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# Frontiers in Gynecological Endocrinology

Volume 4: Pediatric and Adolescent  
Gynecological Endocrinology



INTERNATIONAL SCHOOL  
OF GYNECOLOGICAL  
AND REPRODUCTIVE  
ENDOCRINOLOGY

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Charles Sultan • Andrea R. Genazzani  
Editors

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## Preface

We are delighted to announce the publication of another important issue in the International Society of Gynecological Endocrinology series.

This volume 4 presents important clinical and scientific data on subjects of major clinical and physiological interest, namely adolescent gynecology, presented by international opinion leaders during the ISGRE Summer School organized in Montpellier, France.

The adolescent girl is no longer a child, but not yet an adult and, for this reason, the management of adolescent endocrinology and gynecological diseases is quite specific.

Pediatric and adolescent gynecology is now a well recognized field of medicine and surgery, at the crossroads of most of the specific problems of developmental and reproductive issues. This specific area has always been of particular interest to physicians in the interface of pediatrics, gynecology, and endocrinology.

This volume presents new clinical and scientific data and includes topics of great interest, including anomalies of pubertal development, and other subjects of direct clinical relevance, such as disorders of menstrual cycles, sexuality, contraception, and pregnancy in adolescents.

This is a very well balanced edition. We are convinced that this new addition to the series will prove of great value to all pediatricians, gynecologists, endocrinologists, psychiatrists, surgeons, and public health specialists concerned with adolescent gynecology.

Montpellier, France  
Pisa, Italy

Charles Sultan  
Andrea R. Genazzani

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# Contents

<b>1</b>	<b>Dimorphism of Human Brain: The Basis of the Gender Differences</b> . . . . .	1
	Andrea R. Genazzani, Andrea Giannini, and Tommaso Simoncini	
<b>2</b>	<b>Management of Disorders of Sex Development</b> . . . . .	9
	Charles Sultan, Laura Gaspari, Nicolas Kalfa, and Françoise Paris	
<b>3</b>	<b>Central Precocious Puberty: From Diagnosis to Treatment</b> . . . . .	25
	Juliane Léger and Jean-Claude Carel	
<b>4</b>	<b>Management of Peripheral Precocious Puberty in Girls</b> . . . . .	39
	Charles Sultan, Laura Gaspari, Nicolas Kalfa, and Françoise Paris	
<b>5</b>	<b>Premature Pubarche</b> . . . . .	49
	Charles Sultan, Laura Gaspari, Nicolas Kalfa, and Françoise Paris	
<b>6</b>	<b>Maturation of the Hypothalamic-Pituitary-Ovarian Axis and the Onset of Puberty</b> . . . . .	57
	Françoise Paris, Laura Gaspari, and Charles Sultan	
<b>7</b>	<b>From Primary Hypergonadotropic Amenorrhea to “POI”: Aetiology and Therapy</b> . . . . .	67
	Vincenzina Bruni, Sandra Bucciantini, and Simona Ambroggio	
<b>8</b>	<b>Functional Hypothalamic Amenorrhea as Stress Induced Defensive System</b> . . . . .	111
	Alessandro D. Genazzani, Giulia Despini, Riccardo Bonacini, and Alessia Prati	
<b>9</b>	<b>Amenorrhoea and Anorexia Nervosa in Adolescent Girls</b> . . . . .	119
	Sebastien Guillaume, Laurent Maimoun, Charles Sultan, and Patrick Lefebvre	
<b>10</b>	<b>The Long-Term Cardiovascular Risks Associated with Amenorrhea</b> . . . . .	127
	Tommaso Simoncini, Andrea Giannini, and Andrea R. Genazzani	

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<b>11</b>	<b>Delayed Puberty: Impact on Female Fertility</b> . . . . .	133
	Martin Birkhaeuser	
<b>12</b>	<b>Premature Ovarian Failure in Adolescence and Young Adults: From Diagnosis to Therapy and Follow-up for Fertility Preservation</b> . . . . .	181
	Andrea Giannini, Andrea R. Genazzani, and Tommaso Simoncini	
<b>13</b>	<b>Emergency Contraception</b> . . . . .	189
	Sharon Cameron	
<b>14</b>	<b>Adolescent Pregnancy and Contraception</b> . . . . .	199
	Matan Elami-Suzin and Joseph G. Schenker	
	<b>Index</b> . . . . .	229

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# Dimorphism of Human Brain: The Basis of the Gender Differences

1

Andrea R. Genazzani, Andrea Giannini,  
and Tommaso Simoncini

For mammals, sexual differentiation starts at conception when a fetus inherits a couple of heterologous (XY) or homologous (XX) sex chromosomes. Until the sixth week of gestation embryonal gonadal development is bi-potential, than, according to genetic sex, embryonal gonads differentiate in testes or ovaries. In presence of Y chromosome, fetus will develop testes; hormonal products of the testes, mainly testosterone, then induce the male phenotype by early permanent programming or organizational effects and later transient acute or activational effects, which disappear after withdrawal of the hormones. In the absence of Y chromosome, the fetus develops ovaries, and in the absence of male-like levels of testosterone, the female phenotype emerges. The activating effects of ovarian hormones together with endocrine and exogenous influences enhance female characteristics at puberty and beyond determining phenotyping sex [1].

