidism, and type 1 diabetes mellitus) is higher in males with KS compared to males without KS [114, 115].

The overall malignancy risk is not higher in KS than the general population [116]; however certain types of cancers do seem to be more prevalent than in males without KS, including breast cancer and mediastinal germ cell tumors [117, 118]. While there are many case reports of hematological malignancies in KS, an epidemiological study does not support an increased risk of leukemias or lymphomas [119].

8.3 Testosterone Treatment

8.3.1 Anorchia

Testosterone treatment of boys with anorchia to induce puberty is standard of care. Pubertal virilization in anorchid boys can be achieved by testosterone substitution using oral or transdermal routes of administration or by the use of intramuscular depot injections depending on availability and national practice [120]. In infants with anorchia and micropenis, early treatment may significantly stimulate penile growth [5].

Testosterone replacement therapy in nine anorchid boys virilized the external genitals and stimulated pubertal growth spurt [121, 122]. In these boys, the average testosterone-induced height gain during puberty amounted to 21.7 cm, which was

slightly lower than the expected height gain during puberty (25 cm) [122], suggesting that protocols for androgen therapy during puberty may need to be optimized. A small retrospective study on men with anorchia who were treated with testosterone from adolescence reported normal bone mineral density but mild reductions in cortical area and thickness, despite T treatment [123].

8.3.2 Klinefelter Syndrome

Only limited studies of the effect of testosterone treatment in KS exist, and most studies are retrospective in nature and not based on randomized trials with the inherent risk of biasing the results. There are no evidence-based recommendations for when to start testosterone treatment in KS, but it is widely accepted to consider it as puberty starts and the concentration of LH increases above the normal range for pubertal stage. On average, LH rises above the normal range around 2 years after pubertal onset. Gynecomastia, eunuchoid body proportions, tall stature, and symptoms of hypogonadism may also support the need for initiating treatment. Multiple testosterone formulations are available. Dosing should be titrated to restore serum testosterone concentrations to the middle of the normal range for age and pubertal stage without exceeding the upper limit of the physiologic range. The goals of treatment include developing secondary sexual characteristics, maximizing peak bone mass acquisition (and reducing bone loss), contributing to a more favorable body composition, and supporting psychosocial health. For patients in late adolescence or adulthood, fertility wishes should be discussed prior to initiating testosterone treatment.

Treatment with testosterone prior to puberty has been of interest within the KS community. A short course of testosterone treatment for infancy or children with micropenis has been accepted in the endocrine and urologic communities for many decades, and a minority of boys with KS will meet the criteria for micropenis. Retrospective reports by Samango-Sprouse et al. compared untreated KS boys with KS boys who received testosterone treatment (monthly injection of 25 mg testosterone enanthate for 3 months) because of diminished penis (but not micropenis) at an age ranging from 4 to 15 months. At ages 36 and 72 months, the treated boys had better outcomes in aspects of neuromotor, speech and language, intellectual, and reading function as compared with the untreated boys [124]. A randomized pilot study found that infants with KS who were not treated with testosterone had excess accumulation of adiposity that was significantly higher than typical boys by 5 months of age; however the ten infants receiving a 3-month course of testosterone did not have this increase [125]. Additional prospective studies are needed to evaluate the benefits and potential risks of testosterone treatment during infancy.

To date, only one double-blind, randomized, placebo-controlled study has been conducted in childhood. Ross et al. evaluated treatment with low-dose synthetic oral androgen (oxandrolone) in prepubertal boys aged 4–12 years, [126–128] with a goal to restore normal physiological androgen levels and evaluate effects on motor function/strength, cognition and psychosocial function, cardiometabolic health, and

onset of gonadarche/pubarche. The authors reported improvements in cardiometabolic health with a significant reduction in body fat and triglycerides but also a reduction in high-density lipoprotein (HDL). Measures of anxiety and visual-motor integration improved compared with placebo, but no significant changes in cognition or motor function were observed. Unfortunately, the oxandrolone-treated boys had an unexpected increased risk of early gonadarche and pubarche, as well as advanced bone age, although no significant differences in testosterone and gonadotropin concentrations between treated and untreated boys were found. Samango-Sprouse et al. also recently reported supplementary positive effects of the combination of early (before 5 years) treatment and treatment with testosterone booster between 5 and 10 years as compared with only early treatment. However, this was retrospective, and side effects were not discussed [129]. At this time, caution should be used if considering giving androgen treatment between the minipuberty period (~<6 months of age) and true puberty (~10 years of age), as physiologic levels of testosterone are quite low in typical boys at this age and chronic or high exposure may disrupt the normal hypothalamic-pituitary-gonadal axis and lead to premature puberty.

One double-blind, placebo-controlled study including 13 adult males with KS showed a positive effect of treatment with oral testosterone undecanoate on body composition (total body fat mass and abdominal fat mass) but no effect on glucose homeostasis or free fatty acids [130].

Clinical symptoms of hypogonadism (28%), rising gonadotropin levels (15%), and inadequate testosterone levels (15%) were the most commonly cited reasons for initiation of testosterone replacement therapy reported by 232 experts [89].

In conclusion, congenital hypergonadotropic hypogonadism may be diagnosed in infancy or childhood. While testosterone treatment is standard at the age of male puberty, treatment earlier in childhood is still a research issue. There are many unanswered questions such as when to treat, at what age to start treatment, and what dose and testosterone preparation to use. Ongoing research will begin to address these questions.

References

1. Aynsley-Green A, Zachmann M, Illig R, Rampini S, Prader A. Congenital bilateral anorchia in childhood: a clinical, endocrine and therapeutic evaluation of twenty-one cases. Clin Endocrinol. 1976;5(4):381–91.
2. Smith NM, Byard RW, Bourne AJ. Testicular regression syndrome--a pathological study of 77 cases. Histopathology. 1991;19(3):269–72.
3. Philibert P, Zenaty D, Lin L, Soskin S, Audran F, Leger J, et al. Mutational analysis of steroidogenic factor 1 (NR5a1) in 24 boys with bilateral anorchia: a French collaborative study. Hum Reprod. 2007;22(12):3255–61.
4. Brauner R, Neve M, Allali S, Trivin C, Lottmann H, Bashamboo A, et al. Clinical, biological and genetic analysis of anorchia in 26 boys. PLoS One. 2011;6(8):e23292.
5. Zenaty D, Dijoud F, Morel Y, Cabrol S, Mouriquand P, Nicolino M, et al. Bilateral anorchia in infancy: occurence of micropenis and the effect of testosterone treatment. J Pediatr. 2006;149(5):687–91.

6. Lee MM, Donahoe PK, Silverman BL, Hasegawa T, Hasegawa Y, Gustafson ML, et al. Measurements of serum mullerian inhibiting substance in the evaluation of children with nonpalpable gonads. N Engl J Med. 1997;336(21):1480–6.
7. McEachern R, Houle AM, Garel L, Van VG. Lost and found testes: the importance of the hCG stimulation test and other testicular markers to confirm a surgical declaration of anorchia. Horm Res. 2004;62(3):124–8.
8. De RM, Lupoli G, Mennitti M, Zarrilli S, Mirone V, Lombardi G. Congenital bilateral anorchia: clinical, hormonal and imaging study in 12 cases. Andrologia. 1996;28(5):281–5.
9. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature. 1959;183(4657):302–3.
10. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab. 2003;88(2):622–6.
11. Swerdlow AJ, Higgins CD, Schoemaker MJ, Wright AF, Jacobs PA. Mortality in patients with Klinefelter syndrome in Britain: a cohort study. J Clin Endocrinol Metab. 2005;90(12):6516–22.
12. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet. 2004;364(9430):273–83.
13. Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Birth Defects Orig Artic Ser. 1990;26(4):209–23.
14. Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, et al. Cytogenetics of infertile men. Hum Reprod. 1996;11(Suppl 4):1–24.
15. Aksglaede L, Skakkebaek NE, Almstrup K, Juul A. Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience. Acta Paediatr. 2011;100(6):793–806.
16. Bianchi DW, Wilkins-Haug L. Integration of noninvasive DNA testing for aneuploidy into prenatal care: what has happened since the rubber met the road? Clin Chem. 2014;60(1):78–87.
17. Maiburg M, Repping S, Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. Fertil Steril. 2012;98(2):253–60.
18. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebaek A. Klinefelter syndrome: integrating genetics, neuropsychology, and endocrinology. Endocr Rev. 2018;39(4):389–423.
19. Zinn AR, Ramos P, Elder FF, Kowal K, Samango-Sprouse C, Ross JL. Androgen receptor CAGn repeat length influences phenotype of 47,XXY (Klinefelter) syndrome. J Clin Endocrinol Metab. 2005;14
20. Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E. X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab. 2004;89(12):6208–17.
21. Bojesen A, Hertz JM, Gravholt CH. Genotype and phenotype in Klinefelter syndrome - impact of androgen receptor polymorphism and skewed X inactivation. Int J Androl. 2011;34(6 Pt 2):e642–8.
22. Ferlin A, Schipilliti M, Vinanzi C, Garolla A, Di Mambro A, Selice R, et al. Bone mass in subjects with Klinefelter syndrome: role of testosterone levels and androgen receptor gene CAG polymorphism. J Clin Endocrinol Metab. 2011;96(4):739–45.
23. Wikstrom AM, Painter JN, Raivio T, Aittomaki K, Dunkel L. Genetic features of the X chromosome affect pubertal development and testicular degeneration in adolescent boys with Klinefelter syndrome. Clin Endocrinol. 2006;65(1):92–7.
24. Ross JL, Roeltgen DP, Stefanatos G, Benecke R, Zeger MP, Kushner H, et al. Cognitive and motor development during childhood in boys with Klinefelter syndrome. Am J Med Genet A. 2008;146A(6):708–19.
25. Chang S, Skakkebaek A, Trolle C, Bojesen A, Hertz JM, Cohen A, et al. Anthropometry in Klinefelter syndrome—multifactorial influences due to CAG length, testosterone treatment and possibly intrauterine hypogonadism. J Clin Endocrinol Metab. 2015;100(3):E508–17.

26. Ross NL, Wadekar R, Lopes A, Dagnall A, Close J, Delisi LE, et al. Methylation of two Homo sapiens-specific X-Y homologous genes in Klinefelter's syndrome (XXY). Am J Med Genet B Neuropsychiatr Genet. 2006;141B(5):544–8.
27. Zeger MP, Zinn AR, Lahlou N, Ramos P, Kowal K, Samango-Sprouse C, et al. Effect of ascertainment and genetic features on the phenotype of Klinefelter syndrome. J Pediatr. 2008;152(5):716–22.
28. Stemkens D, Roza T, Verrij L, Swaab H, van Werkhoven M, Alizadeh B, et al. Is there an influence of X-chromosomal imprinting on the phenotype in Klinefelter syndrome? A clinical and molecular genetic study of 61 cases. Clin Genet. 2006;70(1):43–8.
29. Ottesen AM, Aksglaede L, Garn I, Tartaglia N, Tassone F, Gravholt CH, et al. Increased number of sex chromosomes affects height in a nonlinear fashion: a study of 305 patients with sex chromosome aneuploidy. Am J Med Genet A. 2010;152A(5):1206–12.
30. Skakkebaek A, Nielsen MM, Trolle C, Vang S, Hornshoj H, Hedegaard J, et al. DNA hypermethylation and differential gene expression associated with Klinefelter syndrome. Sci Rep. 2018;8(1):13,740.
31. Belling K, Russo F, Jensen AB, Dalgaard MD, Westergaard D, Rajpert-De ME, et al. Klinefelter syndrome comorbidities linked to increased X chromosome gene dosage and altered protein interactome activity. Hum Mol Genet. 2017;26(7):1219–29.
32. Liu X, Tang D, Zheng F, Xu Y, Guo H, Zhou J, et al. Single-cell sequencing reveals the relationship between phenotypes and genotypes of Klinefelter syndrome. Cytogenet Genome Res. 2019;159:55–65.
33. Winge SB, Dalgaard MD, Belling KG, Jensen JM, Nielsen JE, Aksglaede L, et al. Transcriptome analysis of the adult human Klinefelter testis and cellularity-matched controls reveals disturbed differentiation of Sertoli- and Leydig cells. Cell Death Dis. 2018;9(6):586.
34. Winge SB, Dalgaard MD, Jensen JM, Graem N, Schierup MH, Juul A, et al. Transcriptome profiling of fetal Klinefelter testis tissue reveals a possible involvement of long non-coding RNAs in gonocyte maturation. Hum Mol Genet. 2018;27(3):430–9.
35. Huang J, Zhang L, Deng H, Chang L, Liu Q, Liu P. Global transcriptome analysis of peripheral blood identifies the most significantly down-regulated genes associated with metabolism regulation in Klinefelter syndrome. Mol Reprod Dev. 2015;82(1):17–25.
36. Laurentino S, Heckmann L, Di PS, Li X, Meyer Zu HG, Wistuba J, et al. High-resolution analysis of germ cells from men with sex chromosomal aneuploidies reveals normal transcriptome but impaired imprinting. Clin Epigenetics. 2019;11(1):127.
37. Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome. Horm Res. 2005;64(1):39–45.
38. Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. J Clin Endocrinol Metab. 2006;91(4):1254–60.
39. Aksglaede L, Petersen JH, Main KM, Skakkebaek NE, Juul A. High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. Eur J Endocrinol. 2007;157(3):345–50.
40. Cabrol S, Ross JL, Fennoy I, Bouvattier C, Roger M, Lahlou N. Assessment of Leydig and Sertoli cell functions in infants with nonmosaic Klinefelter syndrome: insulin-like peptide 3 levels are normal and positively correlated with LH levels. J Clin Endocrinol Metab. 2011;96(4):E746–53.
41. Lahlou N, Fennoy I, Carel JC, Roger M. Inhibin B and anti-Mullerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. J Clin Endocrinol Metab. 2004;89(4):1864–8.
42. Aksglaede L, Christiansen P, Sorensen K, Boas M, Linneberg A, Main KM, et al. Serum concentrations of anti-Mullerian hormone (AMH) in 95 patients with Klinefelter syndrome with or without cryptorchidism. Acta Paediatr. 2011;100(6):839–45.
43. Fennoy I. Testosterone and the child (0-12 years) with Klinefelter syndrome (47XXY): a review. Acta Paediatr. 2011;100(6):846–50.

44. Ratcliffe SG, Butler GE, Jones M. Edinburgh study of growth and development of children with sex chromosome abnormalities. IV Birth defects. Orig Artic Ser. 1990;26(4):1–44.
45. Aksglaede L, Skakkebaek NE, Juul A. Abnormal sex chromosome constitution and longitudinal growth: serum levels of insulin-like growth factor (IGF)-I, IGF binding protein-3, luteinizing hormone, and testosterone in 109 males with 47,XXY, 47,XYY, or sex-determining region of the Y chromosome (SRY)-positive 46,XX karyotypes. J Clin Endocrinol Metab. 2008;93(1):169–76.
46. Bardsley MZ, Falkner B, Kowal K, Ross JL. Insulin resistance and metabolic syndrome in prepubertal boys with Klinefelter syndrome. Acta Paediatr. 2011;100(6):866–70.
47. Close S, Fennoy I, Smaldone A, Reame N. Phenotype and adverse quality of life in boys with Klinefelter syndrome. J Pediatr. 2015;167(3):650–7.
48. Davis S, Lahlou N, Bardsley M, Temple MC, Kowal K, Pyle L, et al. Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome. Andrology. 2016;4(6):1169–77.
49. Wikstrom AM, Dunkel L, Wickman S, Norjavaara E, Ankarberg-Lindgren C, Raivio T. Are adolescent boys with Klinefelter syndrome androgen deficient? A longitudinal study of Finnish 47,XXY boys. Pediatr Res. 2006;59(6):854–9.
50. Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z, Laron Z. Puberty in 24 patients with Klinefelter syndrome. Eur J Pediatr. 1982;139(1):8–12.
51. Christiansen P, Andersson AM, Skakkebaek NE. Longitudinal studies of inhibin B levels in boys and young adults with Klinefelter syndrome. J Clin Endocrinol Metab. 2003;88(2):888–91.
52. Salbenblatt JA, Bender BG, Puck MH, Robinson A, Faiman C, Winter JS. Pituitary-gonadal function in Klinefelter syndrome before and during puberty. Pediatr Res. 1985;19(1):82–6.
53. Bastida MG, Rey RA, Bergada I, Bedecarras P, Andreone L, del Rey G, et al. Establishment of testicular endocrine function impairment during childhood and puberty in boys with Klinefelter syndrome. Clin Endocrinol. 2007;67(6):863–70.
54. Wikstrom AM, Bay K, Hero M, Andersson AM, Dunkel L. Serum insulin-like factor 3 levels during puberty in healthy boys and boys with Klinefelter syndrome. J Clin Endocrinol Metab. 2006;91(11):4705–8.
55. Ratcliffe SG, Murray L, Teague P. Edinburgh study of growth and development of children with sex chromosome abnormalities. III. Birth defects. Orig Artic Ser. 1986;22(3):73–118.
56. Robinson A, Bender BG, Borelli JB, Puck MH, Salbenblatt JA, Winter JS. Sex chromosomal aneuploidy: prospective and longitudinal studies. Birth Defects Orig Artic Ser. 1986;22(3):23–71.
57. Wikstrom AM, Raivio T, Hadziselimovic F, Wikstrom S, Tuuri T, Dunkel L. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. J Clin Endocrinol Metab. 2004;89(5):2263–70.
58. Mieritz MG, Raket LL, Hagen CP, Nielsen JE, Talman ML, Petersen JH, et al. A longitudinal study of growth, sex steroids, and IGF-1 in boys with physiological gynecomastia. J Clin Endocrinol Metab. 2015;100(10):3752–9.
59. Ratcliffe SG, Bancroft J, Axworthy D, McLaren W. Klinefelter's syndrome in adolescence. Arch Dis Child. 1982;57(1):6–12.
60. Girardin CM, Lemyre E, Alos N, Deal C, Huot C, Van VG. Comparison of adolescents with Klinefelter syndrome according to the circumstances of diagnosis: amniocentesis versus clinical signs. Horm Res. 2009;72(2):98–105.
61. Klinefelter HF, Reifenstein EC, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis without A-leydigism, and increased excretion of follicle-stimulating hormone. J Clin Endocrinol. 1942;2:615–27.
62. Rohayem J, Nieschlag E, Zitzmann M, Kliesch S. Testicular function during puberty and young adulthood in patients with Klinefelter's syndrome with and without spermatozoa in seminal fluid. Andrology. 2016;4(6):1178–86.
63. Foresta C, Bettella A, Vinanzi C, Dabrilli P, Meriggiola MC, Garolla A, et al. A novel circulating hormone of testis origin in humans. J Clin Endocrinol Metab. 2004;89(12):5952–8.

64. Skakkebaek NE. Two types of tubules containing only Sertoli cells in adults with Klinefelter's syndrome. Nature. 1969;223(206):643–5.
65. Aksglaede L, Wikstrom AM, Rajpert-De ME, Dunkel L, Skakkebaek NE, Juul A. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. Hum Reprod Update. 2006;12(1):39–48.
66. Foresta C, Galeazzi C, Bettella A, Marin P, Rossato M, Garolla A, et al. Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. J Clin Endocrinol Metab. 1999;84(10):3807–10.
67. Van SD, Vloeberghs V, Gies I, Mateizel I, Sermon K, De SJ, et al. When does germ cell loss and fibrosis occur in patients with Klinefelter syndrome? Hum Reprod. 2018;33(6):1009–22.
68. Oates RD. The natural history of endocrine function and spermatogenesis in Klinefelter syndrome: what the data show. Fertil Steril. 2012;98(2):266–73.
69. Kitamura M, Matsumiya K, Koga M, Nishimura K, Miura H, Tsuji T, et al. Ejaculated spermatozoa in patients with non-mosaic Klinefelter's syndrome. Int J Urol. 2000;7(3):88–92.
70. Aksglaede L, Jorgensen N, Skakkebaek NE, Juul A. Low semen volume in 47 adolescents and adults with 47,XXY Klinefelter or 46,XX male syndrome. Int J Androl. 2009;32(4):376–84.
71. Corona G, Pizzocaro A, Lanfranco F, Garolla A, Pelliccione F, Vignozzi L, et al. Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2017;23(3):265–75.
72. Vloeberghs V, Verheyen G, Santos-Ribeiro S, Staessen C, Verpoest W, Gies I, et al. Is genetic fatherhood within reach for all azoospermic Klinefelter men? PLoS One. 2018;13(7):e0200300.
73. Garolla A, Selice R, Menegazzo M, Valente U, Zattoni F, Iafrate M, et al. Novel insights on testicular volume and testosterone replacement therapy in Klinefelter patients undergoing testicular sperm extraction. A retrospective clinical study. Clin Endocrinol. 2018;88(5):711–8.
74. Westlander G, Ekerhovd E, Bergh C. Low levels of serum inhibin B do not exclude successful sperm recovery in men with nonmosaic Klinefelter syndrome. Fertil Steril. 2003;79(Suppl 3):1680–2.
75. Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? Hum Reprod. 2004;19(5):1135–9.
76. Okada H, Goda K, Yamamoto Y, Sofikitis N, Miyagawa I, Mio Y, et al. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. Fertil Steril. 2005;84(6):1662–4.
77. Ferhi K, Avakian R, Griveau JF, Guille F. Age as only predictive factor for successful sperm recovery in patients with Klinefelter's syndrome. Andrologia. 2009;41(2):84–7.
78. Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab. 2005;90(11):6263–7.
79. Koga M, Tsujimura A, Takeyama M, Kiuchi H, Takao T, Miyagawa Y, et al. Clinical comparison of successful and failed microdissection testicular sperm extraction in patients with nonmosaic Klinefelter syndrome. Urology. 2007;70(2):341–5.
80. Plotton I, Giscard ES, Cuzin B, Brosse A, Benchaib M, Lornage J, et al. Preliminary results of a prospective study of testicular sperm extraction in young versus adult patients with nonmosaic 47,XXY Klinefelter syndrome. J Clin Endocrinol Metab. 2015;100(3):961–7.
81. Emre BM, Erden HF, Kaplancan T, Ciray N, Bener F, Bahceci M. Aging may adversely affect testicular sperm recovery in patients with Klinefelter syndrome. Urology. 2006;68(5):1082–6.
82. Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. J Urol. 2009;182(3):1108–13.
83. Fullerton G, Hamilton M, Maheshwari A. Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009? Hum Reprod. 2010;25(3):588–97.
84. Van SD, Gies I, De SJ, Tournaye H, Goossens E. Can pubertal boys with Klinefelter syndrome benefit from spermatogonial stem cell banking? Hum Reprod. 2012;27(2):323–30.

85. Nahata L, Yu RN, Paltiel HJ, Chow JS, Logvinenko T, Rosoklija I, et al. Sperm retrieval in adolescents and young adults with Klinefelter syndrome: a prospective, pilot study. J Pediatr. 2016;170:260–5.
86. Franik S, Hoeijmakers Y, D'Hauwers K, Braat DD, Nelen WL, Smeets D, et al. Klinefelter syndrome and fertility: sperm preservation should not be offered to children with Klinefelter syndrome. Hum Reprod. 2016;31(9):1952–9.
87. Gies I, De SJ, Van SD, Anckaert E, Goossens E, Tournaye H. Failure of a combined clinical- and hormonal-based strategy to detect early spermatogenesis and retrieve spermatogonial stem cells in 47,XXY boys by single testicular biopsy. Hum Reprod. 2012;27:998–1004.
88. Mehta A, Paduch DA, Schlegel PN. Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor. Fertil Steril. 2013;100(4):e27.
89. Zganjar A, Nangia A, Sokol R, Ryabets A, Samplaski MK. Fertility in adolescents with Klinefelter syndrome: a survey of current clinical practice. J Clin Endocrinol Metab. 2019;14
90. Robinson A, Bender BG, Linden MG. Summary of clinical findings in children and young adults with sex chromosome anomalies. Birth Defects Orig Artic Ser. 1990;26(4):225–8.
91. Pennington BF, Bender B, Puck M, Salbenblatt J, Robinson A. Learning disabilities in children with sex chromosome anomalies. Child Dev. 1982;53(5):1182–92.
92. Lee NR, Wallace GL, Clasen LS, Lenroot RK, Blumenthal JD, White SL, et al. Executive function in young males with Klinefelter (XXY) syndrome with and without comorbid attention-deficit/hyperactivity disorder. J Int Neuropsychol Soc. 2011;22:1–9.
93. Tartaglia NR, Ayari N, Hutaff-Lee C, Boada R. Attention-deficit hyperactivity disorder symptoms in children and adolescents with sex chromosome aneuploidy: XXY, XXX, XYY, and XXYY. J Dev Behav Pediatr. 2012;33(4):309–18.
94. Van RS, Swaab H. Executive dysfunction and the relation with behavioral problems in children with 47,XXY and 47,XXX. Genes Brain Behav. 2015;14(2):200–8.
95. Ross JL, Roeltgen DP, Kushner H, Zinn AR, Reiss A, Bardsley MZ, et al. Behavioral and social phenotypes in boys with 47,XYY syndrome or 47, XXY Klinefelter syndrome. Pediatrics. 2012;129(4):769–78.
96. Wilson AC, King J, Bishop DVM. Autism and social anxiety in children with sex chromosome trisomies: an observational study. Wellcome Open Res. 2019;4:32.
97. Corona G, Petrone L, Paggi F, Lotti F, Boddi V, Fisher A, et al. Sexual dysfunction in subjects with Klinefelter's syndrome. Int J Androl. 2010;33(4):574–80.
98. El BH, Majzoub A, Al SS, Alnawasra H, Dabbous Z, Arafa M. Sexual dysfunction in Klinefelter's syndrome patients. Andrologia. 2017:49(6).
99. Skakkebaek A, Moore PJ, Chang S, Fedder J, Gravholt CH. Quality of life in men with Klinefelter syndrome: the impact of genotype, health, socioeconomics, and sexual function. Genet Med. 2018;20(2):214–22.
100. Ferlin A, Selice R, Angelini S, Di GM, Caretta N, Cavalieri F, et al. Endocrine and psychological aspects of sexual dysfunction in Klinefelter patients. Andrology. 2018;6(3):414–9.
101. Fisher AD, Castellini G, Casale H, Fanni E, Bandini E, Campone B, et al. Hypersexuality, paraphilic behaviors, and gender dysphoria in individuals with Klinefelter's syndrome. J Sex Med. 2015;12(12):2413–24.
102. Davies GW, Parkinson J. Gender dysphoria in Klinefelter's syndrome: three cases. Australas Psychiatry. 2018;26(3):313–4.
103. Fernandez R, Guillamon A, Gomez-Gil E, Esteva I, Almaraz MC, Cortes-Cortes J, et al. Analyses of karyotype by G-banding and high-resolution microarrays in a gender dysphoria population. Genes Genomics. 2018;40(5):465–73.
104. Kreukels BPC, Cohen-Kettenis PT, Roehle R, van de Grift TC, Slowikowska-Hilczer J, Claahsen-van der Grinten H, et al. Sexuality in adults with differences/disorders of sex development (DSD): findings from the dsd-LIFE study. J Sex Marital Ther. 2019;45(8):688–705.
105. Aksglaede L, Molgaard C, Skakkebaek NE, Juul A. Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome. Arch Dis Child. 2008;93(1):30–4.
106. Baughman FA Jr. Klinefelter's syndrome and essential tremor. Lancet. 1969;2(7619):545.

107. Tartaglia N, Borodyanskya M, Hall DA. Tremor in 48,XXYY syndrome. Mov Disord. 2009;24:2001–7.
108. Tatum WO, Passaro EA, Elia M, Guerrini R, Gieron M, Genton P. Seizures in Klinefelter's syndrome. Pediatr Neurol. 1998;19(4):275–8.
109. Bojesen A, Gravholt CH. Morbidity and mortality in Klinefelter syndrome (47,XXY). Acta Paediatr. 2011;100(6):807–13.
110. Gravholt CH, Jensen AS, Host C, Bojesen A. Body composition, metabolic syndrome and type 2 diabetes in Klinefelter syndrome. Acta Paediatr. 2011;100(6):871–7.
111. Jiang-Feng M, Hong-Li X, Xue-Yan W, Min N, Shuang-Yu L, Hong-Ding X, et al. Prevalence and risk factors of diabetes in patients with Klinefelter syndrome: a longitudinal observational study. Fertil Steril. 2012;98(5):1331–5.
112. Andersen NH, Bojesen A, Kristensen K, Birkebaek NH, Fedder J, Bennett P, et al. Left ventricular dysfunction in Klinefelter syndrome is associated to insulin resistance, abdominal adiposity and hypogonadism. Clin Endocrinol. 2008;69(5):785–91.
113. Salzano A, Arcopinto M, Marra AM, Bobbio E, Esposito D, Accardo G, et al. Klinefelter syndrome, cardiovascular system, and thromboembolic disease: review of literature and clinical perspectives. Eur J Endocrinol. 2016;175(1):R27–40.
114. Seminog OO, Seminog AB, Yeates D, Goldacre MJ. Associations between Klinefelter's syndrome and autoimmune diseases: English national record linkage studies. Autoimmunity. 2015;48(2):125–8.
115. Sawalha AH, Harley JB, Scofield RH. Autoimmunity and Klinefelter's syndrome: when men have two X chromosomes. J Autoimmun. 2009;33(1):31–4.
116. Swerdlow AJ, Schoemaker MJ, Higgins CD, Wright AF, Jacobs PA. Cancer incidence and mortality in men with Klinefelter syndrome: a cohort study. J Natl Cancer Inst. 2005;97(16):1204–10.
117. Volkl TM, Langer T, Aigner T, Greess H, Beck JD, Rauch AM, et al. Klinefelter syndrome and mediastinal germ cell tumors. Am J Med Genet A. 2006;140(5):471–81.
118. Williams LA, Pankratz N, Lane J, Krailo M, Roesler M, Richardson M, et al. Klinefelter syndrome in males with germ cell tumors: a report from the Children's Oncology Group. Cancer. 2018;124(19):3900–8.
119. Keung YK, Buss D, Chauvenet A, Pettenati M. Hematologic malignancies and Klinefelter syndrome. A chance association? Cancer Genet Cytogenet. 2002;139(1):9–13.
120. Rogol AD, Swerdloff RS, Reiter EO, Ross JL, Zumbrunnen TL, Pratt GA, et al. A multicenter, open-label, observational study of testosterone gel (1%) in the treatment of adolescent boys with Klinefelter syndrome or Anorchia. J Adolesc Health. 2014;54:20–5.
121. Moorthy B, Papadopolou M, Shaw DG, Grant DB. Depot testosterone in boys with anorchia or gonadotrophin deficiency: effect on growth rate and adult height. Arch Dis Child. 1991;66(2):197–9.
122. Fouatih K, Belin F, Lambert AS, Bouligand J, Bouvattier C. Pubertal growth spurt in patients with bilateral anorchia after testosterone replacement therapy. Arch Pediatr. 2019;26(6):320–3.
123. Wong SC, Scott D, Lim A, Tandon S, Ebeling PR, Zacharin M. Mild deficits of cortical bone in young adults with Klinefelter syndrome or Anorchia treated with testosterone. J Clin Endocrinol Metab. 2015;100(9):3581–9.
124. Samango-Sprouse CA, Sadeghin T, Mitchell FL, Dixon T, Stapleton E, Kingery M, et al. Positive effects of short course androgen therapy on the neurodevelopmental outcome in boys with 47,XXY syndrome at 36 and 72 months of age. Am J Med Genet A. 2013;161A(3):501–8.
125. Davis SM, Reynolds RM, Dabelea DM, Zeitler PS, Tartaglia NR. Testosterone treatment in infants with 47,XXY: effects on body composition. J Endocr Soc. 2019;3(12):2276–85.
126. Davis SM, Cox-Martin MG, Bardsley MZ, Kowal K, Zeitler PS, Ross JL. Effects of oxandrolone on cardiometabolic health in boys with Klinefelter syndrome: a randomized controlled trial. J Clin Endocrinol Metab. 2017;102(1):176–84.

127. Davis SM, Lahlou N, Cox-Martin M, Kowal K, Zeitler PS, Ross JL. Oxandrolone treatment results in an increased risk of gonadarche in prepubertal boys with Klinefelter syndrome. J Clin Endocrinol Metab. 2018;103(9):3449–55.
128. Ross JL, Kushner H, Kowal K, Bardsley M, Davis S, Reiss AL, et al. Androgen treatment effects on motor function, cognition, and behavior in boys with Klinefelter syndrome. J Pediatr. 2017;185:193–9.
129. Samango-Sprouse C, Lasutschinkow P, Powell S, Sadeghin T, Gropman A. The incidence of anxiety symptoms in boys with 47,XXY (Klinefelter syndrome) and the possible impact of timing of diagnosis and hormonal replacement therapy. Am J Med Genet A. 2019;179(3):423–8.
130. Host C, Bojesen A, Erlandsen M, Groth K, Kritstensen K, Jurik AG, et al. A placebo-controlled randomized study with testosterone in Klinefelter syndrome—beneficial effects on body composition. Endocr Connect. 2019;8:1250–61.

Acquired Testicular Disorders

9

Giulia Izzo, Roberta Pujia, and Antonio Aversa

9.1 Testicular Trauma

The testes are normally protected from traumas by anatomical barriers. Despite their extracorporeal location, the testes are elastic and mobile in the scrotum, and they are equipped with the tunica albuginea, which is a resistant membrane. The cremasteric reflex is also a defense mechanism [1]. Therefore, testicular trauma is uncommon.

9.1.1 Epidemiology

According to retrospective studies, trauma accounts for 10% of acute scrotum cases. Almost 91.0% of genitourinary traumas occur in patients aged between 18 and 64, with an average age of 26 years. Testicular trauma is less common in geriatric men.

9.1.2 Etiology and Pathophysiology

Testicular traumas may be classified into blunt or penetrating trauma according to the pathophysiological mechanism. Clinical approach is different in the two forms.

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Blunt injury is the most common form recurring in the 85% of testicular traumas. It is usually unilateral [2]: in fact, bilateral testicular involvement occurs only in 1.5% of blunt traumas.

Aggression, road accidents, and contact sport are the main causes of blunt injuries. Inguinal hernias and testicular atrophy can be predisposing factors. Blunt scrotal traumas may determine testicular rupture or fracture, hematoma, hematocele, testicular torsion, and spermatic cord lesion. Testicular rupture occurs in 50% of cases, and it is characterized by the rupture of tunica albuginea and the extrusion of scrotal contents [3]. Typically, it occurs as result of a direct blow to the scrotum that presses the testis against the symphysis pubis. Nevertheless, the tunica albuginea is intact in the testicular fracture, while the testicular parenchyma is broken. Testicular rupture can be also associated with hematocele, where the bleeding is confined between the tunica albuginea and the vaginalis, while in hematomas, the bleeding extends beyond the tunica vaginalis. Testicular dislocation occurs when the testis moves in the inguinal canal or in the abdominal cavity.

Penetrating traumas are less common than blunt traumas. They are more frequently bilateral (up to 30% of cases), and they are associated with other injuries in 70% of patients. The most common causes are gunshots and stab wounds.

Finally, cases of genital self-mutilation have also been reported in patients with psychiatric illnesses or drug and alcohol abuse [4].

9.1.3 Clinical Presentation

Testicular trauma usually causes pain, often associated with nausea and vomiting. The affected testis can be tender and swollen, with abrasions, ecchymosis, and subcutaneous hematoma. The ecchymosis may also be present in the surrounding regions. In penetrating lesions, the wound is visible with weapon entry point. Blood vessel injuries could compromise the blood supply. In these cases, it is possible to find signs of local or systemic infection.

9.1.4 Diagnosis

Medical history and physical examination are required for a correct diagnosis. The moment of pain beginning, the pain degree, the mechanism and the force of injury, previous diseases, and local problems (such as hernias) should be investigated. Physical examination should evaluate consistency, size and position of the involved testis compared to the contralateral side, as well the cremasteric reflex and the tunica albuginea integrity. Immediate intervention is mandatory if tunica albuginea rupture is suspected [5]. Sometimes the clinical examination of the scrotum in acute conditions can be difficult and unreliable for different causes (e.g., uncooperative patient due to pain) [2]. Laboratory tests (e.g., complete blood count) may also be performed. Scrotal ultrasonography (US) is the first-line choice in imaging diagnosis and follow-up of testicular trauma [6].

Color Doppler (CD) ultrasound study is necessary for the evaluation of the perfusion within the testes, and it is helpful for establishing vitality of affected testis [7]. US is also used to distinguish testicular rupture from others lesions such as hematoma or hematocele. Typically, in testicular rupture, the hyperechoic line of the tunica albuginea which normally delineates the testis is interrupted, parenchyma echotexture is heterogeneous, and blood flow is decreased on CD US. Conversely, the sonographic features of intratesticular hematoma and hematocele vary over time [1]: in the acute phase, they appear isoechoic, subsequently hypoechoic or anechoic. US has a sensitivity of 64% and a specificity of 75–98% for the diagnosis of testicular trauma [8]. However, US has some limitations in the diagnosis of testicular rupture. In fact, extratesticular or intratesticular hematoma may cause false positive result; instead, a small interruption of the tunica albuginea may cause false negative. In doubtful US cases, magnetic resonance imaging (MRI) may be used [9]. However, MRI is a second-line imaging investigation because of its costs and time required for the examination. On T2-weighted images, the tunica albuginea appears as a dark signal line. In previous studies, MRI had an accuracy in the diagnosis of testicular rupture of 100% [9].

9.1.5 Management and Treatment

The treatment is variable according to the type of trauma. Both the American Urological Association (AUA) and European Association of Urology (EAU) guidelines advised early (within 72 h of trauma) scrotal surgical exploration when there is a suspicion of testicular rupture [10]. The testicular recovery probability is reduced in delayed surgery [9]. Surgical procedures are required to evacuating hematoma, debriding non-vital seminiferous tubules, and closing the tunica albuginea.

Although potentially vital testicular tissues should be saved to preserve endocrine function, orchidectomy is mandatory when the testis cannot be saved. In selected cases, a vascularized flap of the tunica vaginalis can be used to repair a tunica albuginea defect [11]. Extended hematocele and hematomas require a surgical exploration with clot evacuation to prevent ischemia, necrosis, testicular atrophy, or infections. Small (<5 cm) or non-expandable hematomas can be conservatively treated with ice, rest, and elevation of affected testis. Small hematoceles require the same treatment. Spermatic cord should be realigned to restore blood flow when disrupted. Subsequently, a microsurgical vaso-vasostomy may be performed if the testis remains vital. Testicular dislocation can be corrected with manual reduction and orchidopexy. Penetrating injuries require the same clinical management, and their treatment involves surgical exploration and subsequent debridement of necrotic tissue.

Treatment goals are hemostasis, infection prevention, pain resolution, and improvement of recovery times. The main goal is to preserve the patient's fertility, which is the first function to be compromised. Testicular atrophy may occur during follow-up in relation to surgery and trauma. Moreover, orchiectomy or debridement

of nonviable tissues reduces scrotal content with spermatogenesis damage and impairment of hormone production. Therefore, plasma testosterone and antisperm antibodies (ASA) levels should be monitored, and ejaculation and cryopreservation of post-trauma sperm should be evaluated.

Finally, the protective role of sildenafil on the testis has been evaluated after scrotal trauma with encouraging results in rats [12].

9.2 Testicular Torsion

Testicular torsion is a condition caused by a sudden rotation of the spermatic cord around its longitudinal axis in the scrotum. The twisting determines the reduction/interruption of the testicular blood flow to the affected testis and consequent ischemic damage [13]. Therefore, testicular torsion is a surgical emergency requiring prompt diagnosis and treatment because of its deleterious and sometimes irreversible complications.

9.2.1 Epidemiology

Testicular torsion may occur in any age, although it is less frequent in older males. The age distribution is bimodal with the first peak of incidence during the perinatal period and the second during puberty [14]. This condition affects the pediatric population worldwide. The annual incidence is 5 cases per 100,000 males aged <25 years and 3.8 per 100,000 males aged <18 years [15]. Testicular torsion is the etiology of 13–54% of acute scrotum in children and has a rate of orchiectomy of 42% [16, 17].

9.2.2 Etiology and Pathophysiology

There are two types of testicular torsion: the spermatic cord may twist inside the tunica vaginalis (intravaginal torsion) or in association with the tunica vaginalis (extravaginal torsion). The latter is typical of the antenatal or early postnatal period (perinatal testicular torsion), when the whole testis in not tied up to the scrotal wall and the spermatic cord and the tunica vaginalis rotate inside the scrotal sac. The extravaginal torsion is mainly caused by the excessive mobility of the testis within the scrotum due to an immaturity of the testicular anchorage system. It is associated with a poor outcome about testicular vitality and also in case of prompt surgery.

The intravaginal torsion is responsible for the 90% of torsions occurring outside the perinatal period. The main risk factor is an anatomical condition known as bell clapper deformity, characterized by the lack of a normal anchorage of the testis and epididymis to the scrotum. In this context, the testis and the spermatic cord may freely rotate within the tunica vaginalis.

Other putative risk factors include a quick testicular growth at puberty, testicular cancer, repeated traumas, sexual activity, and exposure to cold. These factors are responsible of the asymmetric contraction of the cremaster with consequent occurring of torsion. Moreover, it has been described as genetic predisposition to testicular torsion [18]. About 10% of cases are associated with a positive family history, and the bell clapper deformity is bilateral in about 80% of cases. Evidences reported that testicular torsion tends to occur at the same age in different generations within a family [19].

Therefore, authors speculated a potential role of genetic mutations in the pathogenesis of testicular torsion. In particular, insulin-like hormone 3 (INSL3) gene and its receptor (Relaxin Family Peptide Receptor 2, RXFP2) are essential in the first phase of testicular descent and seem to be involved in the torsion pathogenesis. In fact, INSL3 knockout mice are affected by gubernaculum testis dysgenesis, altered testicular descent, intra-abdominal bilateral cryptorchidism, and presence of mobile testes in the retroperitoneum with a consequent higher percentage of testicular torsion development [20]. However, studies on DNA of males affected by testicular torsion didn't identify significant mutations of these genes [20].

9.2.3 Clinical Presentation

Testicular torsion presents with a sudden onset of unilateral acute scrotal pain joined with nausea and vomiting. Fever and urinary frequency may sometimes be present. Even though the absence of scrotal pain can exclude acute testicular torsion, the specificity is poor (sensitivity 91%; specificity 27%) because pain is present in several clinical conditions. Therefore, acute scrotum differential diagnosis should include epididymitis, orchitis, torsion of appendix testis, scrotal trauma, varicocele, malignant lesions, strangulated inguinal hernia, and hydrocele [21]. In few hours from the beginning of torsion, the scrotal sac presents different degrees of erythema and swelling. A pathognomonic sign of testicular torsion is the lift of the affected testis with respect to the contralateral, due to a shortening of the spermatic cord. Sometimes, the torsion may spontaneously solve and successively relapse, suggesting a less acute onset and an intermittent form.

9.2.4 Diagnosis

Clinical history, signs, and symptoms should be carefully evaluated. Any patient presenting with abdominal pain associated with nausea and vomiting should undergo scrotal examination. A sudden onset of violent, unrestrainable, and unilateral scrotal pain, the presence of nausea and vomiting, and changes of the testicular position are suggestive of testicular torsion. The clinical examination should include a thorough investigation of the abdominal, inguinal, and genital region. Clinical findings indicative of testicular torsion include testicular elevation, increased scrotal consistency, anterior palpation of the epididymis, lift of the testis toward the

external inguinal ring, testicular orientalization (Gouverneur sign), and the absence of cremasteric reflex [22]. Although the cremasteric reflex is often absent in acute torsion, its presence doesn't rule out torsion [22]. Moreover, the absence of cremasteric reflex is more reliable when homolateral to pain (in the presence of contralateral cremasteric reflex), and it is less reliable when bilateral.

The TWIST score may be helpful in the diagnosis of testicular torsion. It is a validated scoring system based on signs and symptoms usually observed in testicular torsion: testicular swelling (2 points), hard testicle (2 points), absent of cremasteric reflex (1 point), nausea/vomiting (1 point), and high-riding testis (1 point). The positive predictive value is 100% for scores >5, and the negative predictive value is 100% for scores <2.

Testicular CD ultrasound is the imaging gold standard in diagnosing testicular torsion. However, when clinical history and physical examination are strongly suggestive of testicular torsion, surgical exploration should immediately be performed [21]. US may reveal a transversally oriented testis, hyperemia, edema, reduction or absence of blood flow at Doppler, spermatic cord twist (whirlpool sign), and vascular congestion of the epididymis and the proximal spermatic cord (pseudotumor sign). Testicular echogenicity may be normal at the disease outset.

However, CD sonography has limitations such as the possibility of false negatives in case of non-complete vascular obstruction [21]. In fact, the twist does not always determine immediate arterial flow obstruction. An initial venous obstruction may occur, followed by diffuse congestion leading finally to arterial interrupted flow. Moreover, sonographic parameters used to diagnose partial, complete, or intermittent forms are strongly variable and misleading.

Recently near-infrared spectroscopy (NIRS) has been investigated as a potentially more reliable imaging diagnostic alternative. Animal studies reported encouraging results regarding the use of NIRS in diagnosis of acute torsion [23]. Human studies are still poor and based on a small sample.

9.2.5 Complications

Testicular atrophy is the most serious consequence of testicular torsion [21]. The testes are indeed very susceptible to ischemic damage because the blood flow is terminal into the scrotum. Furthermore, the tunica albuginea is not elastic, and this limits the compensatory expansion of the testicle during a trauma. Infertility remains one of the most significant sequelae of testicular torsion, whether the testis is removed or saved. The severity of infertility after testicular torsion depends on the extent of the ischemic region and the damage of contralateral testicle. In the monorchid patient or in the bilateral torsion, infertility is due to direct damage to spermatogenesis. In unilateral torsion with a healthy contralateral testicle, the reason of infertility is not entirely clear. The main hypotheses are ischemia/reperfusion injury and autoimmunity response mediated by ASA [24]. There is a correlation between

ASA and testicular torsion and an association between increased levels of ASA and duration of testicular ischemia.

9.2.6 Management and Treatment

When clinical history and physical examination are strongly suggestive of testicular torsion, an immediate surgical exploration followed by an eventual orchidopexy is strongly recommended. Manual detorsion may be utilized when surgery is not immediately available. Manual detorsion should be performed in a clockwise direction since the testicular torsion usually occurs in an anticlockwise direction [21]. However, the torsion may occur in both directions. Following manual detorsion, a CD ultrasound examination should be performed to evaluate the restoration of blood flow. Surgical exploration and orchidopexy with permanent suture to fix the testis into the scrotum are treatment of choice [25]. Orchiectomy should be performed when the affected testicle appears necrotic or nonviable, in 39–71% of cases [26]. Age and delayed diagnosis are risk factors for orchiectomy since prepubertal males have a higher risk of orchiectomy than postpubertal. In fact, prepubertal patients more likely exhibit atypical symptoms resulting in a delayed diagnosis. The severity of testicular torsion depends on the duration of the injury and the degrees of rotation: a greater rotation causes a more rapid onset of ischemia and higher risk of atrophy and orchiectomy [21]. The placement of the testicular prosthesis could be considered at the time of orchiectomy. However, the decision to implant the prosthesis in the pediatric population remains a controversial issue. Psychosexual benefits have not been studied, and the procedure has many complications such as implant extrusion and migration. A decompression of the tunica albuginea consisting of its incision combined with a tunica vaginalis flap may represent an alternative to the conventional surgical approach. The goal is to relieve the testicular compartment by high pressures [27], thus attenuating further vascular alterations [28]. This approach had encouraging results on the outcome of testicular vitality, but its long-term success is not known. The main goal of surgical intervention is to prevent irreversible damage to the testis and to preserve testicular vitality. Therefore, diagnostic and therapeutic timeliness is required. The rates of testicular recovery are indeed associated with the duration of ischemia. Testicular recovery rates can reach 90% if the torsion correction is obtained within 6 h, are reduced to 50% after 12 h, and are lower than 10% after 24 h [29].

9.3 Orchitis

Epididymitis and orchitis are inflammatory processes (with or without infection) involving the epididymis and the testis, respectively [30]. Sexually transmitted infections (STIs) are the main etiological agents in young people, while urinary tract infections (UTIs) are the most frequent cause in older men [31].

9.3.1 Epidemiology and Classification

Epidemiologic data reported an incidence of 2.45 cases per 1000 men in the United Kingdom [32] and a bimodal distribution with the peak of incidence in men aged 16–30 and 51–70 years [30]. Epididymitis is more frequent than orchitis. The last occurs when the epididymal inflammation spreads to the adjacent testicle, in about 58% of men with epididymitis. Instead, isolated orchitis is a rare condition, generally associated with mumps infection in prepubertal boys [30]. According to the duration of the clinical course, epididymo-orchitis (EO) may be classified as acute (<6 weeks), subacute, and chronic (>6 months) [30, 31].

9.3.2 Etiology and Pathophysiology

EO is a frequent cause of intrascrotal inflammation related to the retrograde ascent of uropathogens through the genitourinary tract [30, 33]. Risk factors include urinary tract infections (UTIs) or sexually transmitted infections (STIs), anatomic anomalies (bladder outlet obstruction), prostate/urinary tract surgery or medical procedures, vigorous physical activity, cycling, and prolonged sitting [34–36].

Epididymitis and orchitis may be due to infectious or noninfectious etiologies [31]. The etiology and the pathogens involved vary according to the patients' age [30].

9.3.2.1 Infectious Epididymo-Orchitis

EO may be caused by ascending STI or non-sexually transmitted uropathogens spreading from the urinary tract (Table 9.1) [32]. In 14–35-year-old males, EO is commonly associated with STI by *Neisseria gonorrhoeae*/*Chlamydia trachomatis* [30, 32] or with enteric organisms infections related to anal sex intercourses [32]. In males younger than 14 and older than 35, UTIs induced by *Escherichia coli* may cause epididymitis. Obstructive urinary diseases, urinary tract surgery, or instrumentation are the main risk factors for these gram-negative enteric infections [32]. Moreover, scientific literature reported an increased incidence of UTIs and epididymitis in uncircumcised children [37]. Infectious orchitis usually occurs as a consequence of epididymitis and has the same etiology. A primary testicular inflammation may be due to blood dissemination of systemic infections caused by mumps, Coxsackievirus, Epstein-Barr virus, influenza, and HIV [30, 38]. Mumps orchitis is the most common condition, and it is the consequence of viral parotitis caused by *Rubulavirus*. Recently, an increase in mumps orchitis has been registered among pubertal and postpubertal males as a consequence of the reduction of vaccination. Boys aged 14–24 are more commonly involved by the infection, and the incidence of orchitis in males with postpubertal mumps is about 40% [39].

9.3.2.2 Noninfectious Epididymo-Orchitis

Noninfectious etiologies (Table 9.1) of epididymitis in pediatric and prepubertal males include urinary tract anomalies (e.g., posterior urethral valves or meatal

Table 9.1 Etiologies of epididymo-orchitis

Type	Etiology
Infectious etiologies	
STIs [30, 32]	*Neisseria gonorrhoeae* *Chlamydia trachomatis* *Escherichia coli* (men practicing insertive anal intercourse) *Haemophilus influenza* (less frequently) *Mycoplasma genitalium* (rare and putative etiology)
Non-STIs [30, 32]	*Escherichia coli* Gram-negative enteric infections *Ureaplasma urealyticum* (less frequent) *Proteus mirabilis* (less frequent) *Klebsiella pneumoniae* (less frequent) *Pseudomonas aeruginosa* *Mycobacterium tuberculosis* *Brucella* (in endemic areas) *Candida* [30, 32, 34]
HIV-associated EO [30, 34]	*Cytomegalovirus* *Salmonella* *Toxoplasmosis* *Ureaplasma urealyticum* *Corynebacterium* *Mycoplasma* Mycobacteria Fungi
Infectious primary orchitis [30, 38, 39]	Rubeola virus [39] Coxsackievirus Epstein-Barr virus Influenza HIV
Granulomatous orchitis [38, 42]	Tuberculosis, syphilis, lepromatous leprosy, brucellosis, and schistosomiasis (severe forms, rare in children)
Noninfectious etiologies	
Genitourinary tract abnormalities	Posterior urethral valves [30, 34, 40] Meatal stenosis Anorectal malformations [41] Communications between rectum and urethra [41]
Autoimmune diseases	Primary autoimmune orchitis, caused by ASA direct to the basement membrane or the seminiferous tubules with no evidence of systemic disease [31] Secondary autoimmune orchitis in the presence of systemic diseases such as rheumatic diseases or vasculitis [31]
Systemic diseases	Sarcoidosis Vasculitis (polyangiitis, polyarteritis nodosa, and Henoch-Schönlein purpura causing granulomatous orchitis) [42, 43] Behcet's syndrome [43]
Genitourinary surgery/ medical procedures	Vasectomy [30, 34, 40] Instrumental procedures
Clinical conditions	Trauma [30, 34, 40]. Torsion [30, 34, 40]. Tumors [30, 34, 40]. Cryptorchidism [30, 34, 40]
Medicinal	Amiodarone [44]

STI Sexually transmitted infections, *EO* Epididymo-orchitis, *ASAs* Antisperm antibodies

stenosis determining reflux of urine into the ejaculatory ducts) and a postinfectious inflammatory reaction to pathogens like *Mycoplasma pneumoniae*, enteroviruses, and adenoviruses [30, 34, 40]. Moreover, EO may occur in about 20% of male with anorectal malformation and communication between rectum and urethra [41]. Other etiologies of EO are shown in Table 9.1.