Several studies affirm that gonadal determination may be controlled by various genes, such as gene Sex-determining Region Y (SRY) on short arm of Y chromosome (Yp11) involved in gonadal differentiation to testes. SRY codes for a protein containing sequence High Motility Group box (HMG) which is transient expressed in cells planned to become Sertoli cells. Moreover, gonadal determination seems to be influenced by genes, in particular, Wilm's tumor-related gene-1 (WT-1) and steroidogenic factor-1 (SF-1). Mutations of these genes in both male and female sexes are involved in the pathogenesis of agenesis and dysgenesis gonadal syndrome. It is evident that primordial gonads are inclined to an intrinsic development to female gonads and only the presence of genetic factors on Y chromosome or activated by developing testes are able to masculinize. Currently only one gene coded on X chromosome (Xp21.3), dosage-sensitive sex reversal-adrenal hypoplasia congenital on

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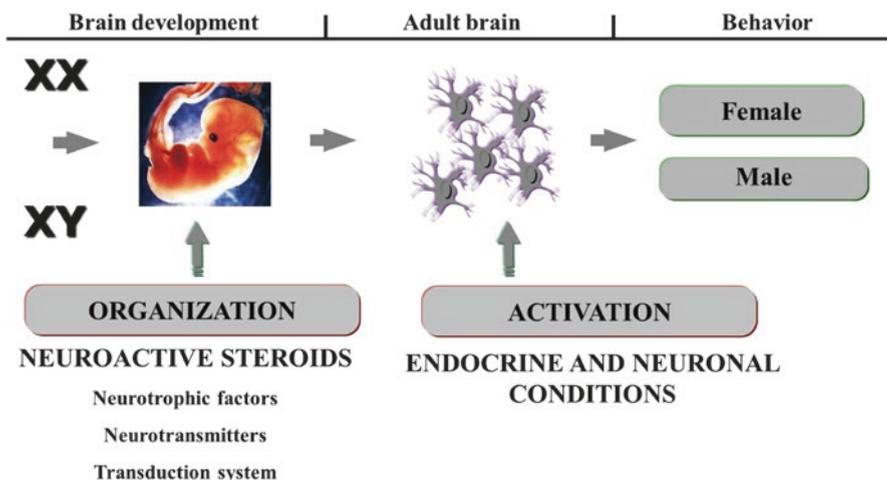
the X chromosome (DAX-1) seems to prevent possible gonadal evolution to testes in female mammals [2].

Sex chromosomes play a direct role in establishing sex differences throughout the body including the brain, thus some differences depend on chromosomal constitution and are gonadal independent. Nonetheless, increasing evidences suggest that for the large majority of neural sex differences, the modifications in gonadal hormones seem to play a dominant role. Sex differences in the central nervous system (CNS), probably may develop to direct actions of gonadal steroids which can determine neural tissue differentiation, moreover, sexual differentiation of CNS occur later than gonadal differentiation thus both processes can be independently influenced [3].

Steroid hormones synthesized by the gonads and adrenal glands easily cross the blood–brain and the blood–nerve barriers and rapidly accumulate within the nervous tissues, except for their conjugated forms such as the steroid sulfates, which cannot easily enter the brain. Neurons and glial cells possess enzymes necessary for progesterone, testosterone, dehydroepiandrosterone (DHEA), and estradiol metabolism (aromatase, 5- $\alpha$  reductase (5 $\alpha$ -R), mainly in neurons, 3- $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ -HSD), mainly in type 1 astrocytes). The activities of these steroid-metabolizing enzymes are strongly influenced by the differentiation process of the precursor stem cells into terminally differentiated CNS cells. Neurons and glial cells coordinately metabolize steroid hormones, thus forming a functional unit; as both the endocrine glands and the local metabolism contribute to the pool of steroids present in the nervous tissues and the sex and age-dependent changes in circulating levels of steroid hormones may reflect changes in brain levels. While steroid-metabolizing enzymes induce the CNS to be able to modify circulating steroids, the CNS is also able to synthesize steroids from cholesterol, at least in part, independently of peripheral steroidogenic glands secretion leading to the production of a series of potent steroidal compounds. These brain-produced steroids have been named “neurosteroids”, and have been found to exert important regulatory actions on neurons and glial cells [4] (Fig. 1.1).

The so called “neurosteroids” influence the neurobiology of sexual function acting by genomic or non-genomic effects. Genomic actions of neurosteroids are carried out directly interacting with their receptors at nuclear membrane level or indirectly throughout their effects on neuropeptides (oxytocin, beta-endorphin, etc.), neurotransmitters (dopamine, serotonin), and neurosteroids metabolites (mainly allopregnanolone). Non-genomic actions are mediated through integrate or associated membrane receptors and the activation of intracellular cascades of events determining rapid neuronal and pituitary activation via biochemical pathways of AMPc and MAP-kinases; thus resulting in a modulation of Ca<sup>2+</sup> channels and exerting neuroprotective effects in contrast to neurotoxins and oxidative stress [5].

Estrogens have long been known to play a crucial role on coordinating many neuroendocrine events that control sexual development, sexual behavior, and reproduction. 17- $\beta$ -estradiol is the primary biologically active form of estrogen in mammals which is critical for sexual differentiation of the brain, indeed it organizes neural circuits and regulates apoptosis of neurons leading to long-term differences



**Fig. 1.1** Brain sexual differentiation is a multisignaling process presenting sex steroids as key modulators in different steps

in the male and the female brain. In addition to its role in development, estradiol prevents neuronal cell death in a variety of brain injury models, modulates learning and memory, and promotes the formation of synapses as well as cellular apoptosis. The physiological effects resulting from estradiol actions in target tissues are mediated primarily by two intracellular receptors ER $\alpha$  and ER $\beta$ . Both estrogen receptors together with progesterone receptors A and B (PR-A, PR-B) and androgens receptors (ARs) are observed in neurons and glia in the brain and are expressed throughout the brain with distinct patterns in different brain regions and with different levels of expression in males and females during development and in adulthood. Consequently, sexual dimorphism of human brain seems to be characterized by functional and structural differences. Functional differences are determined by hormonal and enzymatic actions or pathway modulating masculinization or feminization of different regions of CNS, while structural differences are determined by different distribution of ERs, ARs, PRs, enzymatic isoforms, and neuronal population in different cerebral areas [6].

Relating to functional differences, as previously mentioned, 17- $\beta$ -estradiol is a crucial biologically active form of steroid in mammals involved in sexual differentiation of the brain, nonetheless, several experimental and pre-clinical data suggested that testosterone (T), acting on the brain, seems to regulate reproductive function, sexuality, and emotional behaviors in both sexes in a different gender-related fashion. In addition, T exerts analgesic and anxiolytic properties, affects mood and cognition, and promotes synaptic plasticity in the rat model. T also prevents neuronal death in different experimental models of neurodegeneration, and decreased T levels in plasma may represent a risk factor for the development of neurodegenerative diseases in humans. T brain effects may be directed or modulated by its metabolites, therefore, T can be aromatized to estrogen or metabolized

to dihydrotestosterone (DHT) by 5 $\alpha$ -reductase (5 $\alpha$ -R), and DHT can be further reduced by 3-hydroxysteroid dehydrogenase (3-HSD) to 3-androstanediol (3-diol), a neurosteroid Gamma-Aminobutyric Acid-Aergic (GABA-A) agonist with anxiolytic properties. These two enzymatic pathways, aromatase and 5 $\alpha$ -R-3HSD, are widely distributed in CNS, affecting reproductive (i.e., hypothalamus) and non-reproductive function (i.e., hippocampus, cortex) of gonadal steroids [7].

Interestingly, DHEA and its sulfate metabolite DHEA-S may act on CNS differentiation directly, by modulating several activities in different neuronal populations or as substrate for the conversion in T and DHT in such CNS target regions of androgens and estrogens. In this view, it is remarkable to highlight that brain DHEA and DHEA-S concentrations are 5–6 times higher than peripheral concentrations and several pre-clinical studies demonstrated the presence of steroidal precursors such as cholesterol and lipid derivatives in mammalian brain. The effects of DHEA and DHEA-S on CNS are mediated by direct interaction with GABA-A receptors, thus blocking Cl<sup>-</sup> channels in a dose-dependent manner and resulting in increased neuronal excitability. Experimental data also suggested putative effects of DHEA on N-methyl-D-aspartate (NMDA) and sigma ( $\lambda$ ) receptors. DHEA administration to gonadectomized rats increased concentrations of neurosteroids not related to DHEA metabolism such as allopregnanolone (3-hydroxy-5-pregnan-20-one) (AP), in the hippocampus, in the hypothalamus, in pituitary, and in peripheral circulation and improved mnemonic ability, thus suggesting neurotrophic effects on neurons and glia cells. Moreover, gonadectomy reduced synaptic density on dendritic spines and CA1 pyramidal neurons in both male and female rats while T and DHT administrations are able to reestablish that. In this view, it has been hypothesized that the preservation of physiologic synaptic density may be an androgen-dependent process which can be elicited with different gender-specific mechanisms since that in male rats, contrary to female rats, it is not necessary synthesis of intermediate estrogens. Moreover, the ability to restore hippocampal synaptic density is not directly related to androgenic potency since that it has been demonstrated that DHEA and DHT stimulation activity on dendritic density are similar [8].

Estrogens can also increase the activity of the enzymatic pathway (5 $\alpha$ -R)—3-hydroxysteroid-oxidoreductase, which converts progesterone into 5-dihydroprogesterone and AP, respectively. Progesterone and synthetic progestins can affect brain and peripheral content of AP divergently, both in humans and in experimental animals, suggesting distinct hormonal effects on the enzymatic pathways involved in the synthesis and release of these neurosteroids [9]. AP is a neurosteroid produced by the central nervous system, adrenals, and ovaries. AP is a 3-, 5- reduced metabolite of progesterone by the complex 5 $\alpha$ -R-3HSD. It is a potent endogenous steroid that rapidly affects the excitability of neurons and glia cells through direct modulation of the GABA-A receptors activity. AP exerts neuropharmacological properties with hypnotic/ sedative, anxiolytic, anesthetic, analgesic, and anticonvulsive function [10]. In addition, AP exhibit neurotrophic/neuroprotective actions, reducing cell death, gliosis, and functional deficits after traumatic brain injury in rats and in experimental models of Alzheimer's disease, enhancing myelination/remyelination process. Interestingly, several experimental studies suggest that

AP positively affects all aspects of sociosexual activities, enhancing exploratory, antianxiety, social, and sexual function [11].