9.3.3 Clinical Presentation

EO is usually characterized by pain and swelling (acute scrotum). The inflammation starts gradually and usually unilaterally in the posterior pole of the testis and spreads to the whole scrotum until radiating to the lower abdomen [30, 32]. The pain may reach the adjacent testicle and is often associated with fever, lower UTI symptoms (urgency, frequency, hematuria, dysuria), and penile irritation. Some patient may present mild testicular pain/swelling and light thickening of the peritesticular membranes; moreover, testicular volume and consistency may be reduced [31]. However, in most cases, subacute or chronic orchitis is asymptomatic [31]. Other nonspecific symptoms and signs include nausea, vomiting, tachycardia, and discomfort while sitting [30]. Urethral discharge, hydrocele, erythema, and edema of the scrotum may also be present [32]. Atypically cases of EO may present with bilateral testicular swelling and epididymitis alone or without systemic symptoms [32]. Patients with genitourinary abnormalities show more profound epididymal swelling, pain, voiding disturbances, and recurrent episodes of EO.

Viral orchitis is characterized by a sudden onset of scrotal pain and swelling, primarily unilateral although it may occur bilaterally in 15–30% of cases. In mumps infection, testicular swelling and pain occur from 10 days to 6 weeks after the onset of parotitis [30, 39, 45]. Orchitis is often preceded by aspecific disturbances. Testes are acutely warm and swollen, associated with tenderness and inflammation in the scrotum. Symptoms can progress for about 3 days but tend to resolve within 2 weeks. However, testicular tenderness can persist for several weeks in 20% of cases [39].

Primary AO is usually asymptomatic and associated with infertility, whereas secondary AO is rare and associated with acute orchitis and/or testicular vasculitis in the presence of other autoimmune or systemic diseases [31].

Medications such as amiodarone may cause bilateral testicular involvement without fever or leukocytosis [44], and inflammatory symptoms usually disappear with the treatment withdrawal [32].

9.3.4 Diagnosis

9.3.4.1 History and Physical Examination

An accurate anamnesis is helpful in assessing the inflammation etiology. Physical examination reveals tenderness and swelling of the epididymis/testis and a pain reduction at the scrotal elevation (Prehn's sign) [34]. Hydrocele may be found in the later stages of EO [30]. Moreover, a tender spermatic cord may be suggestive of

epididymitis [34]. Genitalia examination should detect anatomical anomalies such as meatal stenosis, hypospadias, or phimosis [37]. The inguinal area should be also analyzed for hernia or for swollen/tender lymph nodes suggestive of epididymitis and orchitis [30]. Eventual prostatitis causing orchitis should be excluded. EO should be differentiated (Table 9.2) from testicular torsion [30].

Table 9.2 Differential diagnosis of acute scrotum [30, 32]

Epididymitis	Clinical presentation: – Gradual onset of pain radiating to the lower abdomen. – Lower urinary tract infection symptoms (LUTS) – Recurrent pain is unusual Clinical findings: – Localized epididymal tenderness progressing to testicular swelling and tenderness – Normal cremasteric reflex (elicited by stroking the skin of the upper medial thigh) – Positive Prehn's sign – Normal testicular localization Ultrasound findings: – Enlarged, thickened epididymis – Increased flow on color Doppler
Orchitis	Clinical presentation: – Sudden onset of testicular pain – Sometimes urological symptoms Clinical findings: – Testicular swelling and tenderness – Normal cremasteric reflex – Normal testicular localization Ultrasound findings: – Testicular masses or swollen testes – Hypoechoic and hypervascular areas ("inferno sign")
Testicular torsion	Clinical presentation: – Acute and sudden onset of violent pain – Nausea/vomiting without fever or urological symptoms – Recurrent pain (intermittent torsion) – Frequent in perinatal period or in the adolescent period Clinical findings: – Transversely oriented testis – Abnormal cremasteric reflex – Pain with testicular elevation – Reactive hydrocele (common) Ultrasound findings: – Anatomically normal testis – Reduced flow on color Doppler
Torsion of the appendix testis	Clinical presentation: – Recurrent pain is unusual – Frequent in 7–14-year-old boys Clinical findings: – Bluish area in the appendix testis (bluish dot sign) – Normal cremasteric reflex (elicited by stroking the skin of the upper medial thigh)

9.3.4.2 Diagnostic Testing

Urethral swab, urine analysis, and urine culture should be performed [32, 46]. Urine dipstick is useful only as an adjunct to mid-stream urine (MSU) [32, 46], but a negative dipstick test in men should not exclude the diagnosis of UTI [32, 47, 48]. The presence of nitrite and leukocyte esterase suggests UTI in men with urinary symptoms [32, 47, 48], and it helps to differentiate epididymis from testicular torsion [30]. Laboratory investigations should include urethral swab for *Neisseria gonorrhoeae*; polymerase chain reaction (PCR) assays for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or *Mycoplasma genitalium* on urethral swab or urine specimen; and MSU for microscopy and culture [32].

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) increase in epididymitis and are useful in the differential diagnosis of scrotum [32] with a sensitivity and a specificity for epididymitis of 96.2 and 94.2%, respectively [30].

In suspicions of mumps, the virus can be isolated from the saliva, urine, blood, nasopharyngeal swab, and seminal fluid in the first week of symptom onset. In mumps orchitis, white blood cell count and differential counts are usually normal; leukocytosis or leucopenia may occur, and CRP is usually raised over 140 mg/L. Urine analysis, urethral cultures, and MSU are generally negative [39].

In primary AO, ASAs are found in serum/seminal plasma or on sperm surface, even in association with normal seminal parameters [31], while in secondary AO, specific systemic autoimmune diseases are detected [31].

CD US may be helpful in the diagnosis [32]. Although US grayscale signs such as hypoechogenicity and enlarged testis are not specific in the differential diagnosis [49] (Table 9.2), an increased blood flow ("inferno sign") is suggestive of EO. In children, CD examination has a sensitivity of 70% and a specificity of 88% for epididymitis [30]. Moreover, CD US may be helpful in detecting testicular inflammation in mumps orchitis [39, 50].

Finally, further investigations such as renal/bladder US and cystourethrography are recommended in children younger than 5 years presenting epididymitis, while detailed voiding symptom questioning, uroflowmetry, US, and post-void residual urine test are recommended in boys older than 10 years presenting acute and recurrent epididymitis [37].

9.3.5 Complications

EO complications include hydrocele, testicular abscess and infarction, sepsis, extension of the infection, and infertility. These complications tend to be more frequent in EO induced by uropathogens [32] and are more likely associated with therapeutic failure. Risk factors suggesting a lack of response to antibiotics include sepsis, marked scrotal edema, deep testicular pain, and scrotal wall inflammation [51].

Testicular insult may result in reactive hydrocele, which may exert tension effect rarely reducing testicular perfusion. Rare complications include testicular infarction

[51] and testicular abscess formation [52, 53]; the latter condition is common in immunocompromised patients.

The inflammatory damage in EO may be associated with male infertility. Mumps orchitis has been supposed to be associated with transient anomalies of the HPT axis and with congestion, perivascular infiltration of lymphocytes, hyalinization, and necrosis in the seminiferous tubules which may lead to testicular fibrosis and atrophy. Infertility is less frequent and associated with severe cases of bilateral orchitis with atrophy; instead, subfertility occurs in about 22% of patients and shows no association with testicular atrophy [39]. There is a direct correlation between the severity of the orchitis and the seminal anomalies.

Finally, chronic inflammation and atrophy are associated with an increased risk to develop testicular cancer.

9.3.6 Management and Treatment

In case of suspected STI, the patient and his partner should be adequately informed, and sexual abstinence should be advised until the end of the medical treatment and the inflammation symptom resolution [32].

Empiric treatment should be started based on likely pathogens, according to the clinical history and findings. Medical treatment should cure the infection, ameliorate symptoms, prevent transmission, and reduce complications.

In <2-year-old children, the etiology of EO is various, and the antibiotic therapy is required for likely underlying enteric organisms. In children ages 2–14 years without systemic signs such as fever, antibiotic should be used only in case of positive urine analysis or culture, whereas in patients older than 14 years, empiric antibiotics are recommended [34]. To prevent complications, we recommend to begin treatment before laboratory tests are available and base therapy choice on the risk of chlamydia, gonorrhea, or enteric organisms (Table 9.3) [34].

Ofloxacin is a good treatment in *N. gonorrhoeae*, *C. trachomatis*, and most pathogenic infections according to its power to penetrate into the prostate. However, this is not the first-line treatment because of the increasing bacterial resistance to quinolones [32, 54]. In gonorrhea infection, azithromycin should be added to ceftriaxone and doxycycline therapy [32, 54].

Supportive treatments include analgesics, anti-inflammatories, bed rest, hot or cold packs, and scrotal elevation [30, 34]. Two-week course of nonsteroidal anti-inflammatory drugs associated with scrotal icing and elevation is the initial treatments. Pain and inflammation often decrease within 2–3 days of therapy, but a residual pain can persist for several weeks [34]. In persisting pain, tricyclic antidepressant or neuroleptic (gabapentin) may be useful [34].

Patients with intractable pain, vomiting, suspicions of abscesses, failure of other therapies, and suspicions of sepsis should be recovered [30] and treated with intravenous antibiotics [34].

Table 9.3 Treatment of EO

Sexually transmitted EO [32]
First-line therapy – Ceftriaxone 500 mg intramuscular injection twice a day for 10–14 days or doxycycline 100 mg twice a day for 10–14 days. Second-line choice – Ofloxacin 200 mg twice a day for 14 days or levofloxacin 500 mg once a day for 10 days When gonorrhea infection is improbable and there are no risk factors or signs of this infection, the use of ofloxacin and the drop of ceftriaxone should be considered.
Mycoplasma genitalium-induced EO [32] – Moxifloxacin 400 mg once a day for 14 days
Gonorrhea infection [32] – Azithromycin in association with ceftriaxone and/or doxycycline
Enteric organism-related EO [32] – Ofloxacin 200 mg twice daily for 14 days or levofloxacin 500 mg once a day for 10 days
Viral orchitis [30, 34, 39] – Supporting treatment (analgesics, rest, scrotal support, hot (cold pack)) – Antibiotics only in associated bacterial infection – Spermatic cord block with 5 mL of 1% procaine, high-dose steroids, or oxyphenbutazone for analgesic and anti-inflammatory purposes
Autoimmune orchitis [31] – Supportive treatment (analgesics, bed rest, scrotal elevation) – Rarely nerve block – Steroids, immunosuppressive agents, and intravenous immunoglobulin in secondary AO

EO Epididymo-orchitis, *AO* autoimmune orchitis

9.3.7 Follow-Up

Independently from the etiology, the treatment of EO requires a close follow-up. In case of no clinical improvement after 3 days of treatment, the patient should be re-evaluated, and the diagnosis should be reassessed.

For gonococcal EO, a culture should be repeated 3 days after the treatment withdrawal, and the patient should be re-evaluated at 2 weeks to assess the treatment compliance and the clinical course. Moreover, nucleic acid amplification test should be performed 2 weeks after the completion of treatment. At 4 weeks from treatment dropout, a test of cure is required in EO secondary to *C. trachomatis* or *M. genitalium* [32].

In patients suspected/diagnosed for sexually transmitted EO, the screening for all other STIs should be performed, and the partner should be informed and treated.

Scrotal US should be ordered in patient not responding to therapy or in diagnostic doubt.

Finally, hygienic improvement and the use of condom should be advised to reduce the risk of infection.

9.4 Testicular Iatrogenic Damage

Medical regimens and procedures may sometimes cause testicular toxicity leading to gonadal failure or infertility in many patients.

Cancer therapies are the most frequent etiology of iatrogenic testicular damage. However, testicular failure may be induced also by common treatments for nonmalignant diseases. For example, drepanocytosis, thalassemia, idiopathic medullary aplasia, and granulomatous disease may negatively affect testicular function because they require radiotherapy (RT) to deplete the blood stem cell line before the hematopoietic stem cell transplantation (SCT) and hydroxyurea regimens. Moreover, any condition requiring bone marrow transplant is associated with high infertility risk in prepubertal males. Finally, severe autoimmune diseases such as juvenile systemic lupus or systemic sclerosis are treated with high-dose chemotherapy (CT) which may hamper spermatogenesis [55].

Several evidences reported that CT and RT involve the pelvis, testes, head, spine, or total body and may determine possible deleterious effects on puberty and eventually fertility [56] by affecting the tubular and the interstitial components of the testis. The consequent alterations on fertility and sexual function may compromise the quality of life in cancer survivors [57].

The incidence of iatrogenic gonadal dysfunction is likely very high considering that the survival rate for children, adolescents, and adults with cancer has improved in the last years both in Europe and in the USA [57]. In fact, children developing cancer before the age of 15 have a long-term survival in 70–80% of cases [56, 58], and 43% of survivors have late-onset endocrine dysfunctions involving thyroid (22%), sexuality/fertility (22%), and metabolism (6%) [58, 59]. Furthermore, 30% of male childhood cancer survivors become azoospermic adults [55].

The children's age at the moment of treatment, as well as the nature, duration, dose, and combination of treatments, and the individual sensibility may influence the testicular iatrogenic damage [56, 60]. Cancer, CT, and RT may affect the hypothalamic-pituitary-testicular (HPT) axis and the target organs, thus hampering future fertility [58, 61]. Gonadal damage is an acute phenomenon occurring as early as 72 days after the last dose of CT [57]. High-dose therapy determines a syndrome ranging from mild to severe gonadal dysfunction/insufficiency with different possibilities of recovery. Patients with severe gonadal damage present a more severe and permanent gonadal failure in most cases [57]. Moreover, the gonadal damage has a delayed manifestation in people receiving SCT [57]. The risk of infertility after cancer treatment in children and pubertal boys is related to the tumor pathology (Table 9.4) [56, 62].

9.4.1 CT-Associated Testicular Damage

Different risk degrees of gonadal dysfunction or toxicity derived from the various cytotoxic medications (Table 9.5) with nitrogen derivatives, alkylating drugs, and cisplatin may determine the most destructive effect on germ cell proliferation [55, 56].

Spermatogenesis may be altered by DNA alkylating agents such as cisplatin, cyclophosphamide, mechlorethamine, ifosfamide, procarbazine, busulfan, melphalan, and nitrosoureas BCNU (carmustine) and CCNU (lomustine) that cross-link DNA [58].

Table 9.4 Risk of fertility damage after cancer treatment in children and adolescents according to the tumor pathology [56, 62]

Risk degree	Tumor pathology
Low risk	Acute lymphoblastic leukemia Nephroblastoma Soft tissue sarcoma (stage I) Malignant germinal tumor (not treated with radiotherapy) Retinoblastoma Cerebral tumor (treated with surgery or with cranial irradiation <24 Gy)
Intermediate risk	Acute myeloblastic leukemia Hepatoblastoma Osteosarcoma Nonmetastatic Ewing's sarcoma Soft tissue sarcoma (stage II/III) Neuroblastoma Non-Hodgkin's lymphoma Cerebral tumors (treated with cranial irradiation >24 Gy)
Elevated risk	Total body radiation Pelvic or testicular radiotherapy Chemotherapy before bone marrow transplant Hodgkin's lymphoma treated with alkylating agents Soft tissue sarcoma (stage IV) Metastatic Ewing's sarcoma

Table 9.5 Risk of gonadotoxicity related to cytotoxic medications [56, 62]

Risk degree	Cytotoxic medication
Low risk	Vincristine, methotrexate, dactinomycin, bleomycin, mercaptopurine, vinblastine
Intermediate risk	Cisplatin, carboplatin, doxorubicin
High risk	Cyclophosphamide, ifosfamide, chlormethine, busulfan, melphalan, procarbazine, chlorambucil

The cumulative dose of chemotherapeutic drug defines the duration and the width of spermatogenesis impairment. Prolonged azoospermia is associated with the total cumulative dose of cyclophosphamide (single agent >19 ng/m^2; combined with other drugs >7.5 g/m^2), procarbazine (>4 g/m^2), melphalan (>140 mg/m^2), cisplatin (>500 mg/m^2), busulfan (> 600 mg/m^2), and chlorambucil (>1.4 g/m^2) [58]. Moreover, adriamycin, vinblastine, or cytosine arabinoside have additive effects with the above agents in determining prolonged azoospermia, but when not combined, they cause only temporary reduction in the spermatic count [58].

In boys, spermatogonia are more sensitive than Leydig and Sertoli cells to the CT-induced damage because of their elevated mitotic index. Therefore, posttreatment azoospermia ranges from 17% to 82% on the base of the drug used [56]. If spermatogonia are not damaged by CT, spermatogenesis recovers within 12 weeks after the treatment withdrawal [58, 63, 64]. On contrary, factors causing stem cell death may induce azoospermia that lasts more than 12 weeks [58, 65].

It is not known if the prepubertal testis is less sensitive than postpubertal one. Evidences reported that only males <4 years of age at diagnosis were more likely able to achieve fecundation later in life than males 15–20 years old at the diagnosis [58, 66]. Although complete spermatogenesis is not achieved in the prepubertal testis, evidences reported that cytotoxic treatment in prepubertal boys influences their later fertility [58, 67]. Hence, a prepubertal age at diagnosis is not protective against gonadotoxicity of alkylating agents [58, 68].

9.4.2 SCT-Related Testicular Damage

In boys receiving SCT, pretransplant regimens may reduce testicular reserve even preceding the transplantation. Pretransplant protocols include alkylating agents, irradiation, or both and may cause germ cell or Leydig cell damage, generally with cumulative dose-dependent effect. Older age, local RT, or total body irradiation (TBI) allografts may complicate the gonadal insult. SCT- induced gonadal damage is more frequent in postpubertal adults who received pretransplant therapies or TBI allografts [57].

The clinical picture is variable. The high-risk group shows severe gonadal damage with very small possibility of recovery as opposed to subjects treated with CT who show minor injury with greater possibility of reversibility [57].

9.4.3 RT-Associated Testicular Damage

RT treatment may determine harmful effects on exocrine and endocrine gonadal function [56, 62]. In fact, radiations impact on the testicular germ cells in a dose-dependent manner, and spermatogonia are more sensitive than adult spermatozoa (Table 9.6) [58].

Leydig cells are more resistant than spermatogenic cells to RT; however they are sensitive to doses of 20 Gy in prepubertal children and 30 Gy in adolescents [56, 69].

Radiations may also hamper the gonadotropin secretion altering the HPT axis. This effect depends on the irradiation dose and on the target tumor location [58]. Conventional fractioned cranial irradiation (30–50 Gy) determines deficiency of gonadotropins in about 60% of pituitary tumor survivors after 10 years and in >20% of patients with non-pituitary brain tumors [58, 61]. The gonadotropin deficit may

Table 9.6 RT dose-related testicular germ cell damage [58]

Dose	Effect
0.15 Gy	Oligozoospermia
0.30 Gy	Temporary azoospermia
>1 Gy	Reduced number of spermatogonia and preleptotene spermatocytes
>2–3- Gy	Spermatocyte death
>4–6 Gy	Spermatid damage and oligozoospermia

be from subclinical to severe [58]. Clinically significant gonadotropin deficiency is usually a late complication with an incidence of 20–50% on long-term follow-up, regardless of the male age at the radiation [58].

9.4.4 Endocrine Evaluation in Cancer Survivors

Cancer survivors should be evaluated for reproductive function since the pubertal development. Survivors exposed to higher cumulative doses or combination of alkylating agents or radiation >20 Gy to the testes and pelvis and >30 Gy to the cranium before puberty should be evaluated from pubertal development until sexual maturity [58]. Tanner staging, testicular volume, as well gonadotropins, testosterone, and inhibin B should be analyzed. A reduced germinal cell mass is associated with small and soft testes; moreover, azoospermic and oligoasthenozoospermic cancer survivors usually have reduced mean testicular volume and high basal FSH levels. Serum inhibin B is a marker of germ function; however, inhibin B alone or associated with FSH does not reflect normal spermatogenesis in patients treated for cancer in childhood [58].

Sperm cryopreservation may be a successful method for fertility preservation in postpubertal males, whereas in prepubertal children, fertility preservation is more difficult because their spermarche has not yet started. Experimental techniques are based on the cryopreservation of testicular tissue containing SSCs or on the harvest of SSCs from preserved testicular tissue for in vitro maturation and testicular tissue grafting. Moreover, microsurgical epididymal sperm aspiration, testicular sperm extraction, and microscopic testicular sperm extraction (mTESE) have been applied to achieve ICSI [58]. In these cases, the sperm retrieval has been about 37% and the fertilization rate 57.1% [58].

9.4.5 Other Causes of Iatrogenic Testicular Damage

Many environmental endocrine disrupters, food additive, and drugs (etomidate, troglitazone, medroxyprogesterone acetate, and ketoconazole) inhibit testicular 3ß-hydroxysteroid dehydrogenase (HSD3B) interfering with the androgen synthesis [70].

Evidences reported that ketoconazole and theophylline may negatively impact on male gonads during fetal life suppressing testosterone synthesis and androgen action with consequent cryptorchidism or other genitourinary malformations [71].

Moreover, metronidazole at the dose of 500 mg/kg BW/day for 28 days impairs spermatogenic activity, sperm mobility, and fertility in male rats [72].

Evidences reported that substance and drug abuse (alcohol, opioid, anabolic-androgenic steroids) may decrease testosterone production interfering with testicular and HPT axis function (Table 9.7). Moreover, nicotine, cannabis, and amphetamines may damage testes inducing oxidative stress and testicular cell apoptosis with possible deleterious effects on spermatogenesis (Table 9.7). In all these

Table 9.7 Substance abuse and consequent effects on testosterone and spermatogenesis [73]

Substance	Endocrine effects
Cannabis	Significant LH reduction in acute administration LH and T suppression by CB1 receptor agonist ANA Reduced expression of LH receptor on the testis Reduced activity of testicular HSD3B
Cocaine	Induced panhypopituitarism Pituitary infarction by intranasal cocaine abuse Production of HNE-ANCA
Amphetamine, methamphetamine, MDMA (ecstasy)	Inhibition of testosterone production by the adenylate cyclase activation Decreases HSD3B, P450c17, and 17-KR activity Attenuated Ca2+ influx through L-type Ca2+ channel Increases testicular GABA concentration in methamphetamine-treated rats (compensatory responses) Lower expression of GnRH mRNA in adult male Sprague-Dawley rats following acute or chronic MDMA administration
Opioids	OPIAD (reduction of testosterone, libido, and muscle mass, fatigue, and osteopenia) Inhibition of the hypothalamic GnRH secretion, disrupting its normal pulsatility and leading to decreased LH levels Increased prolactin levels in both human and animal models following acute administration of opioids
Anabolic androgenic steroids	Suppression of gonadotropin release from the pituitary gland by a negative feedback mechanism, exerted on both pituitary gland and hypothalamic GnRH-releasing cells Permanent depletion of Leydig cells in animal studies
Substance	*Effects on fertility*
Cannabis	Inhibition of acrosome reaction and sperm capacitation Induction of programmed cell death in Sertoli cells
Cocaine	Ischemic effect Enhanced norepinephrine and epinephrine release with intense vasoconstriction Reperfusion injury
Amphetamine, methamphetamine, MDMA (ecstasy)	Methamphetamine administration decreases significantly cell proliferation and increases apoptosis in both rats' spermatogonia and primary spermatocytes, altering proliferation/apoptosis ratio Hydroxyl radical formation Significant decrease in GSH/GSSG ratio Methamphetamine seems also to reduce in male rats the expression of progesterone and estrogen receptors MDMA-induced hyperthermia could activate apoptosis in rat testicular tissue
Opioids	Effects on μ-, δ-, and κ-opioid receptors, located in the head, in the middle region, and in the tail of the human spermatozoa Lower antioxidant activity and higher sperm DNA fragmentation index Histological degenerative changes in the seminiferous tubules, in Sertoli cells, and in Leydig cells (tramadol) Increased caspase-3 and decreased anti-apoptotic protein Bcl-2 expression in rats chronically treated with tramadol

(continued)

Table 9.7 (continued)

Substance	Endocrine effects
Anabolic androgenic steroids	Reduced spermatogenesis as a consequence of LH reduction, Leydig cell functional arrest, and intratesticular testosterone reduction Reduction of ABP that conveys testosterone in the lumen of seminiferous tubules

LH Luteinizing hormone, *ANA* Anandamide, *HSD3B* 3ß-hydroxysteroid dehydrogenase, *HNE-ANCAs* Human neutrophil elastase-antineutrophil cytoplasmic antibodies, *17-KR* 17-ketosteroid reductase, *GABA* Gamma-aminobutyric acid, *GnRH* Gonadotropin-releasing hormone, *OPIAD* Opioid-induced androgen deficiency, *AAS* Anabolic androgenic steroids, *ABP* Androgen-binding protein

circumstances, the testicular failure may be reversible at the substance withdrawal [73].

9.5 Conclusions

The correct identification of testicular disease in children and adolescent is an important tool for clinicians to prevent reproductive problems in adulthood. Appropriate lifestyle and referral to the andrologist should be recommended at early age and during transitional age to ensure prevention of frequent pathologies (primary andrologic prevention). In adulthood, sign and symptoms are more easily recognized by the patient, and prompt treatment is recommended to prevent fertility problems and sexual dysfunctions associated with reduced testosterone production. Cryopreservation should be recommended in cases of testicular neoplasms prior to chemo- or radiotherapy and in some selected cases of idiopathic infertility, in young adults when fertility declines over time in the absence of any known etiology.

References

1. Bhatt S, Dogra VS. Role of US in testicular and scrotal trauma. Radiographics. 2008;28:1617–29.
2. Morey AF, Metro MJ, Carney KJ, Miller KS, McAninch JW. Consensus on genitourinary trauma: external genitalia. BJU Int. 2004;94(4s):507–15.
3. Ballestero R, Correas GM, Lastra GP, Portillo Martin JA, Zubillaga Guerrero S, Truan Cacho D, et al. Testicular reconstruction after testicular rupture and review of the literature. Arch Esp Urol. 2013;66:372–6.
4. Sherif A, Reynaldo G, McAninch Jack W. Genital self-mutilation. J Urol. 1993;150(4):1143–6.
5. Buckley JC, McAninch JW. Diagnosis and management of testicular ruptures. Urol Clin North Am. 2006;33:111–6.
6. Cubillos J, Reda EF, Gitlin J, Zelkovic P, Palmer LS. A conservative approach to testicular rupture in adolescent boys. J Urol. 2010;184:1733–8.
7. Adlan T, Freeman SJ. Can ultrasound help to manage patients with scrotal trauma? Ultrasound. 2014;22:205–12.
8. Lee SH, Bak CW, Choi MH, Lee HS, Lee MS, Yoon SJ. Trauma to male genital organs: a 10-year review of 156 patients, including 118 treated by surgery. BJU Int. 2008;101(2):211–5.

9. Wang Z, Yang JR, Huang YM, Wang L, Liu LF, Wei YB, et al. Diagnosis and management of testicular rupture after blunt scrotal trauma: a literature review. Int Urol Nephrol. 2016;48(12):1967–76.
10. Redmond EJ, Mac Namara FT, Giri SK, Flood HD. Blunt testicular trauma—is surgical exploration necessary? Ir J Med Sci. 2018;187(4):1109–13.
11. Molokwu CN, Doull RI, Townell NH. A novel technique for repair of testicular rupture after blunt trauma. Urology. 2010;76(4):1002–3.
12. Moeini Moghaddam R, Shalizar Jalali A, Najafi G, Behfar M. Effect of sildenafil in protection of contralateral testis following unilateral blunt testicular trauma in mouse. Horizon Med Sci. 2017;23(1):63–7.
13. Bowlin PR, Gatti JM, Murphy JP. Pediatric testicular torsion. Surg Clin North Am. 2017;97:161–72.
14. Sharp VJ, Kieran K, Arlen AM. Testicular torsion: diagnosis, evaluation, and management. Am Fam Physician. 2013;88:835–40.
15. Zhao LC, Lautz TB, Meeks JJ, Maizels M. Pediatric testicular torsion epidemiology using a national database: incidence, risk of orchiectomy and possible measures toward improving the quality of care. J Urol. 2011;186(5):2009–13.
16. Barbosa JA, Tiseo BC, Barayan GA, Rosman BM, Torricelli FC, Passerotti CC, et al. Development and initial validation of a scoring system to diagnose testicular torsion in children. J Urol. 2013;189(5):1859–64.
17. Liang T, Metcalfe P, Sevcik W, Noga M. Retrospective review of diagnosis and treatment in children presenting to the pediatric department with acute scrotum. AJR Am J Rentgenol. 2013;200(5):W444–9.
18. Cubillos J, Palmer JS, Friedman SC, Freyle J, Lowe FC, Palmer LS. Familial testicular torsion. J Urol. 2011;185:2469–72.
19. Shteynshlyuger A, Yu J. Familial testicular torsion: a meta-analysis suggests inheritance. J Pediatr Urol. 2013;9:683–90.
20. Sozubir S, Barber T, Wang Y, Ahn C, Zhang S, Verma S, et al. Loss of Insl3: a potential predisposing factor for testicular torsion. J Urol. 2010;183(6):2373–9.
21. Osumah TS, Jimbo M, Granberg CF, Gargollo PC. Frontiers in pediatric testicular torsion: an integrated review of prevailing trends and management outcomes. J Pediatr Urol. 2018;14(5):394–401.
22. Beni-Israel T, Goldman M, Bar Chaim S, Kozer E. Clinical predictors for testicular torsion as seen in the pediatric ED. Am J Emerg Med. 2010;28(7):786–9.
23. Hallacoglu B, Matulewicz RS, Paltiel HJ, Padua H, Gargollo P, Cannon G, et al. Noninvasive assessment of testicular torsion in rabbits using frequency-domain near-infrared spectroscopy: prospects for pediatric urology. J Biomed Opt. 2009;14(5):054027.
24. Monga M, Hellstrom WJG. The effects of testicular torsion on fertility. In: Hellstrom WJG, editor. Male infertility and sexual dysfunction. New York, NY: Springer New York; 1997. p. 323–34.
25. Taskinen S, Taskinen M, Rintala R. Testicular torsion: orchiectomy or orchiopexy? J Pediatr Urol. 2008;4(3):210–3.
26. Yang C Jr, Song B, Liu X, Wei GH, Lin T, He DW. Acute scrotum in children: an 18-year retrospective study. Pediatr Emerg Care. 2011;27(4):270–4.
27. Kutikov A, Casale P, White MA, Meyer WA, Chang A, Gosalbez R, et al. Testicular compartment syndrome: a new approach to conceptualizing and managing testicular torsion. Urology. 2008;72:786–9.
28. Arena S, Iacona R, Antonuccio P, Russo T, Salvo V, Gitto E, et al. Medical perspective in testicular ischemia-reperfusion injury. Exp Ther Med. 2017;13:2115–22.
29. Ringdahl E, Teague L. Testicular torsion. Am Fam Physician. 2006;74:1739–43.
30. Trojian TH, Lishnak TS, Heiman D. Epididymitis and orchitis: an overview. Am Fam Physician. 2009;79(7):583–7.
31. Silva CA, Cocuzza M, Carvalho JF, Bonfá E. Diagnosis and classification of autoimmune orchitis. Autoimmun Rev. 2014;13(4–5):431–4.

32. Street EJ, Justice ED, Kopa Z, Portman MD, Ross JD, Skerlev M, et al. The 2016 European guideline on the management of epididymo-orchitis. Int J STD AIDS. 2017;28(8):744–9.
33. Luzzi GA, O'Brien TS. Acute epididymitis. BJU Int. 2001;87(8):747–55.
34. McConaghy JR, Panchal B. Epididymitis: an overview. Am Fam Physician. 2016;94(9):723–6.
35. Kaver I, Matzkin H, Braf ZF. Epididymo-orchitis: a retrospective study of 121 patients. J Fam Pract. 1990;30(5):548–52.
36. Kadish HA, Bolte RG. A retrospective review of pediatric patients with epididymitis, testicular torsion, and torsion of testicular appendages. Pediatrics. 1998;102:73–6.
37. Gkentzis A, Lee L. The aetiology and current management of prepubertal epididymitis. Ann R Coll Surg Engl. 2014;96(3):181–3.
38. Jacobo P. The role of regulatory T cells in autoimmune orchitis. Andrologia. 2018;50(11):e13092.
39. Davis NF, McGuire BB, Mahon JA, Smyth AE, O'Malley KJ, Fitzpatrick JM. The increasing incidence of mumps orchitis: a comprehensive review. BJU Int. 2010;105(8):1060–5.
40. Somekh E, Gorenstein A, Serour F. Acute epididymitis in boys: evidence of a post-infectious etiology. J Urol. 2004;171(1):391–4.
41. Zaccara A, Ragozzino S, Iacobelli BD, Rivosecchi F, Capitanucci ML, Mosiello G, et al. Epidydimo-orchitis and anorectal malformations: when and in whom? Pediatr Surg Int. 2015;31(3):305–9.
42. Jesus LE, Rocha KL, Caldas ML, Fonseca E. Granulomatous orchitis in a pre-pubertal school-aged child: differential diagnosis dilemmas. J Pediatr Urol. 2012;8(5):e51–4.
43. Kanakis MA, Vaiopoulos AG, Vaiopoulos GA, Kaklamanis PG. Epididymo-orchitis in Behcet's disease: a review of the wide spectrum of the disease. Acta Med Iran. 2017;55(8):482–5.
44. Dasu N, Khalid Y, Panuganti S, Daly S. Amiodarone induced epididymo-orchitis. Urol Case Rep. 2019;26:100929.
45. Gemmill I. Mumps vaccine: is it time to re-evaluate our approach? CMAJ. 2006;175:491–2.
46. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The diagnosis of urinary tract infection: a systematic review. Dtsch Arztebl Int. 2010;107(21):361–7.
47. Koeijers JJ, Kessels AG, Nys S, Bartelds A, Donker G, Stobberingh EE, et al. Evaluation of the nitrite and leukocyte esterase activity tests for the diagnosis of acute symptomatic urinary tract infection in men. Clin Infect Dis. 2007;45(7):894–6.
48. Etienne M, Pestel-Caron M, Chavanet P, Caron F. Performance of the urine leukocyte esterase and nitrite dipstick test for the diagnosis of acute prostatitis. Clin Infect Dis. 2008;46(6):951–3.
49. Artul S, Abu Rahmah Y, Abu Shkara H, Yamini A. Inferno: colour Doppler ultrasound sign of orchitis. BMJ Case Rep. 2014;2014:bcr2014203613.
50. Basekim CC, Kizilkaya E, Pekkafali Z, Baykal KV, Karsli AF. Mumps epididymo-orchitis: sonography and color Doppler sonographic findings. Abdom Imaging. 2000;25:322–5.
51. Rhudd A, Moghul M, Reid G. Epididymo-orchitis causing testicular infarction: a serious complication of a common disorder. J Surg Case Rep. 2017;10:rjx207.
52. Agrawal V, Ranjan R. Scrotal abscess consequent on syphilitic epididymo-orchitis. Trop Dr. 2019;49(1):45–7.
53. Yam WL, Ng FC. Spermatic cord abscess: a rare complication of epididymo-orchitis, the diagnosis and management. BMJ Case Rep. 2014;2014:pii: bcr2014205019.
54. Bignell C, Fitzgerald M, Group GD. UK BAfSHaH. UK national guideline for the management of gonorrhoea in adults, 2011. Int J STD AIDS. 2011;22(10):541–7.
55. Onofre J, Baert Y, Faes K, Goossens E. Cryopreservation of testicular tissue or testicular cell suspensions: a pivotal step in fertility preservation. Hum Reprod Update. 2016;22(6):744–61.
56. de Lambert G, Poirot C, Guérin F, Brugières L, Martelli H. Preservation of future fertility in pediatric patients with cancer. J Visc Surg. 2018;155(Suppl 1):S41–6.
57. Chatterjee R, Kottaridis PD. Treatment of gonadal damage in recipients of allogeneic or autologous transplantation for haematological malignancies. Bone Marrow Transplant. 2002;30(10):629–35.
58. Lee SH, Shin CH. Reduced male fertility in childhood cancer survivors. Ann Pediatr Endocrinol Metab. 2013;18(4):168–72.

59. Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. N Engl J Med. 2009;360:902–11.
60. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7:535–43.
61. Darzy KH, Shalet SM. Hypopituitarism following radiotherapy revisited. Endocr Dev. 2009;15:1–24.
62. Wallace WHB, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 2005;6:209–18.
63. Meistrich ML, Wilson G, Mathur K, Fuller LM, Rodriguez MA, McLaughlin P, et al. Rapid recovery of spermatogenesis after mitoxantrone, vincristine, vinblastine, and prednisone chemotherapy for Hodgkin's disease. J Clin Oncol. 1997;15:3488–95.
64. da Cunha MF, Meistrich ML, Haq MM, Gordon LA, Wyrobek AJ. Temporary effects of AMSA (4′-(9-acridinylamino) methanesulfon-m-anisidide) chemotherapy on spermatogenesis. Cancer. 1982;49:2459–62.
65. Meistrich ML. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. Fertil Steril. 2013;100:1180–6.
66. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of male survivors of childhood cancer: a report from the childhood Cancer survivor study. J Clin Oncol. 2010;28:332–9.
67. Wyns C, Curaba M, Vanabelle B, Van Langendonckt A, Donnez J. Options for fertility preservation in prepubertal boys. Hum Reprod Update. 2010;16:312–28.
68. Kenney LB, Cohen LE, Shnorhavorian M, Metzger ML, Lockart B, Hijiya N, et al. Male reproductive health after childhood, adolescent, and young adult cancers: a report from the Children's Oncology Group. J Clin Oncol. 2012;30:3408–16.
69. Wallace WHB. Oncofertility and preservation of reproductive capacity in children and young adults. Cancer. 2011;117(Suppl):2301–10.
70. Zhang S, Mo J, Wang Y, Ni C, Li X, Zhu Q, et al. Endocrine disruptors of inhibiting testicular 3β- hydroxysteroid dehydrogenase. Chem Biol Interact. 2019;303:90–7.
71. Gaudriault P, Mazaud-Guittot S, Lavoué V, Coiffec I, Lesné L, Dejucq-Rainsford N, et al. Endocrine disruption in human fetal testis explants by individual and combined exposures to selected pharmaceuticals, pesticides, and environmental pollutants. Environ Health Perspect. 2017;125(8):087004.
72. Kumari M, Singh P. Tribulus terrestris improves metronidazole-induced impaired fertility in the male mice. Afr Health Sci. 2018;18(3):645–52.
73. Duca Y, Aversa A, Condorelli RA, Calogero AE, La Vignera S. Substance abuse and male hypogonadism. J Clin Med. 2019;8(5):pii:E732.

Risk Factors Affecting Puberty: Environment, Obesity, and Lifestyles

10

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10.1 Introduction

Puberty is the physiological process and crucial developmental stage, typically beginning between 9.5 and 13.5 years of age (average 11.5 years) in boys and lasting normally about 5–6 years, characterized by the gradual transition from childhood to adulthood, with the attainment of appearance of secondary sexual characteristics and mainly of sexual maturity and reproductive capability [1]. Hormonal and physical changes occurring during pubertal development in boys include the onset of growth of pubic and axillary hair (pubarche), the onset of secretion of adrenal androgens (adrenarche), the onset of secretion of sex hormones by the gonads (gonadarche), and the onset of production of spermatozoa (spermarche) [2]. The key physiological process behind the transition from childhood to adulthood is the activation of the hypothalamus–pituitary–gonadal (HPG) axis, and the consequent gonadal stimulation by the two gonadotropins, namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [3].

Physiologically, gonadotropins secretion by the gonadotropic cells of the anterior pituitary is controlled by the gonadotropin-releasing hormone (GnRH)

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synthesized by hypothalamic neurons located in the preoptic area of the brain and released in the pituitary portal circulation in a pulsatile manner. The modulation of GnRH pulses determines the preferential release of gonadotropins; LH is preferentially stimulated at high GnRH pulse frequencies, whereas FSH is preferentially stimulated at low GnRH pulse frequencies [4]. Gonadotropins secretion is modulated by negative feedback mechanisms operated by sex hormones at the hypothalamic and pituitary level. In particular, androgens, mainly testosterone, act on the hypothalamus by reducing GnRH pulsatility frequency and on gonadotropic cells of the pituitary by reducing gonadotropins secretion, whereas estrogens, mainly estradiol, act on the hypothalamus by reducing GnRH pulsatility frequency and on the pituitary by reducing gonadotropic cells sensitivity to GnRH [5, 6]. Moreover, FSH secretion is also regulated by the activin–inhibin–follistatin axis. The gonadal hormones activins and inhibins, belonging to the transforming growth factor-β superfamily of growth and differentiation factors, are produced by Sertoli cells within the testis, and are autocrine and paracrine modulators of Sertoli cell proliferation and germ cell development [7, 8]. Inhibins are heterodimers composed by an α subunit and a βA or βB subunit, forming inhibin A and inhibin B, respectively. Inhibin B is the major form produced by Sertoli cells in the fetal and adult human testis in response to FSH stimulation, and reflects the status of spermatogenesis; indeed, inhibin B concentration positively correlates with Sertoli cell number and function and is therefore a useful clinical marker of spermatogenesis status [6, 9, 10]. Activins are homodimers composed by the same two types of β subunit (βA or βB) of inhibin, and are produced by Sertoli cells and by multiple cells of the anterior pituitary, including the gonadotropic cells [11, 12, 13]. Activins production by Sertoli cells is therefore regulated by inhibins concentration, which determines the availability of β subunits for activins production [7, 8]; contrary to inhibin B, it is therefore still uncertain whether testicular activin production is also directly regulated in response to FSH [11]. Pituitary activins induce the production of their own binding protein, follistatin, and reduce the production of the activin β subunit, therefore self-limiting their own production [13]. Testicular inhibin B and activins inhibits and stimulate, respectively, FSH production at the pituitary level [7, 8, 12]; nevertheless, besides GnRH, the activins locally produced within the pituitary and their modulation by testicular inhibin B represent a major effector in the stimulation of FSH, compared to testicular activins-inhibin B system [11, 12].

Follistatin is produced by the gonadotropic cells and the folliculostellate cells of the anterior pituitary in response to a concerted interplay among a stimulatory effect of GnRH, an inhibitory effect of androgens and a self-limiting intra-pituitary activin tone, which is necessary to maintain follistatin levels but is subjected to a reciprocal activin–follistatin negative feedback loop finalized to FSH modulation [12]. Indeed, follistatin exerts a local control on FSH production, by binding and neutralizing activin action, therefore indirectly decreasing FSH levels [12]. Noteworthy, the inhibin B endocrine feedback on FSH production through pituitary activin modulation appears to be the most physiologically relevant mechanism [12].

Insulin-like 3 (INSL3) is a peptide hormone member of the insulin superfamily specifically produced by the Leydig cells within the testis [14]. During fetal life, INSL3 is involved in testicular descent particularly during the second trimester and immediately after birth [14]. During adult life, INSL3 is involved in the modulation

of androgen production by acting with autocrine manner on Leydig cells, but a role for INSL3 in spermatogenesis has been also hypothesized. However, INSL3 is not regulated by the HPG axis, and is rather a constitutive hormone secreted by Leydig cells, therefore considered as an excellent marker of Leydig cell differentiation and function [14]. Indeed, in the mature adult HPG axis, testosterone feedback leads progressively to a stabilization of LH levels and a correspondingly reduced level of Leydig cell metabolism (stable differentiation status), which is reflected by stable INSL3 levels [14]. After birth, the so-called "mini-puberty" period occurring from the first to the sixth month of life is characterized by a temporary activation of the HPG axis, determining a relevant increase in the secretion of gonadotropins and sex hormones, reaching a peak concentration around the third month of life. Gonadotropins production at this stage is functional to testicular descent and further maturation of testicular cell populations; nevertheless, starting around the third month of life both gonadotropins and sex hormones levels decrease, and HPG axis activity returns inactive and remains in a phase of relative quiescence until puberty [15]. At puberty, the definitive activation of HPG axis induces a sustained increase in the pulsatility of GnRH determining an increase of the amplitude of gonadotropins secretion, particularly LH, and consequent increase of androgens production, particularly testosterone. These hormonal variations are responsible of the changes occurring at puberty in several tissues and organs, including testis enlargement, penile growth, modification of sweat smell, increase of skin sebum production promoting acne, appearance of axillary and pubic hair, increase of the larynx size with lengthening of vocal cords determining voice break, muscle and bone development resulting in growth spurt, changes in the brain with ensuing behavioral changes, and first nocturnal ejaculation as a marker of sexual maturity [3, 16].

Puberty is categorized into five stages according to Tanner classification, which in boys is based on testis volume, pubic hair, penis length and color of scrotal skin [1, 17]. Tanner stage 1 corresponds to the prepubertal status for all development signs with progression to Tanner stage 5, corresponding to the adult status. The specific marker of pubertal onset (first sign of puberty) is the achievement of a testis volume of 4 mL, which is an indicator of gonadotropins production [18]. More specifically, stage 1 is characterized by a testis volume <4 mL or long axis <2.5 cm; stage 2 by a testis volume of 4–8 mL or long axis of 2.5–3.3 cm; stage 3 by a testis volume of 9–12 mL or long axis of 3.4–4.0 cm; stage 4 by a testis volume of 5–20 mL or long axis of 4.1–4.5 cm; stage 5 by a testis volume >20 mL or long axis >4.5 cm [18]. The great part of the testis volume is related to the elongation of the seminiferous cords, and to the increase in seminiferous tubules diameter, reflecting Sertoli cells proliferation [19]. Sertoli cells are essential for adequate sexual differentiation during fetal life, and for spermatogenesis during adult life. In the fetal testis, Sertoli cells are morphologically and functionally immature and release anti-Müllerian hormone (AMH), which triggers the involution of the Müllerian ducts, embryonal precursors of the fallopian tubes, uterus, and upper part of the vagina in the female, therefore contributing to male sex genital differentiation. At pubertal onset, the increasing secretion of testosterone from Leydig cells, which determines a raise of intratesticular testosterone levels, negatively regulates AMH secretion, by mirroring the maturation of Sertoli cells [20]. Inhibin B levels increase early in puberty, and at Tanner stage 2 the adult inhibin B levels have already been reached, and are positively

correlated with LH and testosterone, but not with FSH levels, suggesting a prominent supportive role of Leydig cells in Sertoli cells maturation during early puberty. Conversely, late puberty (from Tanner stage 3) is characterized by a weak negative correlation between inhibin B and LH and by a negative correlation between inhibin B and FSH levels, which is maintained in adults, and no correlation between inhibin B and testosterone, reflecting the accomplishment of HPG axis maturation and full establishment of feedback regulation mechanisms [21]. INSL3 levels decline during childhood, and start to re-increase at pubertal onset under the differentiating action of LH on Leydig cells, until reaching adulthood levels [22]. Lastly, puberty is accompanied by adrenarche, which is defined as the maturation of the adrenal gland with the gradual development of the reticular area of the adrenal cortex, which produces predominantly weak androgens, particularly androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulphate (DHEAS) [23]. Adrenarche generally precedes the gonadal development induced by the maturation of the HPG axis determining full establishment of HPG axis function, and adrenal androgens production continues throughout pubertal stages contributing, along with gonadal testosterone, to great part of the body changes occurring during pubertal progression, including the development of axillary and pubic hair, the achievement of an adult-type sweat smell, and the increase in skin sebum production with appearance of acne [2]. Adrenarche is accompained by the activation of the hypothalamus-pituitary-somatotropic (HPS) axis and of the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) system, which contribute to pubertal development [24].

The onset of puberty is physiologically driven by the pulsatile release of hypothalamic GnRH, although the molecular mechanism underlying GnRH release as puberty approaches are not yet completely understood. GnRH pulses are thought to be regulated by the coordinated activity of different neurons disseminated within the hypothalamus nuclei, known as GnRH pulse generators [2]. In particular, neurons located in the infundibular/arcuate nucleus of the hypothalamus secrete peptides involved in this neuroendocrine network including kisspeptins, neurokinin-B (NKB), and dynorphin (Dyn), and are therefore known as KNDy neurons. Inactivating mutations in genes encoding for kisspeptins and NKB and/or their receptors have been linked to absence of puberty or hypogonadotropic hypogonadism in humans, highlighting a stimulatory effect on GnRH release; conversely, based on studies on experimental models, Dyn has been recognized as a GnRH inhibitor. Moreover, NKB and Dyn also indirectly modulate GnRH release by acting directly on KNDy neurons by autocrine signaling, to positively or negatively modulate kisspeptin secretion, respectively, which in turn modulates the release of GnRH. Lastly, KNDy neurons activity appears to be directly regulated by a negative feedback operated by sex hormones [2]. Many additional factors are implicated in the onset of puberty, and include genes involved in processes upstream of HPG activation, namely, genes involved in migration of the GnRH neurons (GNRHR, KAL-1), and in the proper development of the hypothalamus–pituitary unit (DAX-1) [25]. A graphical overview of the HPG axis and GnRH pulse generator regulation is provided in Fig. 10.1. The current chapter will provide a synoptic overview on the still scarcely deepened state of knowledge linking pathological pubertal

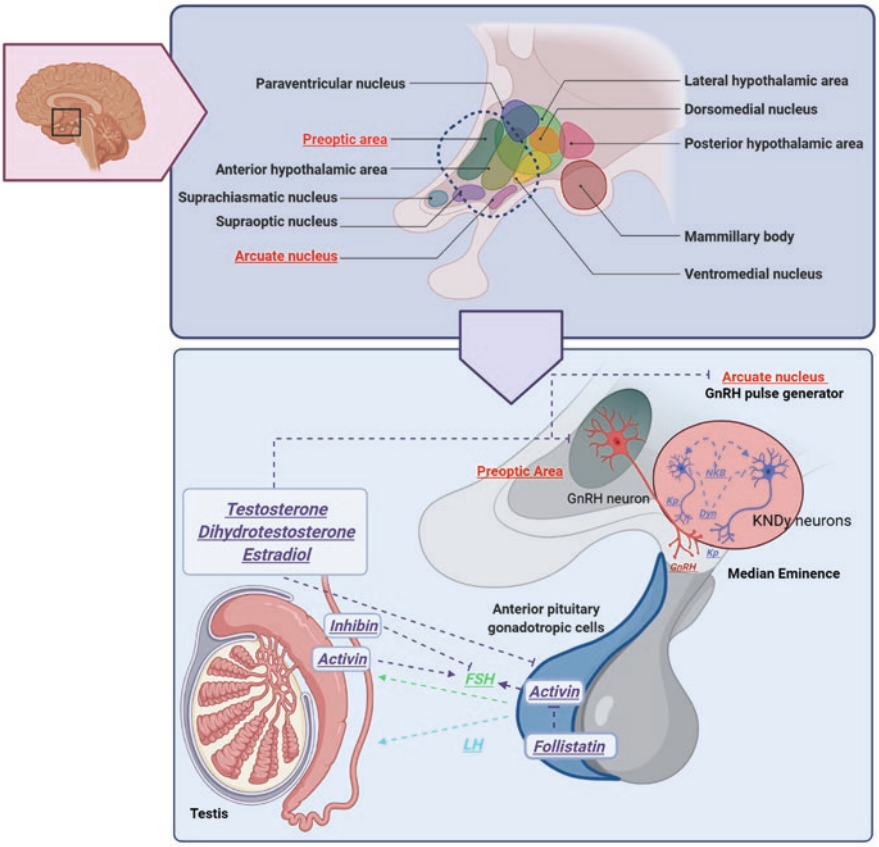

Fig. 10.1 Graphical overview of the hypothalamus–pituitary–gonadal axis and gonadotropin-releasing hormone pulse generator regulation. Gonadotropin-releasing hormone (GnRH) is secreted by GnRH neurons located in the preoptic area of the hypothalamus. The anterior portion of the pituitary gland, particularly, the gonadotropic cells, produce the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn stimulate both steroidogenesis and spermatogenesis within the testis. The main hormone controlling GnRH production is testosterone, which inhibits gonadotropin production by exerting a negative feedback at both the hypothalamic and pituitary level; testosterone may act per se, or after conversion to dihydrotestosterone or estradiol. Inhibin produced by the testis also provides a selective negative feedback on FSH, counteracted by activins produced by the testis and the anterior pituitary, which in turn are modulated by pituitary follistatin. In physiological conditions, pubertal onset is triggered by a sustained increase in the pulsatile release of GnRH from GnRH neurons; a GnRH pulse generator located in the arcuate nucleus of the hypothalamus drives the GnRH pulsatility increase necessary to initiate puberty. Within GnRH pulse generator, a timely regulated neuroendocrine network including kisspeptin (Kp), neurokinin-B (NKB), and dynorphin (Dyn), all secreted by KNDy neurons, is orchestrated to modulate GnRH release. In particular, Kp and NKB stimulate, whereas Dyn inhibits, the GnRH release from GnRH neurons terminals, at the median eminence. Moreover, NKB and Dyn also indirectly modulate GnRH release, by acting back on KNDy neurons to positively or negatively modulate Kp secretion, which in turn stimulates the release of GnRH. Lastly, KNDy neurons activity appears to be directly regulated by estradiol and testosterone by effect of a negative feedback. Figure created with BioRender.com

development to exogenous players related to environment, obesity and lifestyles, including prenatal, postnatal, and pubertal exposure to environmental contaminants with endocrine disrupting activity, obesity, and unhealthy lifestyle-related attitudes with an additional focus on conditions of negative energy balance, such as severe malnutrition and vigorous physical activity.