Genazzani and collaborators, in experimental work on male and female gonadectomized rats model, studied the effects of the administration of subcutaneous T at the dose of 10–100  $\mu\text{g}/\text{kg}/\text{day}$  for female rats, and 1–5  $\text{mg}/\text{kg}/\text{day}$  for male rats, or DHT at the doses of 1–10 and 100  $\mu\text{g}/\text{kg}/\text{day}$  for females, and 0, 1–1 and 5  $\text{mg}/\text{kg}/\text{day}$  for males, or E2V (0.05  $\text{mg}/\text{kg}/\text{day}$ ). Ovariectomy (OVX) and orchidectomy (OCX) induced a significant decrease in AP in frontal and parietal lobe, hippocampus, hypothalamus, anterior pituitary, as well as in serum. In OVX rats, T replacement, as well as E2V, significantly increased AP content in all brain areas and in peripheral circulation, whereas in OCX, T and E2V did not actively result in influencing AP concentration in frontal and parietal lobe, while it produced a significant rise in AP levels in the hippocampus, hypothalamus, anterior pituitary, and serum. Conversely, DHT replacement had no effect on AP levels anywhere or at any administered dose, either in males or in female rats. The author concluded that gender difference and T therapy may affect brain AP synthesis/release during the reproductive aging. This effect becomes particularly evident in the brain of OVX animals, where the content of this specific neurosteroid is much more responsive than male animals to testosterone replacement. Moreover, it has been suggested that T administration should be, at least in part, dependent on a gender difference in the aromatase activity; therefore, a sexually dimorphic activity of aromatase is widely described during the fetal and postnatal life, and also, the expression and the activity of this enzyme were dimorphically affected by gonadectomy and by T replacement, supporting the hypothesis of differential enzymatic regulation also for neurosteroidogenesis [12].

The same group, focusing the attention on the homeostasis of CNS and the role of neurosteroids in the hormonal setting, investigated the gender response of endogenous opioid system to hormonal changes. The endogenous opioid system modulates responses to stress, learning and memory acquisition; it is involved in emotional regulation, pain mechanisms, and the reward system, and it is altered in various pathological states.  $\beta$ -endorphin ( $\beta$ -END) is the endogenous opiate that has received the most attention. It has been speculated that  $\beta$ -END may play a key role in the mechanism of sexual arousal and pleasure in both sexes and its effects seems to be inversely dose-related therefore, the administration of low physiological dose of opiate have facilitative effects and high dose exhibit inhibitory effects. Similarly, the administration of naloxone at low doses to women was able to enhance pleasure during orgasm while higher doses show contrary effects, reducing sexual arousal and orgasmic pleasure. In addition, the administration of exogenous opiates can induce an intense feeling of pleasure which has been associated to orgasm, followed by a state of relaxation and calm. Gonadal steroids are increasingly recognized as crucial factors modulating the endogenous opioid system in both sexes, suggesting the presence of additional hormone-related, neurobiological mechanisms for gender difference in brain function. The administration of above-mentioned doses of T, DHT, and E2V to male and female gonadectomized rats showed relevant results. T administration to OVX rats exerted a powerful impact on the endogenous endorphin system; therefore, it enhanced  $\beta$ -END concentration not only at hypothalamic level but also in several hypothalamic

structures, affecting the activity of endorphinergic neurons in the hippocampus as well as in the frontal and parietal lobes. In contrast, the endorphin content of these hypothalamic structures was not affected in male rats by orchidectomy or by any steroid replacement therapy; thus suggesting that the cerebral structures receiving the endorphinergic peptide exhibit a sex-based difference in opioid system sensitivity to gonadal hormones. Since the effect of estrogen treatment was the same for both sexes, the physiological basis for this sex difference in  $\beta$ -END sensitivity to T therapy might depend, at least in part, on a sex difference in aromatase activity; the authors concluded that sexually dimorphic aromatase activity characterized fetal and postnatal life and this study highlighted that the expression and the activity of the enzyme were dimorphically affected by castration and T replacement [13].

As previously mentioned, structural differences are determined by different distribution of ERs, ARs, PRs, enzymatic isoforms, and neuronal population in different cerebral areas. In particular, ER $\alpha$  and ER $\beta$  are expressed in the amygdala, the hippocampus, in different areas of cerebral cortex, in the cerebellum, and in the hypothalamus where estrogen may determine structural and behavioral sex-specific characteristics.