10.2 Risk Factors Affecting Puberty: Environment, Obesity, and Lifestyles

Physiological variation in age at puberty onset is observed in healthy children. Differences in pubertal development might be related to two main features: onset and progression. Beyond the physiological variation, puberty-associated disorders based on onset are classified into two major types, precocious puberty and delayed puberty, and reflect maturity of the child at the time of puberty, compared to age- and sex-matched peers [26]. Pubertal disorders involving progression concern the velocity of progression from pubertal Tanner Stage 1 throughout Stage 5 [1, 2, 17]. Precocious puberty in boys is defined as the onset of the first sign of puberty and/or appearance of secondary sexual characteristics before 9 years of age, and might be determined by either the premature activation of the HPG axis (central precocious puberty), or a primary disorder of the testis or adrenal gland, independently from HPG axis activation (peripheral or pseudo-precocious puberty). Delayed puberty in boys is defined as the lack of the first sign of puberty and/or secondary sexual characteristics by 14 years of age, and practically might result from a delay in the onset, progression, or completion of pubertal development; delayed puberty might be further classified into pubertal delay and pubertal failure [2].

Divergent secular changes have been pointed out in boys, concerning initial pubertal signs and signs of puberty completion, displaying a skewed age distribution at pubertal signs towards earliness for initial pubertal stages and lateness for final pubertal stages; therefore, extended pubertal range beyond the physiological 5-year period for both the initial and late stages of puberty has emerged [27].

The timely onset of puberty might be influenced by a number of factors, including genetic, endocrine, environmental and nutritional factors, with genetics explaining about 50–80% of pubertal timing [25]. Geographical differences, psychosocial stresses, prenatal and postnatal exposure to endocrine disruptors deriving from environmental pollutants or from chemicals and industrial compounds, as well as unhealthy lifestyles before or during puberty can all influence the normal puberty in boys; indeed, growing consciousness has emerged that pubertal development can no longer be considered under exclusive control of genetic determinants. A relevant challenge in determining pubertal abnormalities in boys, as compared to girls, involves the scarcity of studies with objective clinical inspection of testis volume, and limited simple and reliable self-reported markers for pubertal development, with ensuing problems connected to the correct identification of pubertal onset and/or progression across stages. Indeed, most studies exclusively rely on visual grading of genital development, which might overlook subtle changes, or on questionnaires with drawings of Tanner stages or yes/no items for the presence and/or grade of pubertal signs, all being identification tools prone to proxy reporting. A graphical

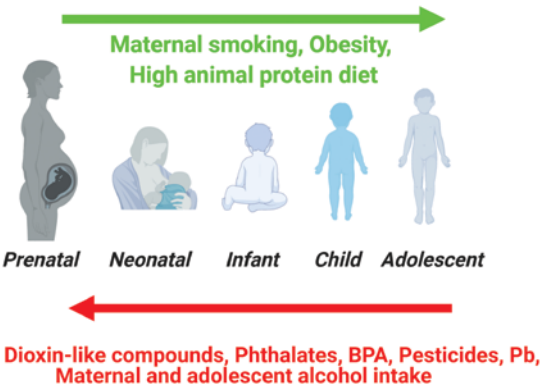

Fig. 10.2 Graphical overview of factors proposed to exert a role in anticipating (green arrow) or delaying (red arrow) pubertal onset and/or progression in boys. Figure created with BioRender.com

overview of factors proposed to exert a role in anticipating or delaying pubertal onset and/or progression in boys is provided in Fig. 10.2.

10.3 Environment

Exposure to environmental pollutants such as endocrine-disrupting chemicals (EDCs) during critical periods of development, characterized by increased susceptibility to endocrine perturbations, may result in disorders of pubertal development and propensity to long-term reproductive consequences, in line with the Barker hypothesis of developmental origin of health and disease [28, 29]. Experimental studies in animal models, mainly of female sex, highlighted that prenatal and neonatal exposure to EDCs determines neuroendocrine imbalance at hypothalamic and pituitary level, as well as peripheral endocrine effects at gonadal level, resulting in an impairement of pubertal development [30]. Nevertheless, despite several clinical studies in humans investigated the potential involvement of prenatal or childhood exposure to EDCs in the development of pubertal disorders in girls, scarce investigation has been performed in boys, due to the lack of a robust marker of puberty, such as menarche in girls, or provide unclear results. However, sparse associations have been highlighted between pubertal development and exposure to selected EDCs, including polychlorinated biphenyls (PCB), dioxin and dioxin-like compounds, phthalates, bisphenol A (BPA), pesticides [organochlorine chemicals, endosulfan, dichloro-diphenyl-trichloroethane (DDT)] and lead (Pb).

Yucheng ("oil-disease") victims were Taiwanese people exposed to PCB from the ingestion of contaminated rice oil in 1978–1979; male descendants of accidentally exposed women had shorter penile length, compared to age-matched unexposed peers, suggesting an impairment of genital development upon prenatal PCB exposure and/or a delayed puberty; nevertheless, puberty was not a specific aim of these studies, and no further investigation was performed concerning major pubertal signs [31, 32]. Consistently, a cross-sectional study on adolescent boys exposed to PCB during pubertal development, with objective evaluation of pubertal stage by physical examination, demonstrated that PCB levels were negatively correlated with pubertal stages; in particular, boys displaying higher PCB levels had an

increased risk of failure to reach the adult stage of genital development and pubic hair growth, therefore corroborating a significant delayed pubertal development in such PCB-exposed boys [33]. Nevertheless, different studies evaluating the relationship between prenatal [34–36], postnatal (during lactation period) [34, 36] and current adolescent [34] exposure to PCB, dioxin and dioxin-like compounds failed to detect an association with stages of pubertal development, assessed by both self-reported pubertal stage [36] and physical examination [34, 35], with the exception of a positive correlation of current exposure with age at first ejaculation [34]. Experimental studies highlighted that PCB inhibits GnRH production, reduces cell viability, with increase of oxidative stress and apoptosis in hypothalamic GnRH-producing cells [37–39], and reduces the expression of antioxidant and steroidogenesis enzymes and LH receptor in Leydig cells [40], with consequent decrease of gonadotropins and testosterone production [40, 41], therefore supporting the hypothesis that PCB exposure prevents the activation or perturbs the function of HPG axis, by acting at both hypothalamic and testicular level.

Exposure to phthalate and BPA displayed some timeframe-dependent anti-androgenic or estrogenic effects on puberty in boys; in particular, exposure during the third trimester of pregnancy was associated with a delayed onset of adrenarche and pubarche and increased peri-pubertal SHBG levels [42], whereas childhood exposure was not associated with a deviation of pubertal onset, although increased SHBG levels and decreased total and free testosterone levels were found [42]. Moreover, clinical signs considered as markers of anti-androgenic or estrogenic exposures, namely, shortening of anogenital distance and gynecomastia, have been highlighted in phthalate-exposed and BPA-exposed boys. Indeed, anogenital distance is a sexually dimorphic landmark established during fetal life in response to the hormonal milieu and is an important clinical surrogate to address endocrine-sensitive outcomes, reflecting potential prenatal exposure to EDCs affecting androgens and/or estrogens signaling [43]. A shortened anogenital distance was associated with parental occupational exposure to phthalate and BPA during the period of pregnancy, displaying a significant dose–response relationship and therefore suggesting a detrimental effect on male genital development in boys with prenatal exposure, by considering either mother occupational exposure, and/or father occupational exposure as a surrogate marker of indirect mother exposure through contaminated clothing, visits to the spouses workplace and residing in the vicinity of the BPA factories. Moreover, although occupational BPA exposure of both mother and father appeared to be significantly correlated with shortened anogenital distance, association was stronger for maternal exposure, possibly implying that there is little or no placental barrier to BPA, with potential ensuing prenatal exposure to high levels of BPA in the male descendants of exposed pregnant women [44]. Similarly, children with pubertal gynecomastia showed significantly higher phthalate levels, compared to children without gynecomastia, although no correlation was found between phthalate levels and gonadotropins and sex hormones levels; these findings might allow to speculate that phthalate exposure may determine pubertal breast enlargement within a timeframe of increased hormonal sensitivity by activating estrogen receptor or intracellular pathways rather than affecting hormone levels [45]. Collectively, available evidence concerning the effects of phthalates and BPA strongly supports an anti-androgenic or estrogenic action of such compounds, potentially affecting pubertal

development. Interestingly, experimental studies in animals demonstrated that phthalates and BPA might induce long-term transgenerational inheritance of reproductive disorders, and that an additional potential mechanism involved in phthalate-induced and BPA-induced reproductive toxicity might be identified in epigenetic changes in the germline. More specifically, exposure of pregnant rats to mixtures of phthalates and BPA determined a significant increase in the incidence of testis pathology and pubertal abnormalities in the third male offspring generation, compared to the third male offspring generation derived from unexposed mothers, together with the evidence of an increase in spermatogenic cell lineage apoptosis. Moreover, in the sperm DNA of the third male offspring generation a significantly different methylation profile was discovered, compared to unexposed animals, which might be associated with adult onset diseases, comprising reproductive abnormalities [46].

A prospective study on boys living in a highly polluted Russian area and subjected to annual physical examinations including pubertal staging and testis volume assessment, demonstrated that peri-pubertal exposure to pesticides of the organochlorine chemicals class and to Pb, measured at enrolment (8–9 years), was associated with delayed pubertal development [47]. In agreement with these findings, history of exposure to endosulfan, a different pesticide, was found as associated with reduced pubic hair as well as testis and penis growth according to Tanner stages, compared to unexposed peers [48], by unraveling delayed sexual maturation; nevertheless, no association was found between maternal DDT levels and height, body mass index (BMI), skeletal age, testosterone or DHEAS in prenatally exposed male descendants [49]. Scarce experimental studies highlighted that organochlorine pesticides increase apoptosis in hypothalamic GnRH-producing cells, inhibiting GnRH production [50, 51], and decrease the expression of testicular steroidogenesis enzymes and testosterone levels [52–54], therefore supporting the hypothesis that this class of pesticides might interfere with the activation or function of HPG axis acting at both hypothalamic and testicular level. Although dearth of robust studies imposes further investigation in this area, precautionary attitude in minimizing EDCs exposures within highly endocrine-sensitive timeframes, particularly prenatal development and early postnatal and pre-pubertal age, and proper assessment of EDCs exposure and potential impact on pubertal development might be recommended, in the management of pubertal disorders. An overview of the main pubertal outcomes and endocrine profile, as reported by observational human studies on EDCs exposure, is provided in Table 10.1.

10.4 Obesity

Obesity is defined, according to the World Health Organization (WHO), as a pathological accumulation of fat into the body with ensuing adverse implications for general health status, and is among the most frequent diseases worldwide [55]. In particular, in 2016, WHO estimates reported that more than 1.9 billion adults over the world were overweight or obese, with a prevalence of overweight of 39% and a prevalence of obesity of 11% in men, whereas 340 million children and adolescents aged 5–19 years were overweight or obese, with a prevalence of overweight of 19%

Table 10.1 Overview of the main pubertal outcomes and hormonal profile, as reported by observational human studies on endocrine disrupting chemicals exposure

	N° subjects/group	Age	Timing of exposure	Type of exposure/ sample for measurement	Main pubertal outcomes[a]	Hormonal profile[a]
Guo YL et al. (2004)	**Group 1:** 55 boys from Yucheng (exposed mothers) **group 2:** 55 boys (non-exposed mothers)	**Group 1:** R 11–14	Prenatal exposure from accidentally intoxicated mothers (contaminated rice oil)	PCBs/maternal serum	↓ Penile length	NA
Den Hond E et al. (2002)	**Group 1:** 40 boys from 2 urban areas **Group 2:** 40 boys from a rural area	**Group 1:** M 17.9; M 17.3 **Group 2:** M 17.1	Current environmental exposure	PCBs/serum	↓ % of boys in genital stage G5 and pubic hair stage PH5 ↓ Testis volume	= TT, FT, SHBG, TE, FE, Inh-B, LH, FSH
Leijs MM et al. (2008)	**Group 1:** 15 boys	**Group 1:** m 14.3	Prenatal, lactational and current environmental exposure	Dioxin and dioxin-like compounds (PCDDs and dl-PCBs)/breast milk—Breast milk multiplied for total breast milk intake—Serum	+ Age at first ejaculation (current dl-PCB) = Genital stage, testis volume, axillary hair development, pubic hair stage, age at first pubic hair development	NA
Mol NM et al. (2002)	**Group 1:** 196 boys	**Group 1:** M 13.9	Prenatal environmental exposure	PCBs/umbilical cord	= Genital stage, testis volume, pubic hair stage	= TT, SHBG, Inh-B, LH, FSH

Gladen BC et al. (2000)	**Group 1:** 278 boys	**Group 1:** R 10–15	Prenatal and lactational environmental exposure	PCB/maternal blood—Cord blood—Placenta—Breast milk	= Age at attainment of pubertal stages	NA
Ferguson KK et al. (2014)	**Group 1:** 278 boys	**Group 1:** R 8–14	Prenatal and current environmental exposure	Phthalates—BPA/maternal third trimester urine—Urine	Prenatal phthalates and BPA: − Adrenarche and pubarche Current phthalates and BPA: = Adrenarche and pubarche	Prenatal phthalates: − Inh-B + SHBG = TT, FT, TE Current phthalate: + SHBG − TT, FT Prenatal BPA: = TT, FT, SHBG, TE, Inh-B Current BPA: + SHBG − TT, FT
Miao M et al. (2011)	**Group 1:** 56 boys (18 exposed mothers, 38 exposed fathers) **Group 2:** 97 boys (non-exposed parents)	**Group 1:** M 4.3; M 5.3 **group 2:** M 6.1	Prenatal exposure from occupationally exposed parents	BPA/parents personal air sample	↓ AGD	NA
Sergeyev O et al. (2017)	**Group 1:** 516 boys from a highly contaminated area	**Group 1:** Age 8–9 years followed-up to 18–19 years of age	Current environmental exposure	OC and Pb/serum and whole blood	+ Age at attainment of testis volume >3, and of pubertal onset	NA

(continued)

Table 10.1 (continued)

	N° subjects/group	Age	Timing of exposure	Type of exposure/ sample for measurement	Main pubertal outcomes[a]	Hormonal profile[a]
Saiyed H et al. (2003)	**Group 1:** 117 boys from a highly contaminated area **Group 2:** 90 boys from a control area	**Group 1 and Group 2:** R 10–19	Current environmental exposure	Endosulfan/serum	↓ Pubic hair, testis and penis development	↓ TT ↑ LH = FSH
Gladen BC et al. (2004)	**Group 1:** 304 boys	**Group 1:** Age 10 years followed-up to 20 years of age	Prenatal environmental exposure	DDT/maternal serum	NA	= TT

[a]Reported changes are intended in the exposed group/cases (group 1), compared to control group (group 2), or correlated to exposure levels: ↑, increased; ↓, decreased; +, positive correlation; −, negative correlation; =, no change/not correlated. *Abbreviations: R* Range, *M* Mean, *m* Median, *PCDDs* Polychlorinated dibenzo-p-dioxins, *dl-PCBs* dioxin-like polychlorinated biphenyls, *BPA* Bisphenol A, *OC* organochlorine compounds, *Pb* lead, *TT* Total testosterone, *FT* Free testosterone, *SHBG* Sex hormone binding globulin, *TE* Total estradiol, *FE* Free estradiol, *Inh-B* Inhibin B, *LH* Luteinizing hormone, *FSH* Follicle stimulating hormone, *AGD* Anogenital distance, *NA* Not applicable/not available

and a prevalence of obesity of 8% in boys [55]. Obesity-associated comorbidities include mainly metabolic, cardiovascular, and psychosocial disorders, together with endocrine disorders, including an impairment of HPG axis; male obesity–induced secondary hypogonadism (MOSH) may be clinically defined as an obesity-induced predominantly secondary form of functional hypogonadism, in men in whom organic and different functional causes of hypogonadism, such as medications, alcohol or drugs abuse, systemic illness, nutritional deficiency or excessive exercise, sleep disorders, and comorbid illness associated with aging, have been excluded [56–58]. Although few studies evaluated body fat distribution in relation to androgenic status, several evidences outline that rather than obesity per se, specific adiposity distribution within the body, and particularly visceral fat deposition, is related to an hypogonadal condition in men [57, 59, 60]. Indeed, visceral adiposity induces the testosterone deficiency, which in turn further promotes visceral adiposity in obese men, determining a typical vicious circle characterizing MOSH [60, 61]. The functional hypogonadism associated with visceral obesity derives from the interplay among several pathogenetic events leading to the impairment of HPG axis function and androgenic status. Adipose tissue is an endocrine organ with abundant aromatase activity, and able to produce inflammatory mediators; therefore, an increased visceral adiposity is associated with an increased aromatase activity and to a chronic systemic low-grade inflammatory status [57]. Moreover, excess visceral adiposity is also associated with a plethora of metabolic abnormalities, generally attributable to the development of insulin resistance (IR) [57]. The inhibition of the HPG axis, in particular the inhibition of gonadotropins and therefore testosterone secretion, may be exerted by the low-grade inflammation, through the inhibition of GnRH neurons function by inflammatory cytokines, as well as by estradiol excess induced by increased aromatase activity. Moreover, the inhibition of the HPG axis may ultimately result from a cascade of events initiated by IR; indeed, IR determines a decrease of SHBG levels, leading to a transient increase of free testosterone levels, which in turn enhances conversion of testosterone to estradiol, leading to estradiol excess, which adds up to the already increased aromatase activity in ultimately inhibiting gonadotropins and, consequently, testosterone production [57, 60]. In summary, compelling evidence highlighted a strict association between visceral obesity and hypogonadism in adult males, mediated by multiple pathogenetic mechanisms interfering at both central and peripheral level. Nevertheless, despite these cornerstones, the significance and relative weight of MOSH and associated mechanisms observed in adults, on pubertal development is unclear, due to lack of focused studies and direct evidence in obese boys. An additional mechanism linking visceral obesity to the impairment of the HPG axis function in adults involves the action of adipokines, cytokines secreted by adipocytes, whose receptors are expressed in the entire series of components of the HPG axis, namely hypothalamus, pituitary, and testis; indeed, the changes in adipokines levels observed in obese patients contribute to HPG axis dysfunction [62]. In this context, a particularly well-recognized player is the major adipokine, leptin, an anorexigenic hormone secreted by adipocytes proportionally to the fat mass and involved in physiological conditions of normal weight in the regulation of energy balance by suppressing the production of the neuropeptide Y (NPY), thereby reducing food intake and inducing weight loss [62]. In physiological conditions of normal weight, leptin exerts a permissive effect on the HPG axis, mediated by the negative

modulation of metabolic-sensing neurons in the arcuate nucleus of the hypothalamus, namely NPY, and the positive modulation of pro-opiomelanocortin (POMC) neurons, which, projecting directly to the GnRH neurons, inhibit and stimulate GnRH secretion, respectively [63, 64]. Moreover, an indirect leptin-induced increase in kisspeptin expression has been also suggested to convey GnRH neurons stimulation. Indeed, considering the lack of functional leptin receptors expression on GnRH neurons and a scattered expression of functional leptin receptors on kisspeptin neurons, different sets of neurons expressing leptin receptor, including NPY and POMC neurons, and neurons located in the ventral premammilary nucleus and preoptic area of the hypothalamus, might mediate the indirect leptin-induced stimulation of kisspeptin [63, 64]. In obese men, obesity-induced leptin resistance, mediated by leptin receptor desensitization at the hypothalamic–pituitary level, results in blunted leptin signaling, which determines central inhibition of the HPG axis [62, 65, 66]. Moreover, a direct peripheral inhibitory effect of hyperleptinemia on testicular steroidogenesis, mediated by leptin receptors expressed on Leydig cells, has also been demonstrated [67, 68]. Indeed, a study including men ranging from normal weight to obese demonstrated that basal and LH-stimulated free and total testosterone levels were reduced and negatively correlated with leptin levels in obese men, independently from gonadotropins, estradiol and SHBG levels, corroborating a direct effect on de novo testicular steroidogenesis; blunted testosterone response was explained by a leptin-related increase in 17-OH-progesterone-to-testosterone ratio, suggesting a defect in the enzymatic conversion of 17-OH-progesterone to testosterone [69]. Hypothesis might be postulated as to whether each component of the obesity-hypogonadism crosstalk might be of major relevance to the onset and/or the progression of male puberty; nevertheless, considering that pre-pubertal boys are indeed in a physiological quiescence of the HPG axis before the onset of puberty, the above mechanisms might be speculated to be more influential on pubertal progression. Nevertheless, considering the relevant and direct permissive role of leptin on the HPG axis and, particularly, on GnRH neurons, changes in leptin levels occurring in obesity might also be directly involved in perturbations of pubertal onset, and generally pubertal timing.

Puberty and the following starting of the reproduction are energy-demanding biological functions; therefore, a tightly controlled coupling between energy reserves and pubertal onset or maintenance of reproductive function exists (metabolic gating), and leptin levels play as a neuroendocrine integrator of such a crosstalk [64]. In physiological conditions of normal weight, in boys, leptin levels rise just before the gonadotropin peak preceding puberty and reach their peak at the onset of puberty; afterwards, leptin levels increase shortly during the early stages of puberty and decline thereafter [62]. The decline of leptin levels in late puberty in boys parallels increased androgens production and most likely reflects the androgen-induced suppression of leptin, together with the direct leptin reduction consequent to a reduced fat mass and relative increase in muscle mass during late puberty in boys [70]. Indeed, leptin levels are negatively modulated by androgens [62], as confirmed by a study demonstrating that pubertal leptin levels were negatively correlated with total testosterone levels in boys [71]. On the other hand, the increase of leptin levels right before puberty exerts a permissive effect on the HPG axis and pubertal onset, mediated by the increased leptin signaling and enhancement of leptin action on metabolic-sensing NPY and POMC neurons in the arcuate nucleus

and of neurons located in the ventral premammilary nucleus and preoptic area of the hypothalamus, ultimately determining the release from inhibitory signals projecting to GnRH neurons, therefore potentially driving the pubertal onset [63, 64].

There is still an ongoing debate regarding whether obesity might impact on the onset and/or progression of puberty, an uncertainty fueled by contrasting evidences provided by dedicated studies in obese boys, often suffering from methodological heterogeneity and pitfalls in the correct identification of pubertal onset and/or proper classification of pubertal stages in males, and fostered by the presence of indirectly hypothesized mechanisms which might favor either an earlier onset/accelerated progression or a delayed onset/decelerated progression of puberty, with absence of clear and unequivocal dissection or prioritization of the relative impacts. Nevertheless, although the relationship between obesity and puberty remains controversial, the majority of studies collectively seem to suggest an association between non-physiological BMI and earlier pubertal development, although BMI is not a direct measure of obesity status nor of adiposity localization or visceral adiposity.

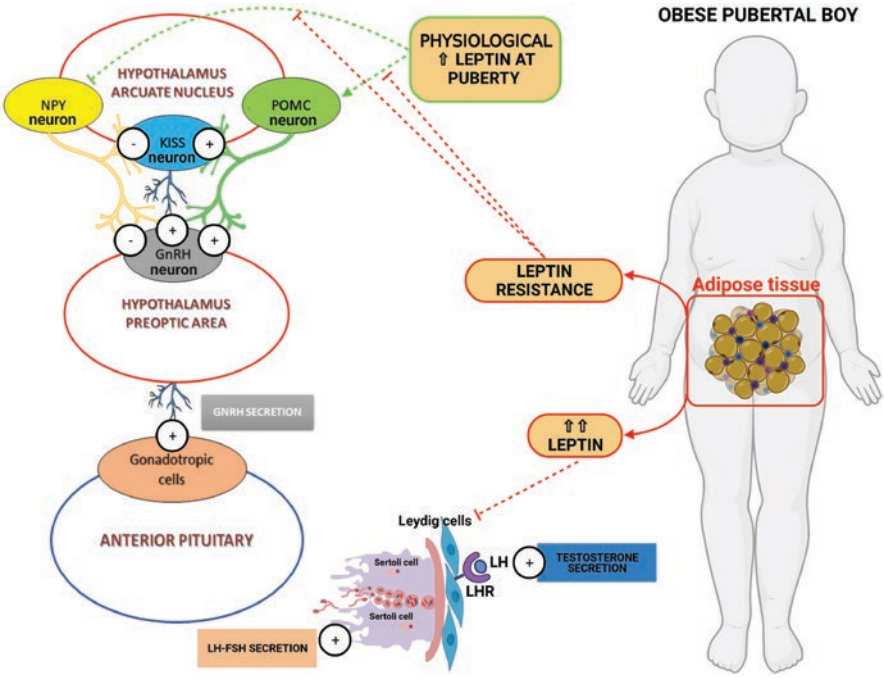

Fig. 10.3 Graphical overview of leptin actions on the hypothalamus–pituitary–gonadal axis at puberty, in physiological condition of normal weight and in obesity. A physiological increase of leptin levels as puberty approaches exerts a permissive effect on pubertal development, mediated by the inhibition of neuropeptide Y (NPY) neurons and stimulation of pro-opiomelanocortin (POMC) neurons, which in turn inhibit and stimulate respectively gonadotropin-releasing hormone (GnRH) secretion, directly by acting on GnRH neurons, or indirectly by means of kisspeptin (KISS) neurons. In obese boys, leptin resistance at the hypothalamus–pituitary level suppresses the beneficial effects of leptin on puberty; moreover, excessively increased leptin levels might directly inhibit testosterone production by Leydig cells, by interference with testicular steroidogenesis enzymes. Figure created with BioRender.com

Contrary to the beneficial effect of the physiological increase of leptin levels on pubertal onset in boys, obesity-induced leptin resistance occurring at the hypothalamic–pituitary level and determining central inhibition of the HPG axis function along with the peripheral hyperleptinemia–induced decrease of testicular response to LH stimulation, collectively resulting in a reduced basal and LH-stimulated testosterone production, might favor the occurrence of delayed pubertal onset and/or to delayed pubertal progression in obese boys [62, 65, 66]. A graphical overview of leptin actions on the HPG axis at puberty, in physiological condition of normal weight and obesity is provided in Fig. 10.3. Moreover, adipokines different from leptin, such as adiponectin, which has a physiological inhibitory function on GnRH secretion, might be involved in pubertal development regulation in obese boys. Indeed, the obesity-related low-grade inflammation status determine the typically reduced adiponectin levels observed in obese boys, compared to normal weight peers, therefore potentially favoring the occurrence of an earlier pubertal onset and/or accelerating pubertal progression in obese boys [62].

Additional mechanisms potentially involved in the pubertal process and therefore potentially impacting on obesity-related disturbances of pubertal development include the interaction between the HPG axis and different endocrine systems, comprising the adrenal androgens and the GH/IGF-1 system. Considering that adrenarche and gonadarche occur independently, it has been suggested that the physiological increase of adrenal androgens levels occurring at adrenarche has an HPG-independent favoring effect on the peripheral androgenic manifestations of puberty [2, 72]. Accordingly, pathological increase of sex hormones levels due to peripheral autonomous premature secretion owing to a primary disorder of the gonads or adrenal gland, including gonadal or adrenal tumors and congenital adrenal hyperplasia, induce pseudo-precocious puberty, manifested independently from the central activation of the HPG axis and hypothalamus–pituitary control [2]. Similarly, obesity might be hypothesized to exert an anticipatory action on the appearance of pubertal signs in boys, mediated by peripheral effects on adrenal androgens; indeed, an increase of the adrenal androgens androstenedione and DHEAS levels has been observed in obese boys, compared to age-matched non-obese peers [73, 74], and obesity was shown to be highly prevalent (47%) in prepubertal boys diagnosed with early adrenarche [75]. Reversible compensatory central activation of the hypothalamus–pituitary–adrenal (HPA) axis likely due to relative hypocortisolism determined by an increased inactivation of cortisol in the liver, by increased 5α-reductase type 1 activity [76, 77] and a stimulatory effect of increased leptin levels on adrenal steroid biosynthesis [78] might be the underlying determinants of such adrenal androgens increase in obese children; these hypotheses are supported by the evidence that substantial weight loss determines a reduction in androstenedione levels, although stably elevated DHEAS levels indicate early irreversible maturation of the adrenal zona reticularis [79, 80]. Considering the physiological contribution of adrenal androgens to the development of pubertal signs independently from HPG axis activation, it might be hypothesized that increased adrenal androgens levels in obese boys might simply accelerate the androgenic manifestations of puberty irrespective of pubertal development and, at the same time, an obesity-induced greater peripheral conversion of adrenal androgens to estradiol might also result in the inhibition of HPG axis after puberty, therefore delaying pubertal completion. Consistently, a longitudinal study

on pre-pubertal healthy boys demonstrated that higher adrenal androgens at adrenarche predicted earlier ages at Tanner stage 2 for pubic hair and genital development, as well as a shorter pubertal growth acceleration period [81], by corroborating the potential anticipatory role on the appearance of pubertal signs exerted by increased adrenal androgens in obese boys. Lastly, the GH-IGF1 system, which is frequently deregulated in cases of obesity, might represent an additional endocrine axis potentially affecting pubertal development in obese boys. HPG and HPS axes are interconnected. Somatotropic cells of the pituitary produce GH, which in turn stimulates hepatic IGF-1 production. IGF-1 exerts multiple actions on the HPG axis, including activation of kisspeptin and GnRH neurons and stimulation of gonadotropic cells to produce gonadotropins; moreover, hepatic IGF-1, along with IGF-1 locally produced within the testis, mainly in response to gonadotropins, are involved in the stimulation of Leydig cells proliferation and testicular steroidogenesis and spermatogenesis [24]. Lasty, growth and development of penis and prostate are also stimulated by IGF-1, and, directly, by GH. On the other side, testosterone per se or, mainly, after conversion to estradiol, might increase pituitary GH pulsatile production, whereas estradiol might inhibit hepatic production of IGF-1 and peripheral IGF-1 response [24]. The mechanisms driving GH/IGF-1 system activation during puberty are not entirely defined, but probably increased hypothalamic release of GH-releasing hormone (GHRH) mediated by testosterone surge, activation of pituitary somatotropic cells by kisspeptin neurons and ensuing increased sensitivity to GHRH stimulation might be concerned [24]. Physiologically, the amplitude of GH secretion bursts together with the overall increase in daily production of pituitary GH at puberty, and both required for linear growth and pubertal progression and development [24], as supported by the evidence that IGF-1 levels progressively increase from Tanner stages 1 to 3, in parallel with the increase in testis volume [24]. Conversely, HPS axis impairment during childhood and puberty might induce delayed pubertal development, as supported by the finding, in pre-pubertal boys with constitutional delay in growth and puberty, of lower IGF-1 levels in patients with hypogonadotropic hypogonadism and higher IGF-1 levels in patients with an earlier puberty [24]. Nevertheless, the major body of evidence demonstrated that GH treatment in short boys did not influence age at pubertal onset nor accelerated pubertal development, despite inducing an increment of testis volume [82]. Collectively, this evidence on the effects of physiological GH and IGF-1 levels and of pathological deficit of these hormones on the HPG axis, support the hypothesis of a beneficial effect of the GH/IGF-1 system in the physiological activation of the HPG axis and pubertal development; moreover, considering the permissive role of the GH-IGF-1 system on the HPG axis activation, and a potential contribution to the onset and acceleration of puberty and appearance of secondary sex characteristics [83–85], defective GH/IGF-1 system with increased IGF-1 levels detected in obese boys might be hypothesized to anticipate pubertal development. Indeed, abnormalities in the GH/IGF-1 system are a common finding in obese, children, and include aberrant GH secretory pattern with decreased GH half-life, frequency of secretory episodes and reduced GH daily production rate, along with increased GH binding protein levels; nevertheless, IGF-1 responsiveness to GH appears to be increased, and increased IGF-1 and IGF binding proteins levels are detected in obese boys, therefore potentially driving an earlier onset of puberty [86, 87, 88]. Noteworthy, the

GH/IGF-1 system abnormalities are reversible, as demonstrated by the positive effect of weight loss [86, 88]. Despite much effort has been put into shedding underlying mechanisms driving pubertal development in obese boys, the relationship between obesity and pubertal onset and/or progression is less linear than that between obesity and HPG axis and androgenic status observed in adult men. Indeed, despite the association between obesity and pubertal timing in girls is well established, with evidence of an earlier onset of puberty in obese and overweight girls [2], clinical evidence is less univocal in boys, with some studies showing an effect of obesity in anticipating puberty, and others showing opposite or no effect [62].

Cross-sectional studies addressing the relationship between BMI and pubertal development are quite inconsistent, with some reporting earlier pubertal development in boys with a higher BMI (overweight and obese), and others failing to denote a consistent relationship, or as opposite reporting delayed pubertal development [89–92]. Conversely, longitudinal studies quite consistently reported an association between higher BMI and earlier pubertal development, heterogeneously assessed by different signs of pubertal onset and/or progression, such as age at peak height velocity, at voice break, and pubic hair or testis development; nevertheless, the majority of studies did not address overweight and obese boys separately, therefore potentially diluting differential trends [62, 93–97]. Few studies specifically assessed the first sign of pubertal onset, in obese boys compared to normal weight peers. A study measuring testis volume by andrological examination demonstrated an earlier onset of puberty, as the occurrence of testis volume ≥4 mL, and an earlier completion of puberty, as the occurrence of a testis volume of 25 mL, and a shorter duration of puberty, in obese boys, compared to normal weight peers [94]. Consistently, a different study on obese boys demonstrated that puberty occurs at an earlier age in obese boys, compared to normal weight peers, particularly highlighted by an earlier occurrence of testis volume ≥4 mL evaluated with Prader orchidometer in obese boys, although no significant differences were detected for either pubarche onset nor genital stage ≥2 [98]. In line with these findings, a school population–based study estimated a significantly higher prevalence of precocious puberty, addressed by testis volume measured with Prader orchidometer, in obese compared to normal weight boys [99]. Lastly, an additional study addressing separately in normal weight, overweight and obese boys the relationship between body fat and testis volume measured with Prader orchidometer, highlighted an earlier pubertal onset and earlier pubertal completion in overweight boys compared to normal weight peers, and a delayed pubertal completion in obese boys compared to overweight and normal weight peers [90], although this was the only study highlighting this difference in overweight and obese boys. Anyhow, these eventual differential trends in overweight versus obese boys might allow to speculate that leptin resistance occurring at higher leptin levels typically observed in obesity could determine a pubertal latency and delayed pubertal attainment by effect of central and peripheral mechanisms, whereas in overweight boys with hyperleptinemia but preserved leptin sensitivity, a greater precocity of pubertal onset could occur. The precise role and effect weight of hypogonadism in obese boys as a factor determining variations in pubertal onset and/or progression, the latter being reasonably the most likely pubertal feature to be possibly impacted, has yet to be exactly determined. Nevertheless, as in adults, increased visceral adiposity might potentially exert peripheral effects; indeed, increased estradiol

production mediated by excessive aromatase activity directly resulting from increased visceral adiposity in obese, but not in overweight boys, as well as resulting by increased aromatase substrate free testosterone levels by effect of reduced SHBG deriving from IR, may suppress the pubertal process by inhibiting HPG axis and, therefore, favor delayed pubertal progression [2, 100]. As potential supportive evidence, obese children have higher estradiol levels from pre-puberty until full sexual maturation, compared to normal weight peers, and body fat is positively associated with markers of estrogens exposure, such as breast development and skeletal age, and negatively associated with pubic hair growth and testis volume [73, 101]. Moreover, in obese children, SHBG levels are substantially lower than in normal weight peers, most likely because of IR and hyperinsulinism; SHBG levels are evenly low independently of pubertal stage, therefore differences with normal weight peers are most pronounced at earlier Tanner stages and decrease during sexual maturation, when SHBG levels physiologically decrease in lean peers [79]. Lastly, consistently with maintained total testosterone despite low SHBG levels, obese children have slightly increased free testosterone, most likely resulting from increased production of adrenal testosterone precursors androstenedione and DHEAS, further corroborated by a positive association of both adrenal androgens with free testosterone [73, 79]. Despite these findings, more studies are needed to understand how and to what extent these peripheral hormonal changes might influence puberty in obese boys.

Controversial results concerning the relationship between obesity and pubertal development in boys might be accounted by a diversity of factors: (1) the paucity of dedicated studies, which mainly include small-sized cohorts; (2) the use of BMI as an indirect marker of obesity; (3) the relatively subjective assessment of pubertal onset in boys compared to menarche onset used in girls; (4) the fact that body fat distribution, rather than body weight, influences the mechanisms potentially involved in the pubertal process.

Childhood obesity is a growing and alarming problem, associated with multiple short- and long-term metabolic and cardiovascular complications; despite uncertainty linking obesity to male pubertal onset, controlling overweight and obesity in children might help prevent the early activation of the HPG axis and the onset of early puberty, along with the occurrence of severe burden of obesity-associated comorbidities.

10.5 Lifestyles

Several clinical studies demonstrated that poor lifestyles and unhealthy habits interfere with pubertal development and, specifically, cigarette smoking, alcohol consumption, nutritional imbalance and poor physical activity might exert a relevant role, with some of these factors being implicated in pubertal disorders following either (or mostly) prenatal or childhood exposure; nevertheless, despite florid evidence exists for girls, fewer studies are focused on boys.

Cigarette smoke contains about 4000 substances belonging to a variety of chemical classes, including polycyclic aromatic hydrocarbons, heavy metals and alkaloids, which are all compounds able to cross the placenta displaying endocrine disrupting properties and reproductive toxicity [102, 103]; therefore, maternal smoking may

affect the intrauterine hormonal environment during pregnancy, and consequent early fetal life exposure may result in detrimental effects on descendants reproductive health. In particular, a significantly increased risk of cryptorchidism was reported in sons of mothers who smoked 20 or more cigarettes/day during pregnancy, compared to non-smoker mothers [104], suggesting an interference of heavy smoking with testis development. Moreover, few studies addressed the relationship between prenatal exposure to cigarette smoke and pubertal development, and highlighted tendency to anticipated puberty. In particular, in a birth cohort follow-up study, information gathered by retrospective questionnaires administered to young men aged 18–21 years concerning the appearance of pubertal indicators, highlighted a tendency to an earlier onset of puberty in men with higher levels of exposure to maternal cigarette smoking during pregnancy, displayed by a trend toward an earlier age at first nocturnal ejaculation, acne and voice break, in men exposed to 15 or more cigarettes/day during fetal life [105]. These results are consistent with a different study demonstrating earlier retrospectively self-reported signs of puberty including pubic hair growth, voice break and penis growth [106], in men prenatally exposed to tobacco smoking, assessed as a dichotomized, non-quantitative variable. Nevertheless, a large birth cohort study failed to detect any correlation between maternal smoking and the height difference in standard deviations, a marker of pubertal timing [107].

Alcohol consumption has been repeatedly reported to exert detrimental, although sometimes equivocal, actions on reproductive function, also mediated by direct and indirect endocrine effects, and alcohol consumption during pregnancy is well known to be linked to an increased risk of adverse pregnancy outcomes and fetal disorders [102, 103]; therefore, maternal alcohol consumption during pregnancy might also be expected to affect descendants reproductive health, including pubertal development. A pregnancy cohort follow-up study performed on the male descendants demonstrated a tendency to delayed self-reported first nocturnal ejaculation and voice break in boys prenatally exposed to 5 or more maternal binge drinking episodes, compared to unexposed peers [108]; nevertheless, although in the same direction, results on weekly alcohol consumption during pregnancy were much weaker. In a different cohort study on male adolescents, higher average daily maternal alcohol consumption was associated with higher adolescent salivary testosterone levels. Nevertheless, although in boys with light-to-no prenatal alcohol exposure testosterone levels were correlated to pubic hair growth, this relationship was lost in boys with moderate-to-heavy prenatal alcohol exposure; similarly, although in boys with light-to-no prenatal alcohol exposure a trend for a correlation was demonstrated between testosterone levels and genital development, no correlation was found in boys with moderate-to-heavy prenatal alcohol exposure, suggesting a decreased responsiveness of testosterone-sensitive tissues in adolescents with higher maternal alcoholic intake [109]. Consistently, alcohol consumption at early stages of reproductive development, namely, pre-pubertal stage, was found to be associated with longer time to occurrence of voice break, and with delayed body and facial hair growth [110]. Moreover, partially in line with prenatal exposure, higher adolescent alcohol consumption was associated with higher estradiol levels, which might indirectly reflect increased activity of the aromatase enzyme, due to higher testosterone levels. It is important to consider the hypothesis of a mutual relationship between sex hormones and alcohol intake, based on the evidence that estradiol levels are

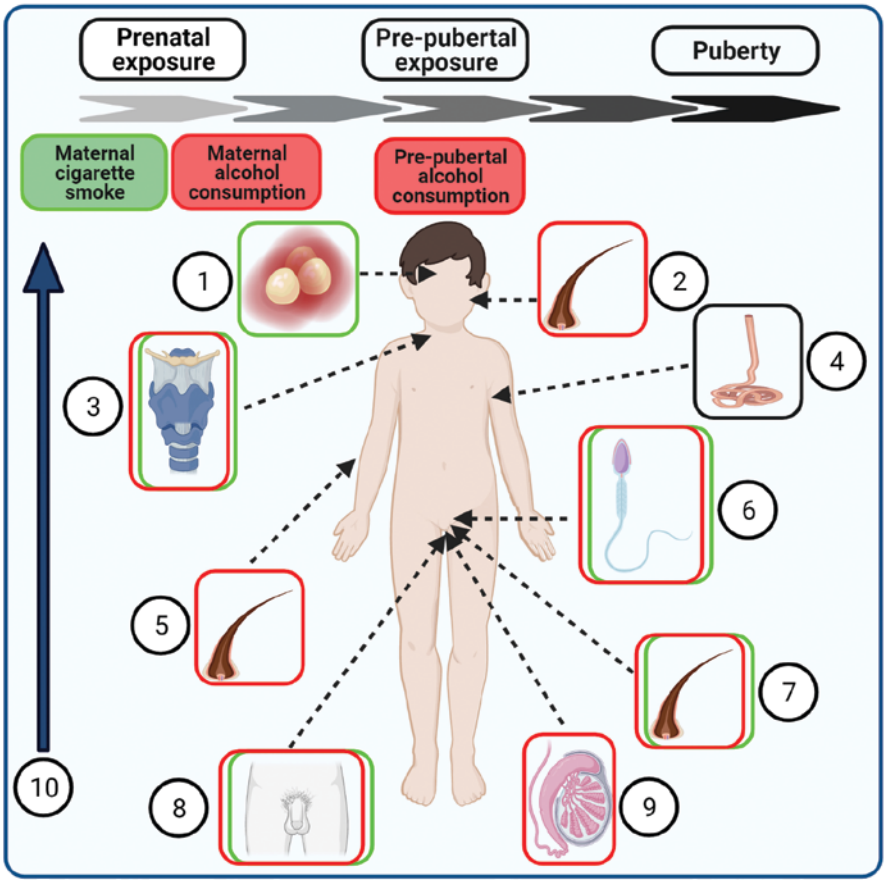

Fig. 10.4 Graphical overview of the pubertal signs proposed to be affected by prenatal exposure to cigarette smoke and alcohol or pre-pubertal alcohol consumption. Pubertal signs: (1) acne; (2) facial hair; (3) voice break; (4) changes in sweat smell; (5) axillary and body hair; (6) first nocturnal ejaculation; (7) pubic hair; (8) penis growth; (9) testis enlargement; (10) growth spurt. Overall, prenatal exposure to cigarette smoke has been associated with an earlier occurrence of pubertal signs (green circle: 1, 3, 6, 7, 8), whereas prenatal exposure to alcohol or pre-pubertal alcohol consumption have been associated with a delayed occurrence of pubertal signs (red circle: 2, 3, 5, 6, 7, 8, 9). Figure created with BioRender.com

related to both the onset and quantity of alcohol use in boys, which may suggest a particular role for sex hormones in promoting alcohol use through stimulation of sensation-seeking behaviors and activation of reward processing brain regions, therefore exacerbating the effects on pubertal development [111]. Moreover, a very recent large survey performed on male high school students, using a validated structured interview on lifestyle attitudes associated with medical examination, pointed out that health risk behaviors during adolescence, such as alcohol consumption, might be associated with andrological disorders, and, particularly, might adversely affect testis development [112]; specifically, a strong association between alcohol consumption and reduced testis volume was highlighted, possibly suggesting testis function impairment and a role for alcohol consumption as a contributor to testis

hypotrophy, with an effect of both moderate and heavy drinking, compared to occasional drinking. An additive effect of alcohol over varicocele toward reduced testis volume was also detected [112]. A graphical overview of the pubertal signs proposed to be affected by prenatal exposure to cigarette smoke and alcohol or prepubertal alcohol consumption is provided in Fig. 10.4.

Nutritional imbalances during pregnancy may be implicated in the reprogramming of fetal metabolism, including the setting of the hypothalamus–pituitary axis on the one hand and of IR and body composition on the other hand, which in turn could trigger subsequent hormonal changes affecting pubertal development. To date, evidence linking prenatal nutritional imbalances to pubertal development is indirect, through the use of birth weight as a surrogate of intrauterine milieu. A range of nutritional factors during pregnancy, from overall malnourishment to single micronutrients deficiencies, vitamin B12 levels, and cervonic acid intake, have been hypothesized to be in relation with birth weight; moreover, a recent study suggested that maternal vitamin D status in early pregnancy might play a role in both birth weight and ensuing growth speed [105]. Despite the indirectly hypothesized role of maternal nutritional environment on pubertal development the impact of specific infant or childhood dietary patterns on male pubertal development has been scarcely addressed. In a large population-based birth cohort study with contemporary breastfeeding data collection, retrospectively collected data on cow milk consumption at about 6 months, 3 years, and 5 years of age, and objective physical examination for pubertal development, neither exclusive and partial (for any length of time) breastfeeding nor cow milk consumption at the various investigated ages was associated with age at pubertal onset [113]. Conversely, higher animal protein dietary intake, but not total fibre intake nor fibre intake from different sources, was associated with earlier pubertal development [114]. Although most of the evidence of a relationship between a specified food category is provided in girls, it would be wisdom less to expect that a given single nutritional factor may contribute for most of pubertal variations; it is much more likely that complex interactions among entire nutritional stimuli or dietary patterns, metabolic processes and other related and/or unrelated influences (i.e., physical activity, potential EDCs contamination in foods) may all take concerted part in pubertal programming. Indeed, it is conceivable that macronutrients, micronutrients, and/or food groups may influence pubertal development through combined effects. In line with this concept, one study addressed the association of overall higher dietary quality in childhood, intended as adherence to specific dietary recommendations (i.e., lower intake of total fat and higher intake of carbohydrates, fibre, and micronutrients) with pubertal development by demonstrating a later pubertal growth spurt in children with pre-pubertal higher dietary quality, compared to peers with lower dietary quality [114].

10.6 Negative Energy Balance: Severe Malnutrition and Vigorous Physical Activity

Consistently with the concept that single food categories may not substantially alter pubertal development, eating disorders with an onset at childhood/adolescence, including the psychiatric disorders anorexia nervosa and bulimia nervosa, might exert a role in pubertal abnormalities, since they involve a progressive overall severe

malnutrition status fueled by extreme methods adopted for weight control, such as fasting, binge eating, vomiting, and excessive exercise, rather than deprivation of specific foods [115]. Indeed, patients with anorexia nervosa display lowered leptin levels and hypogonadotropic hypogonadism with low levels of gonadotropins and testosterone, overall leading to delayed pubertal development [116, 117]. Although disproportionally better characterized in girls, an increase in the prevalence of eating disorders has been described in recent years also in boys. A gender-specific relationship has been pointed out, between puberty and eating disorders; overall, girls who mature early are at higher risk of developing eating disorders, whereas for boys the opposite is true, with boys with delayed pubertal development displaying a higher risk of developing eating disorders [115]. This relationship is bidirectional in nature; indeed, food deprivation is strictly connected to pubertal timing due to a complex crosstalk between leptin and HPA axis with HPG axis, and to the potential downstream effects of an altered function of the hypothalamus–pituitary–thyroid axis and of the GH/IGF-1 system. In pre-pubertal conditions under food restriction, such as in anorexia nervosa, lowered leptin levels determine an increase in NPY activity, which exerts an inhibitory effect on the HPG axis function with reduced gonadotropins and gonadal steroid synthesis and secretion, ultimately interfering with pubertal onset [70]. The release of corticotropin (CRH), which stimulates adrenocorticotropic hormone (ACTH) secretion, is regulated, at least in part, by leptin and insulin; therefore, in anorexia nervosa patients, characterized by lowered leptin levels and hypoinsulinemia due to reduced glucose and amino acid levels, increased cortisol levels might be detected, which inhibit GnRH and gonadotropins release, by contributing to delayed pubertal development [116]. Severe weight loss in anorexia nervosa is characterized by non-thyroidal illness syndrome as an adaptive response to decreased metabolic rate and energy expenditure presenting with low total triiodothyronine (T3) levels, elevated reverse T3 levels due to the peripheral increase in deiodination of thyroxine (T4) to reverse T3, and varying levels of free T4 and TSH, ranging from normal to low-normal [118]. Moreover, in anorexia nervosa patients, a negative correlation between BMI and basal and pulsatile GH levels suggest a link between malnutrition and an altered GH pulsatile secretory pattern; in addition, levels of IGF-binding protein 1 (IGFBP-1) and IGFBP-2 are elevated, due to hypoinsulinemia [119]. Although in anorexia nervosa patients no direct evidence exists on a potential effect of the hypothalamus–pituitary–thyroid axis and the GH/IGF-1 system abnormalities on pubertal development, a contribution to delayed growth at puberty is inferred.

Consistently with the observed effects of severe malnutrition on pubertal development, vigorous physical activity, mainly acting through the effects of a negative energy balance (insufficient caloric intake for the level of physical activity), might delay the onset of normal puberty and pubertal progression, although these effects have been particularly demonstrated in girls. Indeed, in pre-pubertal girls practicing intense physical exercise, delayed menarche and primary amenorrhea may occur; in this setting, negative energy balance, low fat mass and stress act as central drivers by determining decreased leptin levels and increased levels of cortisol, of the orexigenic hormone ghrelin, and of the anorexigenic hormone peptide YY, which overall result in decreased secretion of GnRH followed by hypothalamic amenorrhea, and in suppressed sense of appetite, ultimately further preventing the compensatory increase of energy intake [116, 120].