Two brain regions that show robust estradiol-induced organizational changes are the preoptic area (POA) and medial basal hypothalamus (MBH), both of which are critical for sexual behavior. Estradiol-induced organizational changes are perhaps best exemplified within the POA, an area containing the medial preoptic nucleus (MPN), the sexually dimorphic nucleus of the preoptic area (SDN-POA), and the anteroventral periventricular nucleus (AVPV). The MPN is critical to the control of male sexual behavior and the SDN-POA is a sub-region within this nucleus that has been implicated in partner preference [14]. The AVPV is important for gonadotropin secretion and is believed to be the source of control of the LH surge essential for ovulation in adult females [15]. In the perinatal male brain, estradiol derived from testosterone stimulates opposing events in the SDN and AVPV, protecting neurons within the SDN-POA from apoptosis by enhancing NMDA receptor expression, while provoking expression of proapoptotic proteins such as TRIP, Bad, and Bax to induce apoptosis in the AVPV [16, 17]. In addition to modulating cell death, estradiol also mediates sex differences in synaptic patterning in the MPN by inducing synthesis of the cyclooxygenase enzymes, COX-1 and COX-2, thereby increasing the production of prostaglandin-E2 [18]. Acting via the EP2 and EP4 receptors, PGE2 activates protein kinase A and allows for glutamate-induced activation of AMPA receptors and formation of dendritic spines, the postsynaptic contact points for excitatory synapses [19]. Ultimately, the increased production of PGE2 in the male brain results in a two to three times higher density of dendritic spine synapses compared to females, and interestingly, this higher spine density positively correlates with the degree of masculinization of sexual behavior [20]. Thus, three key cellular responses, cell survival, cell death, and synaptogenesis, are all mediated by estradiol within one brain region, the preoptic area. The divergent effects of estradiol are mediated via the estrogen receptor (ER), in particular the ER $\alpha$  isoform. In addition to exerting organizational effects on the physiology of the POA, estradiol also induces permanent changes in synaptic connectivity in the MBH. The ventromedial nucleus of the hypothalamus (VMN) is a key region for regulating female sexual behavior. Within the VMN, male neurons have

twice the number of dendritic spines and more dendritic branches than females as a result of neonatal hormone exposure. Estradiol produces sex differences in synaptic organization in this region by rapid activation of PI3-kinase which enhances glutamate release from presynaptic cells, thus provoking dendritic spine outgrowth from postsynaptic neurons. Here, too, the ER $\alpha$  isoform is the critical mediator of estradiol action, although the initiating steps in the signal transduction cascade appear to begin at the membrane via rapid activation of PI3 kinase, and more interestingly, the requirement for ER is restricted to the presynaptic membrane despite the induction of changes in neuronal morphology within the postsynaptic neuron. The enduring organizational changes produced by estradiol within the neonatal brain enable circulating gonadal hormones in the adult to activate sexually differentiated brain regions, such as the POA and the MBH, in a sex-specific manner. Thus, in adulthood, estrogens and progesterone act on a female brain to regulate pulsatile LH release and induce estrous cyclicity and female sexual receptivity, whereas testosterone reaches a masculinized adult brain to activate male sexual behavior [19].

As a concluding remark, some sex differences may cause differences in function, in other cases sex differences exist to ensure that function is similar in males and females. In other words, some sex differences compensate for physiological differences that if left unchecked may be maladaptive. Sex differences that perform a compensatory role may become evident when the system is perturbed. A specific example comes from looking at something as apparently basic as cell death programs in neurons. In response to hypoxia, or other conditions mimicking stroke, neurons die in both sexes of rats and mice [21].

A sex difference in a physiological process is one of nature's ways of demonstrating how that process can be modulated. Sex differences in the vulnerability to a disease may similarly reveal factors that are protective in one sex, thereby suggesting strategies to prevent or ameliorate that disease. This is especially true for many neurodevelopmental disorders, where "sex" explains more of the variance than any other known contributing factor. In humans, some treatments are known to be more effective in one sex than the other, and optimal drug doses for men and women may differ. We ignore these things at our peril and we believe that ignoring sex differences in the brain, however they arise, compromises best practices in biology and medicine, in some cases with substantiated negative health effects [22].

A deeper understanding of these mechanisms will ultimately lead to our understanding of the molecular mechanisms underlying differences in the male and female brain, and importantly, differences in how the male and female brain may be able to respond to neuronal insults encountered with injury, neurodegeneration, and normal aging.

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Charles Sultan, Laura Gaspari, Nicolas Kalfa,  
and Françoise Paris

Malformations of the external genitalia, formerly referred to as ambiguous genitalia, are today categorized as disorders of sex differentiation (DSD) [1]. They are secondary to the undervirilization of the 46,XY fetus or the excessive masculinization of the 46,XX fetus. These malformations are usually discovered in the neonatal period during the systematic examination of the newborn's external genital organs. However, they can also be detected during prenatal ultrasonography. These situations are extremely distressful for the families, and optimal management is therefore imperative so that the sex of rearing can be determined as quickly as possible. DSD management is thus the response to a medical emergency. It requires a multidisciplinary team that should include a pediatric endocrinologist, pediatric urological surgeon, geneticist, and psychologist. The clinical, biological, and genetic investigations, along with imaging studies and surgical and psychological management, should suggest the better choice of sex orientation or at least the less onerous one.

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## 2.1 Diagnosis of the Disorders of Sex Differentiation (DSD)

### 2.1.1 Clinical Diagnosis of DSD

A scrupulous clinical examination will permit the diagnosis of a DSD (Fig. 2.1) and will also determine whether it has occurred in a context of malformation. The genital bud, genital ridges (scrotum in boys and labia majora in girls), and urogenital

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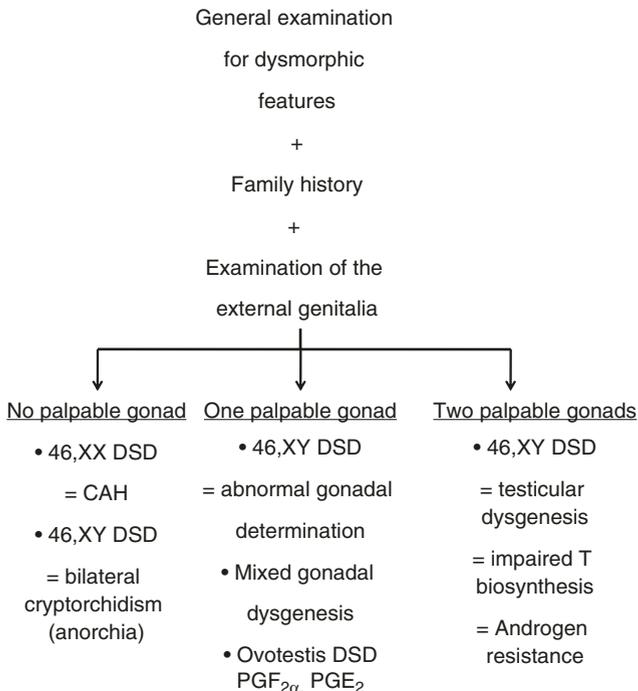
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**Fig. 2.1** Clinical orientation for DSD



sinus (closed in boys and open in girls) should be very carefully examined. It is important to determine whether the family has had other cases of DSD or neonatal salt-wasting.

In some cases, the external genitalia are “ambiguous” and are associated with:

- A genital bud that is midway between a penis and a clitoris
- Genital ridges that are incompletely fused (forked scrotum)
- The urethral opening on the underside of the penis (hypospadias) or a single perineal orifice at the base of the genital bud, between the genital ridges
- Gonads that are impalpable or palpable in the inguinal position

In other cases, the malformation is less clear-cut and attention should be focused on:

- Bilateral cryptorchidism
- Posterior hypospadias
- Cryptorchidism associated with hypospadias
- Micropenis (<2.5 cm)
- Clitoral hypertrophy
- A nonvisible vaginal orifice with posterior fusion of the genital ridges in a newborn with female phenotype

**Table 2.1** The Prader scores

Stage 0
Normal female external genitalia
Stage 1
Female external genitalia with clitoromegaly
Stage 2
Clitoromegaly with partial labial fusion forming a funnel-shaped urogenital sinus
Stage 3
Increased phallic enlargement. Complete labioscrotal fusion forming a urogenital sinus with a single opening
Stage 4
Complete scrotal fusion with urogenital opening at the base or on the shaft of the phallus
Stage 5
Normal male external genitalia

Palpation of a uni- or bilateral mass in the inguinal position or in the labia majora. The degree of the DSD should be evaluated according to the Prader classification (Stage I to V) (Table 2.1).

### 2.1.2 Genetic Investigations

The presence of the SRY gene should be determined with PCR and the results should be ready within 24 h. This will determine whether the case is an undervirilization of a 46,XY fetus or the excessive virilization of a 46,XX fetus. It is essential to determine the karyotype, as this will enable the diagnosis of chromosomal abnormalities like 45X/46,XY mosaicism. However, this type of diagnosis will take from a couple of days to several weeks.

### 2.1.3 Hormonal Investigations

The hormone work-up should be performed between 6 and 36 h after birth. The following measurements are crucial: 17-hydroxyprogesterone (17-OHP), testosterone (T), and anti-Mullerian hormone (AMH). If possible, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and delta4androstenedione ( $\Delta 4A$ ) measurements should be associated.

High 17-OHP suggests congenital adrenal hyperplasia (CAH), which is usually due to a deficiency in 21-hydroxylase that results in a 46,XY DSD.

The values of T and AMH are crucial for evaluating DSD with the 46,XY karyotype. The T, FSH, and LH levels should be reevaluated during the minipuberty (days 15–90), which is the period of activation of the hypothalamic-pituitary-gonadal axis. During other periods, T and its precursors should be evaluated after the human chorionic gonadotropin (hCG) stimulation test. Most often the

**Table 2.2** Comparison between the old and new DSD classification (ESPE Consensus 2006)

Previous classification	New classification
Sexual ambiguities	Disorders of Sexual Development (DSD)
MPH (Male Pseudohermaphrodisms)	46,XY DSD
FPH (Female Pseudohermaphrodisms)	46,XX DSD
True Hermaphroditism	Ovotestis, DSD
46,XX male	46,XX testicular DSD
45,X0, 46,XX Klinefelter Syndrome 47,XXY	DSD with chromosomal abnormalities

long test is used (1500 UI 1d/2 × 7). All these investigations should be able to differentiate 46,XY DSD secondary to insufficient androgen production (gonadal dysgenesis or defective androgen synthesis) from those secondary to androgen resistance associated with normal T production.