10.7 Conclusions

Physiological variation in age at puberty timing is observed in healthy children; nevertheless, pathological variations in pubertal development have been highlighted in secular trends analysis, displaying skewed age distribution at pubertal signs with tendency to earliness for initial and lateness for final pubertal stages, and consequent dilation of pubertal development age window, testifying potential underlying mechanisms with differential effects on anatomical markers of puberty and/or differential sensitive timeframes. Several factors have been claimed to participate to pubertal abnormalities, including environmental exposures from prenatal to adolescent age, obesity and lifestyles, the latter being mainly related to maternal cigarette smoking and alcohol consumption during pregnancy, and nutritional impacts. Although hypothesis have been formulated, based on animal studies, evidence in humans is quite insufficient to draw definitive conclusions in most cases, and causal inference lacks arguments due to unethical experimentation in humans, particularly referred to environmental influences. Notwithstanding, investigation on selected EDCs has provided evidence of sparse and uncertain associations between prenatal to adolescent exposure to PCB, dioxin and dioxin-like compounds, phthalates BPA, pesticides and Pb and delayed pubertal development although definitive findings have not been established. The association of obesity with pubertal development lacks in consistency in boys, partly because of biased cross-sectional studies; indeed, longitudinal studies more consistently report the occurrence of earlier onset of puberty in overweight-obese boys, by using different signs of pubertal onset and/or progression as main outcome. A diversity of mechanisms has been proposed to take part in the modulation of pubertal development in obese boys, some exerting anticipatory and other retarding effects. Leptin resistance occurring at the hypothalamic–pituitary level determines a central inhibition of the HPG axis, therefore preventing the permissive effect of leptin on pubertal onset; moreover, obesity-related hyperleptinemia might be associated with impaired testicular steroidogenesis, therefore potentially further contributing to a delayed pubertal progression. On the other hand, reduced adiponectin levels might favor earlier pubertal onset and/or accelerate pubertal progression. The role of increased adrenal androgens might be dualistic; indeed, although increased adrenal androgens might accelerate the androgenic manifestations of puberty irrespective of HPG axis activation, an excessive peripheral conversion of adrenal androgens to estradiol after pubertal onset might result in the inhibition of HPG axis and delaying pubertal completion. Lastly, derangements of the GH/IGF-1 system might also be hypothesized to potentially take part in anticipating pubertal development in obese boys. Lastly, a quite consistent tendency to an earlier onset of puberty has been associated with higher levels of exposure to maternal cigarette smoking during pregnancy, whereas maternal alcohol consumption during pregnancy has been shown to exert an opposite effect on male descendants pubertal development and a decreased responsiveness of testosterone-sensitive tissues has been hypothesized in adolescents with higher maternal alcoholic intake. Consistently, alcohol consumption in pre-pubertal stage has been associated with testis hypotrophy, possibly suggesting testicular function impairment, and delayed appearance of pubertal signs. Lastly, very scant

evidence put in relation the nutritional impact on pubertal development and seems to suggest an association of higher animal protein dietary intake with earlier puberty in boys, whereas a potential malnutrition-induced and negative energy balance-induced delay in pubertal development might be speculated, based on evidence from girls suffering from anorexia and practicing vigorous physical activity, respectively. Complex interactions are expected to occur, potentially participating in male pubertal maturation, and dissecting individual and independent roles of each involved factor is quite challenging in absence of clear mechanistic insights; more focused research should be performed in order to dissipate inconsistencies, and an appropriate management of pubertal variations should be prone to consider a diverse range of stimuli starting far more in advance than pubertal occurrence.

References

1. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45(239):13–23.
2. Alotaibi MF. Physiology of puberty in boys and girls and pathological disorders affecting its onset. J Adolesc. 2019;71:63–71.
3. Wood CL, Lane LC, Cheetham T. Puberty: normal physiology (brief overview). Best Pract Res Clin Endocrinol Metab. 2019;33(3):101265.
4. Stamatiades GA, Kaiser UB. Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. Mol Cell Endocrinol. 2018;463:131–41.
5. Russell NG, Grossmann M. Estradiol as a male hormone. Eur J Endocrinol. 2019;181:R23–43.
6. Weinbauer GF, Luetjens CM, Simoni M, Nieschlag E. Physiology of testicular function. Berlin: Springer-Verlag; 2010.
7. Meachem SJ, Nieschlag E, Simoni M. Inhibin B in male reproduction: pathophysiology and clinical relevance. Eur J Endocrinol. 2001;145(5):561–71.
8. Wijayarathna R, de Kretser DM. Activins in reproductive biology and beyond. Hum Reprod Update. 2016;22(3):342–57.
9. Pierik FH, Burdorf A, de Jong FH, Weber RF. Inhibin B: a novel marker of spermatogenesis. Ann Med. 2003;35(1):12–20.
10. O'Connor AE, De Kretser DM. Inhibins in normal male physiology. Semin Reprod Med. 2004;22(3):177–85.
11. Bloise E, Ciarmela P, Dela Cruz C, Luisi S, Petraglia F, Reis FM. Activin A in mammalian physiology. Physiol Rev. 2019;99(1):739–80.
12. Bilezikjian LM, Blount AL, Leal AM, Donaldson CJ, Fischer WH, Vale WW. Autocrine/paracrine regulation of pituitary function by activin, inhibin and follistatin. Mol Cell Endocrinol. 2004;225(1–2):29–36.
13. Bilezikjian LM, Blount AL, Corrigan AZ, Leal A, Chen Y, Vale WW. Actions of activins, inhibins and follistatins: implications in anterior pituitary function. Clin Exp Pharmacol Physiol. 2001;28(3):244–8.
14. Ivell R, Heng K, Anand-Ivell R. Insulin-like factor 3 and the HPG axis in the male. Front Endocrinol. 2014;5:6.
15. Kuiri-Hanninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr. 2014;82(2):73–80.
16. Lee MJ, Yang GE, Chueh HW, Park JH, Yoo JH. The effect of first nocturnal ejaculation timing on risk and sexual behaviors of Korean male adolescents. Ann Pediatr Endocrinol Metab. 2017;22(1):43–8.
17. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291–303.
18. Emmanuel M, Bokor BR. Tanner stages. Treasure Island (FL): StatPearls; 2021.

19. Koskenniemi JJ, Virtanen HE, Toppari J. Testicular growth and development in puberty. Curr Opin Endocrinol Diabetes Obes. 2017;24(3):215–24.
20. Aksglaede L, Sorensen K, Boas M, Mouritsen A, Hagen CP, Jensen RB, et al. Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. J Clin Endocrinol Metab. 2010;95(12):5357–64.
21. Andersson AM, Juul A, Petersen JH, Muller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. J Clin Endocrinol Metab. 1997;82(12):3976–81.
22. Johansen ML, Anand-Ivell R, Mouritsen A, Hagen CP, Mieritz MG, Soeborg T, et al. Serum levels of insulin-like factor 3, anti-Mullerian hormone, inhibin B, and testosterone during pubertal transition in healthy boys: a longitudinal pilot study. Reproduction. 2014;147(4):529–35.
23. Mouritsen A, Aksglaede L, Soerensen K, Hagen CP, Petersen JH, Main KM, et al. The pubertal transition in 179 healthy Danish children: associations between pubarche, adrenarche, gonadarche, and body composition. Eur J Endocrinol. 2013;168(2):129–36.
24. Tenuta M, Carlomagno F, Cangiano B, Kanakis G, Pozza C, Sbardella E, et al. Somatotropic-testicular Axis: a crosstalk between GH/IGF-I and gonadal hormones during development, transition, and adult age. Andrology. 2021;9(1):168–84.
25. Topaloglu AK, Kotan LD. Genetics of hypogonadotropic hypogonadism. Endocr Dev. 2016;29:36–49.
26. Ge X, Brody GH, Conger RD, Simons RL, Murry VM. Contextual amplification of pubertal transition effects on deviant peer affiliation and externalizing behavior among African American children. Dev Psychol. 2002;38(1):42–54.
27. Parent AS, Franssen D, Fudvoye J, Pinson A, Bourguignon JP. Current changes in pubertal timing: revised vision in relation with environmental factors including endocrine disruptors. Endocr Dev. 2016;29:174–84.
28. Barker DJ. The fetal and infant origins of adult disease. BMJ. 1990;301(6761):1111.
29. Barker DJ. The developmental origins of adult disease. J Am Coll Nutr. 2004;23(6 Suppl):588S–95S.
30. Fudvoye J, Lopez-Rodriguez D, Franssen D, Parent AS. Endocrine disrupters and possible contribution to pubertal changes. Best Pract Res Clin Endocrinol Metab. 2019;33(3):101300.
31. Guo YL, Lambert GH, Hsu CC, Hsu MM. Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. Int Arch Occup Environ Health. 2004;77(3):153–8.
32. Guo YL, Lai TJ, Ju SH, Chen YC, Hsu CC. Sexual developments and biological findings in Yucheng children. Thirteenth International Symposium on Chlorinated Dioxins and Related Compounds, 24–28 September 1993. Vienna, Austria; 1993.
33. Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect. 2002;110(8):771–6.
34. Leijs MM, Koppe JG, Olie K, van Aalderen WM, Voogt P, Vulsma T, et al. Delayed initiation of breast development in girls with higher prenatal dioxin exposure; a longitudinal cohort study. Chemosphere. 2008;73(6):999–1004.
35. Mol NM, Sorensen N, Weihe P, Andersson AM, Jorgensen N, Skakkebaek NE, et al. Spermaturia and serum hormone concentrations at the age of puberty in boys prenatally exposed to polychlorinated biphenyls. Eur J Endocrinol. 2002;146(3):357–63.
36. Gladen BC, Ragan NB, Rogan WJ. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr. 2000;136(4):490–6.
37. Dickerson SM, Guevara E, Woller MJ, Gore AC. Cell death mechanisms in GT1-7 GnRH cells exposed to polychlorinated biphenyls PCB74, PCB118, and PCB153. Toxicol Appl Pharmacol. 2009;237(2):237–45.

38. Gore AC, Wu TJ, Oung T, Lee JB, Woller MJ. A novel mechanism for endocrine-disrupting effects of polychlorinated biphenyls: direct effects on gonadotropin-releasing hormone neurones. J Neuroendocrinol. 2002;14(10):814–23.
39. Muthuvel R, Venkataraman P, Krishnamoorthy G, Gunadharini DN, Kanagaraj P, Jone Stanley A, et al. Antioxidant effect of ascorbic acid on PCB (Aroclor 1254) induced oxidative stress in hypothalamus of albino rats. Clinica Chimica Acta. 2006;365(1–2):297–303.
40. Murugesan P, Muthusamy T, Balasubramanian K, Arunakaran J. Studies on the protective role of vitamin C and E against polychlorinated biphenyl (Aroclor 1254)–induced oxidative damage in Leydig cells. Free Radic Res. 2005;39(11):1259–72.
41. Matti Viluksela, Päivi Heikkinen, Leo T. M. van der Ven, Filip Rendel, Robert Roos, Javier Esteban, et al. Toxicological profile of ultrapure 2,2′,3,4,4′,5,5′-heptachlorbiphenyl (PCB 180) in adult rats. PLoS ONE 2014;9 (8):e104639.
42. Ferguson KK, Peterson KE, Lee JM, Mercado-Garcia A, Blank-Goldenberg C, Tellez-Rojo MM, et al. Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. Reprod Toxicol. 2014;47:70–6.
43. Sathyanarayana S, Beard L, Zhou C, Grady R. Measurement and correlates of ano-genital distance in healthy, newborn infants. Int J Androl. 2010;33(2):317–23.
44. Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, et al. In utero exposure to bisphenol-A and anogenital distance of male offspring. Birth Defects Res A Clin Mol Teratol. 2011;91(10):867–72.
45. Durmaz E, Ozmert EN, Erkekoglu P, Giray B, Derman O, Hincal F, et al. Plasma phthalate levels in pubertal gynecomastia. Pediatrics. 2010;125(1):e122–9.
46. Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One. 2013;8(1):e55387.
47. Sergeyev O, Burns JS, Williams PL, Korrick SA, Lee MM, Revich B, et al. The association of peripubertal serum concentrations of organochlorine chemicals and blood lead with growth and pubertal development in a longitudinal cohort of boys: a review of published results from the Russian Children's Study. Rev Environ Health. 2017;32(1–2):83–92.
48. Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan H, et al. Effect of endosulfan on male reproductive development. Environ Health Perspect. 2003;111(16):1958–62.
49. Gladen BC, Klebanoff MA, Hediger ML, Katz SH, Barr DB, Davis MD, et al. Prenatal DDT exposure in relation to anthropometric and pubertal measures in adolescent males. Environ Health Perspect. 2004;112(17):1761–7.
50. Andrea C Gore. Organochlorine pesticides directly regulate gonadotropin-releasing hormone gene expression and biosynthesis in the GT1-7 hypothalamic cell line. Mol Cell Endocrinol. 2002;192(1–2):157–70.
51. Leon-Olea M, Martyniuk CJ, Orlando EF, Ottinger MA, Rosenfeld C, Wolstenholme J, et al. Current concepts in neuroendocrine disruption. Gen Comp Endocrinol. 2014;203:158–73.
52. Castellanos CG, Sorvik IB, Tanum MB, Verhaegen S, Brandt I, Ropstad E. Differential effects of the persistent DDT metabolite methylsulfonyl-DDE in nonstimulated and LH stimulated neonatal porcine Leydig cells. Toxicol Appl Pharmacol. 2013;267(3):247–55.
53. Geng X, Shao H, Zhang Z, Ng JC, Peng C. Malathion-induced testicular toxicity is associated with spermatogenic apoptosis and alterations in testicular enzymes and hormone levels in male Wistar rats. Environ Toxicol Pharmacol. 2015;39(2):659–67.
54. Wang N, Xu Y, Zhou XQ, Wu YH, Li SL, Qiao X, et al. Protective effects of testosterone propionate on reproductive toxicity caused by Endosulfan in male mice. Environ Toxicol. 2016;31(2):142–53.
55. World Health Organization. WHO fact sheet No 311. Obesity and overweight; 2018.
56. Saboor Aftab SA, Kumar S, Barber TM. The role of obesity and type 2 diabetes mellitus in the development of male obesity-associated secondary hypogonadism. Clin Endocrinol. 2013;78(3):330–7.
57. Bellastella G, Menafra D, Puliani G, Colao A, Savastano S, Obesity Programs of nutrition, Education, Research and Assessment (OPERA) Group. How much does obesity affect the male reproductive function? Int J Obes Suppl. 2019;9(1):50–64.

58. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, et al. Testosterone therapy in men with hypogonadism: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2018;103(5):1715–44.
59. Kelly DM, Jones TH. Testosterone and obesity. Obes Rev. 2015;16(7):581–606.
60. Pivonello R, Menafra D, Riccio E, Garifalos F, Mazzella M, de Angelis C, et al. Metabolic disorders and male hypogonadotropic hypogonadism. Front Endocrinol. 2019;10:345.
61. Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol. 2005;63(3):280–93.
62. Reinehr T, Roth CL. Is there a causal relationship between obesity and puberty? Lancet Child Adolesc Health. 2019;3(1):44–54.
63. Amstalden M, Alves BR, Liu S, Cardoso RC, Williams GL. Neuroendocrine pathways mediating nutritional acceleration of puberty: insights from ruminant models. Front Endocrinol. 2011;2:109.
64. Vazquez MJ, Velasco I, Tena-Sempere M. Novel mechanisms for the metabolic control of puberty: implications for pubertal alterations in early-onset obesity and malnutrition. J Endocrinol. 2019;242(2):R51–65.
65. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptin, obesity, and leptin resistance: where are we 25 years later? Nutrients. 2019;11(11):2704.
66. Kwon O, Kim KW, Kim MS. Leptin signalling pathways in hypothalamic neurons. Cell Mol Life Sci. 2016;73(7):1457–77.
67. Caprio M, Isidori AM, Carta AR, Moretti C, Dufau ML, Fabbri A. Expression of functional leptin receptors in rodent Leydig cells. Endocrinology. 1999;140(11):4939–47.
68. Ishikawa T, Fujioka H, Ishimura T, Takenaka A, Fujisawa M. Expression of leptin and leptin receptor in the testis of fertile and infertile patients. Andrologia. 2007;39(1):22–7.
69. Isidori AM, Caprio M, Strollo F, Moretti C, Frajese G, Isidori A, et al. Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. J Clin Endocrinol Metab. 1999;84(10):3673–80.
70. Kiess W, Reich A, Meyer K, Glasow A, Deutscher J, Klammt J, et al. A role for leptin in sexual maturation and puberty? Horm Res. 1999;51(Suppl 3):55–63.
71. Xi H, Zhang L, Guo Z, Zhao L. Serum leptin concentration and its effect on puberty in Naqu Tibetan adolescents. J Physiol Anthropol. 2011;30(3):111–7.
72. Klein DA, Emerick JE, Sylvester JE, Vogt KS. Disorders of puberty: an approach to diagnosis and management. Am Fam Physician. 2017;96(9):590–9.
73. Vandewalle S, Taes Y, Fiers T, Van Helvoirt M, Debode P, Herregods N, et al. Sex steroids in relation to sexual and skeletal maturation in obese male adolescents. J Clin Endocrinol Metab. 2014;99(8):2977–85.
74. Cao B, Gong C, Wu D, Liang X, Li W, Liu M, et al. A cross-sectional survey of adrenal steroid hormones among overweight/obese boys according to puberty stage. BMC pediatrics. 2019;19(1):414.
75. Santos-Silva R, Costa C, Castro-Correia C, Fontoura M. Clinical, biochemical and gender characteristics of 97 prepubertal children with premature adrenarche. J Pediatr Endocrinol Metab. 2019;32(11):1247–52.
76. Findling JW, Raff H. Diagnosis of endocrine disease: differentiation of pathologic/neoplastic hypercortisolism (Cushing's syndrome) from physiologic/non-neoplastic hypercortisolism (formerly known as pseudo-Cushing's syndrome). Eur J Endocrinol. 2017;176(5):R205–R16.
77. Scaroni C, Albiger NM, Palmieri S, Iacuaniello D, Graziadio C, Damiani L, et al. Approach to patients with pseudo-Cushing's states. Endocr Connect. 2020;9(1):R1–R13.
78. Biason-Lauber A, Zachmann M, Schoenle EJ. Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. Endocrinology. 2000;141(4):1446–54.
79. Vandewalle S, De Schepper J, Kaufman JM. Androgens and obesity in male adolescents. Curr Opin Endocrinol Diabetes Obes. 2015;22(3):230–7.
80. Reinehr T, Kulle A, Wolters B, Lass N, Welzel M, Riepe F, et al. Steroid hormone profiles in prepubertal obese children before and after weight loss. J Clin Endocrinol Metab. 2013;98(6):E1022–30.

81. Remer T, Shi L, Buyken AE, Maser-Gluth C, Hartmann MF, Wudy SA. Prepubertal adrenarchal androgens and animal protein intake independently and differentially influence pubertal timing. J Clin Endocrinol Metab. 2010;95(6):3002–9.
82. Albin AK, Ankarberg-Lindgren C, Tuvemo T, Jonsson B, Albertsson-Wikland K, Ritzen EM, et al. Does growth hormone treatment influence pubertal development in short children? Horm Res Paediatr. 2011;76(4):262–72.
83. Laron Z, Klinger B. Effect of insulin-like growth factor-I treatment on serum androgens and testicular and penile size in males with Laron syndrome (primary growth hormone resistance). Eur J Endocrinol. 1998;138(2):176–80.
84. Hindmarsh PC, Brook CG. Final height of short normal children treated with growth hormone. Lancet. 1996;348(9019):13–6.
85. Rekers-Mombarg LT, Kamp GA, Massa GG, Wit JM. Influence of growth hormone treatment on pubertal timing and pubertal growth in children with idiopathic short stature. Dutch Growth Hormone Working Group. J Pediatr Endocrinol Metab. 1999;12(5):611–22.
86. Marcovecchio ML, Chiarelli F. Obesity and growth during childhood and puberty. World Rev Nutr Diet. 2013;106:135–41.
87. Bouhours-Nouet N, Gatelais F, Boux de Casson F, Rouleau S, Coutant R. The insulin-like growth factor-I response to growth hormone is increased in prepubertal children with obesity and tall stature. J Clin Endocrinol Metab. 2007;92(2):629–35.
88. Kratzsch J, Dehmel B, Pulzer F, Keller E, Englaro P, Blum WF, et al. Increased serum GHBP levels in obese pubertal children and adolescents: relationship to body composition, leptin and indicators of metabolic disturbances. International journal of obesity and related metabolic disorders: journal of the international association for the study of obesity. 1997;21(12):1130–6.
89. Lee JM, Kaciroti N, Appugliese D, Corwyn RF, Bradley RH, Lumeng JC. Body mass index and timing of pubertal initiation in boys. Arch Pediatr Adolesc Med. 2010;164(2):139–44.
90. Lee JM, Wasserman R, Kaciroti N, Gebremariam A, Steffes J, Dowshen S, et al. Timing of puberty in overweight versus obese boys. Pediatrics. 2016;137(2):e20150164.
91. Lundeen EA, Norris SA, Martorell R, Suchdev PS, Mehta NK, Richter LM, et al. Early life growth predicts pubertal development in south African adolescents. J Nutr. 2016;146(3):622–9.
92. Wang Y. Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. Pediatrics. 2002;110(5):903–10.
93. Boyne MS, Thame M, Osmond C, Fraser RA, Gabay L, Reid M, et al. Growth, body composition, and the onset of puberty: longitudinal observations in Afro-Caribbean children. J Clin Endocrinol Metab. 2010;95(7):3194–200.
94. De Leonibus C, Marcovecchio ML, Chiavaroli V, de Giorgis T, Chiarelli F, Mohn A. Timing of puberty and physical growth in obese children: a longitudinal study in boys and girls. Pediatr Obes. 2014;9(4):292–9.
95. He Q, Karlberg J. BMI in childhood and its association with height gain, timing of puberty, and final height. Pediatr Res. 2001;49(2):244–51.
96. Juul A, Magnusdottir S, Scheike T, Prytz S, Skakkebaek NE. Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. Int J Androl. 2007;30(6):537–42.
97. Monteilh C, Kieszak S, Flanders WD, Maisonet M, Rubin C, Holmes AK, et al. Timing of maturation and predictors of Tanner stage transitions in boys enrolled in a contemporary British cohort. Paediatr Perinat Epidemiol. 2011;25(1):75–87.
98. Busch AS, Hojgaard B, Hagen CP, Teilmann G. Obesity is associated with earlier pubertal onset in boys. J Clin Endocrinol Metab. 2020;105(4):dgz222.
99. Liu Y, Tingting Y, Li X, Pan D, Lai X, Chen Y, Wang X, Yu X, Fu S, Huang S, Lin C, Liu S. Prevalence of precocious puberty among Chinese children: a school population-based study. Endocrine. 2021;72:573–81.
100. Huang A, Reinehr T, Roth CL. Connections between obesity and puberty: invited by Manuel Tena-Sempere, Cordoba. Curr Opin Endocr Metab Res. 2020;14:160–8.
101. Crocker MK, Stern EA, Sedaka NM, Shomaker LB, Brady SM, Ali AH, et al. Sexual dimorphisms in the associations of BMI and body fat with indices of pubertal development in girls and boys. J Clin Endocrinol Metab. 2014;99(8):E1519–29.

102. de Angelis C, Nardone A, Garifalos F, Pivonello C, Sansone A, Conforti A, et al. Smoke, alcohol and drug addiction and female fertility. Reprod Biol Endocrinol. 2020;18(1):21.
103. Sansone A, Di Dato C, de Angelis C, Menafra D, Pozza C, Pivonello R, et al. Smoke, alcohol and drug addiction and male fertility. Reprod Biol Endocrinol. 2018;16(1):3.
104. Jensen MS, Toft G, Thulstrup AM, Bonde JP, Olsen J. Cryptorchidism according to maternal gestational smoking. Epidemiology. 2007;18(2):220–5.
105. Hakonsen LB, Olsen J, Stovring H, Ernst A, Thulstrup AM, Zhu JL, et al. Maternal cigarette smoking during pregnancy and pubertal development in sons. A follow-up study of a birth cohort. Andrology. 2013;1(2):348–55.
106. Ravnborg TL, Jensen TK, Andersson AM, Toppari J, Skakkebaek NE, Jorgensen N. Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index. Hum Reprod. 2011;26(5):1000–11.
107. Brix N, Ernst A, Lauridsen LLB, Parner ET, Arah OA, Olsen J, et al. Maternal pre-pregnancy body mass index, smoking in pregnancy, and alcohol intake in pregnancy in relation to pubertal timing in the children. BMC Pediatr. 2019;19(1):338.
108. Hakonsen LB, Brath-Lund ML, Hounsgaard ML, Olsen J, Ernst A, Thulstrup AM, et al. In utero exposure to alcohol and puberty in boys: a pregnancy cohort study. BMJ Open. 2014;4(6):e004467.
109. Carter RC, Jacobson JL, Dodge NC, Granger DA, Jacobson SW. Effects of prenatal alcohol exposure on testosterone and pubertal development. Alcohol Clin Exp Res. 2014;38(6):1671–9.
110. Davis EM, Peck JD, Peck BM, Kaplan HB. Associations between early alcohol and tobacco use and prolonged time to puberty in boys. Child Care Health Dev. 2015;41(3):459–66.
111. de Water E, Braams BR, Crone EA, Peper JS. Pubertal maturation and sex steroids are related to alcohol use in adolescents. Horm Behav. 2013;63(2):392–7.
112. Gianfrilli D, Ferlin A, Isidori AM, Garolla A, Maggi M, Pivonello R, et al. Risk behaviours and alcohol in adolescence are negatively associated with testicular volume: results from the Amico-Andrologo survey. Andrology. 2019;7(6):769–77.
113. Kwok MK, Leung GM, Lam TH, Schooling CM. Breastfeeding, childhood milk consumption, and onset of puberty. Pediatrics. 2012;130(3):e631–9.
114. Cheng G, Buyken AE, Shi L, Karaolis-Danckert N, Kroke A, Wudy SA, et al. Beyond overweight: nutrition as an important lifestyle factor influencing timing of puberty. Nutr Rev. 2012;70(3):133–52.
115. McNicholas F, Dooley B, McNamara N, Lennon R. The impact of self-reported pubertal status and pubertal timing on disordered eating in Irish adolescents. Eur Eat Disord Rev. 2012;20(5):355–62.
116. Munoz-Calvo MT, Argente J. Nutritional and pubertal disorders. Endocr Dev. 2016;29:153–73.
117. Misra M, Katzman DK, Cord J, Manning SJ, Mendes N, Herzog DB, et al. Bone metabolism in adolescent boys with anorexia nervosa. J Clin Endocrinol Metab. 2008;93(8):3029–36.
118. Schorr M, Miller KK. The endocrine manifestations of anorexia nervosa: mechanisms and management. Nat Rev Endocrinol. 2017;13(3):174–86.
119. Munoz-Calvo MT. Anorexia nervosa: an endocrine focus and procedure guidelines. J Pediatr Endocrinol Metab. 2005;18(Suppl 1):1181–5.
120. Munoz MT, de la Piedra C, Barrios V, Garrido G, Argente J. Changes in bone density and bone markers in rhythmic gymnasts and ballet dancers: implications for puberty and leptin levels. Eur J Endocrinol. 2004;151(4):491–6.

Sexually Transmitted Infections and Risk Behaviors in the Adolescence

11

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11.1 Introduction

Sexually transmitted infections (STIs), previously known as "sexually transmitted diseases" (STDs) or "venereal diseases," are represented by a multitude of different conditions that recognize bacterial, viral, or parasitic etiology. Nowadays, the name STIs is preferred in respect to STDs because the term "disease" would imply the presence of active signs or symptoms, while sexually transmitted infections are often asymptomatic for a long period of time, potentially leading to complications, such as pelvic inflammatory disease, and long-term consequences, such as ectopic pregnancies in female and inability to procreate in both sexes.

Since adolescents are at particularly high risk for STIs for a multitude of reasons, healthy sexual activity and knowledge of STIs risk is fundamental in this population.

11.2 Epidemiology

STIs represent a major health problem that involves primarily young individuals, not only in developing countries but also in industrialized nations. The epidemiology of STIs differs in respect to sex, sexual practices, and geography (Fig. 11.1). Despite this, in a scenario characterized by a general decline of age at first sexual intercourse with increasing proportions of individuals reporting sexual activity before the age of 16, the incidence of STIs is on the rise in both sexes among

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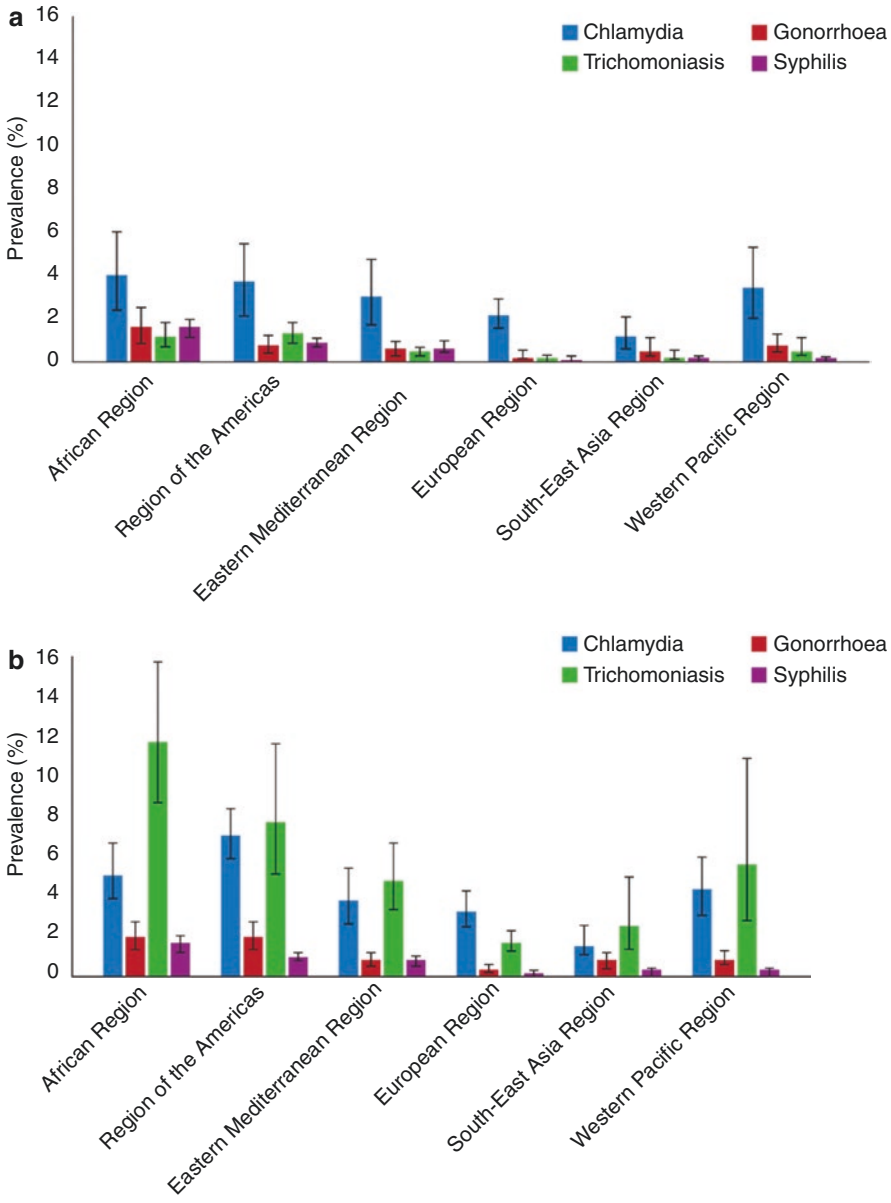

Fig. 11.1 Geographical distribution of the four most common STIs in women aged 15–49 (**a**) and men aged 15–49 (**b**) between 2009 and 2016. (Adapted from "Report on global sexually transmitted infection surveillance 2018" of the World Health Organization)

adolescents. Inconsistent use of condoms and lack of perception of STIs risk are among the predominant reasons for this observed trend [1].

It has been estimated that 357.4 million new cases of the four most common STIs occur each year worldwide: 142.6 million cases of trichomoniasis, 130.9 million cases of chlamydial infection, 78.3 million cases of gonorrhea, and 5.6 million cases of syphilis. The Centers for Disease Control and Prevention reports that in the United States half of the STIs cases are acquired by individuals during adolescence (15–24 years) [2]. In Europe in 2016, 403.807 cases of chlamydial infection were registered in subjects aged 15–25 years. It has been estimated that one out of four sexually active adolescent females present an STI, with *Chlamydia trachomatis* and Human Papilloma Virus (HPV) being among the most common STIs in this population. Other most frequent STIs in the adolescent population are represented by gonorrhea, syphilis, Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), and trichomoniasis; hepatitis B infection (HBV) showed an important decrease after introduction of preventive vaccination strategy, but it still a major problem in developing countries.

In recent years in the United States, the rate of genital chlamydial infection increased by 4% among female adolescent and by 15% among males adolescent, while a decrease in rates of gonorrhea have been observed among females. In the same period, syphilis rate increased in both sexes by around 24% among adolescents aged 15–19 years and 25% in individuals aged 20–24 years [3].

HPV infection is the most common STI worldwide. It has been estimated that 8 out of 10 sexually active women will be exposed to HPV. HPV 6 and 11 accounts for 90% of all genital warts and HPV 16 and 18 are involved in 70% of cervical cancer cases. Time of exposure to HPV is between 3 and 7 years after first sexual contact, with a prevalence of 29% among female aged 14–19 years old and 58.7% in females aged 20–24 years. It is to be noted that HPV epidemiology among adolescents greatly varies upon local vaccination programs [4, 5].

Around 21% of all new HIV diagnoses in 2018 in the United States have been made in individuals aged 13–24 years old, 86% of which occurred among young men. The vast majority (92%) of new HIV infections in young men involved gay and bisexual men; on the other hand, most new HIV diagnosis (85%) in young females were consequent to heterosexual contacts [6].

HSV infection is common among both, young females and men; the estimated rate of HSV-1 is around 49% in adolescent females and 53% in adolescent males, while prevalence of HSV-2 infection have been estimated around 15% in adolescent females and 12% in males [7].

Despite trichomoniasis represents the most common nonviral STI worldwide with an estimated 248 million cases each year, the epidemiology of this infection is unclear due to lack of established surveillance programs. Prevalence of this infection among adolescents in the United States has been estimated around 3%, while some studies highlighted rates as high as 14%, with a high rate of reinfection. Trichomoniasis among males ranges between 2% in high-risk subjects and 73% among partners of infected females [8].

Reinfection is common in the setting of STIs: studies show that every year 40% of new cases of chlamydial and gonococcal infections in adolescent individuals occur within few months from a previous diagnosis of the same STI [9]. Since HIV transmission among adolescents occurs mainly through the sexual route, the presence of an STI other than HIV (in particular HSV, syphilis, and trichomoniasis) increase the likelihood of HIV transmission and repeated STIs episodes represent a risk factor for subsequent acquisition of HIV infection.

11.3 Risk Factors for STIs in Adolescents

Incidence rate of STIs among the adolescent population is particularly high due to the presence of peculiar risk factors that can be mainly classified as biological or behavioral. From the biological perspective, young women appear to be particularly vulnerable to the acquisition of STIs due to specific developmental conditions of the genital tract:

- During youth the epithelial lining of the cervix appears immature and predominantly composed by thin, thus vulnerable, columnar epithelium (cervical ectopy).
- Local mucus production at the cervical–vaginal site, that represents an important defensive barrier against external pathogens, is reduced during young age in respect to what observed in adults. Moreover, studies showed that cervical epithelial cells are major actors of the local innate and adaptive mucosal immune response through the expression of Toll-like receptors and the secretion of cytokines, and that the quantitative and qualitative profile of cytokines secretion differs between young and adult women, although the clinical impact of this observation needs to be elucidated [10]. In addition, vaginal microbiome could play a role in the risk of STIs acquisition through its ability to modulate local inflammation and to compete with pathogens colonization: as an example, women with local inflammation due to bacterial vaginosis are three times more likely to transmit HIV infection to their partners when compared with women which local flora is dominated by acid producers *Lactobacilli* [11].

Sexual behavior plays a major role in the definition of the risk of STIs acquisition, especially among the adolescent population [12]:

- The age of sexual debut represents an important risk factor for STIs acquisition. Early age is associated with lower perception of the risk of getting infected with STIs. Nowadays about 11% of adolescents worldwide start sexual intercourses before 15 years old and reduced age at the time of first sexual contact, in particular before the age of 13, have been independently associated with the presence of STIs.
- Time since first sexual contact represents another important risk factor for the presence of STIs; the diagnosis of a STI, most commonly chlamydia or HPV,

often occurs within the first year of sexual activity with common reinfection episodes, thus screening programs in adolescents, which are generally not implemented, should start early in life and continue with regular and frequent follow-up.

- Inconsistent use of condoms is frequent among adolescent and represents a major risk factor for STIs transmission and acquisition; surveys show that the use of condom during oral sex is infrequent among adolescent women, heterosexual men, and gay men and that the use of condom is far from being optimal among these groups when considering insertive and receptive vaginal intercourses and insertive and receptive anal intercourses among gay men, with the exception of receptive anal intercourse in women, although the purpose of using condom reported among adolescents is prominently for preventing pregnancy rather than STIs.
- The use of alcohol and/or drugs with sex is associated with an increased risk of STIs; the practice of consuming those substances for sexual contacts reduces the perception of the risk and is linked to more frequent unprotected intercourses [13].
- Having multiple sex partners is a risk factor for acquisition of STIs; in multiple studies, the number of recent partners, as well as partners during lifetime, have been recognized as a risk factor for acquiring sexually transmitted pathogens.
- Sending or receiving sexually explicit messages ("sexting") and the use of apps and social media to find sexual partners are increasing among adolescents; these practices have been identified as risk factors for STIs and frequent sexting have also been linked with having multiple partners and to unprotected sex. In the setting of STIs in the adult population, the experimental studies showed that social networks could offer a resource to screen and control STIs [14, 15].
- As for adults, performing enemas or douching before anal receptive sex could tear and inflame the rectal mucosal barrier, thus leading to an increased risk of STIs acquisition, especially in the case of unprotected intercourses [16].
- Having sex with a person with a penis is a risk factor for STIs in the adolescent female transgender population.

Other recognized risk factors for STIs are as follows:

- Mood disorders (consequently to the increased risk of substances abuse)
- Being in a detention facility
- History of maltreatment or sexual assault
- "Survival sex" (e.g., giving sex in exchange of money, food, shelter, or drugs)
- Street involvement or homelessness

11.4 Screening for STIs in Adolescents

11.4.1 Investigation of Sexual History

The first step to provide high quality STIs-related care is represented by a thorough investigation of sexual history and sexual activity of the individual, together with

STIs risk assessment; it is fundamental that this evaluation is conducted straightforward and with a nonjudgmental attitude. A complete sexual history evaluation should follow the "five Ps":

- **Partners**: Assumption about patient's sexual orientation should not be made; number and gender of patient's partners should always be investigated (e.g., "In recent months how many partners have you had?" "Are your sex partners men, women or both?"). Duration of the relationship, condom use, and partner's risk factors should be always included in the interview.
- **Practices**: The investigation of sexual practices together with condom use will guide in assessment of risk for STIs and help identify anatomical sites from which to collect specimens to investigate the presence of STIs (e.g., "What kind of sexual contact do you have? Genital, anal, oral?" "What kind of sexual contact have you had?").
- **Protection**: Protection measures adopted by the patient and his partner or partners represents a necessary topic to discuss during the evaluation (e.g., "Do you and your partner(s) use any protection against STIs?" "What kind of protection do you use against STIs?" "How often do you use protection against STIs?"). Knowledge of the perception of STIs risk and adopted protective measures will guide the clinician in assessing the need for STIs risk reduction counseling.
- **Past history of STIs**: If your patient received a diagnosis of STI in the past, he or she could be at greater risk for STIs in the present. If your patient have been diagnosed with an STI in the past, it is fundamental to get information about when the diagnosis was made, the treatment that the patient received, testing for other STIs at the time of diagnosis, the presence of recurring symptoms, STIs of the partner or partners, and treatments of partner or partners (e.g., "Have you ever been diagnosed with an STI? When and how were you treated?" "Have you ever been tested for HIV or other STIs?" "Has your partner or former partners ever been diagnosed or treated for a STI? Where you tested for the same STI?").
- **Prevention of pregnancy**: Assessing the desire of pregnancy or becoming a parent is especially important when caring for adolescents and will guide the clinician in defining the need for counseling about contraception or birth control and reduction of STIs risk (e.g., "Are you concerned about getting pregnant or getting your partner pregnant?" "Are you using contraception or practicing birth control?").

Additional questions to evaluate risk for HIV and viral hepatitis should investigate if the patient or partner(s) made current or previous use of injective drugs, if the patient or partner(s) ever exchanged money or drugs for sex [17, 18], etc.

11.4.2 Physical Examination

Physical examination represents the second fundamental step in the evaluation of STIs, allowing the provider to identify signs related to STIs that the patient might

have not noted. During physical examination, physician should pay particular attention to the skin, oral cavity, pharynx, anogenital area. Presence of ulcerations and/or discharge should be carefully assessed. Lymph nodes and neurologic system should be also taken into account.

Skin should be thoroughly assessed for the presence of rash, lumps, bumps, ulcers, smell, presence of lice in the pubic hair:

- HPV-related anogenital warts represent the most common skin manifestation of STIs; most common sites of manifestation are the vulva, anal and perianal skin, penis, perineum, groin, and sovrapubic skin. Warts are usually asymptomatic and can present as single or multiple lesions with a wide array of size, from 1 mm to several centimeters, and appearance: flat, raised, cauliflower shaped, smooth, verrucous, pedunculated; color of the lesion can also range from white to hyperpigmented. Warts can involve the cervix and anal canal, thus the observation of warts on external skin should address to gynecologic evaluation and, in young men who have sex with men (YMSM), to investigation of the anal canal preferably through high-resolution anoscopy [19].
- Primary syphilis is typically characterized by the presence of a not swollen and not painful ulcer with raised indurated margins (also known as "chancre") that appears at the site of contact with the pathogen (usually involves penis, but can also appear on vaginal, anorectal, or oropharyngeal mucosa). Chancre usually resolves spontaneously within 3–6 weeks; weeks to months after the resolution of chancre, one out of four subjects shows signs of secondary syphilis which can present as: (i) diffuse and symmetric macular or papular rash on trunk and extremities with involvement of palms and soles; (ii) pustular presentation, less commonly; (iii) wart-like lesion ("condylomata lata") may also be present; (iv) secondary syphilis can occasionally present as "moth eaten" alopecia [20].
- Skin manifestations are present in approximately 75% of subjects with disseminated gonococcal infection; typical lesions are represented by painless pustulae or vesicopustulae on the extremities that spontaneously resolve within few days, although vesicles, bullae, or nodules can less commonly be present. Lesions are usually between 2 and 10 in number. Skin manifestations in the setting of disseminated gonococcal infection can be associated to tenosynovitis and polyarthralgia (arthritis–dermatitis syndrome).
- Classic scabies skin lesions are represented by multiple and small erythematous papulae often distributed to one or more sites between: fingers, wrists, elbows, axillary folds, periareolar skin, umbilical skin, waist, scrotum, penile shaft, glans, knees, buttocks, feet. Scabies is characterized by the presence of intense pruritus as a result of hypersensitivity reaction to the mite.
- Pruritus also characterizes pubic pediculosis. Diagnosis of pediculosis is made by the demonstration of lice or nits. Pubic pediculosis can also involve other body areas with hairs. In the case of prolonged infestation, blue macules can be observed on the lower part of the abdomen, buttocks, or thighs as a result of injection of anticoagulant factors during feeding of the parasite [21].

Oral cavity and pharynx should be examined when investigating the presence of signs of STIs:

- Clinical manifestation of primary syphilis (chancre, previously described) can involve the lips, oral mucosa, tongue, or tonsils. Secondary syphilis can cause little multiple painless erosions on the surface of the tongue (mucous patches).
- Extragenital gonococcal infection can present as gonococcal pharyngitis. This infection is usually asymptomatic or it can cause aspecific manifestations such as sore throat and/or cervical lymphadenitis; pharyngeal exudate can be present, and it can resemble aphthous stomatitis [22].
- HSV-1 infection, and less commonly HSV-2, typically causes gingivostomatitis or pharyngitis. In the first case, manifestations are characterized by the presence of painful vesicular lesions that can involve the lips, soft palate, tongue, buccal mucosa, the floor of the mouth, or pharyngeal mucosa. HSV pharyngitis is characterized by the presence of pharyngeal edema, tonsillar exudate, and tonsillar ulcerative lesions.
- Oral HPV infection has been linked to higher numbers of sex or open mouth kissing partners, smoke, and older age; it can be associated to the onset of benign warty lesions that can affect tongue or the oral mucosa, the possible presentations of such lesions can resemble those described in respect to the skin. Focal hyperplasia of the oral mucosa, also known as Heck's disease, can be another benign manifestation. HPV infection of the oropharyngeal cavity is also associated to the onset of squamous cell carcinoma [23].

Genital ulcers are not invariably caused by infectious agents and can be part of clinical presentations of systemic diseases such as Beçhet's disease, inflammatory bowel diseases such as Crohn's disease or can be present as satellite manifestation of viral illnesses. Since the presence of genital ulcers increases the risk for STIs, subjects with genital ulcerations should be offered testing for the most common STIs (syphilis, chlamydia, gonorrhea, HIV) [24]. Characteristics that should always be evaluated include number of lesions, presence of pain, presence of urinary symptoms (e.g., chlamydial or gonococcal urethritis), presence of systemic symptoms (e.g., secondary syphilis or primary HSV infection):

- HSV-2 infection, and less commonly HSV-1, represent the most common cause of genital ulcer among adolescent population. The clinical presentation of genital HSV infection starts as grouped vesicles with erythema of the surrounding epithelium, vesicles rapidly break causing ulceration. Vesicles can undergo unnoticed and ulcer can be the first perceived manifestation. In the case of HSV infection, ulcer can be single or multiple.
- Primary syphilis represents a common cause of infectious genital ulceration (syphilitic chancre); most often the presentation involves a single lesion.
- The clinical presentation of primary stage of lymphogranuloma venereum (LGV, caused by serovars L1, L2, and L3 of *Chlamydia trachomatis*) is characterized

by the onset of a single, painless, and often small genital ulcer in the site of inoculation of the causative organism. Since the primary stage is often asymptomatic, patients frequently present when secondary manifestations are present: inguinal nodes swelling and eventually rupture (this manifestation is less common among females), rectal pain, tenesmus, constipation, proctocolitis with rectal discharge, hemorrhagic proctocolitis, rectal inflammatory mass. Although often paucisymptomatic, anogenital strictures could represent the presentation of secondary stage of LGV. Unrecognized LGV should also be taken into account as a possible cause of infertility.

- Chancroid (*Haemophilus ducreyi* infection) is characterized by the onset of single or multiple genital suppurative ulcer that cause pain to the patient. The lesion starts as a papule that rapidly progresses to pustule and to ulcer. Ulceration caused by *Haemophilus ducreyi* is usually wider and deeper that syphilitic chancre. It has been estimated that outside endemic areas a proportion as high as 15% of genital ulcers can be caused by this pathogen [25].
- Granuloma inguinale (donovanosis) is nowadays rare in Europe and United States. It affects the genital area in 90% of cases and inguinal area in 10% of cases, although it can rarely affect lips, cheeks, palate, or pharynx. Granuloma inguinale most commonly presents as an ulcerogranulomatous disease with single or multiple exuberant red ulcers that easily bleed if touched. In other cases, it can present with a hypertrophic verrucous, necrotic, or sclerotic appearance [26].

Discharge is a characteristic of some STIs, although it might be unnoticed:

- Gonorrhea causes purulent discharge that is usually noted by male patients in case of urethritis but it can be unnoticed in women or in the case of gonococcal proctitis or pharyngitis.
- Although women with cervicitis due to Mycoplasma genitalium are frequently asymptomatic, when symptoms are present mucopurulent discharge represents the most common one. Similarly, *Mycoplasma genitalium* urethritis in men can cause purulent or mucopurulent discharge, although is often asymptomatic [27].
- Chlamydial infection of the cervix is often asymptomatic but it can be associated to mucopurulent endocervical discharge. Chlamydial urethritis can cause dysuria but rarely discharge. In chlamydial proctitis mucopurulent discharge is occasionally observed [28].
- The presence of white, thick, clumpy, and odorless discharge is suggestive of Candida vulvovaginitis; discharge is usually associated with burning, dysuria, dyspareunia, local erythema, and excoriation.
- Trichomoniasis in women is asymptomatic in around 80% of cases. When symptomatic, thin smelly discharge together with pruritus, burning, dysuria, or dyspareunia can be present. Symptoms tend to worsen during menstruation. Men with urethritis caused by *Trichomonas vaginalis* can infrequently experience mucopurulent discharge and dysuria.

11.5 Prevention of HIV Risk: Preexposure Prophylaxis

HIV Preexposure Prophylaxis (PrEP) represents a highly effective intervention for the prevention of HIV transmission. PrEP is based on the daily administration of the combination of tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) or tenofovir alafenamide/emtricitabine (TAF/FTC). These medications are often used in combination with other antiretroviral drugs to treat HIV-infected individuals; their administration alone demonstrated preventive efficacy among HIV-negative subjects at high risk for HIV acquisition. The American Food and Drug Administration first approved the daily administration of TDF/FTC as part of the strategy to prevent sexual transmission of HIV among high-risk adolescents weighing at least 35 kg and recently approved TAF/FTC with the same purpose, with the exception of those at risk for acquiring HIV through receptive vaginal sex [29]. The efficacy of PrEP among adolescents has been evaluated in two main studies that involved, respectively, 67 young MSM aged 15–17 years and 148 young individuals aged 15–19 years (98 females and 50 males). The efficacy of PrEP is dependent upon adherence to treatment and data from these studies highlighted that adolescents may benefit from closer follow-up visits during PrEP to ensure daily intake of TDF/FTC. Patient counselling is mandatory before and during the course of PrEP. Females should use an effective method of contraception during PrEP. Periodic testing for HIV and other STIs is mandatory while on PrEP (Table 11.1). Several factors can limit the use of PrEP in adolescents, among these the most significant could be represented by: the great variability between countries or, more often, the complete lack of a legislation on this topic; the eventual need for a parental or guardian consent that would imply the disclosure of sexual activity and sexual orientation; physicians may not be comfortable with PrEP [30].

Table 11.1 Interventions and screening strategy for individuals on PrEP

First MONTH	• Pregnancy test • Fourth-generation HIV test (HIV ab/ag) • Counselling on adherence and reduction of sexual behavior risk
EVERY 3 MONTHS	• Pregnancy test • Fourth-generation HIV test (HIV ab/ag) • Counselling on adherence and reduction of sexual behavior risk[a]
EVERY 6 MONTHS	• Estimation of creatinine and glomerular filtration rate • Screening for STIs
EVERY 12 MONTHS	• Evaluation of HIV risk and indication to continue PrEP

[a]Monthly counselling may be indicated for adolescents to ensure optimal adherence to PrEP

11.6 Confidentiality in Adolescent Patient–Physician Relationship

Confidentiality represents a fundamental part of the patient–physician relationship and usually involves two actors. On the contrary, when interacting with adolescent patients, confidentiality represents a more complicated issue that may involve parents as third actor, which potentially undermines the relationship and confidence between adolescent and physician [31]. Studies showed that adolescents are more likely to seek for medical care if they are confident that doctor will keep for himself the information discussed during visits; on the other hand, concerns about confidentiality could create barriers in the communication between the adolescent patient and healthcare provider and discourage the adolescent from seeking medical care and counseling. Topics such as sexual health and STIs may easily lead to concerns about confidentiality in young patients. Regulations on informed consent and confidentiality are different between countries and physicians should have knowledge of laws in their own region [32].

It is important to make a clear statement with the adolescent patient about circumstances when confidentiality might become conditional and continuous encouragement for the patient to directly talk to parents could be important.

References

1. American Sexual Health Association. STDs/STIs. http://www.ashasexualhealth.org/stdsstis/.
2. Satterwhite CL, Torrone E, Meites E, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187–93.
3. Sexually Transmitted Disease Surveillance. Division of STD prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention. Sep 2017; 2016.
4. Moscicki AB, Ellenberg JH, Vermund SH, et al. Prevalence of and risks for cervical human papillomavirus infection and squamous intraepithelial lesions in adolescent girls impact of infection with human immunodeficiency virus. Arch Pediatr Adolesc Med. 2000;154:127.
5. Herrero R, Hildesheim A, Bratti C, et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst. 2000;92:464.
6. CDC. Diagnoses of HIV infection in the United States and dependent areas, 2018. HIV Surveillance Report. 2019:30.
7. Auslander BA, Biro FM, Rosenthal SL. Genital herpes in adolescents. Semin Pediatr Infect Dis. 16(1):24–30. https://doi.org/10.1053/j.spid.2004.09.008.
8. Kissinger P. Epidemiology and treatment of trichomoniasis. Curr Infect Dis Rep. 2015;17(6):484.
9. Wikström E, Bloigu A, Ohman H, et al. Increasing proportion of reported chlamydia trachomatis infections are repeated diagnoses. Sex Transm Dis. 2012;39(12):968–72.
10. Hwang LY, Scott ME, Ma Y, Moscicki AB. Higher levels of cervicovaginal inflammatory and regulatory cytokines and chemokines in healthy young women with immature cervical epithelium. J Reprod Immunol. 2011;88(1):66. Epub 2010 Nov 3.

11. Cone RA. Vaginal microbiota and sexually transmitted infections that may influence transmission of cell-associated HIV. J Infect Dis. 2014;210(Suppl 3):S616–21.
12. Oskar AA, Mar VG, Montserrat RS, et al. Risk factors associated with sexually transmitted infections and HIV among adolescents in a reference Clinic in Madrid. PLoS One. 2020;15(3):e0228998.
13. Evers YJ, Van Liere GAFS, Hoebe CJPA, et al. Chemsex among men who have sex with men living outside major cities and associations with sexually transmitted infections: a cross-sectional study in the Netherlands. PLoS One. 2019;14(5):e0216732.
14. Rice E, Craddock J, Hemler M, et al. Associations between sexting behaviors and sexual behaviors among mobile phone-owning teens in Los Angeles. Child Dev. 2018;89(1):110.
15. Madigan S, Ly A, Rash CL, et al. Prevalence of multiple forms of sexting behavior among youth. A systematic review and meta-analysis. JAMA Pediatr. 2018;172(4):327–35.
16. Peiyang L, Tanwei Y, Fitzpatrick T, et al. Association between rectal douching and HIV and other sexually transmitted infections among men who have sex with men: a systematic review and meta-analysis. Sex Transm Infect. 2019;95(6):428–36.
17. Barrow RY, Faruque A, et al. Recommendations for providing quality sexually transmitted diseases clinical services, 2020. MMWR Recomm Rep. 2020;68(5):1–20.
18. Workowski KA, Bolan GA. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2015. MMWR Recomm Rep. 2015;64(RR-03):1–137.
19. Yanofsky VR, Patel RV, Goldenberg G. Genital warts A comprehensive review. J Clin Aesthet Dermatol. 2012;5(6):25–36.
20. Hook EW 3rd. Syphilis. Lancet. 2017;389:1550–7.
21. Flores AR, Caserta MT. Pharyngitis. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 2015:753–759.e2.
22. Komaroff AL, Aronson MD, Pass TM, et al. Prevalence of pharyngeal gonorrhea in general medical patients with sore throats. Sex Transm Dis. 1980;7(3):116.
23. Pickard RK, Xiao W, Broutian TR, et al. The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18-30 years. Sex Transm Dis. 2012;39(7):559.
24. Maliyar K, Mufti A, Syed M, et al. Genital ulcer disease: a review of pathogenesis and clinical features. Cutan Med Surg. 2019;23(6):624–34.
25. Lewis DA. Epidemiology, clinical features, diagnosis and treatment of Haemophilus ducreyi - a disappearing pathogen? Expert Rev Anti-Infect Ther. 2014;12(6):687–96.
26. O'Farrell N, Hoosen A, Kingston M. 2018 UK national guideline for the management of donovanosis. Int J STD AIDS. 2018;29(10):946–8.
27. Tosh AK, Van Der Pol B, Fortenberry JD, et al. Mycoplasma genitalium among adolescent women and their partners. J Adolesc Health. 2007;40(5):412. Epub 2007 Feb 15.
28. Falk L, Fredlund H, Jensen JS. Symptomatic urethritis is more prevalent in men infected with mycoplasma genitalium than with chlamydia trachomatis. Sex Transm Infect. 2004;80(4):289–93.
29. Tanner MR, Miele P, Carter W, et al. Preexposure prophylaxis for prevention of HIV acquisition among adolescents: clinical considerations, 2020. MMWR. 2020;69(3):1–12.
30. Hosek S, Celum C, Wilson CM, et al. Preventing HIV among adolescents with oral PrEP: observations and challenges in the United States and South Africa. J Int AIDS Soc. 2016;19(7 Suppl 6):21107.
31. Adolescent Health Care, Confidentiality. AAFP web site: https://www.aafp.org/about/policies/all/adolescent-confidentiality.html.
32. Ford CA, Millstein SG, Halpern-Felsher BL, et al. Influence of physician confidentiality assurances on adolescents' willingness to disclose information and seek future health care. A randomized controlled trial. JAMA. 1997;278(12):1029.

Sexual Disorders in Adolescents and Young Adults

12

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12.1 Introduction

The development of sexuality is a process encompassing the entire arc of life, and it is characterized by several bio-psycho-social systems and relational factors [1]. However, from a historical point of view, the theory of sexuality by Sigmund Freud represents a landmark for all the studies on human sexology.

With a language at its times considered scabrous, the father of psychoanalysis centered, into the *Three Essays on the Theory of Sexuality* (1905) [2], the development of human sexuality in the infancy, from the oral phase to genital phase in adolescence, and therefore the adult life. The polymorphous perversity characterizes, in the original Freudian theory, the infantile sexuality, through the separate and

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peculiar ontological phases named the oral, the anal, and the phallic (Fig. 12.1). The journey throughout the three phases may lead to "perverse" and/or bisexual residues that may remain in the adult, or genital, final phase. The psychosexological healthy adult is one who sublimized (a specific term selected by Freud from physical chemistry) his/her perverse residues. On the contrary, both the neurosis or perversion (paraphilia, in the modern language) are explained, in the original Freud's research, as fixation or regression to three above-mentioned early phases [3, 4]. Freud, moreover, identified the sexual instinct on libido, and the relationship between sexual development and Ego as central for the development of personality in a normal or a pathological pattern.

On the other hand, psychoanalyst Wilhelm Reich pointed out on psychosexological development during infancy as a fundamental phase for the formation of character.

In the famous book *Character Analysis* (1933), Reich affirmed that the origin of neurosis is due to a familiar and patriarchal education characterized by the repression of sexuality, highlighting also the central role of Oedipal phase [5] (Fig. 12.2).

For these and other reasons, peculiar attention toward sexual health in the first phase of life is fundamental. The adolescence is characterized by several biological, anatomical, hormonal, and, therefore, phenotypical changes in males and females. Sexuality plays a pivotal role in these bodily changes, as in the development of secondary sexual characters. Moreover, adolescence is the period of the first relationships and sexual relationships, together with the consolidation of sexual identity. At the light of these intrinsic characteristic of the adolescence, it is more correct to

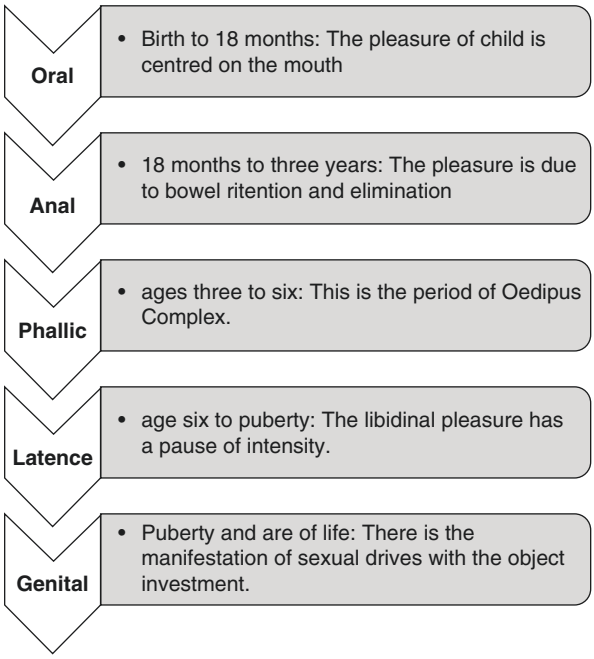

Fig. 12.1 Psychosexual development Genital phase is the last step of psychosexual development, in which the personality and its relational patterns are successfully developed. According to psychodynamic perspective the achievement of genital phase is fundamental for the structuration of a healthy personality. A fixation to a previous phase, as oral, anal, or phallic is indicative of a pathological structure of personality

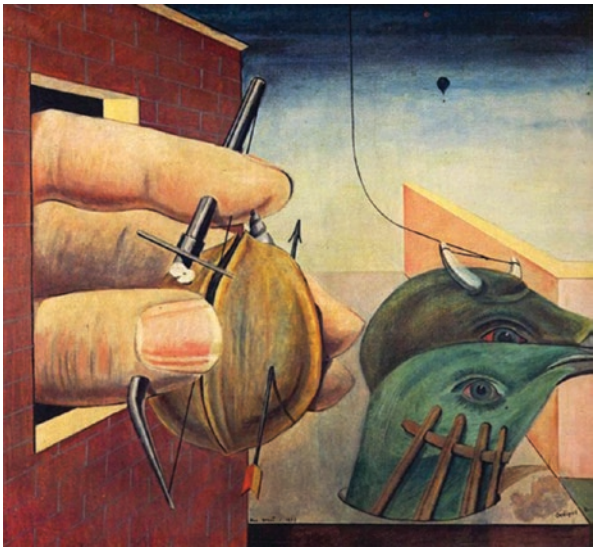

Fig. 12.2 **Oedipus Complex** (Max Ernst, *Oedipus Rex*, 1922) Oedipus Complex represents the main moment of the internal representation of relational world, and it is fundamental for the constitution of reality exam for the personality. During the Oedipal period, Superego is structured in the psychic system as also the internalization of main figures of attachment in terms of femininity and masculinity. During the Oedipus phase, child changes from dual relational condition to triadic, and this phase is also central for the mating strategies and relational life in the adulthood, as also was affirmed by the Attachment Theory [90]

study and eventually treat the sexual and also affective development of individuals transiting in this delicate period of life. In this regard, traditional theories consider adolescence a controversial phase characterized by an internal dualism of people: dependence versus independence, from paternal bonds to intimate and social relationships [6].

In this period, therefore, biological and psychological factors strongly condition the behavior and a psychoendocrine perspective is suitable to better describe adolescents. Hormones and the attachment styles, in fact, play together a central role to drive sexual behavior and all related factors.

This chapter describes the main characteristics of sexual behavior, from a normal to pathological continuous in the adolescents and young adults, with particular attention to the more recent investigations as also to peculiar social phenomena regarding sexuality.

12.2 Sexual Dysfunctions in Adolescence

The topic of sexual health in adolescents has rarely been addressed by the scientific literature, and even in slightly older (young adult) populations research has been extremely narrow in scope, investigating only small parts of sexuality and mostly

focusing on the psychological factors involved in sexual dysfunctions. This is surprising given that in most Western countries many adolescents have already had their first sexual intercourse by the age of 18: Only 10–40% of young men and women have not had intercourse by that time, and this prevalence steadily decreases over time, with only 3% and 5% for females and males, respectively, between 25 and 29 years of age [7]. It has long been established that "early" sexual intercourse may lead to severe consequences on quality of life, especially when triggered by peer pressure, by partners, or by more complex scenarios, such as coercion [8]. Gender differences have also been investigated: Boys often consider their first intercourse as an "achievement," whereas girls are seemingly more likely to engage in intercourse to strengthen the relationship with their partner [9, 10]. However, the precise definition of "early" is uncertain. Several studies have suggested that during early and middle adolescence, teenagers are less likely to be "cognitively ready" for their first sexual experiences as well as less aware of the potential risks associated with unprotected sex [11]. There is a worrying association between risky behaviors, such as alcohol and drug use, and both early and unprotected intercourse [12, 13].

An ever-growing body of evidence supports the notion that most sexual dysfunctions occur later in life, as clearly reported by the increasing prevalence of erectile dysfunction in elderly men [14] or by sexual symptoms associated with menopause [15]. However, sexual dysfunction can occur in adolescents as well. It is not uncommon for adolescents to request a specialist consultation for premature ejaculation, painful intercourse, or even difficulty in achieving orgasm, although physicians expert in the developmental sexology are very few [16]. It is generally assumed that some of these symptoms could be age-dependent: Premature ejaculation is indeed more common in adolescents, young adults, and sexually naïve men [17, 18], and vulvodynia is similarly highly prevalent in young girls as also primary vaginismus [19, 20]. Performance anxiety is among the most common sexual complaints and is highly prevalent in subjects who are starting to explore their and their partner's sexuality [21]. Inhibition of sexual desire, premature ejaculation, and erectile dysfunction are common consequences of this condition [21, 22], and they can greatly affect sexual function in younger subjects. Additionally, it can be speculated that younger subjects are less likely to be able to cope with sexual dysfunction [23], resulting in worsened sexual health. Additionally, sexual performance anxiety can induce and maintain a "vicious circle" with progressively worsening sexual symptoms: in boys as well as in adult men, inadequate erection may lead to shorter time to ejaculation, and at the same time the attempts to delay ejaculation may lead to reduced control over erectile function.

The high prevalence of psycho-relational, apparently nonorganic risk factors has led many clinicians to overlook other possible organic components of sexual dysfunction; however, while undoubtedly several risk factors are closely tied to increasing age, such as endothelial dysfunction and late-onset hypogonadism [24–26] for erectile dysfunction, organic risk factors should never be excluded a priori. Endocrine disorders, as well as drug-related conditions and cardiovascular diseases, can also be observed in young patients, and a careful medical history and physical examination are therefore mandatory in any clinical evaluation [14]. In these

regards, it is worth remembering that erectile dysfunction, sharing the same risk factors, has been recognized as a predictor of cardiovascular disease [27]. In young adults, and even more in adolescents, clinicians should never overlook cardiovascular risk factors or conditions suggestive of cardio-metabolic derangements in subjects complaining of erectile dysfunction, despite their age [28].

Young patients with a history of onco-hematological malignancies deserve an additional mention in the context of the assessment of sexual dysfunctions. Hypogonadism and infertility occur in these patients once they reach adult age, and they frequently also develop sexual dysfunctions, both for the treatment and for the psychological burden associated with their medical history [29]. Tailored treatment is of utmost importance to provide normal sexual and psychological development, and a psycho-sexological approach should always be suggested to maximize the treatment potential.

The treatment of sexual dysfunctions in adolescents follows the same steps suggested for adults, starting from the identification and removal of any risk factors or health-risk behaviors, such as smoking, alcohol consumption, and drug abuse. Such behaviors are common in adolescents: Despite laws restricting, the sale of alcohol and tobacco products to underage subjects, a recent nationwide survey endorsed by the Italian Ministry of Health and the Italian Society of Andrology and Sexual Medicine reported that occasional alcohol consumption is up to 80% of male high school students, and 51% of interviewed subjects also reported having tried smoking [12]. Such behaviors are likely to result in impaired sexual development, especially when they start during adolescence, which is a vulnerable time period for the development and maturation of the genitourinary and reproductive tract [30].

Once health-risk behaviors have been ruled out, if the sexual symptoms still persist, drug treatment can be proposed; however, guidelines from different scientific societies do not provide any information concerning the benefits or the risks of the available treatments in the adolescent population. The absence of any specific indication does not mean, however, that such treatments are not sought after by young subjects. Indeed, despite the lack of any clinical evidence (and often without any prescription), recreational use of phosphodiesterase type 5 inhibitors (PDE5i) by young, healthy men has been reported in the literature and is seemingly more prevalent than expected [31–33]. Several hypotheses can be developed in these regards: While it is clearly possible that some of these PDE5i users have reasons to take these medications, it has also been suggested that a lack of confidence in erectile function once again, possibly due to sexual performance anxiety, could be the main determinant for their use [33]. PDE5is are safe for use in children, as proven in trials on pulmonary hypertension [34, 35]. As such, it can be assumed that the "physical" safety of PDE5i is not an issue in adolescents; although, whether such treatments should be considered first-line therapy in underage subjects is a matter open to discussion. Treating sexual performance anxiety with anxiolytics/antidepressants requires caution since some drugs most notably, some GABAergic drugs, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors, antidopaminergic antipsychotics, and anticonvulsants [21] can have negative effects on erectile function. Additionally, most of these drugs have not been extensively investigated in

adolescents. Integrating a psychological approach with pharmacological treatment is often advised to improve erectile function since the two main components, organic and psychogenic, should not be considered two separate entities but rather two sides of the same coin [36]. Given the little evidence available for drug treatment, a cautionary approach involving psychological and psychosexual behavioral therapy can be suggested as a first-line treatment before eventually administering a PDE5i. Concerning premature ejaculation, the only approved oral treatment is dapoxetine [37], a SSRI useful for on-demand treatment due to its short half-life and quick absorption [38]. Dapoxetine is safe and effective for the treatment of lifelong premature ejaculation [39, 40], and, as such, it is hypothetically able to improve ejaculation latency times in adolescents, as well. Nevertheless, no studies have been designed so far to assess its efficacy and safety in subjects under the age of 18. It can be assumed that treating either or both sexual dysfunctions in a young boy could provide better outcomes in terms of sexual development by reducing anxiety, without severe side effects [38]. However, on the other hand, the lack of any solid evidence in these regards suggests that any treatment should be carefully evaluated on an individual basis. Combination treatment with dapoxetine and psychological and psychosexual behavioral therapy is generally considered to provide greater benefits to the patient rather than either treatment alone [39, 41]. Whether drug therapy can be delayed in favor of behavioral treatment in younger populations is a matter open to debate.

12.3 Sexual Dysfunctions in Young Adults

The period of young adulthood is characterized by several psychosocial modifications, which may impact and determine the future development of the person. If we consider all the changes during this important life period (the beginning of independency, work position, possible cohabitation or marriage, etc.), we can speculate that from a psychological point of view the presence of a sexual dysfunction may represent an expression of a general psychological disease, generated by different psychosocial and relational stressors, maybe related to the increase in accountability. However, this perspective does not consider all organic variables, which may impact sexual well-being, also in this specific young timeframe. In fact, it is common to think about young people as "immortal" and not subjected to any kind of dysfunction or disease. Hence, this paragraph aims to deal with both the organic and nonorganic variables bearing on the general male and female psychosexual well-being, trying to give to the reader the most possible broader overview on the sexual dysfunctions that characterize young adulthood. When we refer to an organic etiology of sexual dysfunctions, we consider all possible organic variables that explain the presence of an impaired sexual life. The most impacting organic variable bearing on young sexuality is the presence of cancer, whether testicular or breast cancer, which is strictly related to sexuality. There are a lot of literature evidences showing as cancer diagnosis and cancer treatments worsen patients' sexual life [42–46]. On the other hand, there are a lot of the cancer treatment guaranteeing a high probability of

survivorship. For this reason, after the cancer eradication, the clinicians ask about the global quality of life, specifically investigating also the quality of sexual functioning. A cancer diagnosis may have an indirect impact on sexuality through a psychological reaction toward the same diagnosis and the consequent necessity to rearrange her or his global functioning [47]. The "physiological" development in the first phases after diagnosis, of a depressive mood, a mood disorder, or an adjustment disorder [48] surely bear on sexual well-being, inducing the person to live a low sexual desire, or, in extreme cases, to avoid sexuality [49]. In addition to diagnosis, curative treatments also affect sexuality. Recently, studies have indicated impaired female sexual functioning in young women after treatment for breast cancer and the development of erectile dysfunction in men after treatment for testicular cancer [50]. Interestingly, the presence of sexual dysfunctions has also been indicated in survivors of childhood cancer [51]. Hence, in this last case, the impact of cancer on sexual well-being is mediated by physical and psychological aspects; in particular, for what concerns the physical problems, they are related to surgery side effects and late effects of chemotherapy and/or radiation therapy such as vaginal dryness/tightness, pain, and fatigue. For what concerns the psychological aspects, the patients refer the worry about the interruption of adolescent psychosocial development, the altered perceptions of body image, the concerns about fertility, and the presence of inadequate clinical support [52]. In addition, the impact of testicular cancer on male sexual functioning has also been evaluated in a 5-year follow-up study after the interruption of treatment [42]. The authors found that, compared to the controls, the study group had lower scores in erectile function, sexual desire, intercourse satisfaction, and overall satisfaction in almost all long-term follow-ups (from time 0 to 5 years after diagnosis). Beyond cancer, many other organic conditions affect the sexuality of young people and couples. In this phase of life, when is presumable to suppose that a reproductive desire has a prerogative on the other life's projects, sexual problems seem to have a central role in determining the general personal and couple well-being. In this regard, we can suppose that genito-pelvic pain due to endometriosis or vulvodynia in females and premature ejaculation for males may play a disruptive role in sexuality and the quality of a relationship.

Endometriosis and the associated dyspareunia affect about 10% of women of reproductive age [53], with significant levels of chronic pain in about 50–70% of cases. If, on the one hand, a painful sexual experience is a distressing factor for females, leading to the avoidance of sexual intercourse in some cases, on the other hand, endometriosis seems to induce in male partners greater sexual and relational dissatisfaction [53].

The other side of the coin, for what concerns the sexual dysfunctions in the young population, may be represented by premature ejaculation (PE). If PE may guarantee reproduction, it worsens the quality of one's sexual life, inducing distress in their partner [54], and, specifically, the impossibility to obtain orgasm in the female partner. This condition makes this couple asynchronous [55]. For a long time, PE has been considered a sexual condition with a psychogenic etiology, especially for acquired forms [22]. However, literature evidence more frequently shows the impact of specific organic variables inducing the development of PE [37, 56].

Among the most accredited variables, there is evidence of the role of genetic diathesis in the central serotoninergic pathway, prostate inflammation/infection, or comorbidity with other sexual dysfunctions, such as erectile dysfunction (ED) [37].

During the assessment of any sexual dysfunction, the individuation of specific organic variables makes the comprehension of the nature of that symptom clearer. Organic and nonorganic etiology is, in fact, often copresent in individuals. However, we can reflect on the specific nonorganic etiology of sexual dysfunctions. In the literature, we can find specific macro areas, which can be summarized as the following: sexual dysfunctions due to another sexual condition (e.g., low sexual desire) [57], sexual dysfunctions due to the presence of a psychopathology (mood/anxiety disorders, eating disorders, personality disorders) [58], and sexual dysfunctions due to relational problems [59] or substance abuse [60]. Among these variables, maybe the less intuitive condition in young people is the presence of low sexual desire, which, in some cases, assumes the characteristics of a hypoactive sexual desire disorder (HSDD). It is a sociocultural belief that young people always desire sexual intercourse. However, it is not properly true. Low sexual desire in younger people is often present in the comorbidity of another psychopathological problem, which may be represented by an anxiety/mood disorder. Alternatively, relational problems and the use of hormonal contraceptive birth control pills may also modulate sexual desire [61, 62]. For example, the use of combined oral contraceptives does not seem to influence sexual desire, but it induces a reduction in sexual frequency [62].

The clinician in the field of sexual medicine should accurately evaluate all possible variables that explain the presence of a specific sexual dysfunction to establish its etiology. In light of the literature, it seems that the best treatment option for all sexual problems is a combination of treatments (pharmacotherapy and sex therapy, or psychotherapy, if necessary) [41]. In support of this consideration, it is important to bring in mind that an organic etiology is always, or almost always, associated with a nonorganic etiology. The organic and nonorganic variables generate a vicious cycle that may invalidate the treatment tentative if the clinician does not take care of both these two aspects. This approach is the basis of the bio-psycho-social model.

12.4 Internet-Related Sexual Disorders in Youths

Some social phenomena also involve sexual behavior in its pathological declinations. In this regard, the use of specific Internet content is related to dysfunctional aspects of sexuality; although, only in vulnerability conditions and in more fragile personalities Internet use could negatively condition sexual health. Therefore, it is necessary to consider the pathological use of the Internet and its impact on sexuality as a consequence of primary psychosocial suffering and not a cause.

After this fundamental premise, we mainly focused on four Internet phenomena involving sexual behavior of adolescents and young adults: pornography, meeting app use, sexting, and sextortion.

With respect to the first phenomenon, it has been demonstrated not substantially harmful in the large majority of adult users [63], across the religions and the

cultures, men and women as well as young, middle-aged, and mature adults. However, some authors have recently found that better quality family bond is associated with a lower likelihood of pornography access, and the time spent with one's own parents appears to delay the debut of pornography use in adolescents [64]. Serious non-ideologically driven surveys on the possible risks of pornography use in young generations are currently lacking, although some stereotypical models of pornography are considered in correlation with gender-based beliefs of sexuality [65].

Another important issue regards violent pornography and its possible association with sexual behavior and sexual relationships. Pornography, as every technological medium, plays a central role in education and, in many cases, represents a model for adolescents where other institutional media is lacking. To date, (wrong) knowledge about sex and sexuality is almost totally obtained in the porno context, being laic, well-controlled, scientifically sound sex, and affective education (see later) lacking in many societies. This severe bias limits prevention strategies also toward violence.

A cross-sectional study suggested that exposure to violent pornography is a possible risk factor for violent dating among adolescents. Male adolescents who were exposed to violent pornography were over three times as likely to perpetrate sexual teen violent dating (TDV), while females exposed to violent pornography were over one-and-a-half times as likely to perpetrate physical and threatening TDV [66]. These findings, coupled to the tremendously large, if not universal, diffusion of porno at all ages we are currently facing demonstrated two things: (i) the absence of institutional sexual education (and not pornography, even fetishist or violent, itself) is most probably and in the vast majority of cases the real offender, the culprit of leaving alone the young generations facing wrong information about sexual rights, needs, and behaviors; (ii) as the Bordeaux is not producing alcoholism (but the alcoholics should not drink Bordeaux), pornography may negatively impact on weak personalities primitively predisposed to have dysfunctional behaviors.

Similarly, another risk related to Internet use is addictive personalities and, therefore, sexual addiction, hypersexual behaviors, and, more specifically, porn addiction. Porn addiction can be defined as a sexual compulsion when masturbating through the viewing of adult content [67, 68]. Individuals suffering from porn addiction are completely absorbed by their stereotyped sexual activities to find the most self-suitable porn content, dedicating up to several hours per day in researching and viewing the erotic material [67, 68].

Moreover, some evidence has revealed that pornography could indirectly contribute to an early debut of sexual activities in adolescence [69, 70].

Another dark side of porn movies on websites is sexual risk behaviors and lack of use of condom. In fact, rarely do porn videos present sexual frames with the use of condoms. This specific aspect could also be considered a possible negative example to prevent sexually transmitted infections (STIs) among adolescents and young adults [71] and further advocate for an intelligent sex education.

On the other hand, when an individual uses pornography on websites, he/she plays a passive role, while when the same individual becomes a meeting app (MA) user, he/she plays an active role. The MA users tend to find a possible and real

sexual partner on the Internet, posting own photos, inserting own characteristics, preferences, and hobbies to exalt their own social profile. There exist numerous MAs used by many millions of people. The virtual place of MAs represents a contemporary way for dating, with sexual scopes in most cases. However, the diffusion and use of MAs has both positive and negative consequences on sexual and relational life. First of all, the main aspect to take into consideration is related to sexual health and safe sex. In some specific cases, MA users are more exposed to sexual risk behaviors, especially when sexual activities are associated with the use or abuse of substance and alcohol in heterosexuals and in males having sex with males [72, 73].

These elements should be strongly considered when treating sexuality in adolescents who are using MA for dating. It is also suitable, during the anamnestic process, to investigate the possible use of MAs as a fundamental part to know the sexual behavior of our young patients. Clinicians assessing sexuality and sexual behaviors are obligated to evaluate several factors that concern life and social environment, as also the most common deviant behaviors associated with risky sexual activities.

However, the use of MAs, in addition to the large use of social networks, represents a new way to meet and date new people, and interesting research has revealed that more than a quarter of real encounters after a match on Tinder, the most used among MAs, evolved into committed relationships, confuting the common bias about the association between MA use and casual sex [74, 75].

As MA phenomenon is largely diffused and easily accessible to everyone due to smartphone use, the more restricted phenomenon of sexting is also strictly related to smartphone use. Sexting consists of sending, receiving, or forwarding sexually explicit materials with a technological device, generally a smartphone. There exists a further subdivision of primary and secondary sexting. Primary sexting is composed of sending sexual materials, such as photos, in a consensual manner between two individuals. Secondary sexting is characterized by the forwarding of sex materials beyond the intended recipient [76, 77].

Recent research has demonstrated that the need for popularity is present in both types of sexting among adolescents, without gender differences, and no negative aspects were found in individuals involved in the short term; although, depressive and anger traits are related to secondary sexting in girls [78]. On the other hand, a relevant metanalytic study concluded that sexting is associated with mental risk factors and several dangerous or deviant behaviors, such as a lack of contraception use and substance use among younger adolescents [79].

Other relevant problems sometimes related to sexting are revenge porn and sextortion, which are assimilable phenomena characterized by the diffusion of sexual content without consent or for the aim of procuring other images, sexual acts, or money [80]. A large survey of American youth found that about 5% of students reported that they had been the victim of sextortion, highlighting in an empirical manner dramatic deviant behavior among adolescents [81]. Other research has revealed that almost 60% of victims of sextortion knew the perpetrators, sometimes found to be the romantic partners, and about one-third of those who were involved

in these deviant acts were threatened with physical assault, demonstrating that sextortion can be considered a violent behavior or a strong risk factor for violence [82].

In light of the Internet-related phenomena described above, that is, pornography, MAs, sexting, and sextortion, we found several pathological declinations related to the relationship between sexuality and Internet devices. Moreover, there seems to be a link between the four described phenomena, but the Internet is only a medium. The deviant or pathological use of Internet for the sexual behavior is most likely a result of primary problems regarding psychosexological, relational, and social health of individuals [83].

12.5 Sex Education

Affective and sexual education traits the emotional, cognitive, physical, social, and relational aspects of sexuality. Moreover, affective and sexual education is considered an important landmark in adolescent and young age, allowing people to acquire awareness about their body, their sexual life, and their sexual well-being. The promotion of sex education means guaranteeing sexual rights. The most critical time for sexual behavior in adolescence, characterized by body changes, which start in puberty [84]. During this period, it is very hard for an adolescent to self-recognize when and how their own body changes every day. The first sexual signals, such as erection, are something new. Anxiety, fear of making mistakes, and fear of judgment are just a few psychological aspects that could influence sexual intercourse. Therefore, importance of sexual education becomes stronger in a particular population at risk. For example, it has been shown that young men who have sex with men usually have inappropriate sexual habits and this has a strong impact on HIV transmission among this subpopulation; good use of sex education for these young people was effectively associated a reduction in risky sexual behavior and risky habits [78]. Sexual education can help alleviate sexual dysfunctions. In fact, an interesting study demonstrated improvement in women with sexual desire disorders, with an increase in sexual health after 6 weeks [85]. In general, young people are more protected during sexual intercourse if they have acquired the needed social skills to approach romantic and sexual relationships, which are developed through sexual expertise [80]. Adolescents spend the biggest part of their time at school. For this reason, this is the best place for affective and sexuality education [86]. Unfortunately, sex education is not regularly taught in all countries. The role of the family, which should be considered essential, could be more dangerous than useful for the young's psychosexual development, particularly in the environments lacking institutional sex education. It is, in fact, far of being easy the honest, open dialogue, without prejudices and taboo which is the assumption of a domestic sex education [84]. To date, the Internet is the main source of sexual information for adolescents, with negative and positive impacts on sexuality. It is the most common research engine to obtain medical information, and it is characterized by the so called "Triple A": Accessibility, Access, Anonymity [87]. In recent years, surfing the web for sexual activity has become normal and ordinary, with peculiarities associated with age and

gender difference, and adolescents are exposed to greater risk. Acceptance and sense of belonging are essential for adolescents, and web and social networks can give the (fake) impression that they are part of a large community where they can be whoever they want; the different, the disable, and the outcast do not exist behind fake social media profiles, but instead, all is possible. Lesbian, gay, bisexual, and transgender (LGBT) people prefer this hidden aspect of the Internet, especially to establish dates and occasional relationships. In a nonvirtual reality, LGBT people are forced to come out, where they are submitted to strong prejudice. At the same time, sexting and pornographic material has grown. Sexting, as previously stated, can represent a significant problem when the exchange is not authorized, especially when users are minor. Pornography, instead, is not always negative. In fact, pornography allows one to better understand own body and sexual intercourse. The search for sexual information on the web demonstrates that the quality can be lacking, but, on the other hand, young people can critically evaluate and check this information [88]. A valid sex education program should improve cognitive, emotional, and relational skills to promote responsible and positive behavior. The World Health Organization [89] identifies the following 10 life skills: The emotive area involves empathy, self-awareness, emotion management, and stress management; the relational area involves effective communication and skills for interpersonal relationships; and the cognitive area involves decision-making, problem-solving, critical sense, and creativity. It is necessary to improve socio-emotive skills, increasing self-esteem, decision-making, and problem-solving. Intended as an important and strong prevention tool, sexual education promotes sexuality as a positive element of human potential and as a source of enrichment for the intimate relationships that people experience. In this regard, a major attention toward the role of social media also for the sex education could be considered a current challenge of studies in for the sexual health.

12.6 Conclusions

Sexuality in adolescence represents a delicate aspect of life for several reasons and, therefore, clinicians should be very careful to assess the sexual function and behavior in adolescents. Healthcare professionals, physicians, and educators should considerate the peculiar aspects of the sexuality of the young patients because sexual health strictly depends on lifestyles and general health. In general, sexuality can be, in fact, considered an optimal indicator of psychophysical wellness [1], and when there exists a psychological or medical problem, also sexual function suffers about this. However, also the social conditions influence sexual behavior, and therefore systems, or bio-psycho-social approach is always indispensable when assessing or treating sexuality, above all in adolescents and in young. If the sexual dysfunctions and sexual disorders can be considered as a central aspect for youth with this problem, also other behaviors linked to the relationship between sexuality and new technologies, as some aspects related to Internet use, should be seriously taken into consideration.

In conclusion, major attention on juvenile sexuality is necessary, as well as a major collaboration between health and education institutions. Reproductive and Sexual health is a central part of public health and it represents both a medical and a social challenge for contemporary society.

References

1. Jannini EA. SM = SM: the Interface of systems medicine and sexual medicine for facing non-communicable diseases in a gender-dependent manner. Sex Med Rev. 2017;5(3):349–64.
2. Freud S. Three essays on the theory of sexuality. New York: Basic Books; 1962.
3. Kernberg OF. Mature love: prerequisites and characteristics. J Am Psychoanal Association. 1974;22(4):743–68.
4. Lingiardi V, McWilliams N. Psychodynamic diagnostic manual, second edition (PDM-2). New York, NY: Guilford Press; 2017.
5. Reich W. Character analysis. In: New York. Inc.: Strauss & Giroux; 1949.
6. Sanders RA. Adolescent psychosocial, social, and cognitive development. Pediatr Rev. 2013;34(8):354–8; quiz 358-9.
7. Boislard MA, van de Bongardt D, Blais M. Sexuality (and lack thereof) in adolescence and early adulthood: a review of the literature. Behav Sci (Basel). 2016:**6**(1).
8. Rouche M, et al. Feelings about the timing of first sexual intercourse and health-related quality of life among adolescents. BMC Public Health. 2019;19(1):408.
9. Guggino JM, Ponzetti JJ Jr. Gender differences in affective reactions to first coitus. J Adolesc. 1997;20(2):189–200.
10. Cotton S, et al. Adolescent girls perceptions of the timing of their sexual initiation: "too young" or "just right"? J Adolesc Health. 2004;34(5):453–8.
11. Dixon-Mueller R. How young is "too young"? Comparative perspectives on adolescent sexual, marital, and reproductive transitions. Stud Fam Plan. 2008;39(4):247–62.
12. Gianfrilli D, et al. Risk behaviours and alcohol in adolescence are negatively associated with testicular volume: results from the Amico-Andrologo survey. Andrology. 2019;7(6):769–77.
13. Bellis MA, et al. Sexual uses of alcohol and drugs and the associated health risks: a cross sectional study of young people in nine European cities. BMC Public Health. 2008;8(1):155.
14. Shamloul R, Ghanem H. Erectile dysfunction. Lancet. 2013;381(9861):153–65.
15. Jannini EA, Nappi RE. Couplepause: a new paradigm in treating sexual dysfunction during menopause and andropause. Sex Med Rev. 2018;6(3):384–95.
16. O'Sullivan LF, et al. A longitudinal study of problems in sexual functioning and related sexual distress among middle to late adolescents. J Adolesc Health. 2016;59(3):318–24.
17. Jannini EA, Lenzi A. Epidemiology of premature ejaculation. Curr Opin Urol. 2005;15(6):399–403.
18. Mitchell KR, et al. Sexual function in 16- to 21-year-olds in Britain. J Adolesc Health. 2016;59(4):422–8.
19. Hersh JE. Vulvodynia in adolescents. Curr Opin Obstet Gynecol. 2018;30(5):293–9.
20. Ciocca G, et al. Alexithymia and vaginismus: a preliminary correlation perspective. Int J Impot Res. 2013;25(3):113–6.
21. Pyke RE. Sexual performance anxiety. Sex Med Rev. 2020;8:183–00.
22. McMahon CG, et al. The pathophysiology of acquired premature ejaculation. Transl Androl Urol. 2016;5(4):434–49.
23. Corona G, et al. Sexual function of the ageing male. Best Pract Res Clin Endocrinol Metab. 2013;27(4):581–601.
24. Sansone A, et al. Endocrine evaluation of erectile dysfunction. Endocrine. 2014;46(3):423–30.
25. Isidori AM, et al. A critical analysis of the role of testosterone in erectile function: from pathophysiology to treatment—a systematic review. Eur Urol. 2014;65(1):99–112.

26. Maiorino MI, et al. From inflammation to sexual dysfunctions: a journey through diabetes, obesity, and metabolic syndrome. J Endocrinol Investig. 2018;41(11):1249–58.
27. Kloner RA. Erectile dysfunction as a predictor of cardiovascular disease. Int J Impot Res. 2008;20(5):460–5.
28. Rastrelli G, Maggi M. Erectile dysfunction in fit and healthy young men: psychological or pathological? Transl Androl Urol. 2017;6(1):79–90.
29. Rose SR, et al. Late endocrine effects of childhood cancer. Nat Rev Endocrinol. 2016;12(6):319–36.
30. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4(3):254–64.
31. Korkes F, et al. Recreational use of PDE5 inhibitors by young healthy men: recognizing this issue among medical students. J Sex Med. 2008;5(10):2414–8.
32. Bechara A, et al. Recreational use of phosphodiesterase type 5 inhibitors by healthy young men. J Sex Med. 2010;7(11):3736–42.
33. Harte CB, Meston CM. Recreational use of erectile dysfunction medications and its adverse effects on erectile function in young healthy men: the mediating role of confidence in erectile ability. J Sex Med. 2012;9(7):1852–9.
34. Cohen JL, et al. Sildenafil use in children with pulmonary hypertension. J Pediatr. 2019;205:29–34.e1.
35. Olguín HJ, et al. Pharmacokinetics of sildenafil in children with pulmonary arterial hypertension. World J Pediatr. 2017;13(6):588–92.
36. Jannini EA, et al. Organic vs. psychogenic? The Manichean diagnosis in sexual medicine. J Sex Med. 2010;7(5):1726–33.
37. Jannini EA, et al. Premature ejaculation: old story, new insights. Fertil Steril. 2015;104(5):1061–73.
38. Russo A, et al. Efficacy and safety of dapoxetine in treatment of premature ejaculation: an evidence-based review. Int J Clin Pract. 2016;70(9):723–33.
39. Gillman N, Gillman M. Premature ejaculation: aetiology and treatment strategies. Med Sci. 2019;7(11):102.
40. De Hong C, et al. The role of dapoxetine hydrochloride on-demand for the treatment of men with premature ejaculation. Sci Rep. 2014;4(1):7269.
41. Ciocca G, et al. Integrating psychotherapy and pharmacotherapy in the treatment of premature ejaculation. Arab J Urol. 2013;11(3):305–12.
42. Pallotti F, et al. Long-term follow up of the erectile function of testicular cancer survivors. Front Endocrinol. 2019;10
43. Nichols PE, et al. Patient decision-making and predictors of genital satisfaction associated with testicular prostheses after radical orchiectomy: a questionnaire-based study of men with germ cell tumors of the testicle. Urology. 2019;124:276–81.
44. Wiklander M, et al. Feasibility of a self-help web-based intervention targeting young cancer patients with sexual problems and fertility distress. Support Care Cancer. 2017;25(12):3675–82.
45. Mutsch J, et al. Sexuality and cancer in adolescents and young adults-a comparison between reproductive cancer patients and patients with non-reproductive cancer. BMC Cancer. 2019;19(1):828.
46. Congard A, et al. The self-reported perceptions of the repercussions of the disease and its treatments on daily life for young women with breast cancer and their partners. J Psychosoc Oncol. 2019;37(1):50–68.
47. Williams F, Jeanetta SC. Lived experiences of breast cancer survivors after diagnosis, treatment and beyond: qualitative study. Health Expect. 2016;19(3):631–42.
48. Lu D, Andersson TML, Fall K. Clinical diagnosis of mental disorders immediately before and after cancer diagnosis: a Nationwide matched cohort study in Sweden (vol 2, pg 1188, 2016). JAMA Oncol. 2016;2(9):1244.
49. Jackson SE, et al. Sexuality after a cancer diagnosis: a population-based study. Cancer. 2016;122(24):3883–91.

50. Bandak M, et al. Sexual function in a nationwide cohort of 2,260 survivors of testicular cancer after 17 years of followup. J Urol. 2018;200(4):794–800.
51. Twitchell DK, et al. Psychological impacts of male sexual dysfunction in pelvic cancer survivorship. Sex Med Rev. 2019;7(4):614–26.
52. Frederick NN, et al. Sexual dysfunction in young adult survivors of childhood cancer. Pediatr Blood Cancer. 2016;63(9):1622–8.
53. Hammerli S, et al. Does endometriosis affect sexual activity and satisfaction of the man partner? A comparison of partners from women diagnosed with endometriosis and controls. J Sex Med. 2018;15(6):853–65.
54. Limoncin E, et al. Premature ejaculation results in female sexual distress: standardization and validation of a new diagnostic tool for sexual distress. J Urol. 2013;189(5):1830–5.
55. Jannini EA, Porst H. A practical approach to premature ejaculation. Introduction: the asynchronous couple. J Sex Med. 2011;8(Suppl 4):301–3.
56. Jannini EA, et al. Genetics of human sexual behavior: where we are, where we are going. Sex Med Rev. 2015;3(2):65–77.
57. Gunst A, et al. A network analysis of female sexual function: comparing symptom networks in women with decreased, increased, and stable sexual desire. Sci Rep. 2018;8(1):15,815.
58. Barata BC. Affective disorders and sexual function: from neuroscience to clinic. Curr Opin Psychiatry. 2017;30(6):396–401.
59. Carvalho J, Nobre P. Gender issues and sexual desire: the role of emotional and relationship variables. J Sex Med. 2010;7(7):2469–78.
60. Bhatia D, Mikulich-Gilbertson SK, Sakai JT. Prescription opioid misuse and risky adolescent behavior. Pediatrics. 2020;145(2):e20192470.
61. Elaut E, et al. Cycle-related changes in mood, sexual desire, and sexual activity in oral contraception-using and nonhormonal-contraception-using couples. J Sex Res. 2016;53(1):125–36.
62. Malmborg A, Brynhildsen J, Hammar M. A survey of young women's perceptions of the influence of the Levonorgestrel-intrauterine system or copper-intrauterine device on sexual desire. Sex Reprod Healthc. 2019;21:75–80.
63. Rowland DL, Uribe D. Pornography use: what do cross-cultural patterns tell us? In: Rowland DL, Emmanuele AJ, editors. Cultural differences and the practice of sexual medicine. Cham: Springer; 2020.
64. Shelby S, Nathan Leonhardt N, Willoughby B, et al. J Sex Res. 2019;57:1089–99.
65. Peter J, Valkenburg PM. Adolescents and pornography: a review of 20 years of research. J Sex Res. 2016;53(4–5):509–31.
66. Rostad WL, Gittins-Stone D, Huntington C, Rizzo CJ, Pearlman D, Orchowski L. The association between exposure to violent pornography and teen dating violence in grade 10 high school students. Arch Sex Behav. 2019;48(7):2137–47.
67. Blais-Lecours S, Vaillancourt-Morel MP, Sabourin S, Godbout N. Cyberpornography: time use, perceived addiction, sexual functioning, and sexual satisfaction. Cyberpsychol Behav Soc Netw. 2016;19(11):649–55.
68. Mollaioli D, Sanaone A, Romanelli F, Jannini EA. Sexual dysfunctions in the internet era. In: Jannini E, Siracusano A, editors. Sexual dysfunctions in mentally ill patients. Trends in andrology and sexual medicine. Cham: Springer; 2018.
69. Braithwaite S, Coulson G, Keddington K, Fincham FD. The influence of pornography on sexual scripts and hooking up among emerging adults in college. Arch Sex Behav. 2015;44(1):111–23.
70. Fisher WA, et al. Pornography, sex crime, and paraphilia. Curr Psychiatry Rep. 2013;15(6):362.
71. Lim M, Carrotte ER, Hellard ME. The impact of pornography on gender-based violence, sexual health and well-being. What do we know? J Epidemiol Community Health. 2016;70:3–5.
72. Chan PA, et al. A network analysis of sexually transmitted diseases and online hookup sites among men who have sex with men. Sex Transm Dis. 2018;45(7):462–8.
73. Boonchutima S, Kongchan W. Utilization of dating apps by men who have sex with men for persuading other men toward substance use. Psychol Res Behav Manag. 2017;10:31–8.

74. Timmermans E, Courtois C. From swiping to casual sex and/or committed relationships: exploring the experiences of tinder users. Inf Soc. 2018;34(2):59–70.
75. Holtzhausen N, Fitzgerald K, Thakur I, Ashley J, Rolfe M, Pit SW. Swipe-based dating applications use and its association with mental health outcomes: a cross-sectional study. BMC Psychol. 2020;4(8):1–22.
76. Calvert C. Sex, cell phones, privacy and the first amendment: when children become child pornographers and the Lolita effect undermines the law. Comm Law Conspec. 2009;18:1–65.
77. Walker K, Sleath E. A systematic review of the current knowledge regarding revenge pornography and non-consensual sharing of sexually explicit media. Aggress Violent Behav. 2017;36:9–24.
78. Del Rey R, Ojeda M, Casas JA, Mora-Merchán JA, Paz E. Sexting among adolescents: the emotional impact and influence of the need for popularity. Front Psychol. 2019;10:1828.
79. Mori C, Temple JR, Browne D, Madigan S. Association of sexting with sexual behaviors and mental health among adolescents: a systematic review and meta-analysis. JAMA Pediatr. 2019;173:770–9.
80. Fido D, Harper CA, Davis MA, Petronzi D, Worrall S. Intrasexual competition as a predictor of women's judgments of revenge pornography offending. Sex Abus. 2021;33:295–320.
81. Patchin J, Hinduja S. Sextortion among adolescents: results from a National Survey of U.S. Youth. Sex Abus. 2020;32(1):30–54.
82. Wolak J, et al. Sextortion of minors: characteristics and dynamics. J Adolesc Health. 2018;62(1):72–9.
83. Ciocca G, Robilotta A, Fontanesi L, Sansone A, D'Antuono A, Limoncin E, Nimbi F, Simonelli C, Di Lorenzo G, Siracusano A, Jannini EA. Sexological aspects related to tinder use: a comprehensive review of the literature. Sex Med Rev. 2020;8:367–78.
84. Dawson R. Adolescent sexual health and education: where does the pediatrician's responsibility fall? Pediatr Ann. 2018;47(4):e136–9.
85. Kaviani M, et al. The effect of education on sexual health of women with hypoactive sexual desire disorder: a randomized controlled trial. Int J Community Based Nurs Midwifery. 2014;2(2):94–102.
86. Dawson RS. Adolescent sexual health and education: where does the pediatrician's responsibility fall? Pediatr Ann. 2018;47(4):136–e139.
87. Cooper A. Sexuality and the internet: surfing into the new millennium. Cyberpsychol Behav. 1998;2:475–9.
88. Simon L, Daneback K. Adolescents' use of the internet for sex education: a thematic and critical review of the literature. Int J Sex Health. 2013;25(4):305–19.
89. World Health Organization. Division of Mental Health. Life skills education for children and adolescents in schools. Pt. 1, Introduction to life skills for psychosocial competence. Pt. 2, Guidelines to facilitate the development and implementation of life skills programmes, 2nd rev. World Health Organization; 1994.
90. Biton-Bereby L, Mikulincer M, Shaver PR. Attachment and the oedipus complex: attachment orientations moderate the effects of priming oedipal representations on the construal of romantic relationships. Psychoanal Psychol. 2020;37:324–34.

Diagnosis and Management of Testicular Tumours in Children and Adolescents

13

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13.1 General Introduction

Testicular tumours comprise a number of neoplasms that differ significantly from each other with regard to the cell of origin, pathogenesis, typical age at presentation and incidence, histology, and clinical course. This huge variety and heterogeneity of phenotypes constitutes a diagnostic conundrum. In most cases the diagnosis depends on the histological evaluation of the tumour tissue and must be supported by specific biological markers. As presented in this review, careful diagnosis is essential for choosing the most appropriate treatment modality. In addition, testicular cancer occurs predominantly at a young age, hence reproductive issues and endocrine late effects are of particular importance. The contribution of andrologists and endocrinologists to the management of young cancer patients, who are usually under the care of urologists and oncologists, is therefore essential, especially concerning fertility issues, possible hypogonadism and sexual function. These issues are the focus of this review.

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13.2 Histopathology, Biological Features, and Diagnostic Markers

The testis is a complex organ and comprises several different cell types that can give rise to a tumour. The most important distinction is the cell of origin: the most common are testicular germ cell tumours (TGCTs), but there are also numerous tumour types that originate from testicular somatic cells, grouped under the name of sex cord–stromal tumours. The most recent classification of testicular malignancies, which was substantially revised by the World Health Organization in 2016 [1], is presented in Table 13.1. The tumour types that occur predominantly in children and adolescents are highlighted.

13.2.1 Testicular Germ Cell Tumours

TGCTs are the most common tumours in the testis and can occur at any age [2]. During childhood and extended adolescence—from birth to 18 years of age—the distribution of TGCTs is bimodal, with a small peak in infants and toddlers, then a quiescent period, followed by a sharply rising incidence coinciding with puberty [3, 4]. Hence, 11 years has been suggested as a cut-off age for clinical studies [3], although in older publications the cut-off age was often 14–15, or even 18. The bulk (more than 90%) of TGCT cases occur in post-pubertal adolescents and young adults, usually in the age interval between 15 and 45 years, but these tumours can also occur in older men. Importantly, these common TGCTs are associated with a pre-malignant precursor lesion, the germ cell neoplasia in situ, GCNIS [5]. A third, very rare TGCT type, the spermatocytic tumour, which is derived from post-pubertal spermatogonia, never occurs in children and adolescents (median age of diagnosis >50 years) [2, 6]. Because of the focus on young patients, the spermatocytic tumour will not be discussed in this review.

13.2.1.1 Childhood/Paediatric/Infantile TGCTs

Childhood TGCTs typically occur at the age interval of 0–4 years, with occasional cases up to 6 years, and the incidence of these tumours has been stable over last decades [7]. No specific risk factors have been identified. Importantly, the childhood TGCTs are not associated with GCNIS [8], and – in contrast to GCNIS-associated post-pubertal TGCTs, in the tumour karyotype there is no isochromosome 12p, which is pathognomonic for TGCTs in adolescents [9]. These distinct biological features are consistent with a different pathogenesis of childhood TGCTs. Most likely, there is an underlying gene defect causing disturbed differentiation of primordial germ cells, but the mechanisms of malignant transformation remain unknown [10, 11].