### 2.1.4 Imaging

Fetal ultrasonography sometimes reveals a uterus. Genitography can show evidence of Mullerian derivatives. Urogenital endoscopy under general anesthesia can be used to specify the implantation height of the Mullerian cavity when it is present.

## 2.2 Causes of DSD

According to the 2006 consensus statement of the European Society for Paediatric Endocrinology (ESPE) [1] (Table 2.2), we differentiate between 46,XX DSD, 46,XY DSD, DSD with ovotestis, and DSD associated with chromosome abnormalities.

### 2.2.1 46,XX DSD

46,XX DSD is due to the masculinization of the 46,XX fetus because of excessive exposure to androgens during intrauterine life, whether of endogenous or exogenous origin. It may also be due to abnormal ovarian determination.

#### 2.2.1.1 Fetal Hyperandrogenism

In the great majority of cases (75%), the 46,XX fetus has been overexposed to fetal androgens in the context of congenital adrenal hyperplasia (CAH). The excessive adrenal androgens produced upstream of an enzymatic block are peripherally converted to T and dihydrotestosterone (DHT) and cause fetal virilization (Fig. 2.2).

DHT deficiency is the most frequent (90–95%) of the enzymatic blocks, accounting for 1/14,000 births. Systematic screening with blotting paper is performed on the third day of life. A salt-wasting condition with hyponatremia may be associated with



the hepatic 3 $\beta$  HSD. An HSD3B2 gene abnormality will confirm the diagnosis, since transmission is recessive [5]. Substitutive therapy with gluco- and mineralocorticoids should be prescribed.

More rarely, the virilization of the 46,XX fetus may be caused by a lipoid adrenal hyperplasia secondary to a mutation of the StAR gene, which codes for the steroidogenic acute regulatory protein (StAR). Transmission is autosomal recessive. The StAR protein is involved in the cholesterol transport in mitochondria, which is the first step in adrenal and gonadal steroidogenesis. Lipoid adrenal hyperplasia is thus characterized by a major deficit in adrenal and gonadal steroidogenesis, evidenced by severe adrenal insufficiency and a female phenotype in both 46,XX and 46,XY fetuses. However, less severe forms – characterized by adrenal insufficiency but less pronounced undervirilization – have recently been reported [6].

The P450-oxidoreductase (POR) deficit is a possible etiology of 46,XY DSD, as well as 46,XX DSD [7]. These forms are dealt with more extensively in the chapter on 46,XY DSD.

### 2.2.1.2 Exogenous Hyperandrogenism

This type of fetal hyperandrogenism may be secondary to placental aromatase gene mutation, which is recessively transmitted. This abnormality is rare and is characterized by maternal virilization in the third trimester of pregnancy with spontaneous regression after delivery [8]. Another cause of exogenous androgens is one of the rare ovarian tumors, such as luteoma of pregnancy or maternal adrenal tumors [9].

### 2.2.1.3 Abnormalities in Gonadal Determination

Abnormalities in gonadal determination lead to 46,XX testicular DSD, and affected individuals were formerly termed XX males. These patients may present genital abnormalities during the neonatal period or a normal male phenotype. In the latter case, the diagnosis is made in adult life, frequently because of infertility. Since about 10% of patients are SRY negative, other genes are probably involved in testis determination. It has been hypothesized that these abnormalities are secondary to either an underexpression of ovary-determining genes or an overexpression of testis-determining genes [10], and both hypotheses have been reinforced by evidence [11]. Moreover, Camerino et al. reported an RSPO1 gene mutation in the family of a 46,XX patient with male phenotype associated with palmoplantar hyperkeratosis [11].

Regarding the potential role of the testis-determining genes, the overexpression of the Sox9 gene—which has its major role during testicular development—was reported in a 46,XX man [12, 13].

## 2.2.2 46,XY DSD

46,XY DSD refers to the case of 46,XY newborns with undermasculinization. One or both gonads are usually palpated at birth. The hormonal levels of T, AMH, FSH, and LH, as well as the presence of Mullerian derivatives at pelvic ultrasonography, will differentiate gonadal dysgenesis (associated with insufficient gonadal secretion of T and AMH) from T production defects or T insensitivity.

### 2.2.2.1 Gonadal Dysgenesis

Gonadal dysgenesis is a defect in testis determination characterized by a variable alteration in Leydig and Sertoli cell function. This disorder may be secondary to mutations in any of the several genes taking part in the differentiation process of the primitive gonad to a testis.

#### SRY Gene Abnormalities

SRY gene abnormalities express with a clinical picture of 46,XY sex reversal with female phenotype. If gonads are not palpated at birth, it is probable that the diagnosis will be made in the pubertal period in the context of primitive amenorrhea associated with pubertal delay. However, some patients may present partial pubertal development, often caused by an association with a secreting gonadoblastoma [14]. This picture of 46,XY sex reversal is associated with a SRY gene mutation in 20% of the cases.