The most common paediatric TGCT is mature teratoma, including dermoid and epidermoid cysts (40–50%), followed by pure yolk sac tumour (YST) (10–15%) [12]. The two types can also occur mixed together. Histological features of childhood TGCTs are generally similar to the post-pubertal equivalents, but with some

Table 13.1 WHO 2016 classification of tumours of the testis [1]

- **Germ cell tumours derived from germ cell neoplasia in situ (GCNIS)**
  - *Non-invasive neoplasia*
    - Germ cell neoplasia in situ
    - Specific forms of intratubular germ cell neoplasia
  - *Pure tumours*
    - Seminoma
    - Non-seminomatous germ cell tumours
      - Embryonal carcinoma
      - Yolk sac-tumour, post-pubertal type
      - Trophoblastic tumours
        - Choriocarcinoma
        - Placental site trophoblastic tumour
        - Epithelioid trophoblastic tumour
        - Cystic trophoblastic tumour
      - Teratoma, post-pubertal type
      - Teratoma with somatic-type malignancy
  - *Non-seminomatous germ cell tumours of more than one type (mixed)*
- **Germ cell tumours unrelated to GCNIS**
  - Spermatocytic tumour
  - Teratoma, prepubertal-type
    - Dermoid cyst
    - Epidermoid cyst
    - Monodermal teratoma(differentiated neuroendocrine tumour)
  - Mixed teratoma and yolk sac tumour, prepubertal-type
  - Yolk sac tumour, prepubertal-type
- **Sex cord-stromal tumours**
  - *Pure tumours*
    - Leydig cell tumour
      - Malignant Leydig cell tumour
    - Sertoli cell tumours
      - Sertoli cell tumour, NOS
      - Malignant Sertoli cell tumour
      - Large cell calcifying Sertoli cell tumour
      - Intratubular large cell hyalinizing Sertoli cell neoplasia
    - Granulosa cell tumour
      - Adult granulosa cell tumour
      - Juvenile granulosa cell tumour
    - Tumours in the fibroma-thecoma group
  - *Mixed and unclassified sex cord-stromal tumours*
- **Tumour containing both germ cell and sex cord-stromal elements**
  - Gonadoblastoma
- **Miscellaneous tumours of the testis**
  - Ovarian epithelial-type tumours
  - Juvenile xanthogranuloma
  - Haemangioma
- **Haematolymphoid tumours**
  - Diffuse large B-cell lymphoma
  - Follicular lymphoma, NOS
  - Extranodal NK/T-cell lymphoma, nasal-type
  - Plasmacytoma
  - Myeloid sarcoma
  - Rosai-Dorfman disease (sinus histiocytosis)
- **Tumours of collecting duct and rete testis**
  - Adenoma
  - Adenocarcinoma

Tumours that occur in children and adolescents are highlighted in yellow; the tumours that occur predominantly in young pre-pubertal boys are underlined. Tumours never reported in children are shaded in grey. Very rare subtypes of unclassified and miscellaneous tumours are not listed. NOS: not otherwise specified

distinct features [13]. Childhood-like teratomas, without GCNIS, can apparently also occur in older adolescents or young adults but these cases are very rare [14].

Teratomas of pre-pubertal type are usually unilateral, have benign clinical course and do not metastasize. The tumours contain well differentiated somatic tissues, including ectodermal, mesenchymal, and endodermal subtypes, and cellular atypia is absent. Dermoid and epidermoid cysts have specialized skin-like organoid appearance, often with hair follicles and squamous epithelium. Diagnosis is based on the histology and absence of serum markers typical for other tumour types, and there are no specific immunohistochemical markers.

Yolk sac tumour (YST) of pre-pubertal type, previously called endodermal sinus tumour, usually occurs in young children, with the peak incidence in the second year of life. YST usually occurs in a pure form and resembles morphologically extra-embryonic structures, with typical microcystic, glandular, perivascular, papillary, solid, or vitelline patterns. In contrast to teratoma, YST has malignant features, often with haemorrhage and necrotic areas, and the tumours can metastasize. This tumour has to be differentiated from juvenile granulosa cell tumour, which can have a similar morphological appearance. The most helpful in differential diagnosis is serum α-fetoprotein (AFP), an embryonic glycoprotein, produced first by the yolk sac and later mainly by the foetal liver [15]. Histopathological diagnosis of the tumour tissue should also be supported by immunohistochemical staining for AFP, which is a pathognomonic marker, with SALL4 staining as another useful marker in childhood YST [16].

13.2.1.2 TGCTs of Adolescents and Young Adults

These are the most common tumours, also known as TGCTs type II, which start appearing in adolescents at late puberty, from around 15 years onward. A special feature of the pathogenesis of TGCTs of adolescents and young adults is that these tumours are derived from a precursor lesion, currently termed GCNIS and previously known as *carcinoma in situ testis* [5, 13]. GCNIS cells are considered transformed foetal gonocytes and GCNIS-derived tumours have been associated with ***testicular dysgenesis syndrome (TDS)***, which comprises disorders with a link to disturbed early gonadal development [17]. Hence, it is important to be aware of the possible incipient testicular malignancy in children and adolescents with specific high-risk conditions. A high risk of malignancy is associated with disorders of sex development (DSD), previously termed the intersex syndrome, and specifically with those that contain some Y chromosome material, thus allowing testicular development. Among the DSD disorders, the greatest risk of TGCT is in phenotypic males with mixed gonadal dysgenesis (45,X/46,XY) and with partial androgen insensitivity syndrome (AIS), usually caused by mutations in the androgen receptor gene (*AR*) or in the downstream signalling pathway [18–20]. TDS disorders identified in early childhood (usually at birth) include undescended testis (cryptorchidism) and hypospadias, and these common disorders partly overlap with DSD, as the latter often present with genital malformations [17, 19]. Cryptorchidism is the strongest known risk factor for testicular cancer with a relative risk for TGCT in patients with a history of cryptorchidism estimated as fourfold to sixfold [21]. The relative risk is

greatest in the undescended testis (RR = 6.3) but is also slightly increased in the normally descended testis in males with unilateral cryptorchidism [22]. Some data suggested that orchidopexy before puberty can lower the risk of TGCT [23, 24], but other studies did not confirm this [25, 26].

The incidence of TGCT derived from GCNIS has been increasing worldwide, predominantly among white populations, but with changing temporal trends clearly suggestive of the primary importance of environmental factors [27]. The aetiology behind this rise remains unknown, but the epidemiological and biological evidence implicates factors acting early in life and disturbing the development of immature germ cells [17, 28]. Despite a predominantly environmental pathogenesis, testicular cancer has a relatively high heritability and familial risk, which is greater for the brothers (eightfold to tenfold) than the sons (fourfold to sixfold) [29]. The genetic predisposition is complex and polygenic, without specific oncogenic driver mutations, but instead a constellation of numerous predisposing variants, among which KITLG is the strongest [30]. Genetic features of the tumours are also complex, with polyploidization (hypotetraploidy) and amplification of 12p, often in the form of isochromosome 12p, as the most consistent features [31, 32].

TGCTs of adolescents and young adults are divided into two main groups; *seminoma* and *non-seminoma*; the latter being a histologically heterogeneous group of tumours, comprising pure or mixed components of embryonal carcinoma (EC), teratoma (post-pubertal type), yolk sac tumour (YST post-pubertal type) and choriocarcinoma (CHC) [2, 13]. In some patients TGCTs contain mixed seminoma and non-seminoma types, and these tumours should be clinically classified as non-seminomas. In adolescent boys, a mixed non-seminoma is the most frequent tumour type, with relatively greater proportions of pure EC and CHC, but seminoma can also occur [33]. Only rarely these tumours can be detected at *the precursor stage of GCNIS,* usually in patients from risk groups, including infertility or history of cryptorchidism. Tubules with GCNIS cells have characteristic appearance with GCNIS cells located in the place of spermatogonia, and these tubules contain usually only Sertoli cells, which can be normal or have immature appearance. Occasionally, microcalcifications can be seen inside or outside tubules. GCNIS cells are larger than spermatogonia; have abundant cytoplasm; and have characteristic large nuclei with irregular clumps of chromatin [5].

Morphological heterogeneity of TGCTs requires careful histological assessment of the tumour and the adjacent testicular parenchyma, which usually harbours GCNIS and often other dysgenetic features, such as poorly differentiated tubules, immature Sertoli cells, and microcalcifications. In DSD and dysgenetic cases, gonadoblastoma can be present, a lesion similar to GCNIS but with the presence of undifferentiated somatic cells, resembling foetal Sertoli cells or granulosa cells [19]. See also the description of gonadoblastoma in section *'Other tumours'*. Early seminoma can grow intratubularly or microinvasive tumour spread can be seen. Overt seminoma is a homogeneous tumour, with cells morphologically similar to GCNIS cells. One of the characteristic features of seminoma is a prominent lymphocytic infiltrate [34, 35].

There is a range of ***IHC markers*** that have been well studied and validated for the identification of practically all tumour subtypes and histological components

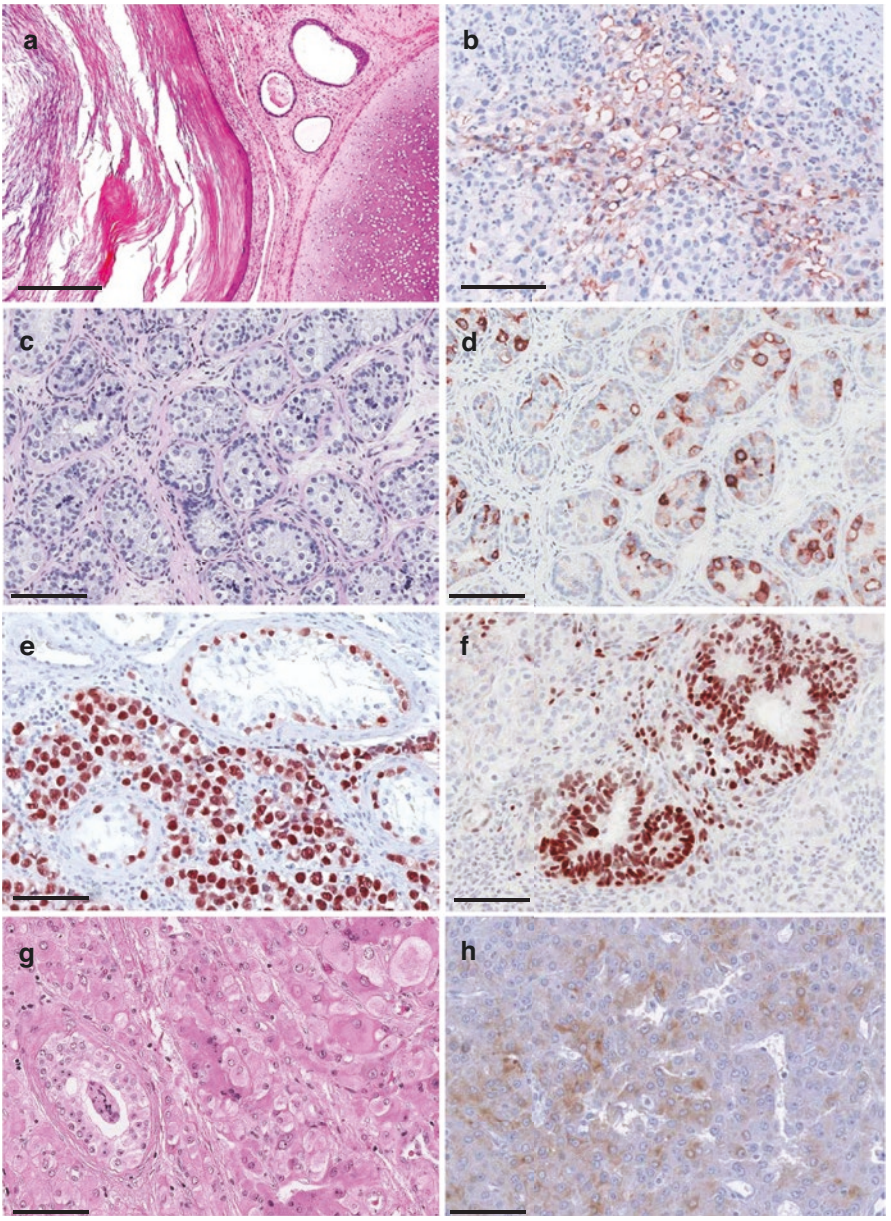

Fig. 13.1 Examples of typical histology and selected IHC markers of testicular tumours which occur in children and adolescents. Scale bar = 100 μm. (**a**) Mature teratoma with well-differentiated epidermoid (left) and cartilage-like tissue (right), (**b**) Yolk sac tumour stained for AFP, (**c**, **d**) Germ cell neoplasia in situ (GCNIS) in a 13-year-old boy with mixed gonadal dysgenesis (45,X/46,XY), in (**d**) GCNIS cells visualised with PLAP, (**e**) Seminoma with a few GCNIS tubules, malignant cells marked by nuclear expression of OCT4, (**f**) Embryonal carcinoma (EC) component of a nonseminomatous tumour, EC cells positive for SOX2, (**g**) Leydig cell tumour in a 7.5-year old boy, with tumour cells showing heterogeneous staining for INSL3 in image (**h**)

that occur in children and adolescents [36], some examples are showed in Fig. 13.1. ***GCNIS and seminoma*** have the transcriptome and immunohistochemical marker profile very similar to foetal gonocytes, hence facilitating diagnosis in the testis tissue [37]. The most robust and clinically applicable IHC markers for GCNIS and seminoma include embryonic pluripotency factors: OCT4 [38, 39], NANOG [40, 41], LIN28 [42], and other immature germ cell markers: placental-like alkaline phosphatase (PLAP) [43]; AP-2 gamma/TFAP2C [44] and podoplanin (PDPN, D2–40 antigen) [45, 46]. Regarding PDPN, it is important to remember that protein is also a marker of the lymphatic vessels, and immature Sertoli cells, present in the prepubertal testis [45].

Non-seminomas are morphologically very heterogeneous but contain some typical histological features, and detection of different components is helped by IHC markers. Post-pubertal YST has the same morphology and expression profile as the pre-pubertal type, with AFP as the best marker, and Glypican-3 (GPC3) and SALL4 as additional IHC markers [11, 16, 47]. Above-mentioned pluripotency markers OCT4 and NANOG are useful for recognition of EC, but to distinguish it from seminoma, additional markers specific for EC are needed (e.g. CD30 [48] or SOX2 [49]). Teratomas, both mature and immature forms, contain somatically differentiated components for which no specific markers exist, therefore the diagnosis is based on morphological features and the absence of elevation of serum markers. Differentiated somatic elements within teratomas have expression profiles of various tissue lineages and no longer express pluripotency factors or germ cell–specific genes. CHC resembles foetal trophoblast and secretes β-hCG, which is measurable in serum and can be detected in tissues by IHC [13]. It is important to remember that seminoma in 10–20% of cases can contain syncytiotrophoblastic cells which are detected by IHC for β-hCG.

13.2.2 Sex Cord–Stromal Tumours

Tumours arising from testicular somatic cells are collectively known as sex cord–stromal tumours, and comprise a heterogeneous group of neoplasms [13]. In prepubertal children, gonadal stromal cell tumours are relatively more common than in adults and represent around 8–15% of all TGCTs, being is the second largest group after TGCTs [12, 50]. Among them the most common are Leydig and Sertoli cell tumours, granulosa cell tumour, and pure stromal tumour. Some types of tumours derived from Sertoli cells can be a part of rare cancer syndromes.

13.2.2.1 Leydig Cell Tumours

Leydig cell tumours (LCTs) are the most common sex cord–stromal tumours of the testis and account for approximately 3% of all testicular neoplasms in recent series [51–54]. LCTs show a wide age range at presentation, with one peak in childhood (5–10 years, mean 70 months) and a second peak in adulthood (20–50 years) [55–57].

In children and adolescents LCTs often manifest as small functional tumours, which may present with isosexual gonadotropin-independent precocious puberty caused by androgen excess and sometimes with gynecomastia due to oestrogen

production. The clinical picture can also comprise accelerated skeletal growth and advanced bone age, increased penis size, or premature isolated pubarche, while impotence or loss of libido has been reported in some young men. These symptoms often prompt further investigations thus leading to diagnosis, although a number of LCTs are found incidentally at ultrasound (US) examinations performed for other indications [58]. A history of ipsilateral cryptorchidism or testicular hypotrophy may be present in some cases; therefore, TDS has been hypothesized to be associated with LCT predisposition [59–61]. A higher frequency of LCTs is also encountered in children with activating mutations of the luteinizing hormone (LH) receptor, in adult patients referred for infertility, in Klinefelter syndrome (alongside with Leydig cell hyperplasia) and in patients with germline fumarate hydratase (N64T) mutation, hereditary leiomyomatosis and renal cell carcinoma [62, 63]. Somatic activating mutation (R201S) in *GNAS* is occasionally encountered in LCTs and is thought to result in tumour development, inhibin alpha overexpression and hyperactivity of the testosterone biosynthetic pathway [64].

While malignant, locally invasive and metastasizing LCTs have been reported in literature with a frequency of around 10%, no such cases have been reported in children and adolescents [55, 56, 60].

LCTs usually appear as circumscribed lesions, with a mean diameter of approximately 1 cm and most frequently present as unilateral non-palpable lesions [59, 65–68]. Multifocal cases can be encountered and are especially frequent in Klinefelter syndrome.

These neoplasms appear to be often encapsulated, less than 5 cm in size and homogeneously yellow or golden brown. Growth pattern is usually diffuse [69, 70] and the neoplastic element is commonly represented by medium to large round or polygonal cells with abundant granular eosinophilic cytoplasm [71]. Cells may appear vacuolated, with some presenting as multinucleated. Intracytoplasmatic, nuclear, or extracellular Reinke crystals, a pathognomonic feature, are present in only 25% of cases. Mitoses are rare, mild nuclear atypia is not uncommon and psammoma bodies are occasionally present.

Malignant forms of LCT are characterized by the presence of at least two among the following features: frequent mitoses (>3/10 HPF), necrosis, significant cytological atypia, size greater than 5 cm, vascular invasion, an infiltrative margin or extension beyond the testis and aneuploidy [69, 72–75].

Cytology is rarely performed as no cytological feature allows to discriminate between LCTs and Leydig cell hyperplasia, nor benign from malignant LCTs [76]. On IHC, LCTs usually test positive for inhibin, calretinin, Melan A, vimentin and WT1, androgen and other steroid hormones, SF-1 (nuclear staining), chromogranin (in >90% of cases), synaptophysin (in 70% of cases), and CD99 (membranous staining); conversely they stain negative for nuclear β-catenin, the epithelial membrane antigen (EMA), and SALL4 [77–80].

Some notable differential diagnoses for LCTs include Leydig cell hyperplasia, which presents as a nodular growth pattern with nodules <0.5 cm and is often bilateral or multifocal; seminoma, which unlike LCTs presents intratubular germ cell involvement, lymphocytic infiltrate, fibrous septae and a different staining profile;

testicular adrenal rest tumours (TARTs) in congenital adrenal hyperplasia (CAH) patients, which are usually bilateral, accompanied by an endocrine profile with high ACTH and 17αOH-progesterone levels and usually show regression after medical therapy [81–84] and large cell calcifying Sertoli cell tumour (SCT), which is associated with Carney syndrome and shows extensive calcification with variable tubular or intratubular growth [13].

13.2.2.2 Sertoli Cell Tumours

SCTs are less common than LCTs, and account for only 1% of all testicular tumours. The most common presentation is with a testicular mass (58% of cases) or testicular enlargement (31% of cases), usually not associated with scrotal pain (89% of cases). They are less likely to be hormonally active, but gynaecomastia has been described (15% of cases). Incidental finding during US examination for other reasons, or during work-up for hydrocele, infertility, varicocele, or abdominal pain is rare (<5% altogether) [85].

Although historically approximately one-third of SCT cases has been reported in children, this figure may have included juvenile granulosa cell tumours and a more recent estimate of the median age at diagnosis of cases reported in literature (n = 435) attests to 29 years (range 0.8–86 years); as such SCTs are rare in childhood and quite uncommon during adolescence [85–87].

Two histological variants are recognized by the WHO: The large-cell calcifying SCT (23% of cases), which can occur sporadically or in association with Carney complex, and the intratubular large cell hyalinizing Sertoli cell neoplasia (2% of cases), which has been linked to Peutz–Jeghers syndrome [1]. ***Large-cell calcifying SCTs*** are frequently bilateral and multifocal when they occur on an inherited basis; being linked to Carney complex (harbouring a distinct germline *PRKAR1A* mutation) they frequently present in patients affected by cardiac myxomas, spotty skin hyperpigmentation, and primary pigmented nodular adrenocortical disease. ***Intratubular large-cell hyalinizing Sertoli cell neoplasias*** are linked to Peutz–Jeghers syndrome (harbouring a characteristic germline mutation in *STK11*) and are often associated with gynecomastia, bilateral and multifocal. Outside of genetic conditions, the most frequently encountered somatic mutation in benign SCTs is in the *CTNNB1* gene [88].

Malignant forms account for around 10% of all cases, although they have never been described in childhood. SCTs may occur more frequently in undescended testes and in patients with complete or partial androgen insensitivity syndrome (AIS) as well as in Klinefelter syndrome [89].

These neoplasms appear as little nodules of fibrous consistency, with a homogenously greyish-white to yellow appearance. Necrosis is uncommon, while haemorrhages can be seen. Neoplastic elements are arranged in trabeculae forming ribbon- and tubule-like structures. Cytoplasm appears eosinophilic to vacuolated for the presence of lipids. Tumour cells appear as uniform and round, with oval elongated nuclei; mild nuclear atypia and pleomorphism may be encountered. Fibrous stroma is present, although in absence of inflammatory cells, and containing blood vessels. Calcifications are present in the stroma in a quarter of cases, usually minor

in extension. Mitoses are uncommon [85]. Large-cell calcifying SCT present Sertoli cells with abundant eosinophilic cytoplasm and a variable number of calcifications. Intratubular large-cell hyalinizing Sertoli cell neoplasia presents with almost exclusive intratubular Sertoli cells proliferation with eosinophilic cytoplasm.

Malignant forms have been traditionally identified by the presence of at least two among the following: size greater than 5 cm, frequent mitoses (>5/10 HPF), nuclear pleomorphism with presence of nucleoli, vascular invasion, and necrosis [90]. Recently, additional risk factors for metastatic disease at staging or follow-up have been identified: age >27.5 years, tumour diameter >2.4 cm, presence of necrosis, extension to the spermatic cord, angiolymphatic invasion, and a high mitotic index [85].

SCTs frequently lack a specific IHC marker, although the cells variably stain positive for: cytokeratin, nuclear β-catenin, vimentin, inhibin, and S100; staining for chromogranin, synaptophysin, and CD99 is inconsistent, while cells stain negative for: AFP, β-hCG, and PLAP [54, 80, 91].

Occasionally a SCT may be mistaken for a juvenile granulosa cell tumour, which however grows in a follicular rather than tubular pattern; seminoma, which presents a different growth pattern, inflammatory elements in the stroma, granulomas, and a different staining pattern; distant metastases from epithelial cancers.

13.2.2.3 Other Tumours

The granulosa cell tumour category includes adult and juvenile variants, both of which appear morphologically similar to their ovarian counterparts, with the latter being more frequent. ***Juvenile granulosa cell tumours*** are the most common tumours of the testis in the first 6 months of life (mean age 1.5 months) and are rare outside of childhood. They usually show solid and follicular growth patterns with tumour cells characterized by immature nuclei, which stain positively for inhibin and CD99 [91, 92] and may occur in cryptorchid testes or dysgenetic gonads (with Y chromosome structural abnormalities reported in some cases). It is considered a benign entity, hormonally inactive, with no metastatic cases reported in this age group. ***Adult granulosa cell tumours*** occur in a broader age range, from teenagers to elderly individuals, and are composed of small cells with pale nuclei, often showing grooves, that typically grow in sheets in a fibrocollagenous or oedematous background. No known epidemiological associations exist for them.

A mixed tumour containing both germ cells and sex cord–stromal elements is termed ***gonadoblastoma***. It is mostly observed post-pubertally (and under the age of 20), is rarely associated with androgen production, and is bilateral in approximately one-third of cases (mostly in DSD gonads) [56]. Undescended abdominal testes are at risk for gonadoblastoma development [93, 94]. Morphologically it comprises discrete, round nests of germ cells (resembling GCNIS, seminoma cells or large spermatogonia) and small sex cord–stromal cells resembling immature Sertoli or granulosa cells. If left untreated, it will progress to seminoma in approximately 50% of cases and to a non-seminomatous tumour in around 8% of cases. A precursor lesion to gonadoblastoma, termed undifferentiated gonadal tissue, is often observed adjacent to it in DSD gonads [54].

Ovarian epithelial-type tumours include tumours resembling the homonymous surface ovarian epithelial tumours. They have been reported in adolescent to adult

patients. Serous borderline tumours are the most frequent, followed by mucinous tumours. Unlike what is seen with carcinomas, no metastases or recurrences have been observed with borderline tumours [54, 95].

Juvenile xanthogranuloma is a rare neoplastic histiocytic disorder occurring in children <13 months, capable of extending beyond the testis and composed of mononuclear histiocyte-like cells, growing in a diffuse and infiltrative pattern, positive for CD68 and S100.

Testicular haemangioma is a rare vascular tumour occurring from infants to adults. It presents as a small and circumscribed lesion showing an invariably benign behaviour.

13.3 Clinical Management of Testicular Tumours

13.3.1 Diagnostic Procedures

Any enlargement of a testicle requires careful and comprehensive diagnostic work-up, first to exclude possible malignancy, and subsequently for a recognition of the tumour type, which is essential for proper management and staging. Overt testicular tumours are usually easy to recognize because of their location and are often reported by the patients themselves or parents of young boys. Differential diagnosis of testicular tumours includes a variety of approaches that comprise careful anamnesis to establish possible family history, physical examination, scrotal and extragonadal imaging, measurements of serum markers, hormone profiles and tumour histology, which together are used to select the most appropriate management.

13.3.1.1 Imaging

Ultrasound (US) is the most common technique for the evaluation of testiculare tumours. US examination major aid consists in its ability to distinguish intratesticular from extratesticular lesions with a 98–100% accuracy [96], as most extratesticular masses are benign in nature, while intratesticular masses are more likely to be malignant [97, 98]. Intratesticular masses can be then categorized as solid, cystic, or mixed lesions: cystic lesions usually being benign, and solid and mixed lesions, with few exceptions, usually neoplastic in nature. Their US appearance reflects gross morphology and histological characteristics. Most malignant testicular tumours prove to be hypoechoic to the surrounding testicular parenchyma, although a number of benign intratesticular processes can mimic malignancy. Some tumours are heterogeneous and can be partially cystic. Focal changes within tumours—such as haemorrhage, necrosis, calcification, or fatty changes—all produce areas of increased echogenicity and confer a heterogeneous appearance to these lesions. In a hypoechoic testis, such as a prepubertal or atrophic testis, tumours can be isoechoic to surrounding parenchyma. In these cases, colour Doppler ultrasound (CDU) and power Doppler US prove to be useful. Flow studies demonstrate increased vascularity in most malignant tumours and help to better define testicular involvement, margins, cystic degeneration, and any necrotic areas of the lesion. However, the presence of hypervascularity is not specific enough for a diagnosis of malignancy.

There are few US features that can point to a differential diagnosis between seminomatous and non-seminomatous GCT, but exceptions to the typical appearance in these tumours are common. In general, seminomatous tumours are mostly homogeneous and more hypoechoic, whereas non-seminomatous tumours are heterogenous even when small, and frequently show cystic or calcified parts with alternating hypo- and hyperechoic areas. Both tend to have neat margins; however, large seminomatous tumours can appear non-uniform and mixed tumours can present with polycyclic borders. Some examples of US imaging in adolescent testicular tumours are presented in Fig. 13.2.

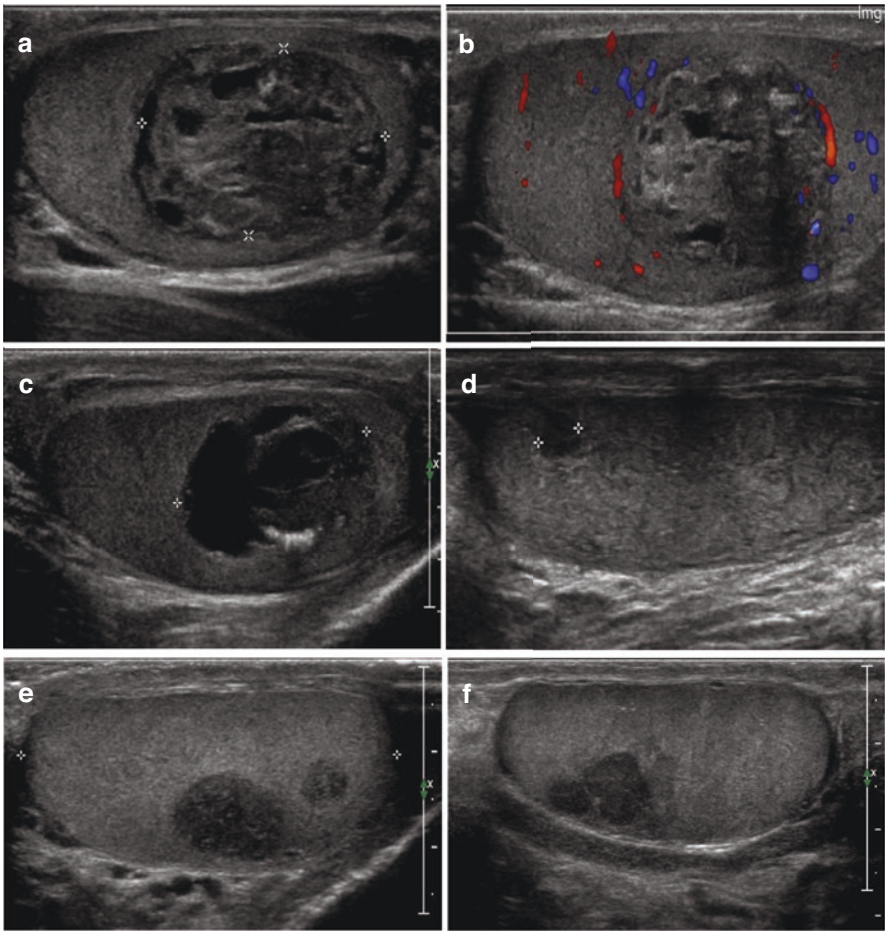

Fig. 13.2 Examples of ultrasound examinations of testicular neoplasia in children and adolescents. (**a**, **b**) Mature post-pubertal teratoma, in a 16-year-old patient, appearing as a complex solid lesion, with cystic components and hyperechoic spots, in (**b**) peripheral vascularization on colour Doppler ultrasound examination, (**c**) Post-pubertal teratoma with epithelial malignancy in a 14 years old, appearing as a complex, predominantly cystic lesion with a solid component and hyperechoic foci with acoustic shadowing, (**d**) Leydig cell tumour in a 10 years old, appearing as a well-demarcated small, hypoechoic and homogenous lesion, (**e**) Multifocal pure seminoma in a 15 years old, appearing as hypoechoic and homogenous solid lesions, (**f**) Pure seminoma with polycystic lobulated margins in a 14 years old

Contrast-enhanced ultrasound (CEUS) is a new US technique which employs intravenous microbubble contrast to provide information on vascular characteristics of testicular lesions and as such offers additional information in the differential diagnosis of testicular lesions, being superior to non-contrast US. In particular, its quantitative evaluation improves the diagnostic accuracy of non-enhanced US up to 93%. Both benign and malignant tumours tend to enhance strongly and this helps in the differential diagnosis against non-neoplastic lesions, such as ischemia, abscesses, and cysts. Furthermore, malignant lesions are characterized by rapid enhancement and by a rapid washout, whereas benign tumours feature a more prolonged washout pattern and the latter seems to be a distinctive feature of sex cord–stromal tumours [51].

A further development is the concept of tissue elastography, which allows the assessment of the hardness of a specific tissue. This technique provides non-invasive, real-time tissue characterization via the analysis of the shear waves generated by an ultrasound burst; the propagation speed of the shear waves directly correlates with tissue stiffness and the information is displayed as a colour-coded representation of the lesion alongside a semi-quantitative characterization, providing a measure of the lesion's stiffness [99].

Although, generally speaking, no US finding is pathgnomonic by itself, CEUS and elastography are helpful in better characterizing intratesticular lesions. The increased lesion understanding derived from their use may allow a personalized follow-up strategy or for a targeted US-guided excision biopsy, when feasible, thus potentially reducing the number of unnecessary radical orchiectomies (RO).

Magnetic resonance imaging (MRI) may offer additional diagnostic information in selected cases, while computed tomography (CT) is mainly used for staging purposes at diagnosis and follow-up [100, 101].

LCTs usually appear on US examination as a solid, homogeneous and hypoechoic or weakly hypoechoic lesion, with well-defined margins and internal vascularization, while intralesional calcifications are uncommon. Some larger lesions may show cystic areas resulting from haemorrhages and necrosis. Radiology cannot distinguish between GCTs and LCTs or between benign and malignant LCTs. On contrast-enhanced ultrasound (CEUS) examination 85% of LCT show a rapid enhancement of the lesion (wash-in) and a delayed washout compared to the surrounding parenchyma [51, 59]. On elastography most lesions (83%) show medium (ES2) or hard (ES3) elasticity [59]. On MRI 68% of LCT show a markedly hypointense signal on T2-WI and a rapid and marked wash-in followed by a prolonged washout [59, 101].

SCTs on US examination appear as single or multiple masses, either hypo- or hyperechoic, with possible calcification and a median diameter of 2 cm. The large-cell calcifying SCT, most often seen in paediatric patients affected by Carney complex, can be recognized by its diffusely heterogeneous pattern, with increased echogenicity and large areas of calcification [102].

Although much progress has been made in the radiological evaluation of intratesticular lesions, no combination of imaging techniques guarantees 100% accuracy in preoperatively distinguishing GCTs from sex cord–stromal tumours or other testicular neoplasms, and the final diagnosis remains histological.

13.3.1.2 Tumour Markers in Blood

As soon as a testicular malignancy is suspected, an obligatory procedure is to measure biochemical tumour markers of GCTs in serum, since GCTs are the most common and clinically aggressive cancer type in patients of all ages. These markers include β-hCG, AFP, and lactate dehydrogenase (LDH) [15, 103, 104]. LDH is an enzyme mainly produced by seminoma. β-hCG is secreted by the CHC component of non-seminomatous tumours and syncytiotrophoblastic cells in mixed TGCTs. AFP is an early embryonal protein, produced also by yolk sac tumours (YST), both of the prepubertal and adult type. It is important to remember that small infants can have physiologically detectable AFP levels, hence clinical decision-making in infants should be based on serial measurements [15]. In boys over 2 years of age, a serum AFP falls to normal adult levels of <10 kU/L (approximately 100 ng/mL), so higher levels will be suggestive of a YST or a teratoma with focal YST. Very high serum AFP levels (≥10,000 ng/mL) at diagnosis are associated with a worse prognosis.

These serum markers are raised in only approximately 60% of patients with TGCTs at the time of primary diagnosis, and their interpretation in seminoma, EC, and teratoma can be difficult [104, 105]. Furthermore, these markers are negative in pre-malignant germ cell lesions, GCNIS, and gonadoblastoma.

A new sensitive blood test, based on the detection of GCT-specific micro-RNAs (miR-371-3 cluster), which are detectable in all TGCTs, except teratomas, is currently under development and clinical trials are in progress, but the test is not yet routinely available [106–108].

As far as the sex cord–stromal tumours are concerned, there are currently no clinically suitable markers measurable in blood samples. Although rarely, some SCTs can present with mildly elevated TGCT serum markers. However, the absence of elevation of the above-listed tumour markers and alterations in the reproductive hormonal profile can be suggestive of a non-GCT.

13.3.1.3 Hormone Profiles

LCTs can be manifested by typical signs of steroid hormone excess and also high aromatase activity in some of the tumours. According to some authors, an hCG stimulation test may be helpful in the diagnosis by showing a higher increase in serum total testosterone and oestradiol levels at 24–48 h compared to healthy controls and seminoma patients [59, 109, 110]. In young boys, androgen secretion by LCTs often causes precocious puberty, manifested as appearance of pubic hair, penis enlargement, accelerated growth, and pubertal-like skin changes with adult-type sweat odour. In addition, excess of oestrogens can cause gynecomastia in boys, adolescents, and young men, in whom the main sign of a LCT is in fact usually gynecomastia. It is important to bear in mind that in about half of all adolescent boys gynecomastia is a benign transient disorder caused by a quick rise in oestradiol at puberty and that obese boys can have pseudogynecomastia [111–113]. Several other conditions are associated with frequent gynecomastia, including Klinefelter syndrome and partial AIS; the latter carrying an increased risk of TGCT, therefore a careful differential diagnosis must be performed in each case [113].

In the suspicion of a sex cord-stromal tumour the initial evaluation should include the assessment of the serum levels of LH, FSH, total testosterone, oestradiol, as well as 17-OHP and androstenedione.

13.3.1.4 Histopathological Assessment

The final diagnosis of testicular tumour must be confirmed by the histological analysis of tissue. Because of histological heterogeneity, the IHC analysis is very helpful, and has become mandatory in most centres. The typical histological features and clinically useful IHC markers are described in this review in section: *Histopathology, biological features, and diagnostic markers*. Recognition of different components of non-seminoma is important, because of differences in prognosis: in pre-pubertal children, the presence of pure YST is associated with poor prognosis, whereas in adolescents and young adults, the presence of CHC may worsen the prognosis, while the presence of pure EC is a risk factor of an early relapse [114].

13.3.2 Surgery, Staging, and Oncological Treatment

13.3.2.1 Childhood (Paediatric) Tumours

Orchiectomy is a treatment of choice for malignant TGCT, mainly YST, which can be distinguished by highly increased serum AFP. After careful histopathological description of the tumour, each patient must be classified into a clinical stage. Staging is based on tumour spread and serum markers. In boys up to 6 years of age the marker evaluation is focused primarily on AFP dynamics. A commonly accepted staging system prepared by the Children Oncology Group (COG) from the USA is presented in Table 13.2.

The YST diagnosis has the worst prognosis among childhood testicular tumours, even though only about 20% of the cases are metastatic, while the vast majority of cases are cured by chemotherapy [47, 115]. An unfavourable outcome is associated with a lymphovascular invasion, large primary tumour size (>4.5 cm in largest diameter), invasion of rete testis or epididymis, and the presence of necrosis within the tumour [115].

All other histological types of TGCT, mature teratoma, and (epi)dermoid cysts, which are usually benign and are never associated with GCNIS, should be treated by testis-sparing surgery (TSS), unless the tumour has overgrown testicular parenchyma. TSS is also the treatment of choice for sex cord–stromal tumours, including juvenile granulosa cell tumours [92] and haemangiomas.

Table 13.2 Staging of paediatric testicular germ cell tumours, recommended by the Children Oncology Group (USA) and WHO [1]

Stage I	Tumour is limited to testis. There is no evidence of disease beyond the testis by clinical, histological, or radiographical examination. An appropriate decline in serum AFP has occurred (*the half-life of AFP is 5 days*)
Stage II	Microscopic disease is located in the scrotum or high in the spermatic cord (<5 cm from the proximal end). Retroperitoneal lymph node involvement is present (≤2 cm). Serum AFP is persistently elevated
Stage III	Retroperitoneal lymph node involvement is present (>2 cm). There is no visible evidence of visceral or extra-abdominal involvement
Stage IV	Distant metastases are present, including liver

13.3.2.2 Testicular Germ Cell Tumours in Adolescents

Management and treatment of TGCTs in adolescents is the same as in young adults. Immediately after diagnosis, careful evaluation of disease diffusion must be performed to help assessing a possible spread of malignancy. In adolescent boys, the diagnosis is often delayed, hence the metastatic disease is already present at the first presentation in approximately 20–30% of cases [33]. After RO, which is the treatment of choice in the vast majority of patients presenting with a TGCT, prognostic staging must be performed in each case. The staging includes the levels of the above-mentioned circulating serum tumour markers (STM), primary tumour type and size, and the presence of metastases. The commonly used classification is postsurgical and pathological (p)TNM (pT = primary tumour, pN = regional lymph nodes, M = distant metastasis) [116]. Primary tumour size and metastatic tumour size in lymph nodes are measured, vascular lymphatic invasion, and invasion of surrounding tissues are also assessed, for example, epididymis, tunica albuginea, scrotum, spermatic cord. The patients should be staged and stratified to the good, intermediate, or poor prognosis groups according to the system developed for the adults by the International Germ Cell Consensus Classification (IGCCC) [117] (Table 13.3). Adaptation of existing staging systems for specific needs of adolescent patients with GCT, has been advocated by the Malignant Germ Cell International Consortium (MaGIC) [3, 118].

In general, pure seminoma has a better prognosis than non-seminoma. Patients with stage I seminoma (confined to the testis) are usually treated by orchiectomy and surveillance alone, whereas adjuvant chemotherapy can be considered to treat patients with stage I non-seminoma. Adjuvant radiotherapy should not be used in adolescent patients [50]. The treatment of patients with disseminated or recurrent disease comprises combined cisplatin chemotherapy regimens with various forms of adjuvant treatment and salvage surgery that should be carefully adapted to the needs of the individual patient. Detailed regimens widely accepted in Europe can be found in the latest guidelines of the European Association of Urology (EAU), and the European Society for Medical Oncology (ESMO) [50, 114]. The typical chemotherapy regimens in the good prognosis TGCT start from three cycles of cisplatin, bleomycin, and etoposide (PEB, 90% 5-year survival rate), through four cycles of this combined therapy in patients with intermediate prognosis, to intensified

Table 13.3 Prognostic staging system for young adult TGCT based on IGCCC recommendation

	Good	Intermediate	Poor
Seminoma	Absence of non-pulmonary visceral metastasis	Presence of non-pulmonary visceral metastasis	Not applicable
Non-seminoma	Gonadal or retroperitoneal primary site	Gonadal or retroperitoneal primary site	Mediastinal primary site
	Absence of non-pulmonary visceral metastasis	Absence of non-pulmonary visceral metastasis	Presence of non-pulmonary visceral metastasis
	S0 or S1 STM:	S2 STM:	S3 STM:
βHCG	<5000 IU/mL	5000–50,000 IU/mL	>50,000 IU/mL
AFP	<1000 ng/mL	1000–10,000 ng/mL	>10,000 ng/mL
LDH	1.5 × ULN	1.5–10 × ULN	>10 × ULN

combination chemotherapy with addition of etoposide and ifosfamide in poor prognosis patients (reviewed in [6]).

Some modifications of chemotherapy regimens specifically for adolescent patients from the age of 11 have been proposed by the MaGIC consortium, for example, substituting cisplatin with carboplatin, or including weekly bleomycin, which was previously used sparingly in young patients [3].

13.3.2.3 Sex Cord–Stromal Tumours in Adolescents

Although initial laboratory and radiological evaluation may strongly point towards a benign sex cord–stromal tumour, current guidelines recommend a surgical approach, nonetheless. However, a 'wait-and-see' approach can be reasonably pursued in the case of a patient refusing surgery, when initial evaluation points towards a sex cord–stromal tumour (no STM elevation, compatible US features, absence of distant metastases). In this case, the follow-up should be based on serial US examinations every 3 months for at least 18 months and a putative diagnosis would require absence of growth or resolution of the lesion(s). Diagnosis is intra-operatory and should be entrusted to expert pathologists able to confirm the sex cord–stromal nature of the tumour on frozen section, thus guiding towards TSS when tumour size and topography make it feasible. If frozen section examination determines the suspicion of malignancy, surgery shall be converted to RO.

While orchiectomy remains the treatment of choice for tumours characterized by a large volume, TSS has been proven to be efficacious and associated with excellent prognosis in smaller size tumours, preserving fertility, and testicular endocrine function, while not being associated with relevant local or distant recurrence rates. After testicular surgery, in absence of GCTs risk factors, follow-up is recommended on a semestral basis; it may include evaluation of the retroperitoneum (US) and of the thorax (CT, yearly for the first 3–4 years) and the endocrine assessment of the residual testicular function (LH, FSH, total testosterone, oestradiol, SHBG, inhibin B).

13.3.2.4 Fertility Preservation in Children and Adolescents

As treatment of testicular cancer, both surgical and medical, will invariably determine reduction in the fertile potential of the patient, efforts should be made by the clinicians to obtain spermatozoa for cryopreservation prior to treatment. This is particularly challenging considering the age of patients, the maturation of the spermatogenic machinery until late adolescence and the increased prevalence of subfertility/infertility in testicular cancer patients [119–122].

Based on age, pre-pubertal boys can be proposed to undergo sperm retrieval via penile vibratory stimulation or the more invasive electro-ejaculation via placement of a rectal probe under general anaesthesia, although the success rate is variable [123–125]. Experimental attempts at testicular tissue or spermatogonial stem cells cryopreservation in these patients are promising and so far have proven to successfully restore fertility upon reimplantation in animal models [126].

Adolescents, based on pubertal status, should be proposed a semen analysis through masturbation at the time of diagnosis, before testis surgery (whether TSS or RO), especially if there is suspicion of contralateral testicular dysfunction, and

sperm banking through cryopreservation should be encouraged [127]. The same holds true in the post-surgical setting, if adjuvant chemo- and/or radio-therapy are to be employed or if retroperitoneal surgery is being considered, for the risk of infertility due to retrograde ejaculation (although in this setting a post-ejaculatory urine specimen often contains enough sperm for cryopreservation) [128, 129].

Another possible attempt is sperm retrieval during surgery, termed onco-TESE (Testicular Sperm Extraction), with ex-vivo dissection of spermatozoa in the contralateral and/or ipsilateral testis, which proves particularly useful for azoospermic patients, although studies are few and success can vary [130, 131].

After completion of treatment, clinicians should refer patients to semen analysis at 12 months and discourage attempts to naturally conceive before 24 months, for the increased rate of sperm aneuploidy up to that time [132].

13.4 Long-Term Consequences of Testicular Tumours in Childhood and Adolescence

The treatment of testicular cancer can result in reduced quality of life in some patients, especially in those who receive four cycles or more of chemotherapy with cisplatin and etoposide combined with either bleomycin or ifosfamide [133]; these patients have been reported to show increased rates of chronic fatigue and anxiety disorders [134, 135].

Hypogonadism is observed in 13–33% of patients, and has been linked to depression, sexual problems, and diminished well-being observed in these patients [136]. As such, all patients undergoing treatment for testicular cancer should be monitored for their residual testicular endocrine function and, when appropriate, be prescribed testosterone replacement therapy (TRT).

Retroperitoneal lymph node dissection (RPLND), especially if performed after chemotherapy, can result in loss of antegrade ejaculation, which can determine infertility and the need to recur to assisted reproduction techniques (ART) [137, 138].

Exercise programmes have been shown to moderately improve quality of life in testicular cancer survivors, as well as emotional well-being, social functioning, anxiety, and fatigue upon exercise [139].

Adolescents and young men who have experienced testicular cancer possess a 16.2 times higher risk for a second testicular cancer and a 2.9 times higher risk for prostate cancer than matched controls [140]. Furthermore, testicular cancer survivors show a 6% higher non-cancer mortality than the general population [141, 142] which rises to 26% if previously treated with chemotherapy; this risk seems to be attributable mostly to infectious diseases, gastrointestinal diseases, and cardiovascular diseases, especially among young men.

References

1. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO classification of tumours of the urinary system and male genital organs-part a: renal, penile, and testicular tumours. Eur Urol. 2016;70(1):93–105.

2. Oosterhuis JW, Looijenga LHJ. Human germ cell tumours from a developmental perspective. Nat Rev Cancer. 2019;19(9):522–37.
3. Fonseca A, Frazier AL, Shaikh F. Germ cell tumors in adolescents and young adults. J Oncol Pract. 2019;15(8):433–41.
4. Calaminus G, Schneider DT, von Schweinitz D, Jurgens H, Infed N, Schonberger S, et al. Age-dependent presentation and clinical course of 1465 patients aged 0 to less than 18 years with ovarian or testicular germ cell tumors; data of the MAKEI 96 protocol revisited in the light of prenatal germ cell biology. Cancers (Basel). 2020;12(3):611.
5. Skakkebaek NE. Possible carcinoma-in-situ of the testis. Lancet. 1972;2(7776):516–7.
6. Rajpert-De Meyts E, McGlynn KA, Okamoto K, Jewett MA, Bokemeyer C. Testicular germ cell tumours. Lancet. 2016;387(10029):1762–74.
7. Poynter JN, Amatruda JF, Ross JA. Trends in incidence and survival of pediatric and adolescent patients with germ cell tumors in the United States, 1975 to 2006. Cancer. 2010;116(20):4882–91.
8. Jørgensen N, Müller J, Giwercman A, Visfeldt J, Møller H, Skakkebaek NE. DNA content and expression of tumour markers in germ cells adjacent to germ cell tumours in childhood: probably a different origin for infantile and adolescent germ cell tumours. J Pathol. 1995;176(3):269–78.
9. Zhang C, Berney DM, Hirsch MS, Cheng L, Ulbright TM. Evidence supporting the existence of benign teratomas of the postpubertal testis: a clinical, histopathologic, and molecular genetic analysis of 25 cases. Am J Surg Pathol. 2013;37(6):827–35.
10. Mosbech CH, Rechnitzer C, Brok JS, Rajpert-De Meyts E, Hoei-Hansen CE. Recent advances in understanding the etiology and pathogenesis of pediatric germ cell tumors. J Pediatr Hematol Oncol. 2014;36(4):263–70.
11. Murray MJ, Nicholson JC, Coleman N. Biology of childhood germ cell tumours, focussing on the significance of microRNAs. Andrology. 2015;3(1):129–39.
12. Pohl HG, Shukla AR, Metcalf PD, Cilento BG, Retik AB, Bagli DJ, et al. Prepubertal testis tumors: actual prevalence rate of histological types. J Urol. 2004;172(6 Pt 1):2370–2.
13. Ulbright TM, Amin MB, Balzer B, Berney DM, Epstein JI, Guo C, et al. Germ cell tumours. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE, editors. WHO classification of tumours of the urinary system and male genital organs. Lyon: IARC Press; 2016. p. 189–226.
14. Oosterhuis JW, Stoop JA, Rijlaarsdam MA, Biermann K, Smit VT, Hersmus R, et al. Pediatric germ cell tumors presenting beyond childhood? Andrology. 2015;3(1):70–7.
15. Murray MJ, Nicholson JC. alpha-Fetoprotein. Arch Dis Child Educ Pract Ed. 2011;96(4):141–7.
16. Mosbech CH, Svingen T, Nielsen JE, Toft BG, Rechnitzer C, Petersen BL, et al. Expression pattern of clinically relevant markers in paediatric germ cell- and sex-cord stromal tumours is similar to adult testicular tumours. Virchows Arch. 2014;465(5):567–77.
17. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson AM, Eisenberg ML, et al. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev. 2016;96(1):55–97.
18. van der Zwan YG, Biermann K, Wolffenbuttel KP, Cools M, Looijenga LH. Gonadal maldevelopment as risk factor for germ cell cancer: towards a clinical decision model. Eur Urol. 2015;67(4):692–701.
19. Jørgensen A, Lindhardt Johansen M, Juul A, Skakkebaek NE, Main KM, Rajpert-De Meyts E. Pathogenesis of germ cell neoplasia in testicular dysgenesis and disorders of sex development. Semin Cell Dev Biol. 2015;45:124–37.
20. Hersmus R, van Bever Y, Wolffenbuttel KP, Biermann K, Cools M, Looijenga LH. The biology of germ cell tumors in disorders of sex development. Clin Genet. 2017;91(2):292–301.
21. Dieckmann KP, Pichlmeier U. Clinical epidemiology of testicular germ cell tumors. World J Urol. 2004;22(1):2–14.
22. Akre O, Pettersson A, Richiardi L. Risk of contralateral testicular cancer among men with unilaterally undescended testis: a meta analysis. Int J Cancer. 2009;124(3):687–9.

23. Pettersson A, Richiardi L, Nordenskjold A, Kaijser M, Akre O. Age at surgery for undescended testis and risk of testicular cancer. N Engl J Med. 2007;356(18):1835–41.
24. Walsh TJ, Dall'Era MA, Croughan MS, Carroll PR, Turek PJ. Prepubertal orchiopexy for cryptorchidism may be associated with lower risk of testicular cancer. J Urol. 2007;178(4 Pt 1):1440–6; discussion 6.
25. Weidner IS, Moller H, Jensen TK, Skakkebaek NE. Risk factors for cryptorchidism and hypospadias. J Urol. 1999;161(5):1606–9.
26. Myrup C, Schnack TH, Wohlfahrt J. Correction of cryptorchidism and testicular cancer. N Engl J Med. 2007;357(8):825–7; author reply −7.
27. Gurney JK, Florio AA, Znaor A, Ferlay J, Laversanne M, Sarfati D, et al. International trends in the incidence of testicular cancer: lessons from 35 years and 41 countries. Eur Urol. 2019;76(5):615–23.
28. Rajpert-De Meyts E. Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. Hum Reprod Update. 2006;12(3):303–23.
29. Hemminki K, Chen B. Familial risks in testicular cancer as aetiological clues. Int J Androl. 2006;29(1):205–10.
30. Litchfield K, Levy M, Huddart RA, Shipley J, Turnbull C. The genomic landscape of testicular germ cell tumours: from susceptibility to treatment. Nat Rev Urol. 2016;13(7):409–19.
31. de Jong B, Oosterhuis JW, Castedo SM, Vos A, te Meerman GJ. Pathogenesis of adult testicular germ cell tumors. A cytogenetic model. Cancer Genet Cytogenet. 1990;48(2):143–67.
32. Shen H, Shih J, Hollern DP, Wang L, Bowlby R, Tickoo SK, et al. Integrated molecular characterization of testicular germ cell tumors. Cell Rep. 2018;23(11):3392–406.
33. Cost NG, Lubahn JD, Adibi M, Romman A, Wickiser JE, Raj GV, et al. A comparison of pediatric, adolescent, and adult testicular germ cell malignancy. Pediatr Blood Cancer. 2014;61(3):446–51.
34. Hvarness T, Nielsen JE, Almstrup K, Skakkebaek NE, Rajpert-De Meyts E, Claesson MH. Phenotypic characterisation of immune cell infiltrates in testicular germ cell neoplasia. J Reprod Immunol. 2013;100(2):135–45.
35. Klein B, Haggeney T, Fietz D, Indumathy S, Loveland KL, Hedger M, et al. Specific immune cell and cytokine characteristics of human testicular germ cell neoplasia. Hum Reprod. 2016;31(10):2192–202.
36. Rajpert-De Meyts E, Nielsen JE, Skakkebaek NE, Almstrup K. Diagnostic markers for germ cell neoplasms: from placental-like alkaline phosphatase to micro-RNAs. Folia Histochem Cytobiol. 2015;53(3):177–88.
37. Sonne SB, Almstrup K, Dalgaard M, Juncker AS, Edsgard D, Ruban L, et al. Analysis of gene expression profiles of microdissected cell populations indicates that testicular carcinoma in situ is an arrested gonocyte. Cancer Res. 2009;69(12):5241–50.
38. Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, van Roozendaal KE, et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res. 2003;63(9):2244–50.
39. Jones TD, Ulbright TM, Eble JN, Cheng L. OCT4: a sensitive and specific biomarker for intratubular germ cell neoplasia of the testis. Clin Cancer Res. 2004;10(24):8544–7.
40. Hoei-Hansen CE, Almstrup K, Nielsen JE, Brask Sonne S, Graem N, Skakkebaek NE, et al. Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma in situ and germ cell tumours. Histopathology. 2005;47(1):48–56.
41. Hart AH, Hartley L, Parker K, Ibrahim M, Looijenga LH, Pauchnik M, et al. The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. Cancer. 2005;104(10):2092–8.
42. West JA, Viswanathan SR, Yabuuchi A, Cunniff K, Takeuchi A, Park IH, et al. A role for Lin28 in primordial germ-cell development and germ-cell malignancy. Nature. 2009;460(7257):909–13.
43. Jacobsen GK, Norgaard-Pedersen B. Placental alkaline phosphatase in testicular germ cell tumours and in carcinoma-in-situ of the testis. An immunohistochemical study. Acta Pathol Microbiol Immunol Scand A. 1984;92(5):323–9.

44. Hoei-Hansen CE, Nielsen JE, Almstrup K, Sonne SB, Graem N, Skakkebaek NE, et al. Transcription factor AP-2gamma is a developmentally regulated marker of testicular carcinoma in situ and germ cell tumors. Clin Cancer Res. 2004;10(24):8521–30.
45. Sonne SB, Herlihy AS, Hoei-Hansen CE, Nielsen JE, Almstrup K, Skakkebaek NE, et al. Identity of M2A (D2-40) antigen and gp36 (Aggrus, T1A-2, podoplanin) in human developing testis, testicular carcinoma in situ and germ-cell tumours. Virchows Arch. 2006;449(2):200–6.
46. Idrees M, Saxena R, Cheng L, Ulbright TM, Badve S. Podoplanin, a novel marker for seminoma: a comparison study evaluating immunohistochemical expression of podoplanin and OCT3/4. Ann Diagn Pathol. 2010;14(5):331–6.
47. Nogales FF, Quinonez E, Lopez-Marin L, Dulcey I, Preda O. A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac. Histopathology. 2014;65(1):51–9.
48. Herszfeld D, Wolvetang E, Langton-Bunker E, Chung TL, Filipczyk AA, Houssami S, et al. CD30 is a survival factor and a biomarker for transformed human pluripotent stem cells. Nat Biotechnol. 2006;24(3):351–7.
49. de Jong J, Stoop H, Gillis AJ, van Gurp RJ, van de Geijn GJ, Boer M, et al. Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. J Pathol. 2008;215(1):21–30.
50. Albers P, Albrecht W, Algaba F, Bokemeyer C, Cohn-Cedermark G, Fizazi K, et al. Guidelines on testicular cancer: 2015 update. Eur Urol. 2015;68(6):1054–68.
51. Isidori AM, Pozza C, Gianfrilli D, Giannetta E, Lemma A, Pofi R, et al. Differential diagnosis of nonpalpable testicular lesions: qualitative and quantitative contrast-enhanced US of benign and malignant testicular tumors. Radiology. 2014;273(2):606–18.
52. Lagabrielle S, Durand X, Droupy S, Izard V, Marcelli F, Huyghe E, et al. Testicular tumours discovered during infertility workup are predominantly benign and could initially be managed by sparing surgery. J Surg Oncol. 2018;118(4):630–5.
53. Paffenholz P, Held L, Loosen SH, Pfister D, Heidenreich A. Testis sparing surgery for benign testicular masses: diagnostics and therapeutic approaches. J Urol. 2018;200(2):353–60.
54. Idrees MT, Ulbright TM, Oliva E, Young RH, Montironi R, Egevad L, et al. The World Health Organization 2016 classification of testicular non-germ cell tumours: a review and update from the International Society of Urological Pathology Testis Consultation Panel. Histopathology. 2017;70(4):513–21.
55. Thomas JC, Ross JH, Kay R. Stromal testis tumors in children: a report from the prepubertal testis tumor registry. J Urol. 2001;166(6):2338–40.
56. Coppes MJ, Rackley R, Kay R. Primary testicular and paratesticular tumors of childhood. Med Pediatr Oncol. 1994;22(5):329–40.
57. Ross JH, Kay R. Prepubertal testis tumors. Rev Urol. 2004;6(1):11–8.
58. Maizlin ZV, Belenky A, Kunichezky M, Sandbank J, Strauss S. Leydig cell tumors of the testis: gray scale and color Doppler sonographic appearance. J Ultrasound Med. 2004;23(7):959–64.
59. Pozza C, Pofi R, Tenuta M, Tarsitano MG, Sbardella E, Fattorini G, et al. Clinical presentation, management and follow-up of 83 patients with Leydig cell tumors of the testis: a prospective case-cohort study. Hum Reprod. 2019;34(8):1389–403.
60. Fankhauser CD, Grogg JB, Hayoz S, Wettstein MS, Dieckmann KP, Sulser T, et al. Risk factors and treatment outcomes of 1,375 patients with testicular leydig cell tumors: analysis of published case series data. J Urol. 2020;203:949–56.
61. Mameli C, Selvaggio G, Cerini C, Bulfamante G, Madia C, Riccipetitoni G, et al. Atypical Leydig cell tumor in children: report of 2 cases. Pediatrics. 2016;138(5):e20160151.
62. Liu G, Duranteau L, Carel JC, Monroe J, Doyle DA, Shenker A. Leydig-cell tumors caused by an activating mutation of the gene encoding the luteinizing hormone receptor. N Engl J Med. 1999;341(23):1731–6.

63. Carvajal-Carmona LG, Alam NA, Pollard PJ, Jones AM, Barclay E, Wortham N, et al. Adult Leydig cell tumors of the testis caused by germline fumarate hydratase mutations. J Clin Endocrinol Metab. 2006;91(8):3071–5.
64. Libe R, Fratticci A, Lahlou N, Jornayvaz FR, Tissier F, Louiset E, et al. A rare cause of hypertestosteronemia in a 68-year-old patient: a Leydig cell tumor due to a somatic GNAS (guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1)-activating mutation. J Androl. 2012;33(4):578–84.
65. Bozzini G, Picozzi S, Gadda F, Colombo R, Decobelli O, Palou J, et al. Long-term follow-up using testicle-sparing surgery for Leydig cell tumor. Clin Genitourin Cancer. 2013;11(3):321–4.
66. Elert A, Olbert P, Hegele A, Barth P, Hofmann R, Heidenreich A. Accuracy of frozen section examination of testicular tumors of uncertain origin. Eur Urol. 2002;41(3):290–3.
67. Giannarini G, Mogorovich A, Bardelli I, Manassero F, Selli C. Testis-sparing surgery for benign and malignant tumors: a critical analysis of the literature. Indian J Urol. 2008;24(4):467–74.
68. Sheynkin YR, Sukkarieh T, Lipke M, Cohen HL, Schulsinger DA. Management of nonpalpable testicular tumors. Urology. 2004;63(6):1163–7; discussion 7.
69. Kim I, Young RH, Scully RE. Leydig cell tumors of the testis: a clinicopathological analysis of 40 cases and review of the literature. Am J Surg Pathol. 1985;9(3):177–92.
70. Billings SD, Roth LM, Ulbright TM. Microcystic Leydig cell tumors mimicking yolk sac tumor: a report of four cases. Am J Surg Pathol. 1999;23(5):546–51.
71. Ulbright TM, Srigley JR, Hatzianastassiou DK, Young RH. Leydig cell tumors of the testis with unusual features: adipose differentiation, calcification with ossification, and spindle-shaped tumor cells. Am J Surg Pathol. 2002;26(11):1424–33.
72. Al-Agha OM, Axiotis CA. An in-depth look at Leydig cell tumor of the testis. Arch Pathol Lab Med. 2007;131(2):311–7.
73. Colecchia M, Nistal M, Gonzalez-Peramato P, Carmignani L, Salvioni R, Nicolai N, et al. Leydig cell tumor and hyperplasia: a review. Anal Quant Cytol Histol. 2007;29(3):139–47.
74. McCluggage WG, Shanks JH, Arthur K, Banerjee SS. Cellular proliferation and nuclear ploidy assessments augment established prognostic factors in predicting malignancy in testicular Leydig cell tumours. Histopathology. 1998;33(4):361–8.
75. Reznik Y, Rieu M, Kuhn JM, Mandard JC, Bottet P, Lemonnier D, et al. Luteinizing hormone regulation by sex steroids in men with germinal and Leydig cell tumours. Clin Endocrinol. 1993;38(5):487–93.
76. Ortiz DJ, Silva J, Abad M, Garcia-Macias MC, Bulon YA. Leydig cell tumour of the testis: cytological findings on fine needle aspiration. Cytopathology. 1999;10(3):217–8.
77. McCluggage WG, Shanks JH, Whiteside C, Maxwell P, Banerjee SS, Biggart JD. Immunohistochemical study of testicular sex cord-stromal tumors, including staining with anti-inhibin antibody. Am J Surg Pathol. 1998;22(5):615–9.
78. Augusto D, Leteurtre E, De La Taille A, Gosselin B, Leroy X. Calretinin: a valuable marker of normal and neoplastic Leydig cells of the testis. Appl Immunohistochem Mol Morphol. 2002;10(2):159–62.
79. Busam KJ, Iversen K, Coplan KA, Old LJ, Stockert E, Chen YT, et al. Immunoreactivity for A103, an antibody to melan-a (Mart-1), in adrenocortical and other steroid tumors. Am J Surg Pathol. 1998;22(1):57–63.
80. Iczkowski KA, Bostwick DG, Roche PC, Cheville JC. Inhibin a is a sensitive and specific marker for testicular sex cord-stromal tumors. Mod Pathol. 1998;11(8):774–9.
81. Pierre P, Despert F, Tranquart F, Coutant R, Tardy V, Kerlan V, et al. Adrenal rest tissue in gonads of patients with classical congenital adrenal hyperplasia: multicenter study of 45 French male patients. Ann Endocrinol (Paris). 2012;73(6):515–22.
82. Rutgers JL, Young RH, Scully RE. The testicular "tumor" of the adrenogenital syndrome. A report of six cases and review of the literature on testicular masses in patients with adrenocortical disorders. Am J Surg Pathol. 1988;12(7):503–13.

83. Ashley RA, McGee SM, Isotaolo PA, Kramer SA, Cheville JC. Clinical and pathological features associated with the testicular tumor of the adrenogenital syndrome. J Urol. 2007;177(2):546–9; discussion 9.
84. Aycan Z, Bas VN, Cetinkaya S, Yilmaz Agladioglu S, Tiryaki T. Prevalence and long-term follow-up outcomes of testicular adrenal rest tumours in children and adolescent males with congenital adrenal hyperplasia. Clin Endocrinol. 2013;78(5):667–72.
85. Grogg J, Schneider K, Bode PK, Kranzbuhler B, Eberli D, Sulser T, et al. Sertoli cell tumors of the testes: systematic literature review and meta-analysis of outcomes in 435 patients. Oncologist. 2020;25(7):585–90.
86. Gabrilove JL, Freiberg EK, Leiter E, Nicolis GL. Feminizing and non-feminizing Sertoli cell tumors. J Urol. 1980;124(6):757–67.
87. Goswitz JJ, Pettinato G, Manivel JC. Testicular sex cord-stromal tumors in children: clinicopathologic study of sixteen children with review of the literature. Pediatr Pathol Lab Med. 1996;16(3):451–70.
88. Necchi A, Bratslavsky G, Shapiro O, Elvin JA, Vergilio JA, Killian JK, et al. Genomic features of metastatic testicular sex cord stromal tumors. Eur Urol Focus. 2019;5(5):748–55.
89. Young S, Gooneratne S, Straus FH, Zeller WP, Bulun SR, Rosenthal IM. Feminizing Sertoli cell tumors in boys with Peutz-Jeghers syndrome. Am J Surg Pathol. 1995;19(1):50–8.
90. Young RH, Koelliker DD, Scully RE. Sertoli cell tumors of the testis, not otherwise specified: a clinicopathologic analysis of 60 cases. Am J Surg Pathol. 1998;22(6):709–21.
91. Kommoss F, Oliva E, Bittinger F, Kirkpatrick CJ, Amin MB, Bhan AK, et al. Inhibin-alpha CD99, HEA125, PLAP, and chromogranin immunoreactivity in testicular neoplasms and the androgen insensitivity syndrome. Hum Pathol. 2000;31(9):1055–61.
92. Kao CS, Cornejo KM, Ulbright TM, Young RH. Juvenile granulosa cell tumors of the testis: a clinicopathologic study of 70 cases with emphasis on its wide morphologic spectrum. Am J Surg Pathol. 2015;39(9):1159–69.
93. Talerman A, Dlemarre JF. Gonadoblastoma associated with embryonal carcinoma in an anatomically normal man. J Urol. 1975;113(3):355–9.
94. Scully RE. Gonadoblastoma. A review of 74 cases. Cancer. 1970;25(6):1340–56.
95. Burger T, Schildhaus HU, Inniger R, Hansen J, Mayer P, Schweyer S, et al. Ovarian-type epithelial tumours of the testis: immunohistochemical and molecular analysis of two serous borderline tumours of the testis. Diagn Pathol. 2015;10:118.
96. Dogra VS, Gottlieb RH, Oka M, Rubens DJ. Sonography of the scrotum. Radiology. 2003;227(1):18–36.
97. Geraghty MJ, Lee FT Jr, Bernsten SA, Gilchrist K, Pozniak MA, Yandow DJ. Sonography of testicular tumors and tumor-like conditions: a radiologic-pathologic correlation. Crit RevDiagn Imaging. 1998;39(1):1–63.
98. Thava V, Cooper N, Egginton JA. Yolk sac tumour of the testis in childhood. Br J Radiol. 1992;65(780):1142–4.
99. Pozza C, Gianfrilli D, Fattorini G, Giannetta E, Barbagallo F, Nicolai E, et al. Diagnostic value of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions. Andrology. 2016;4(6):1193–203.
100. Manganaro L, Saldari M, Pozza C, Vinci V, Gianfrilli D, Greco E, et al. Dynamic contrast-enhanced and diffusion-weighted MR imaging in the characterisation of small, non-palpable solid testicular tumours. Eur Radiol. 2018;28(2):554–64.
101. Manganaro L, Vinci V, Pozza C, Saldari M, Gianfrilli D, Pofi R, et al. A prospective study on contrast-enhanced magnetic resonance imaging of testicular lesions: distinctive features of Leydig cell tumours. Eur Radiol. 2015;25(12):3586–95.
102. Krone KD, Carroll BA. Scrotal ultrasound. Radiol Clin N Am. 1985;23(1):121–39.
103. Milose JC, Filson CP, Weizer AZ, Hafez KS, Montgomery JS. Role of biochemical markers in testicular cancer: diagnosis, staging, and surveillance. Open Access J Urol. 2011;4:1–8.
104. Ehrlich Y, Beck SD, Foster RS, Bihrle R, Einhorn LH. Serum tumor markers in testicular cancer. Urol Oncol. 2013;31(1):17–23.

105. Dieckmann KP, Richter-Simonsen H, Kulejewski M, Ikogho R, Zecha H, Anheuser P, et al. Testicular germ-cell tumours: a descriptive analysis of clinical characteristics at first presentation. Urol Int. 2018;100(4):409–19.
106. Murray MJ, Huddart RA, Coleman N. The present and future of serum diagnostic tests for testicular germ cell tumours. Nat Rev Urol. 2016;13(12):715–25.
107. Nappi L, Nichols C. MicroRNAs as biomarkers for germ cell tumors. Urol Clin North Am. 2019;46(3):449–57.
108. Almstrup K, Lobo J, Morup N, Belge G, Rajpert-De Meyts E, Looijenga LHJ, et al. Application of miRNAs in the diagnosis and monitoring of testicular germ cell tumours. Nat Rev Urol. 2020:17;201-213.
109. Bandak M, Jorgensen N, Juul A, Lauritsen J, Gundgaard Kier MG, Mortensen MS, et al. Preorchiectomy Leydig cell dysfunction in patients with testicular cancer. Clin Genitourin Cancer. 2017;15(1):e37–43.
110. Zarrilli S, Lombardi G, Paesano L, Di Somma C, Colao A, Mirone V, et al. Hormonal and seminal evaluation of Leydig cell tumour patients before and after orchiectomy. Andrologia. 2000;32(3):147–54.
111. Mieritz MG, Raket LL, Hagen CP, Nielsen JE, Talman ML, Petersen JH, et al. A longitudinal study of growth, sex steroids, and IGF-1 in boys with physiological gynecomastia. J Clin Endocrinol Metab. 2015;100(10):3752–9.
112. Sansone A, Romanelli F, Sansone M, Lenzi A, Di Luigi L. Gynecomastia and hormones. Endocrine. 2017;55(1):37–44.
113. Kanakis GA, Nordkap L, Bang AK, Calogero AE, Bartfai G, Corona G, et al. EAA clinical practice guidelines-gynecomastia evaluation and management. Andrology. 2019;7(6):778–93.
114. Honecker F, Aparicio J, Berney D, Beyer J, Bokemeyer C, Cathomas R, et al. ESMO consensus conference on testicular germ cell cancer: diagnosis, treatment and follow-up. Ann Oncol. 2018;29(8):1658–86.
115. Cornejo KM, Frazier L, Lee RS, Kozakewich HP, Young RH. Yolk sac tumor of the testis in infants and children: a clinicopathologic analysis of 33 cases. Am J Surg Pathol. 2015;39(8):1121–31.
116. Sobin L, Gospodarowicz MK, Wittekind C, editors. International Union Against Cancer (UICC) TNM classification of malignant tumors. 7th ed. Oxford, UK: Wiley-Blakwell; 2009.
117. International Germ Cell Consensus Classification: a prognostic factor-based staging system for metastatic germ cell cancers. J Clin Oncol. 1997;15(2):594–603.
118. Frazier AL, Hale JP, Rodriguez-Galindo C, Dang H, Olson T, Murray MJ, et al. Revised risk classification for pediatric extracranial germ cell tumors based on 25 years of clinical trial data from the United Kingdom and United States. J Clin Oncol. 2015;33(2):195–201.
119. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972–8.
120. Rives N, Perdrix A, Hennebicq S, Saias-Magnan J, Melin MC, Berthaut I, et al. The semen quality of 1158 men with testicular cancer at the time of cryopreservation: results of the French National CECOS Network. J Androl. 2012;33(6):1394–401.
121. Williams DH. Sperm banking and the cancer patient. Ther Adv Urol. 2010;2(1):19–34.
122. Djaladat H, Burner E, Parikh PM, Beroukhim Kay D, Hays K. The association between testis cancer and semen abnormalities before orchiectomy: a systematic review. J Adolesc Young Adult Oncol. 2014;3(4):153–9.
123. Fode M, Ohl DA, Sonksen J. A step-wise approach to sperm retrieval in men with neurogenic anejaculation. Nat Rev Urol. 2015;12(11):607–16.
124. Gat I, Toren A, Hourvitz A, Raviv G, Band G, Baum M, et al. Sperm preservation by electroejaculation in adolescent cancer patients. Pediatr Blood Cancer. 2014;61(2):286–90.
125. Berookhim BM, Mulhall JP. Outcomes of operative sperm retrieval strategies for fertility preservation among males scheduled to undergo cancer treatment. Fertil Steril. 2014;101(3):805–11.

126. Onofre J, Baert Y, Faes K, Goossens E. Cryopreservation of testicular tissue or testicular cell suspensions: a pivotal step in fertility preservation. Hum Reprod Update. 2016;22(6):744–61.
127. Sabanegh ES Jr, Ragheb AM. Male fertility after cancer. Urology. 2009;73(2):225–31.
128. Ku JY, Park NC, Jeon TG, Park HJ. Semen analysis in cancer patients referred for sperm cryopreservation before chemotherapy over a 15-year period in Korea. World J Mens Health. 2015;33(1):8–13.
129. Heidenreich A, Pfister D. Retroperitoneal lymphadenectomy and resection for testicular cancer: an update on best practice. Ther Adv Urol. 2012;4(4):187–205.
130. Schrader M, Muller M, Sofikitis N, Straub B, Krause H, Miller K. "Onco-tese": testicular sperm extraction in azoospermic cancer patients before chemotherapy-new guidelines? Urology. 2003;61(2):421–5.
131. Furuhashi K, Ishikawa T, Hashimoto H, Yamada S, Ogata S, Mizusawa Y, et al. Onco-testicular sperm extraction: testicular sperm extraction in azoospermic and very severely oligozoospermic cancer patients. Andrologia. 2013;45(2):107–10.
132. Tempest HG, Ko E, Chan P, Robaire B, Rademaker A, Martin RH. Sperm aneuploidy frequencies analysed before and after chemotherapy in testicular cancer and Hodgkin's lymphoma patients. Hum Reprod. 2008;23(2):251–8.
133. Kerns SL, Fung C, Monahan PO, Ardeshir-Rouhani-Fard S, Abu Zaid MI, Williams AM, et al. Cumulative burden of morbidity among testicular cancer survivors after standard cisplatin-based chemotherapy: a multi-institutional study. J Clin Oncol. 2018;36(15):1505–12.
134. Mykletun A, Dahl AA, Haaland CF, Bremnes R, Dahl O, Klepp O, et al. Side effects and cancer-related stress determine quality of life in long-term survivors of testicular cancer. J Clin Oncol. 2005;23(13):3061–8.
135. Orre IJ, Fossa SD, Murison R, Bremnes R, Dahl O, Klepp O, et al. Chronic cancer-related fatigue in long-term survivors of testicular cancer. J Psychosom Res. 2008;64(4):363–71.
136. Wiechno P, Demkow T, Kubiak K, Sadowska M, Kaminska J. The quality of life and hormonal disturbances in testicular cancer survivors in cisplatin era. Eur Urol. 2007;52(5):1448–54.
137. Baniel J, Sella A. Complications of retroperitoneal lymph node dissection in testicular cancer: primary and post-chemotherapy. Semin Surg Oncol. 1999;17(4):263–7.
138. Heidenreich A, Thuer D, Polyakov S. Postchemotherapy retroperitoneal lymph node dissection in advanced germ cell tumours of the testis. Eur Urol. 2008;53(2):260–72.
139. Mishra SI, Scherer RW, Snyder C, Geigle P, Gotay C. Are exercise programs effective for improving health-related quality of life among cancer survivors? A systematic review and meta-analysis. Oncol Nurs Forum. 2014;41(6):E326–42.
140. Chao C, Bhatia S, Xu L, Cannavale KL, Wong FL, Huang PS, et al. Incidence, risk factors, and mortality associated with second malignant neoplasms among survivors of adolescent and young adult cancer. JAMA Netw Open. 2019;2(6):e195536.
141. Fossa SD, Gilbert E, Dores GM, Chen J, McGlynn KA, Schonfeld S, et al. Noncancer causes of death in survivors of testicular cancer. J Natl Cancer Inst. 2007;99(7):533–44.
142. Kvammen O, Myklebust TA, Solberg A, Moller B, Klepp OH, Fossa SD, et al. Long-term relative survival after diagnosis of testicular germ cell tumor. Cancer Epidemiol Biomark Prev. 2016;25(5):773–9.

Gender Dysphoria: Management in the Transition age

14

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14.1 Introduction

Gender incongruence (GI) is defined by a marked and persistent incongruence between an individual's experienced gender and the assigned sex at birth [1]. When this condition leads to a clinically significant distress or impairment in social, occupational, or other important areas of functioning, we refer to it as Gender Dysphoria (GD) [1]. Individuals whose gender identity does not completely and/or permanently match their sex characteristics may describe themselves as *trans* or *transgender* [1]. In particular, we use the term *trans men* for those assigned female at birth who identify as men, and *trans women* for those assigned males who identify as women. GI/GD represent a dimensional phenomenon that can occur with different degrees of intensity, of which the most extreme form is accompanied by a desire for gender-affirming treatment, including hormonal treatment and/or surgical interventions.

GI/GD may develop during childhood and remit in most prepubertal children [2, 3]. Afterwards, it can persist during adolescence and adulthood only in a minority of cases. The percentage of "*persisters*" appears to be between 10% and 27% [2, 4, 5].

During the management of children and adolescents with GI/GD, health care professionals should broadly conform to the Standards of Care of the World

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Professional Association for Transgender Health (WPATH) [6] and to the Endocrine Society's guidelines [7]. These are both mainly inspired by the pioneering work of Delamarre-van de Waal and Cohen-Kettenis [8] who described for the first time the "Dutch Approach," a clinical protocol for the management of GI/GD during adolescence. This protocol is characterized by a "Combined Approach," including both psychological support and medical intervention, structured in three different phases: (i) a first diagnostic phase without medical interventions; (ii) the extended diagnostic phase, characterized by puberty suspension with GnRH analogues (GnRHa);` and [3] induction of puberty congruent with gender identity.

In the first phase, a psychological support and evaluation is offered, with the aim to assess the presence and intensity of GI/GD [1] and to evaluate the presence of interfering psychological and/or social conditions. In this phase, the mental health professional (MHP) should also evaluate the presence of adequate psychological and social support, actively including parents. In line with WPATH recommendations, this phase should be conducted by a MHP with a specific training in child and adolescent developmental psychopathology and skilled in GI/GD [6].

During the establishment of the therapeutic relationship, the clinician should maintain a neutral attitude regarding any possible outcome in order to help the adolescent to explore his/her gender identity openly. Finally, in this phase the MHP should inform the adolescent and the family accurately regarding different treatment options, and consequences (including those related to fertility preservation) in order to prevent unrealistic expectations.

In case GD persists, the MHP's role is also to assess the presence of criteria for pubertal suppression with GnRHa, as reported in Table 14.1.

In particular, it is recommended to start puberty suppression with GnRHa not earlier than Tanner stage G2/B2, in case the early pubertal modifications lead to a worsening of GD feelings [6, 7]. In fact, emotional reactions to the undesired body changes—in the opposite direction from the experienced gender identity—have an important diagnostic value for the MHP.

Table 14.1 Eligibility criteria for gonadotropin-releasing hormone analogues (GnRHa) and cross-sex hormones

Adolescents are eligible for GnRHa treatment if they:

1. Meet the DSM 5 criteria for gender dysphoria (GD)
2. Have experienced puberty at least up to Tanner stage 2
3. Have an increase in GD at the arrival of early pubertal changes
4. Do not suffer from psychiatric comorbidities that interfere with the diagnostic work-up or treatment
5. Have adequate psychological and social support during treatment
6. Demonstrate knowledge and understanding of the expected outcomes, risks, and benefits of therapy
7. Have parents' consent to the treatment and give adequate support during treatment

Adolescents are eligible for cross-sex hormone treatment if they:

1. Meet the criteria for GnRH analogues
2. Are 16 years or older
3. Have parents' consent to the treatment and give adequate support during treatment

14.2 Puberty Suspension

During the *extended diagnostic phase*, the administration of GnRHa allows to suspend pubertal development. In fact, these long-acting analogues temporarily suppress the endogenous production of sex steroids by GnRH receptor desensitization, after an initial increase of gonadotropins during 10 days after the first and the second injection [9]. Despite several GnRHa being available, triptorelin is the most studied in GD/GI adolescents [8]. The most used protocol contemplates the administration of triptorelin with a monthly formulation (3.75 mg every 28 days). In case gonadotropins are well suppressed, after 6 months, it is possible to switch to a trimestral formulation (11.25 mg). In transboys (assigned female at birth adolescents) the initial interval may be shortened, repeating the second injection (3.75 mg) after 12 days, in order to avoid menstrual bleeding risk.

GnRH antagonist could represent a potential alternative to GnRHa, since they immediately suppress pituitary gonadotropin secretion [10, 11]. This could represent an advantage through the elimination of the initial "flare" in gonadotropic axis activation. However, the absence of evidences regarding the safety and efficacy of GnRH antagonists does not allow their use in GD/GI adolescents.

GnRHa effectively suspend pubertal development, leaving these adolescents in a "limbo" in which they can explore their gender identity, without the distress derived by the undesired body modifications. In fact, already at the onset of Tanner 2 puberty, body changes—such as breast development in transboys and increasing testicular size in transgirls (assigned male at birth adolescents)—may become unbearable. With the progression of puberty, other modifications occur, such as menarche/menses in transboys and deepening of voice, virilizing hair pattern, development of facial dimorphic characteristics, and spontaneous erections in transgirls. These undesired body modifications are perceived as devastating and humiliating. At this point, GD/GI adolescents realize that they cannot avoid the natural expression of their biological sex, which is incongruent with their gender identity. In fact, the onset of puberty is usually associated in transgender youth with a worsening of GD, distress, and psychological functioning and well-being [12].

During GnRHa treatment, slight development of secondary sex characteristics may regress or stop. Among the effects, we can mention breast atrophy, menses cessation in transgirls, and decrease in body hair distribution and in testicular volume, as well as reduction of spontaneous erections in transgirls [13].

Because of the suppression of pubertal development, GnRHa treatment immediately reduces the subject's suffering and may prevent emotional and psychological impairment [14, 15]. Furthermore, GnRH treatment extends the diagnostic phase, by leaving the body in a neutral early pubertal state. During this period the adolescent can continue to self-explore his/her gender identity, without the distress caused by pubertal modifications [7], allowing the clinicians to "gain time" during the extended diagnostic phase.

An important advantage of GnRHa is the reversibility of the intervention. If the adolescent decides not to follow with the transition path, pubertal block can be

discontinued. Spontaneous pubertal development will resume immediately and the subject can resume the maturation in the biological direction [16].

Furthermore, physical treatment outcome in adolescents treated with GnRHa is more satisfactory compared to treatment started later in puberty, when secondary sex characteristics have already been developed [17, 18]. Starting earlier medical treatment may reduce the invasiveness of future medical and surgical interventions [19]. For example, electrolysis hair removal, voice therapy, or surgery could not be necessary in transwomen individuals, as well as chest surgery in transmen.

Psychological support to the adolescent is crucial during all this delicate phase. Therefore, meetings with a skilled MHP are encouraged in order to evaluate all the aspects of psychological and social functioning, as well as exploring GD/GI and its possible outcomes [20].

Furthermore, during GnRHa treatment, guidelines recommend measuring gonadotropins and sex steroids levels to confirm adequate gonadal axis suppression [7]. In case gonadal axis is not completely suppressed, the time between GnRHa administrations can be shortened or the dose increased. Moreover, it is important to monitor negative effects of delaying puberty, such as halted growth spurt and impaired bone mineral accretion.

14.2.1 Criticalities of GnRHa Treatment

Overall, GnRHa appear to be an effective and safe treatment to block endogenous pubertal development in GD/GI adolescents. However, most of the evidences are based on studies in which GnRHa treatment was used to suppress precocious puberty and long-term follow-up studies are still quite limited.

The main risk of pubertal suppression in GD/GI adolescents may include an impairment of bone mineralization. In fact, puberty is the most important period in life for correct bone mass achievement, since about 85–90% of the total bone mass will have been acquired at the end of puberty [21]. Few data are available on the effect of GnRHa on bone mineral density (BMD) in adolescents with GD/GI. Contrasting evidences come from studies in different settings. For example, some studies reported that men with constitutionally delayed puberty have decreased BMD in adulthood [22], while others showed a normal BMD in this population [23, 24]. Similarly, a decrease of BMD is described in children with central precocious puberty treated with GnRHa in some studies [25], while others did not confirm these findings [26].

Concerning GnRHa treatment in GD/GI adolescents, data demonstrated no change of absolute areal BMD during GnRHa treatment, but a decrease in BMD Z-scores, mainly the lumbar spine [8, 27, 28]. The subsequent hormonal treatment to induce puberty led to an increase of BMD Z-score in both transmen and transwomen; however, even after 24 months of gender-affirming hormonal treatment, pretreatment Z-score values were not reached in most of transgender adolescents [28]. Considering these evidences, dual-energy X-ray absorptiometry (DXA) scans remain important in follow up of bone health of transgender adolescents. Besides, calcium and vitamin D supplementation in case of deficiency may improve bone

health during GnRHa treatment, as well as an appropriate lifestyle (including physical activity and smoking avoidance/cessation). With regard to body composition, GnRHa have not been associated with a change in body mass index (BMI) standard deviation score in GD/GI adolescents [27]. However, GnRHa treatment seems to modify body composition leading to an increase in fat mass and a decrease in lean body mass percentage [13]. Comparable results have been observed also in girls treated for precocious puberty with GnRHa [29, 30].

Another criticality of GnRHa treatment is represented by the potential effects on brain development. Puberty has been suggested to represent a second organizational period during brain development both in animals and humans [31, 32], especially regarding the development of executive functioning. The question arises if pubertal suppression with GnRHa affects the development of this task. Limited data are available regarding this aspect. A single cross-sectional study [33], comparing the performance on the Tower of London (ToL) task of adolescents under GnRHa treatment with control boys and girls, demonstrated no compromise of executive function. Unexpectedly, when evaluating brain activation patterns during ToL performance, GnRHa treated adolescents with GD showed sex differences in neural activation similar to their natal sex control group.

An additional side effect reported in a few girls treated with GnRHa for precocious/early puberty is arterial hypertension [34, 35]. Similar evidences have not yet been reported in GD/GI adolescents treated with GnRHa, however blood pressure monitoring before and during treatment is recommended [7].

Furthermore, treated adolescents may also experience hot flashes, fatigue, and mood alterations as a consequence of pubertal suppression and reduction of sex steroids levels. Though, there is no consensus on treatment of these side effects in this context.

Finally, fertility issues should be adequately explored before the start of GnRHa treatment. Puberty suppression can pause the maturation of germ cells, and thus, affect fertility potential. However, data on fertility outcome after GnRHa treatment are lacking and fertility preservation options in these individuals are still investigational.

14.3 Puberty Induction

Clinicians can start gender-affirming hormones after a multidisciplinary team has confirmed the persistence of gender dysphoria/gender incongruence in adolescents with a sufficient mental (and legal) capacity to give informed consent to this partially irreversible treatment (Table 14.2).

Although in many countries adolescents are able to start treatments since 16 years old, the timing of sex hormones starting in transgender adolescents remains under debate. The Endocrine Society guidelines supports the initiation of treatment at the age of 16 years and even earlier in selected cases evaluated by a multidisciplinary team with expertise in gender identity development in children [7]. However, minimal data supporting an earlier use currently exist. When GnRHa treatment is started

Table 14.2 Protocol induction for puberty

Induction of female puberty:
– *Transdermal 17b-estradiol* (matrix patch, 25 mcg/24 h patch, changing the patch as directed once or twice weekly): Start with 3 mcg, applied for 12 h per day for 6 months, than 3 mcg for 24 h. double the dose every 6 months in a stepwise fashion: 6 mcg, 12.5 mcg, up to 25 mcg, 50 mcg/d, 75 mcg/d, up to the adult dose (100 mcg/d).
– *Oral 17b-estradiol*: Start with 5 mcg/kg/d, increasing the dose every 6 months in a stepwise way by 5 mcg/kg/d, up to adult dose (2–4 mg/d).
– *Oral estradiol valerate*: Start with 8 mcg/kg/d (0.5 mg/d), increasing the dose every 6 months in a stepwise way by 8 mcg/kg/d, up to adult dose (2–4 mg/d).
– *Oral estradiol hemihydrate*: Start with 6 mcg/kg/d (0.4 mg/d) increasing the dose every 6 months in a stepwise way by 6 mcg/kg/d, up to adult dose (1.5–3 mg/d).
Induction of male puberty:
Intramuscular testosterone esters, increasing the dose every 6 months:
25 mg/m^2 every 2 week im
50 mg/m^2 every 2 week im
75 mg/m^2 every 2 week im
100 mg/m^2 every 2 week im

in the *early stages* of pubertal development, a puberty congruent with the experienced gender identity is induced with a dose scheme characterized by a progressive increase of hormonal treatment doses in order to simulate a physiological puberty, such as in hypogonadal patients. Alternatively, in *late puberty* gender-affirming treatment can be given at higher doses until the expected adult dose [7].

This gradually increasing schedule of sex steroid doses does not allow to effectively suppress endogenous sex steroid secretion. For this reason, the continuation of GnRHa treatment is advised until gonadectomy, even if further studies are necessary to evaluate its long-term effects.

Furthermore, transgender adolescents and their parents/other caretakers need to be clearly informed about the potential loss of fertility (impaired spermatogenesis and oocyte maturation) induced by hormonal therapy to make a reasoned and balanced decision and potentially access to fertility preservation clinic.

14.3.1 Transgender Girls

Treatment for induction of a female puberty in transgirls consist in oral or transdermal estrogens formulations.

The oral administration of 17β-estradiol should be started at a dosage of 5 μg/kg/d, increasing the dose every 6 months of 5 μg/kg (to 20 μg/kg/d) until a maintenance dosage of 2 mg is reached.

The transdermal 17β-estradiol may be an alternative for the oral one with an initial dosage from 6.25–12.5 μg/24 h to 37.5 μg/24 h until the adult dose (50–200 μg/24 h) is achieved, raising the dose every 6 months. The use of transdermal alternative is increasing, but the absence of specific low-dose estrogen patches may be uncomfortable: Individuals need to cut the patches themselves to obtain a size corresponding to the appropriate dosage, which is sometimes difficult to

calculate and realize; the patches glue may cause allergic reactions; new patches need to be placed every 3.5 day at the same hour of the day [36].

After a period of gonadal suppression varying from 3 to 6 months, estrogens can be given at a daily start dosage of 1 mg and increased to 2 mg after 6 months [7].

The effects of the addition of 17b-estradiol were studied prospectively in 28 transgender girls [37]. Estrogen treatment was started at a median age of 16 years after a median duration of 24.8 months of GnRHa monotherapy. When the adult dose of 2 mg of estradiol daily was reached during a median duration of 2 years, the median serum estradiol was 27 pg/mL (100 pmol/L) [range, 6.5–103 pg/mL (24–380 pmol/L)] and no changes in prolactin levels, hemoglobin, hematocrit, glycated hemoglobin, liver enzymes, and creatinine were seen [38]. Furthermore, physical changes were observed such as the breast development (started within 3 months, and after 1 year median Tanner breast stage was 3 progressing to 5 after 3 years), the increase of hip circumference and the decrease of waist circumference [38]. Although BMI increased, BMI SD scores did not: absolute BMD and Z-scores in the lumbar spine (not in the hip) increased [27, 28], but after 2 years of estrogens *Z*-scores were still below those of age- and sex-assigned–matched norms [28].

Studies regarding treatment with estrogens on pubertal development and short-term safety demonstrate feminization of the body without adverse events [38], although data on long-term outcomes are still sparse.

14.3.2 Transgender Boys

For male pubertal induction the use of testosterone ester injections is recommended. The initial dose is 25 mg/m^2 IM every 2 weeks, progressively increasing 25 mg/m^2 every 6 months. The maintenance dosages vary from 100 to 200 mg per 2 weeks for testosterone monoesters, such as testosterone enanthate, to 250 mg per 3 to 4 weeks for testosterone ester mixtures. For transgender boys who started treatment in late puberty, testosterone can be started at 75 mg IM every 2 weeks, followed by the maintenance dosage after 6 months [7]. With androgens treatment, virilization of the body occurs in the first 3–6 months, including lowering of the voice, facial, and body hair growth, muscular development (particularly in the upper body), and clitoral growth [7, 8]. In post-menarche adolescent transboys, a progestogen can be added to stop or decrease menses frequency; instead, the adult dosage alone will be sufficient to suppress gonadal axis.

Prospective data on combined GnRHas and androgens are still scarce. The clinical effects, including those metabolic parameters, in transgender boys have been investigated retrospectively in two studies, one single-center study ($n = 42$) [39] and one multicenter study center ($n = 72$) [40]. Only the single-center study reported on side effects, which were fatigue and acne. Clinically, there was a weight gain as both BMI [40] and BMI SD scores increased [39]. Although testosterone preparation and dosages differed, both studies reported an increase in both hemoglobin and hematocrit and in alanine aminotransferase, aspartate aminotransferase, and creatinine (even if they remained in the normal range), a worsening of lipid profile (cholesterol

and low-density lipoprotein increase, high-density lipoprotein decrease); glucose homeostasis parameters (HbA1c, insulin, glucose, homeostatic model assessment index) were not affected [41]. In transgender boys the bone density and *Z*-scores of the lumbar spine and the femoral region increased after 2 years of testosterone therapy even if they don't reach the pretreatment values [28, 42, 43].

14.4 Conclusions

Knowledge regarding the treatment of gender dysphoria and nonconforming youth has steadily advanced during the past 10 years [44, 45]. The current available research on transgender adolescents treatment is based mostly on cross-sectional studies with limited longitudinal data as well as paucity of information on diverse ethnic and socioeconomic populations (mostly from Western Europe and higher-income countries where many participants undergo surgical procedures and drop the follow-up). Nevertheless, the few somatic data available in adolescents are favorable and hither to support the fact that the proven psychological benefits of early medical intervention in transgender adolescents effectively outweigh the potential medical risks [36].

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-5). Arlington, VA: American Psychiatric Publishers; 2013.
2. Wallien MS, Cohen-Kettenis PT. Psychosexual outcome of gender-dysphoric children. J Am Acad Child Adolesc Psychiatry. 2008;47:1413–23.
3. Zucker KJ, Bradley S. Gender identity disorder and psychosexual problems in children and adolescents. Guilford: New York, NY; 1995.
4. Drummond KD, Bradley SJ, Peterson-Badali M, Zucker KJ. A follow-up study of girls with gender identity disorder. Dev Psychol. 2008;44:34–45.
5. Steensma TD, McGuire JK, Kreukels BP, et al. Factors associated with desistence and persistence of childhood gender dysphoria: a quantitative follow-up study. J Am Acad Child Adolesc Psychiatry. 2013;52:582–90.
6. Standard of care, 2011 W.P.A.T.H. (World Professional Association of Transgender Health).
7. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, Rosenthal SM, Safer JD, Tangpricha V, T'Sjoen GG. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. Endocr Pract. 2017;23(12):1437.
8. Delemarre-van de Waal HA, Cohen-Kettenis PT. Clinical management of gender identity disorder in adolescents: a protocol on psychological and paediatric endocrinology aspects. Eur J Endocrinol. 2006;155(suppl 1):S131–7.
9. Roth CL, Brendel L, Rückert C, Hartmann K. Antagonistic and agonistic GnRH analogue treatment of precocious puberty: tracking gonadotropin concentrations in urine. Horm Res. 2005;63(5):257–62.
10. Roth C. Therapeutic potential of GnRH antagonists in the treatment of precocious puberty. Expert Opin Investig Drugs. 2002;11(9):1253–9.
11. Tuvemo T. Treatment of central precocious puberty. Expert Opin Investig Drugs. 2006;15(5):495–505.

12. Fisher AD, Ristori J, Castellini G, Sensi C, Cassioli E, Prunas A, Mosconi M, Vitelli R, Dèttore D, Ricca V, Maggi M. Psychological characteristics of Italian gender dysphoric adolescents: a case-control study. J Endocrinol Investig. 2017;40(9):953–65.
13. Schagen SE, Cohen-Kettenis PT, Delemarre-van de Waal HA, Hannema SE. Efficacy and safety of gonadotropin-releasing hormone agonist treatment to suppress puberty in gender dysphoric adolescents. J Sex Med. 2016;13(7):1125–32.
14. Cohen-Kettenis PT, Delemarre-van de Waal HA, Gooren LJG. The treatment of adolescent transsexuals: changing insights. J Sex Med. 2008;5:1892–7.
15. De Vries AL, Steensma TD, Doreleijers TA, Cohen-Kettenis PT. Puberty suppression in adolescents with gender identity disorder: a prospective follow-up study. J Sex Med. 2011;8(8):2276–83.
16. Manasco PK, Pescovitz OH, Feuillan PP, Hench KD, Barnes KM, Jones J, Hill SC, Loriaux DL, Cutler JRGB. Resumption of puberty after long term luteinizing hormone-releasing hormone treatment of central precocious puberty. J Clin Endocrinol Metab. 1988;67:368–72.
17. Cohen-Kettenis PT, van Goozen SH. Sex reassignment of adolescent transsexuals: a follow-up study. J Am Acad Child Adolesc Psychiatry. 1997;36:263–71.
18. Smith YL, van Goozen SH, Cohen-Kettenis PT. Adolescents with gender identity disorder who were accepted or rejected for sex reassignment surgery: a prospective follow-up study. J Am Acad Child Adolesc Psychiatry. 2001;40:472–81.
19. Cohen-Kettenis PT, Pfafflin F. Transgenderism and intersexuality in childhood and adolescence. London: Sage Publications, Making Choices; 2003.
20. Coleman E, Bockting W, Botzer M, et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. Int J Transgend. 2011;13:165–232.
21. Van Coeverden SC, Netelenbos JC, Roos JC, de Ridder CM, Delemarre-van de Waal HA. Reference values for bone mass in Dutch white pubertal children and their relation to pubertal maturation characteristics. Ned Tijdschr Geneeskd. 2001;145(38):1851–6.
22. Finkelstein JS, Klibanski A, Neer RM. A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty. J Clin Endocrinol Metab. 1996;81(3):1152–5.
23. Bertelloni S, Baroncelli GI, Ferdeghini M, Perri G, Saggese G. Normal volumetric bone mineral density and bone turnover in young men with histories of constitutional delay of puberty. J Clin Endocrinol Metab. 1998;83(12):4280–3.
24. Darelid A, Ohlsson C, Nilsson M, Kindblom JM, Mellström D, Lorentzon M. Catch up in bone acquisition in young adult men with late normal puberty. J Bone Miner Res. 2012;27(10):2198–207.
25. Saggese G, Bertelloni S, Baroncelli GI, Battini R, Franchi G. Reduction of bone density: an effect of gonadotropin releasing hormone analogue treatment in central precocious puberty. Eur J Pediatr. 1993;152(9):717–20.
26. Neely EK, Bachrach LK, Hintz RL, Habiby RL, Slemenda CW, Feezle L, Pescovitz OH. Bone mineral density during treatment of central precocious puberty. J Pediatr. 1995;127(5):819–22.
27. Klink D, Caris M, Heijboer A, van Trotsenburg M, Rotteveel J. Bone mass in young adulthood following gonadotropin-releasing hormone analog treatment and cross-sex hormone treatment in adolescents with gender dysphoria. J Clin Endocrinol Metab. 2015;100(2):E270–5.
28. Vlot MC, Klink DT, den Heijer M, Blankenstein MA, Rotteveel J, Heijboer AC. Effect of pubertal suppression and cross-sex hormone therapy on bone turnover markers and bone mineral apparent density (BMAD) in transgender adolescents. Bone. 2017;95:11–9.
29. Pasquino AM, Pucarelli I, Accardo F, Demiraj V, Segni M, Di Nardo R. Long-term observation of 87 girls with idiopathic central precocious puberty treated with gonadotropin-releasing hormone analogs: impact on adult height, body mass index, bone mineral content, and reproductive function. J Clin Endocrinol Metab. 2008;93(1):190–5.
30. Magiakou MA, Manousaki D, Papadaki M, Hadjidakis D, Levidou G, Vakaki M, Papaefstathiou A, Lalioti N, Kanaka-Gantenbein C, Piaditis G, Chrousos GP, Dacou-Voutetakis C. The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. J Clin Endocrinol Metab. 2010;95(1):109–17.

31. Juraska JM, Sisk CL, DonCarlos LL. Sexual differentiation of the adolescent rodent brain: hormonal influences and developmental mechanisms. Horm Behav. 2013;64:203–10.
32. Romeo RD. Puberty: a period of both organizational and activational effects of steroid hormones on neurobehavioural development. J Neuroendocrinol. 2003;15:1185–92.
33. Staphorsius AS, Kreukels BPC, Cohen-Kettenis PT, Veltman DJ, Burke SM, Schagen SEE, Wouters FM, Delemarre-van de Waal HA, Bakker J. Puberty suppression and executive functioning: an fMRI-study in adolescents with gender dysphoria. Psychoneuroendocrinology. 2015;56:190–9.
34. Calcaterra V, Mannarino S, Corana G, Codazzi AC, Mazzola A, Brambilla P, Larizza D. Hypertension during therapy with triptorelin in a girl with precocious puberty. Indian J Pediatr. 2013;80(10):884–5.
35. Siomou E, Kosmeri C, Pavlou M, Vlahos AP, Argyropoulou MI, Siamopoulou A. Arterial hypertension during treatment with triptorelin in a child with Williams-Beuren syndrome. Pediatr Nephrol. 2014;29(9):1633–6.
36. T'Sjoen G, Arcelus J, Gooren L, Klink D, Tangpricha V. Endocrinology of transgender medicine. Endocr Rev. 2019;40(1):97–117.
37. Laron Z, Kauli R, Zeev ZB, Comaru-Schally AM, Schally AV. D-TRP5-analogue of luteinizing hormone releasing hormone in combination with cyproterone acetate to treat precocious puberty. Lancet. 1981;2(8253):955–6.
38. Hannema SE, Schagen SEE, Cohen-Kettenis PT, Delemarre-van de Waal HA. Efficacy and safety of pubertal induction using 17b-estradiol in transgirls. J Clin Endocrinol Metab. 2017;102(7):2356–63.
39. Tack LJ, Craen M, Dhondt K, Vanden Bossche H, Laridaen J, Cools M. Consecutive lynestrenol and cross-sex hormone treatment in biological female adolescents with gender dysphoria: a retrospective analysis. Biol Sex Differ. 2016;7(1):14.
40. Jarin J, Pine-Twaddell E, Trotman G, Stevens J, Conard LA, Tefera E, Gomez-Lobo V. Cross-sex hormones and metabolic parameters in adolescents with gender dysphoria. Pediatrics. 2017;139(5):e20163173.
41. Crowley WF Jr, Comite F, Vale W, Rivier J, Loriaux DL, Cutler GB Jr. Therapeutic use of pituitary desensitization with a long-acting LHRH agonist: a potential new treatment for idiopathic precocious puberty. J Clin Endocrinol Metab. 1981;52(2):370–2.
42. De Roo C, Lierman S, Tilleman K, Peynshaert K, Braeckmans K, Caanen M, Lambalk CB, Weyers S, T'Sjoen G, Cornelissen R, De Sutter P. Ovarian tissue cryopreservation in female-to-male transgender people: insights into ovarian histology and physiology after prolonged androgen treatment. Reprod Biomed Online. 2017;34(6):557–66.
43. Lierman S, Tilleman K, Braeckmans K, Peynshaert K, Weyers S, T'Sjoen G, De Sutter P. Fertility preservation for trans men: frozen-thawed in vitro matured oocytes collected at the time of ovarian tissue processing exhibit normal meiotic spindles. J Assist Reprod Genet. 2017;34(11):1449–56.
44. Cohen-Kettenis PT, Schagen SE, Steensma TD, de Vries AL, Delemarre-van de Waal HA. Puberty suppression in a gender-dysphoric adolescent: a 22-year follow-up. Arch Sex Behav. 2011;40(4):843–7.
45. Cohen-Kettenis PT, Klink D. Adolescents with gender dysphoria. Best Pract Res Clin Endocrinol Metab. 2015;29(3):485–95.

Preserving Fertility in Adolescents

15

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15.1 Introduction

Antineoplastic treatments almost always have a transient or permanent effect on spermatogenesis. In young adults, the recovery of spermatogenesis depends on the type of treatment used. Semen quality in Hodgkin's Lymphoma (HL) patients treated with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) reaches pre-treatment levels within 2 years after ending the treatment, while patients treated with other chemotherapy regimens such as bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine and prednisone (BEACOPP); cyclophosphamide, vincristine, procarbazine, prednisone (COPP)/doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD); or mechlorethamine, vincristine, procarbazine, vincristine (MOPP) generally have permanent azoospermia, with some recovery seen 3–5 years post-treatment only with a smaller number of chemotherapy cycles [1]. Chemo- and radiotherapy protocols for testicular tumours have the most detrimental effect on spermatogenesis within 3–6 months of the treatment.

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Spermatogenesis recovery is a function of the time since the end of the therapy, with 97% of chemotherapy patients and 94% of radiotherapy patients showing good recovery after 24 months [2]. From the currently available published studies, it is impossible to predict a priori which patients will recover spermatogenesis and which will remain azoospermic, and there is no sperm index which might help predict which patients will remain permanently sterile. However, malignant tumours have a significant impact on adolescents and are the second most common cause of death before the age of 19 years. The incidence before the age of 14 years is 200,166 cases a year (10.2/100,000), and between 15 and 29 years it is 397,000 cases a year (22.1/100,000) [3]. Leukaemia is the most common cancer in the under-14 s, and lymphoma in 15–19-year-olds (Fig. 15.1).

The development of diagnostic and therapeutic procedures has led to a considerable improvement in the prognosis and survival of these patients, with 5-year survival now above 80%. For this reason, and given the youth of these patients, the medium- and long-term complications of antineoplastic therapies, including impaired fertility, have aroused great concern in recent years.

Only a handful of studies to date have described pre- and post-treatment semen quality in adolescent cancer patients. Some investigated pre-treatment semen quality, while others only evaluated the hormone profile of the pituitary–testicular axis. These studies are often limited by grouping older and younger patients together in a single 'reproductive age' group, thus complicating the identification of any issues specific to the adolescent population [4]. The characteristics of adolescents are different to those of children and adults, not least in terms of psychological development and social integration.

In any case, fertility may also need to be preserved in non-oncological patients, such as those requiring haemopoietic stem cell transplants, and hence the use of

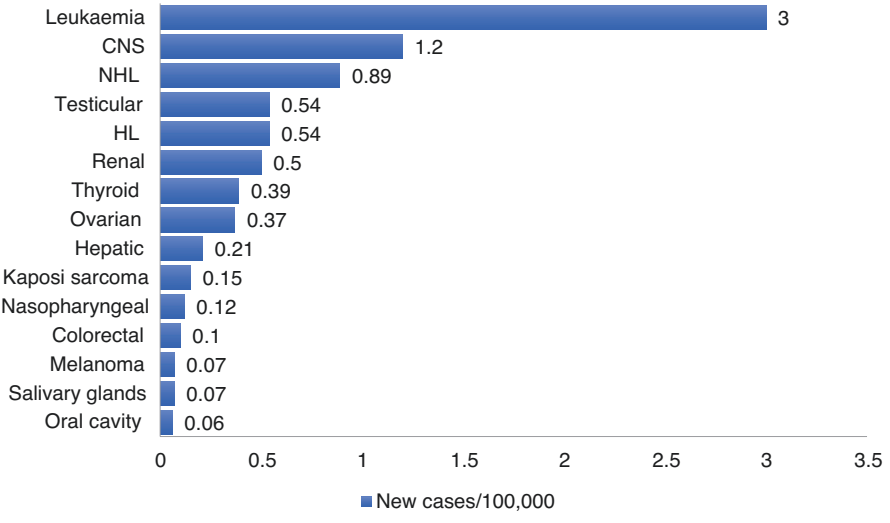

Fig. 15.1 Incidence of new cases of cancer in the 0–19 year age group in 2018 [International Agency for Research on Cancer data]. The values refer to the number of new cases per 100,000 subjects. *CNS* Central nervous system, *NHL* Non-Hodgkin's lymphoma, *HL* Hodgkin's lymphoma

myeloablative chemotherapy agents, as in the case of bone marrow aplasia or thalassaemia. Spermatogenesis can also be affected by other drugs, such as those used for male to female gender reassignment. In this case, medical therapy leads to changes that may be at least partly reversible on stopping the treatment, whereas bilateral orchiectomy is of course an irreversible procedure [5].

In all the above cases, the impairment of andrological health in adolescents is iatrogenic in nature, and hence associated with medical and/or surgical treatments enacted to protect the patient's physical and psychological well-being, to the detriment of his reproductive health. However, in some cases the testicular damage may be induced by the condition itself. The progressive nature of Klinefelter syndrome, for example, means that cryopreservation of seminal fluid or testicular tissue should be carried out while the patient is still young.

15.2 Spermatogenesis

Spermatogenesis is regulated by the hypothalamic–pituitary–gonadal (HPG) axis, which is already active in the foetus, remains active in early postnatal life and peaks at 3 months, leading to the so-called mini-puberty. It is then relatively quiescent until puberty, when it is reactivated by the hypothalamic pulsatile secretion of gonadotropin-releasing hormone (GnRH) (Fig. 15.2). This condition seems to depend on the action of kisspeptin, the peptide product of the KISS1 gene located on chromosome 1, a G-protein coupled receptor ligand for GPR54. The bond between kisspeptin and its receptor stimulates the release of GnRH by the hypothalamic neurons and plays a crucial part in the initiation of puberty [6].

GnRH is a peptide hormone produced in the preoptic area of the hypothalamus, secreted in the median eminence. It binds receptors expressed on the plasma

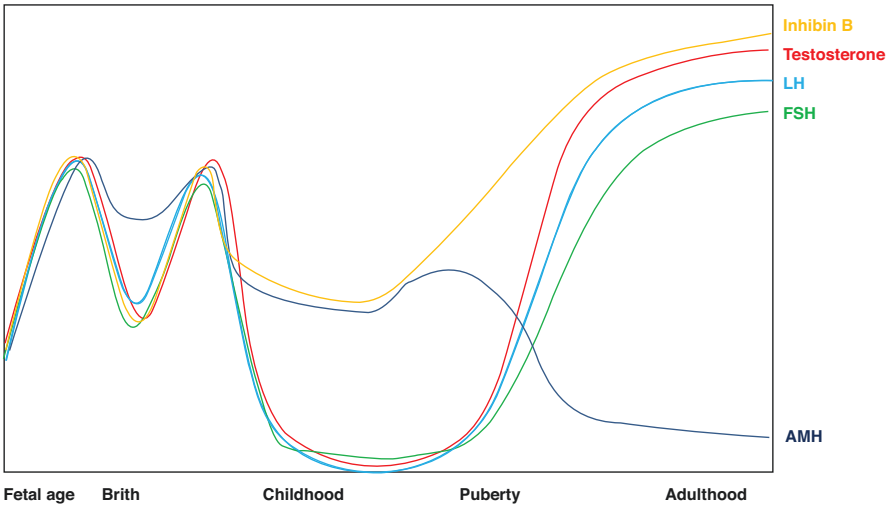

Fig. 15.2 Hormone variations in male subjects. *LH* Luteinizing hormone, *FSH* Follicle stimulating hormone

membrane of the gonadotropic cells in the anterior pituitary and stimulates the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In turn, LH stimulates the androgen-producing Leydig cells and FSH stimulates the cells of the seminiferous tubules, thus modulating spermatogenesis [7].

The predominant androgen produced by the testicles is testosterone, but androstenedione and dehydroepiandrosterone sulphate (DHEAS) are also produced. The active metabolite of testosterone is dihydrotestosterone (DHT), produced by the 5α-reductase-mediated peripheral conversion of testosterone. Testosterone and DHT have a major role in virilization through the activation of androgen receptors (ARs), and are responsible for the development of secondary sexual characteristics. The Leydig cells also produce insulin-like factor 3 (INSL-3), responsible for testicular descent. The Sertoli cells produce inhibin B, the release of which increases in two phases: during mini-puberty and then during puberty. In adults, inhibin B exerts a negative feedback on FSH and its concentration offers an indirect marker of spermatogenesis. Anti-Müllerian hormone (AMH) is secreted by the Sertoli cells during foetal life, causing the regression of the Müllerian ducts.

Reactivation of the HPG axis is determined by genetic, ethnic, nutritional and environmental aspects and may occur over a variable period of time, conventionally considered normal in boys of 9–14 years of age [8]. Puberty is therefore not a specific moment but a period with a variable start time and duration in which physical, psychological and reproductive changes take place. Full reproductive potential is only achieved at the end of puberty, but spermatogenesis begins in a very early stage and precedes the ability to ejaculate.

It is difficult to establish the exact time of the spermarche, as it is not associated with any clinical markers. Generally, sperm are already being produced before or during the development of pubic hair and when the testicular volume has only increased slightly. Plasma concentrations of gonadotropins and testosterone are not effective markers of spermarche. A more reliable marker could be the detection of sperm in urine: sperm are found in 5% of clinically prepubertal boys and in half of those in Tanner stage II–III.

Complete sperm maturation is modulated by the reactivation of the HPG axis on puberty, but the spermatogenesis process itself begins during foetal life. The primordial germ cells originate from the extraembryonic tissue surrounding the yolk sac. Between the third and fifth week of development they migrate along the gonadal ridge and differentiate into gonocytes, which stop at stage G0 of the cell cycle, remaining inactive until birth. In the first 6 months of life, the gonocytes differentiate into spermatogonia and remain quiescent until the age of 5–7 years, when they multiply through mitotic division. Spermatogonia are classified as type A, which may be dark (Ad) or pale (Ap), and type B. The spermatogonial reserve is maintained by type Ad spermatogonia which, following mitotic division, produce both new stem cells (type Ad) and more differentiated spermatogonia (type Ap). Mitotic division of type Ap gives rise to type B spermatogonia, from which further mitotic division produces primary spermatocytes. There is no further development of germ cells until the beginning of puberty, when rising serum levels of gonadotropins and androgens induce the resumption of spermatogenetic activity [9]. The

spermatogenetic process in humans, from spermatogonium to mature sperm, requires around 74 days and involves complex interactions between the germ cells and the Sertoli cells. The cytoplasmic extensions of the Sertoli cells are interconnected by tight junctions and comprise the so-called blood–testicle barrier [10], hence ensuring the specific microenvironment required for the correct development of germ cells within the seminiferous tubules.

As the cells progress through the spermatogenetic process, they are pushed by the tubular fluid towards the lumen of the seminiferous tubules, culminating, at the end of spermiogenesis, in the release of mature spermatozoa into the lumen, known as spermiation [11]. Finally, the sperm progress from the lumen of the seminiferous tubules through the tubuli recti, the rete testis, and the efferent ducts to the epididymis.

When they reach the head of the epididymis, the sperm have no progressive movement or fertilizing ability. These two properties derive from a complex process that begins during epididymal transit and is completed in the female genital tract. Functional maturation takes place in the epididymis over 12–15 days and is the result of intrinsic sperm processes and interactions with the epithelium of the epididymal duct. The sperm collect in the epididymis and are only united with the seminal plasma—the product of the combined secretions of the accessory glands of the male genital tract (seminal vesicles, prostate, bulbourethral glands, vas deferens and ampullae)—on ejaculation. Seminal fluid thus contains a corpuscular fraction (the sperm) and a liquid fraction, namely the seminal plasma in which the sperm are suspended.

During transit in the female genital tract, the sperm undergo a further functional maturation called capacitation. This, by removing cholesterol from the membrane, makes them suitable for the acrosomal reaction and hence for fecundation. The full attainment of this capacity can be considered as defining the completion of puberty and the start of adolescence. There is no absolute classification of these categories, but it is generally agreed that adolescence can be considered as 15–19 years of age and hence full adulthood begins at 20 [12].

15.3 Gonadotoxic Treatments: Mechanisms of Damage

Neoplastic diseases: Blood cancers and testicular tumours are the most common neoplastic diseases in the under-18 s. For stage I seminomas, the post-orchiectomy strategy may be watchful waiting or, depending on the risk factors, treatment with a carboplatin cycle, which has shown non-inferiority in relation to radiotherapy on ipsilateral paraaortic and iliac lymph nodes with a total dose of 30 Gray (Gy) divided into 15 fractions. For other stage I tumours, the treatment option could be a cisplatin, etoposide, bleomycin (PEB) cycle or retroperitoneal lymphadenectomy. For more advanced seminomas and non-seminomas alike, the treatment strategy is three or four PEB cycles, possibly associated with lymphadenectomy and/or radiotherapy, depending on the risk factors [13]. Cisplatin and carboplatin are derived from platinum and act as inhibitors of DNA synthesis, etoposide is a type II topoisomerase inhibitor and bleomycin is a glycopeptide that binds single or double DNA strands, thus impeding cell division [2, 14].

For localized (stage I–II) HL, two cycles of ABVD should be used, followed by 20 Gy of radiotherapy. ABVD (up to eight cycles), standard BEACOPP or dose-escalated BEACOPP is used for more advanced stages [13]. Adriamycin is an anthracycline that blocks DNA transcription and acts as a type II topoisomerase inhibitor, vinblastine and vincristine are indole alkaloids that bind to microtubular proteins, dacarbazine and procarbazine are alkylating agents and prednisone is a synthetic steroid with a predominantly glucocorticoid action. Other chemotherapy regimens are also available to treat HL in paediatric patients [15], including vincristine, etoposide, prednisone, doxorubicin (OEPA), cyclophosphamide, vincristine, prednisone, dacarbazine (COPDAC) and doxorubicin, etoposide, cyclophosphamide, vincristine, prednisone, dacarbazine (DECOPDAC). Cyclophosphamide is an alkylating agent.

Treatments for non-HL (NHL) are more varied. The most common chemotherapy regimen is rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP). However, the need for an autologous or allogenic bone marrow transplant is more common in this group. This requires a myelosuppressive treatment, most often with varying combinations of carmustine, busulfan, cyclophosphamide, melphalan, etoposide and fludarabine or with total body irradiation [16].

Classic osteosarcoma is a rare cancer (0.2% of all malign tumours) but is typically seen in young patients, with age of onset varying from 10 to 25 years. Ewing's sarcoma is more common than osteosarcoma but is also typically seen in paediatric patients. In both cases, the chemotherapy protocol involves doxorubicin, ifosfamide, methotrexate and cisplatin [17]. Methotrexate is a folic acid antagonist, while ifosfamide is structurally equivalent to cyclophosphamide and hence an alkylating agent. It has been shown that ifosfamide is less aggressive than cyclophosphamide on spermatogenesis and Leydig cell function [18].

Non-neoplastic diseases: Myeloablative therapy followed by hemopoietic stem cell transplant is not only reserved for oncological diseases. It is also used in blood disorders such as sickle cell anaemia, Fanconi's anaemia, bone marrow aplasia, thalassaemia, Blackfan–Diamond anaemia, Wiskott–Aldrich syndrome and congenital immunodeficiency syndromes as well as in metabolic diseases caused by enzyme disorders such as Gaucher's disease, congenital glycosylation disorders, adrenoleukodystrophy, oligosaccharidosis and mucopolysaccharidosis. In gender identity disorders, medical treatments involve the use of anti-androgens such as cyproterone acetate and spironolactone alongside oestrogens, usually oestradiol valerate and oestradiol hemihydrate. Spironolactone is an anti-aldosterone diuretic; its chemical structure means that it can compete for both dihydrotestosterone and aldosterone receptors. Cyproterone acetate can also compete for androgen receptors, as well as having a central antigonadotropin effect. Oestrogens suppress the production of pituitary gonadotropins through a negative feedback mechanism, thus impairing spermatogenesis and testosterone production [19]. HPG axis suppression can also be achieved with GnRH analogues. This type of treatment is indicated in patients with Tanner stage I or II and blocks the development of secondary sexual characteristics that are incongruous with the patient's gender identity [20].

15.4 Gonadotoxic Treatments: Clinical Effects

Neoplastic diseases: Chemo- and radiotherapy can affect fertility directly by damaging the germ cells, or indirectly by damaging the Sertoli or Leydig cells or hypothalamic–pituitary function. Their impact on fertility depends on the type of neoplasia and the treatment type, duration and dose, as well as the patient's age [21, 22].

Germ cells: Low doses of chemo- or radiotherapy can deplete the pool of differentiated spermatogonia without destroying the spermatogonial stem cells. The recovery of spermatogenesis after cytotoxic damage depends on the capacity of the mitotically quiescent spermatogonia to survive the treatment and reactivate the mitotic processes that lead to the production of differentiated spermatogonia. If the damage is severe, such as in the case of exposure to high cumulative doses of alkylating agents, the apoptotic pathway is activated in Ad cells, leading to persistent azoospermia.

It is difficult to establish the threshold dose of alkylating agents and platinum derivatives that will trigger irreversible damage. Green et al. [23] studied 214 adult survivors treated with alkylating agents at a median age of 7.7 years (range 0.01–20.3 years), finding that a cumulative dose below 4000 mg/m^2 did not affect spermatogenesis in adulthood. Azoospermia was found in the 25% of patients receiving a mean cumulative cyclophosphamide equivalent dose (CED) dose of 10,830 mg/m^2, oligozoospermia in the 27.5% of cases receiving a mean CED of 8480 mg/m^2 and normozoospermia in the 47.5% receiving a mean CED of 6626 mg/m^2. While damage from alkylating agents may be difficult to predict, despite its dose-dependent trend, damage induced by radiotherapy is easier to establish. Even single doses of less than 0.1 Gy lead to a transient arrest of spermatogenesis by damaging the differentiated spermatogonia. Cumulative doses of 2–3 Gy can affect the Ap cells, inducing long-term azoospermia, while doses above 6 Gy generally cause persistent azoospermia, involving the Ad spermatogonia [24].

Radiotherapy can also affect the circulatory system. The small vessels are sensitive to irradiation, which accelerates the atherosclerosis process, thus causing degeneration of the smooth muscles, intimal and subintimal fibrin accumulation, fibrosis of the tunica adventitia, accumulation of foamy histiocytes in the vessel wall and obliteration of the vasa vasorum [25]. These effects are well known in adults undergoing radiotherapy, but there is a lack of data on the effects of radiation on testicular vascularization in pubertal patients.

Leydig cells: Leydig cell damage is less common. Most adolescents do not develop hypogonadism after antineoplastic treatments, and most prepubertal patients subsequently undergo normal pubertal development. Leydig cells therefore seem less vulnerable to damage. Leydig cell insufficiency, which may be clinically evident or identifiable through a compensatory increase in LH production with normal or slightly reduced testosterone levels, has been documented in cases of combined procarbazine/bendamustine therapy and combined busulfan/cyclophosphamide therapy, especially in the setting of chemotherapy protocols actuated prior to a bone marrow transplant [26].

Sertoli cells: Quantification of Sertoli cell damage is more complex, and few studies have been conducted in humans [27]. De Rooij et al. [28] analysed the testicles and epididymis of Rhesus macaques after single-dose or fractionated total body irradiation. The testicular weight was considerably lower than in subjects of the same age that had not been exposed to radiation.

Spermatogenesis was present in some seminiferous tubules in most of the irradiated monkeys, indicating the ability to repopulate these structures with germ cells. By correlating the percentage of repopulated seminiferous tubules with testicular weight, it was hypothesized that the weight reduction could be attributed to death of the Sertoli cells, which, having a limited proliferative capacity, were unable to counteract the damage. In fact, only immature Sertoli cells are able to proliferate, and the definitive number of Sertoli cells is reached before adulthood.

For this reason, if the immature Sertoli cells are damaged in prepuberty, this leads to a reduced testicular volume—or in other words, a reduction in the number of mature Sertoli cells—leading to impaired spermatogenesis.

Few studies have investigated semen quality in adolescent cancer patients. One of the first, by Kliesh et al. (1996) [29], evaluated the hormone profile and semen quality in 12 patients aged 14–17 years compared with 17 patients aged 18–20 years and 210 aged over 20 years. This study found differences in hormone concentrations, with LH values significantly lower in the adolescents than in the adults. Testosterone levels, testicular volume, sperm concentration, the percentage of motile sperm and the percentage of abnormal forms were similar in all three groups. It should be noted that this study was limited by its small caseload.

Bahadur et al. (2002) [30] studied 238 patients with different neoplastic conditions aged between 12 and 19 years. They found a similar sperm concentration in all patients except those with acute myeloid leukaemia, in whom it was lower. The semen volume was also similar for all diseases except Ewing's sarcoma, which was associated with a higher volume, and acute lymphatic leukaemia, associated with a lower volume. Classification by age revealed that semen volume and sperm concentration both increased with increasing age.

Daudin et al. (2014) [31] conducted a retrospective multicentre study of 4345 patients aged 11 to 20 years with various types of neoplasia, namely lymphoma, leukaemia, germ cell tumours and osteosarcomas. A pre-treatment semen analysis revealed that the total sperm number was directly correlated with the patient's age. However, this was true only for patients with leukaemia, lymphoma or osteosarcoma, whereas there was an inverse correlation in patients with germ cell tumours, that is, the total sperm number decreased with increasing age. The percentage of motile sperm increased in proportion to age in both lymphoma and germ cell tumour patients, in line with the physiological maturation of the HPG axis.

A study conducted at the Division of Oncology at the Children's Hospital of Philadelphia [32] also evaluated pre-treatment semen quality in 261 adolescents with neoplastic diseases. Most azoospermic patients were found in the youngest age group (35% in patients aged 11–14 years), whereas 19.5% of 15-to-17-year-olds and 9.7% of 18-to-30-year-olds were azoospermic prior to treatment. There was no significant difference in the percentage of azoospermia between the different

diseases considered in the study. In contrast, in non-azoospermic patients, the sperm concentration was significantly lower in testicular tumour patients (17.8×10^6/mL) than in HL patients (45.5×10^6/mL). A recent retrospective study [33] investigated 197 adolescents aged between 11 and 19 years and 95 adults (aged over 19 years) with cancer. It found that 8.6% of the adolescents were azoospermic, and the prevalence of azoospermia dropped with age: only 3.2% of the 95 adult cancer patients were azoospermic. In the non-azoospermic patients, the median sperm concentration was 30 million/mL in adolescents and 39 million/mL in adults, while the median percentage of motile sperm was 39% in adolescents and 45% in adults (Table 15.1).

Table 15.1 Semen quality before treatment in adolescent cancer patients

Author	Year	Total patients	Patients <18 years	Age range	Baseline assessment	Post-treatment assessment	Results
Kliesch et al.	1996	239	12	>14	12 aged 14–17 years; 17 aged 18–20 years; 210 pts >20 years	N/A	LH significantly lower in adolescents than adults; testosterone values and testicular volumes similar in both groups, as are sperm concentration, motility and morphology.
Bahadur et al.	2002	238	123	>12	238 pts <20 yreas; 71 healthy donors	N/A	>85% of adolescents provided a semen sample adequate for future ART; semen parameters worse than those of healthy donors.
Daudin et al.	2015	4345	N/A	11–20	11–14 years; 15–17 years; 18–20 years	N/A	Leukaemias, lymphomas and sarcomas: Total sperm number directly associated with age group. Germ cell tumours: Total sperm number inversely associated with age group. Motility directly associated with age group only for testicular tumours and lymphomas.

(continued)

Table 15.1 (continued)

Author	Year	Total patients	Patients <18 years	Age range	Baseline assessment	Post-treatment assessment	Results
DiNofia et al.	2016	339	140	11–30	11–14 years; 15–17 years; 18–30 years	N/A	Azoospermic patients: 35% of 11–14-year-olds, 19.5% of 15–17-year-olds, 9.7% of 18–30-year-olds. Non-azoospermic patients: Sperm concentration lower in testicular tumour patients (17.8×10^6/mL) than in HL patients (45.5×10^6/mL).
Halpern et al.	2019	292	167	>11	11–19 years; >19 years	N/A	Azoospermic patients: 8.6% of adolescents vs. 3.2% of adults. Non-azoospermic patients: Median sperm concentration 30 million in adolescents vs. 39 million in adults; median motility 39% in adolescents vs. 45% in adults.

There is little information on pre- and post-antineoplastic treatment semen quality in adolescent patients. An early study by Meistrich et al. (1989) [34] investigated the post-treatment semen parameters of 37 osteosarcoma patients, but only six of these had also undergone pre-treatment semen analysis. Of these six, just two were aged 18 years or under, and their sperm concentration was within normal limits. Post-therapy analysis of all 37 patients was carried out at least 2 years after the end of a cisplatin, adriamycin, dacarbazine (PADIC) regimen; 13/37 were aged 18 years or under at the time of the therapy and of these, seven were normozoospermic, five oligozoospermic and one azoospermic. It should be stressed that the study is limited by the low caseload and the lack of data on pre-treatment semen quality.

In 2008, van Casteren et al. investigated pre- and post-treatment semen quality in 80 pubertal patients with various types of neoplastic disease [35]. The age range was 13.9–18.7 years. Semen samples suitable for cryopreservation were taken from 53 patients (66.7%), while 14 patients were azoospermic. Thirteen (16.3%) were unable to produce a sample by masturbation on the first attempt. This percentage is in line with other studies, which reported a masturbation failure rate in adolescents of 8.9–13.9% [30, 36, 37].

The sperm concentration in the 53 patients with acute lymphoblastic leukaemia was higher than in patients with HL, acute myeloid leukaemia, autoimmune diseases, brain tumours and solid tumours. The post-treatment semen quality was evaluated in 10 patients (median follow-up 1.1 year). Of these, four (two with sarcoma and two with HL) were azoospermic and the other six (three with germ cell tumour, two with HL and one with acute myeloid leukaemia) had motile sperm in their seminal fluid. The study therefore suggests that all boys aged 12 years or over who need to undergo gonadotoxic treatment should undergo semen cryopreservation, as they have entered the first phases of development (Table 15.2).

In some studies, semen quality was only investigated after treatment. Lopez Andreu et al. (2000) [38] investigated the semen quality of 43 adolescent cancer survivors at a median period of 13.6 years post-treatment. Azoospermia was found in 19% and severe oligoasthenoteratospermia in 5% of patients; only 37% were normozoospermic. Testicular hypotrophy and significantly increased serum FSH values were found in only half of the azoospermic patients. This suggests that cancer survivors should be routinely offered semen quality testing to establish their fertility regardless of their testicular volume or hormone concentrations.

Wilhelmsson et al. (2014) [39] also investigated post-treatment testicular function in a group of haemopoietic stem cell transplant patients after a mean of 13 years since the transplant. The patients were all under 18 years at the time of the transplant. Twenty-one of the 31 patients undergoing semen quality testing were azoospermic, and the remaining 10 had a mean total sperm number of 53 ± 76.9 × 10^6/ejaculate. Two of these 10 patients had had leukaemia, and the other eight non-malignant diseases. The 10 non-azoospermic patients were compared against the azoospermic patients for testicular volume (19 ± 7.4 mL vs. 9 ± 4.9 mL) and FSH concentration (9 ± 4.4 IU/L vs. 22 ± 20.5 IU/L). The authors therefore suggested that a 10-year-post-treatment testicular volume of 15 ml or more could be a marker for the presence of sperm in the seminal fluid, with a sensitivity of 80% and a specificity of 91%.

A larger testicular volume and low serum FSH can also be seen in patients treated with cyclophosphamide or busulfan conditioning in comparison with those treated with total body irradiation, as observed by Servitzoglou et al. (2015) [40] who investigated 171 patients with lymphoma a median of 9.3 years post-treatment. The authors measured FSH, LH and total testosterone, finding FSH above the upper limit of normal (fixed at 10 IU/L) in 42.1% of patients. Chemotherapy protocols containing cyclophosphamide, procarbazine and lomustine induced a significant increase in FSH, while no correlation was found between FSH or LH levels and the pubertal state at the time of diagnosis. HL patients showed an increased FSH correlated with exposure to procarbazine and influenced by the number of MOPP/OPPA chemotherapy cycles, thus highlighting that the gonadotoxic effect of alkylating agents is dose-dependent.

Non-neoplastic diseases: Zhao et al. (2019) [41] investigated, at least 1 year post-treatment, the hormone profile and semen parameters of five patients aged between 11 and 19 years who had undergone haemopoietic stem cell transplants for non-neoplastic diseases. Two patients were azoospermic, two were

Table 15.2 Semen quality before and after gonadotoxic treatment in adolescent cancer patients

Author	Year	Total subjects	Subjects <18 years	Age range	Baseline assessment	Post-treatment assessment[a]	Results
Meistrich et al.	1989	37	10	16–47	2 pts. 16–18 years; 4 pts >18 years	13 pts 16–18 years 24 pts >18 years	Baseline: 2 patients with osteosarcoma aged under 18 years, both with sperm concentrations within normal limits. Post-treatment: 13 patients with osteosarcoma aged under 18 years at time of treatment (at least 2 years post-treatment) of whom 1 azoospermic, 5 oligozoospermic and 7 normozoospermic.
Van Casteren et al.	2008	80	N/A	13.7–18.9	68 pts 13–18 years	10 pts 13–18 years	Baseline: Azoospermic or with immotile sperm: 17.5%; in non-azoospermic patients the sperm concentration was higher in patients with acute lymphoblastic leukaemia than in patients with HL, acute myeloid leukaemia, autoimmune diseases, brain tumours and solid tumours. Post-treatment: 11 patients recruited (median follow-up 3.4 years): 4 azoospermic (2 with sarcoma and 2 with HL), 7 non-azoospermic (3 with germ cell tumour, 2 with HL, 1 with acute myeloid leukaemia).

[a]Age at start of treatment

oligoasthenoteratozoospermic and one oligoteratozoospermic. Four of the patients were prepubertal or in puberty at the time of the treatment. This study demonstrated that stem cell transplants do not affect pubertal development but may have a severe impact on spermatogenesis.

Studies investigating the preservation of fertility in transgender patients treated in adolescence are also rare. Chen et al. (2017) [42] evaluated 13 patients who had received a specialist assessment concerning the preservation of fertility. Seven of these were male to female, of whom only four underwent semen cryopreservation. This is in line with other published studies, which show that semen cryopreservation is not common in adolescent transgender patients [43]. The World Professional Association for Transgender Health standards of care clearly recommend discussing reproductive options with patients before treatment. It should be noted that for male to female gender transitions, semen may still be cryopreserved after hormone therapy has begun, as long as the treatment is suspended for an adequate period.

15.5 Cryopreservation

Given all the above, cryopreservation of seminal fluid is a fundamental tool for preserving the fertility of patients of reproductive age who must undergo antineoplastic treatments that could impair spermatogenesis. It can also offer valuable psychological comfort to those about to undergo therapeutic protocols that may significantly affect their quality of life [44]. Cryopreservation enables cells and tissues to remain viable for an indefinite period of time through the use of cryogenic temperatures (−196 °C) at which all metabolic processes are suspended. Cell integrity depends on the simultaneous interaction of different biochemical reactions, the equilibrium of which is regulated by homeostatic control mechanisms. Long-term cell preservation is thus made possible by reducing these reactions to a minimum. Achieving such low temperatures stops chemical reactions from taking place, creating a state of ‘suspended animation’.

Specific procedures must be followed to avoid irreversible damage and death of the cryopreserved cells. Cell damage can be avoided by using various cryoprotective solutions. These have different chemical compositions but they are all water soluble and all have a concentration-dependent toxicity. They act directly on the cell membrane, establishing electrostatic interactions to lower the solution’s freezing point, modifying both the intra- and extra-cellular environment. Cryoprotectants are used to protect sperm from dehydration, higher salt concentrations and thermal shock, to safeguard the integrity of the cell membrane, especially its lipoprotein component, and to optimize osmolarity in the extracellular fluids. It is essential to avoid excessively rapid temperature rises during thawing, to enable the cells to recover their normal biological activities. The cell structure of human sperm enables them to tolerate a series of temperature variations. They can withstand damage caused by fast initial freezing (cold shock) due to their high membrane fluidity, itself due to polyunsaturated acids in the double lipid layer, and their very low cytoplasmic content and, hence, low water content (around 50%) [45].

15.6 Prepuberty

All the fertility preservation methods discussed above refer to patients who have already reached puberty and, hence, a sufficiently advanced sexual development to ensure adequate spermatogenesis. Preservation of fertility in prepubertal patients is a different matter. The damage induced by antineoplastic drugs affects sperm due to their rapid replication. Chemotherapeutic agents work by acting on mitotic division, causing sufficient damage to induce apoptosis. The greater the mitotic activity of the cell line, the greater the action of the chemotherapeutic agent.

Prepuberty is a period of hormonal quiescence and low testicular activity, so should be correlated with greater resistance to antineoplastic damage. However, impaired spermatogenesis has also been observed in adults who were treated with antineoplastics between the ages of 2 and 10 years. Aubier et al. (1989) [46] studied 30 cancer patients, of whom 19 were prepubertal at the time of gonadotoxic therapy. On carrying out a semen analysis a median of 9 years post-treatment, 12 of the 19 prepubertal patients were azoospermic. Dhabhar et al. (1993) [47] investigated semen quality in 26 HL patients who had been treated with COPP/MOPP prior to puberty, finding, a median of 6 years post-treatment, that 18 of these patients were azoospermic.

These two studies demonstrate that spermatogenesis is damaged even with prepubertal exposure to gonadotoxic therapies. It could therefore be hypothesized that there is some testicular activity even before puberty, because if the quiescence were absolute, exposure to antineoplastic treatments should not cause such marked damage. In this field too, relatively few published studies have investigated prepubertal testicular function and related hormone regulation mechanisms, and they are mainly based on animal models. Kelnar et al. (2002) [48] compared the testicles of marmosets treated with GnRH antagonists against untreated controls, beginning the treatment at the 25th week of life. This phase can be equated to the period of pituitary–gonadal axis quiescence in humans. The treated monkeys presented a reduced level of testicular maturation, demonstrating that quiescence was relative, not total. This leads to the hypothesis that the gonadal axis is blocked in prepuberty to make the Sertoli and Leydig cells less active and, consequently, less vulnerable to antineoplastic damage. However, the American Society of Clinical Oncology (ASCO) 2018 guidelines [49] do not recommend the use of hormone therapy in humans to preserve fertility.

The use of antiapoptotic agents such as sphingosine-1-phosphate [50] or immunomodulators such as AS101 have not proven effective in combatting gonadotoxicity [51]. The only possible strategy is therefore cryopreservation of the testicular tissue (TT), with the aim of reimplanting the spermatogonial stem cells (SSC) or immature testicular tissue, or using the cells after *in vitro* maturation [52]. SSC transplantation, TT grafting and recent advances in *in vitro* spermatogenesis reached in animals, have opened new possibilities to restore fertility in humans. However, these techniques are still experimental [53] and their efficiency depends on the amount of SSCs available for fertility restoration. Therefore, maintaining the number of SSCs is a critical step in human fertility preservation. Standardizing a successful cryopreservation method for TT and testicular cell suspensions is most important before any clinical application of fertility restoration could be successful [54].

15.7 Discussion

Cryopreservation of seminal fluid is the only validated method to ensure access to assisted reproduction techniques for all patients who have to undergo antineoplastic therapies. It is routinely offered to adults, but is less often suggested to pubertal patients. There are many reasons for this, including unawareness of medical staff, ethical and religious motives, objections of parents/guardians, doubts over prepubertal semen quality, and inability to masturbate. The available studies in the literature demonstrate that in most cases, even in young patients, there may be normozoospermia or in any case the presence of enough sperm to enable cryopreservation. The possibility that young patients are unable to collect the sample due to their disease or embarrassment is marginal, and should not prevent the procedure from being suggested. The need for rapid diagnosis and initiation of treatment often means that the protection of fertility is only a secondary consideration, but young patients and their families should be offered adequate counselling so that a decision on whether or not to cryopreserve the patient's semen can be made in full awareness of the facts. It has been demonstrated that there is a greater chance of collecting a cryopreservable semen sample not only from adolescents with a more advanced Tanner stage but also from those who have received adequate specialist counselling and feel supported by their family [55]. The 2018 ASCO guidelines [49] reiterate this concept, emphasizing that medical staff must describe the risks of the treatment for fertility to both adult and paediatric patients and, if the patient is interested, refer him to a specialist in reproductive medicine.

15.8 Conclusions

Semen cryopreservation should be offered to all patients about to undergo potentially gonadotoxic treatments, regardless of their age at the time of diagnosis, as it is currently the only strategy to preserve their future fertility. In most cases, the quality of seminal fluid in young patients is sufficient to provide an adequate sample. There is still little information on the effect of antineoplastic treatments during puberty and this aspect should be further investigated, to understand if the damage and potential recovery of spermatogenesis in paediatric patients is similar to that seen in young adults.

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References

1. Paoli D, Rizzo F, Fiore G, Pallotti F, Pulsoni A, Annechini G, Lombardo F, Lenzi A, Gandini L. Spermatogenesis in Hodgkin's lymphoma patients: a retrospective study of semen quality before and after different chemotherapy regimens. Hum Reprod. 2016;31(2):263–72.
2. Gandini L, Sgrò P, Lombardo F, Paoli D, Culasso F, Toselli L, Tsamatropoulos P, Lenzi A. Effect of chemo- or radiotherapy on sperm parameters of testicular cancer patients. Hum Reprod. 2006;21(11):2882–9. Epub 2006 Sep 22.
3. International Agency for Research on Cancer. Globocan; 2018. Available at http://gco.iarc.fr
4. Levine J, Canada A, Stern CJ. Fertility preservation in adolescents and young adults with cancer. J Clin Oncol. 2010;28(32):4831–41.
5. Mattawanon N, Spencer JB, Schirmer DA III, Tangpricha V. Fertility preservation options in transgender people: a review. Rev Endocr Metab Disord. 2018;19(3):231–42. https://doi.org/10.1007/s11154-018-9462-3.
6. Trevisan CM, Montagna E, de Oliveira R, Christofolini DM, Barbosa CP, Crandall KA, Bianco B. Kisspeptin/GPR54 system: what do we know about its role in human reproduction? Cell Physiol Biochem. 2018;49(4):1259–76. https://doi.org/10.1159/000493406. Epub 2018 Sep 11.
7. McLachlan RI. The endocrine control of spermatogenesis. Baillieres Best Pract Clin Endocrinol Metab. 2000;14:345–62.
8. Villanueva C, Argente J. Pathology or normal variant: what constitutes a delay in puberty? Horm Res Paediatr. 2014;82(4):213–21. https://doi.org/10.1159/000362600. Epub 2014 Jul 7.
9. Gandini L, Lenzi A. Biotecnologie della riproduzione umana: "Assetto morfologico strutturale e substrutturale dello spermatozoo". Carocci Editore. 2013:113–7.
10. Meinhardta A, Hedgerb MP. Immunological, paracrine and endocrine aspects of testicular immune privilege. Mol Cell Endocrinol. 2011;335(1):60–8.
11. O'Donnel L. Mechanisms of spermiogenesis and spermiation and how they are disturbed. Spermatogenesis. 2015;4(2):e979623. eCollection 2014 May–Aug.
12. Barr RD, Ferrari A, Ries L, Whelan J, Bleyer WA. Cancer in adolescents and young adults: a narrative review of the current status and a view of the future. JAMA Pediatr. 2016;170(5):495–501. https://doi.org/10.1001/jamapediatrics.2015.4689.
13. Associazione Italiana di Oncologia Medica. Manuale metodologico, linee guida AIOM 2019; 2019.
14. Ghezzi M, Berretta M, Bottacin A, Palego P, Sartini B, Cosci I, Finos L, Selice R, Foresta C, Garolla A. Impact of Bep or carboplatin chemotherapy on testicular function and sperm nucleus of subjects with testicular germ cell tumor. Front Pharmacol. 2016;7:122. https://doi.org/10.3389/fphar.2016.00122. eCollection 2016.
15. European Network-Pediatric Hodgkin Lymphoma Study Group. Second international intergroup study for classical Hodgkin lymphoma in children and adolescent; 2015.
16. Zahid U, Akbar F, Amaraneni A, Husnain M, Chan O, Riaz IB, McBride A, Iftikhar A, Anwer F. A review of autologous stem cell transplantation in lymphoma. Curr Hematol Malig Rep. 2017;12(3):217–26. https://doi.org/10.1007/s11899-017-0382-1.
17. Meyers PA. Systemic therapy for osteosarcoma and Ewing sarcoma. Am Soc Clin Oncol Educ Book. 2015:e644–7. https://doi.org/10.14694/EdBook_AM.2015.35.e644.
18. Garolla A, Pizzato C, Ferlin A, Carli MO, Selice R, Foresta C. Progress in the development of childhood cancer therapy. Reprod Toxicol. 2006;22(2):126–32.
19. Unger CA, et al. Transl Androl Urol. 2016;5(6):877–84. https://doi.org/10.21037/tau.2016.09.04.
20. Cheng PJ, Pastuszak AW, Myers JB, Goodwin IA, Hotaling JM. Fertility concerns of the transgender patient. Transl Androl Urol. 2019;8(3):209–18. https://doi.org/10.21037/tau.2019.05.09.

21. Brignardello E, Felicetti F, Castiglione A, BiasinE NA, Ciccone G, Fagioli F, Corrias A. Gonadal status in long-term male survivors of childhood cancer. J Cancer Res Clin Oncol. 2016;142:1127–32.
22. Ghezzi M, De Toni L, Palego P, Menegazzo M, Faggian E, Berretta M, Fiorica F, De Rocco PM, Foresta C, Garolla A. Increased risk of testis failure in testicular germ cell tumor survivors undergoing radiotherapy. Oncotarget. 2017;9(3):3060–8.
23. Green DM, Liu W, Kutteh WH, Ke RW, Shelton KC, Sklar CA, Chemaitilly W, Pui CH, Klosky JL, Spunt SL, Metzger ML, Srivastava D, Ness KK, Robison LL, Hudson MM. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. Lancet Oncol. 2014;15:1215–23.
24. Rowley MJ, Leach DR, Warner GA, Heller CG. Effect of graded doses of ionizing radiation on the human testis. Radiat Res. 1974;59:665–78.
25. Fajardo LF. Is the pathology of radiation injury different in small vs large blood vessel? Cardiovascular radiation medicine. 1999.
26. Bramswig JH, Heimes U, Heriermann E, Schlegel W, Nieschlag E, Schellong G. The effects of different cumulative doses of chemotherapy on testicular function. Results in 75 patients treated for Hodgkin's disease during childhood or adolescence. Cancer. 1990;65:1298–302.
27. Stukenborg JB, Jahnukainen K, Hutka M, Mitchell RT. Cancer treatment in childhood and testicular function: the importance of the somatic environment. Endocr Connect. 2018;7(2):R69–87. https://doi.org/10.1530/EC-17-0382. Epub 2018 Jan 19.
28. de Rooij DG, van de Kant HJ, Dol R, Wagemaker G, van Buul PP, van Duijn-Goedhart A, de Jong FH, Broerse JJ. Long-term effects of irradiation before adulthood on reproductive function in the male rhesus monkey. Biol Reprod. 2002;66(2):486–94.
29. Kliesch S, Behre HM, Jürgens H, Nieschlag E. Cryopreservation of semen from adolescent patients with malignancies. Med Pediatr Oncol. 1996;26:20–7.
30. Bahadur G, KLE L, Hart R, Wafa R, Ashraf A, Jaman N, Mahmud S, Oyede AW. Semen quality and cryopreservation in adolescent cancer patients. Hum Reprod. 2002;17(12):3157–61.
31. Daudin M, Rives N, Walschaerts M, Drouineaud V, Szerman E, Koscinski I, Eustache F, Saïas-Magnan J, Papaxanthos-Roche A, Cabry-Goubet R, Brugnon F, Le Lannou D, Barthélémy C, Rigot JM, Fréour T, Berthaut I, Giscard d'Estaing S, Touati F, Mélin-Blocquaux MC, Blagosklonov O, Thomas C, Benhamed M, Schmitt F, Kunstmann JM, Thonneau P, Bujan L. Sperm cryopreservation in adolescents and young adults with cancer: results of the French national sperm banking network (CECOS). Fertil Steril. 2015;103(2):478–86.e1. https://doi.org/10.1016/j.fertnstert.2014.11.012. Epub 2014 Dec 17.
32. DiNofia A, Wang X, Yannekis G, Ogle S, Hobbie WL, Carlson CA, Ginsberg JP. Analysis of semen parameters in a young cohort of cancer patients. Pediatr Blood Cancer. 2017;64(2):381–6. https://doi.org/10.1002/pbc.26221. Epub 2016 Sep 13.
33. Halpern JA, Thirumavalavan N, Kohn TP, Patel AS, Leong JY, Cervellione RM, Keene DJB, Ibrahim E, Brackett NL, Lamb DJ, Ramasamy R. Distribution of semen parameters among adolescent males undergoing fertility preservation in a multicenter international cohort. Urology. 2019;127:119–23. https://doi.org/10.1016/j.urology.2019.01.027. Epub 2019 Feb 13.
34. Meistrich ML, Chawla SP, Da Cunha MF, Johnson SL, Plager C, Papadopoulos NE, Lipshultz LI, Benjamin RS. Recovery of sperm production after chemotherapy for osteosarcoma. Cancer. 1989;63:2115–23.
35. van Casteren NJ, Dohle GR, Romijn JC, de Muinck Keizer-Schrama SM, Weber RF, van den Heuvel-Eibrink MM. Semen cryopreservation in pubertal boys before gonadotoxic treatment and the role of endocrinologic evaluation in predicting sperm yield. Fertil Steril. 2008;90(4):1119–25. Epub 2007 Oct 1.
36. Muller J, Sonksen J, Sommer P, Schmiegelow M, Petersen PM, Heilman C, Schmiegelow K. Cryopreservation of semen from pubertal boys with cancer. Med Pediatr Oncol. 2000;34:191–4.
37. Postovsky S, Lightman A, Aminpour D, Elhasid R, Peretz M, Arush MW. Sperm cryopreservation in adolescents with newly diagnosed cancer. Med Pediatr Oncol. 2003;40(6):355–9.

38. Lopez Andreu JA, Fernandez PJ, Ferris i Tortajada J, Navarro I, Rodriguez-Ineba A, Antonio P, Muro MD, Romeu A. Persistent altered spermatogenesis in long-term childhood cancer survivors. Pediatr Hematol Oncol. 2000;17:21–30.
39. Wilhelmsson M, Vatanen A, Borgstrom B, Gustafsson B, Taskinen M, Saarinen-pihkala UM, Winiarski J, Jahnukainen K. Adult testicular volume predicts spermatogenetic recovery after allogeneic HSCT in childhood and adolescence. Pediatr Blood Cancer. 2014;61:1094–100.
40. Servitzoglou M, et al. Dose-effect relationship of alkylating agents on testicular function in male survivors of childhood lymphoma. Pediatr Hematol Oncol. 2015;32:613–23.
41. Zhao J, Beebe K, Magee K, Salzberg D, Stahlecker J, Miller HK, Adams RH, Lipskind S, Walsh A, Mirea L, Ngwube A. Adolescent male fertility following reduced-intensity conditioning regimen for hematopoietic stem cell transplantation in non-malignant disorders. Pediatr Transplant. 2019;23(6):e13496. https://doi.org/10.1111/petr.13496. Epub 2019 May 23.
42. Chen D, Simons L, Johnson EK, Lockart BA, Finlayson C. Fertility preservation for transgender adolescents. J Adolesc Health. 2017;61(1):120–3. https://doi.org/10.1016/j.jadohealth.2017.01.022. Epub 2017 Mar 28.
43. Nahata L, Tishelman AC, Caltabellotta NM, Quinn GP. Low fertility preservation utilization among transgender youth. J Adolesc Health. 2017;61(1):40–4. https://doi.org/10.1016/j.jadohealth.2016.12.012. Epub 2017 Feb 1.
44. Pacey AA, Merrick H, Arden-Close E, Morris K, Barton LC, Crook AJ, Tomlinson MJ, Wright E, Rowe R, Eiser C. Monitoring fertility (semen analysis) by cancer survivors who banked sperm prior to cancer treatment. Hum Reprod. 2012;27(11):3132–9. https://doi.org/10.1093/humrep/des300. Epub 2012 Aug 27.
45. Lenzi A. Lombardo F. Biotecnologie della riproduzione umana; 2012.
46. Aubier F, Flamant F, Brauner R, Caillaud JM, Chaussain JM, Lemerle J. Male gonadal function after chemotherapy for solid tumors in childhood. J Clin Oncol. 1989;7:304–9.
47. Dhabar BN, Malhotra H, Joseph R, Garde S, Bhasin S, Sheth A, Advani SH. Gonadal function in prepubertal boys following treatment for Hodgkin's disease. Am J Pediatr Hematol Oncol. 1993;15:306–10.
48. Kelnar CJ, McKinnell C, Walker M, Morris KD, Wallace WH, Saunders PT, Fraser HM, Sharpe RM. Testicular changes during infantile 'quiescence' in the marmoset and their gonadotrophin dependence: a model for investigating susceptibility of the prepubertal human testis to cancer therapy? Hum Reprod. 2002;17(5):1367–78.
49. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, Wallace WH, Wang ET, Loren AW. Fertility preservation in patients with cancer: ASCO clinical practice guideline update. J Clin Oncol. 2018;36(19):1994–2001. https://doi.org/10.1200/JCO.2018.78.1914. Epub 2018 Apr 5.
50. Suomalainen L, Hakala JK, Pentikäinen V, Otala M, Erkkilä K, Pentikäinen MO, Dunkel L. Sphingosine-1-phosphate in inhibition of male germ cell apoptosis in the human testis. J Clin Endocrinol Metab. 2003;88:5572–9.
51. Carmely A, Meirow D, Peretz A, Albeck M, Bartoov B, Sredni B. Protective effect of the immunomodulator AS101 against cyclophosphamide induced testicular damage in mice. Hum Reprod. 2009;24:1322–9.
52. Radaelli MRM, Almodin CG, Minguetti-Câmara VC, Cerialli PMA, Nassif AE, Gonçalves AJ. A comparison between a new vitrification protocol and the slow freezing method in the cryopreservation of prepubertal testicular tissue. JBRA Assist Reprod. 2017;21(3):188–95. https://doi.org/10.5935/1518-0557.20170037.
53. Jurewicz M, Hillelsohn J, Mehta S, Gilbert BR. Fertility preservation in pubertal and pre-pubertal boys with cancer. Pediatr Endocrinol Rev. 2018;15(3):234–43. https://doi.org/10.17458/per.vol15.2018.jhmg.fertilitypubertalboys.
54. Onofre J, Baert Y, Faes K, Goossens E. Cryopreservation of testicular tissue or testicular cell suspensions: a pivotal step in fertility preservation. Hum Reprod Update. 2016;22(6):744–61.
55. Klosky JL, Lehmann V, Flynn JS, Su Y, Zhang H, Russel KM, Schenck LAM, Schover MR. Patient factors associated with sperm cryopreservation among at-risk adolescent newly diagnosed with cancer. Cancer. 2018;124:3567–75.