#### Abnormalities in Other Sex Determination Genes

About 80% of the cases of gonadal dysgenesis are not caused by a SRY gene abnormality. They may be secondary to abnormalities in the other genes that take part in testis determination, however, and they are autosomal or X-linked.

Some cases of gonadal dysgenesis have been linked to SF1 gene mutation. This gene is involved in the development of male gonads and the adrenal glands [15]. The phenotype is variable, from severe expression [16] with isolated clitoral hypertrophy to moderate expression with hypospadias or isolated micropenis [17]. Adrenal insufficiency may be associated but is not systematically observed [18].

In some patients, the gonadal dysgenesis is associated with renal dysfunction. In these cases, the diagnosis of Drash syndrome—defined as Wilms tumor associated with renal insufficiency—or Frasier syndrome—which is proteinuria secondary to focal glomerular sclerosis—may be made. Both syndromes are due to WT1 gene abnormalities that are nevertheless quite specific for each syndrome. In particular, heterozygous mutations in the open reading frame have been associated with Drash syndrome [19], while intron mutations leading to splicing abnormalities have been found in Frasier patients [20].

Sox9 gene abnormalities have been reported. Sox9 is a key gene in early male sex determination [15]. Several mutations have been identified in patients with severe skeletal malformations like campomelic dysplasia, associated in some cases with sex reversal and gonadal dysgenesis [21–23].

Homozygous or composite heterozygous mutations of the desert hedgehog (DHH) gene, which is involved in testis differentiation and perineal development, have been identified. The virilization defect is frequently severe, the phenotype is often female, and a neuropathy may be associated.

A linkage study recently identified an MAP3K1 mutation in two families with several cases of 46,XY DSD, thus indicating another player in male sex determination [24].

In addition, duplications in the short arm of the X chromosome [dosage sensitive sex reversal (DSS) locus, DAX1 gene] have been reported in several cases of gonadal dysgenesis. A DSS locus duplication was found in 46,XY DSD patients with female phenotype (46,XY complete dysgenesis). Both the DAX1 (DSS-AHC critical region

on X chromosome, gene 1) and NROB1 (nuclear receptor OB1) genes have been identified in this locus. The NROB1 gene belongs to the nuclear receptor family and, through its linkage with other transcription factors such as SF1, it has an “anti-testis” effect during the process of male sex determination, proportional to the gene dosage. Thus, the overexpression of the DAX gene, as in the case of duplication in a 46,XY patient, may contrast with normal testis differentiation, leading to gonadal dysgenesis [25].

Last, a chromobox homolog 2 (CBX2) gene mutation was recently identified in a newborn with complete female phenotype but a 46,XY karyotype, which had been determined in the prenatal period because of concerns about maternal age. This testis differentiation abnormality nevertheless differed from the previously presented cases of gonadal dysgenesis in that ovaries with primordial follicles were detected [26]. A mutation in the CBX2 gene, which is known for activating SF1 transcription, was identified in this patient. The CBX2 gene thus seems to actively repress ovarian development in 46,XY gonads.

### **2.2.2.2 Defects in Testosterone Production**

Defects in T production are rare and are characterized by variable degrees of external genital undervirilization. Conversely, no Mullerian derivatives are present because AMH is normally secreted by the Sertoli cells. These defects are due to an enzymatic defect in T biosynthesis or they may be secondary to an LH receptor gene abnormality.

#### **Defect in 3-Beta-Hydroxysteroid Dehydrogenase**

This defect is associated with a variable but insufficient virilization of the 46,XY fetus, ranging from a female phenotype to minor forms of DSD, such as isolated micropenis or salt-wasting conditions. The biological and genetic investigations are the same as for 46,XX DSD.

#### **Defect of 17-Alpha-Hydroxylase**

The phenotype in cases of a 17-alpha-hydroxylase defect may also be extremely variable. In some individuals, the diagnosis is made only in the pubertal period because of pubertal delay or stagnation associated with gynecomastia. A DOC excess causes hypertension during puberty. The plasma levels of pregnenolone, progesterone, and corticosterone are elevated, which contrasts with the low values of T and D4 that are unresponsive to stimulation. The genetic abnormality concerns the CYP17 gene with recessive transmission.

#### **Defect of 17-Beta-Hydroxysteroid Reductase**

This is a rare testicular block that causes a deficit in testicular T production. The phenotype is more frequently female at birth. The diagnosis is based on a striking elevation in plasma D4 contrasting with a low T level. The mutation involves the 17 $\beta$ -HSD type 3 gene, which is expressed only in testis, and its transmission is recessive. Virilization occurs at puberty associated with gynecomastia.

#### **P450-Oxydoreductase (POR) Deficit**

A POR deficit may cause 46,XY DSD as well as 46,XX DSD [7]. The cytochrome P450 oxydoreductase protein enables the electron transport from NADPH to P450 cytochromes localized in microsomes. Several cytochromes take part in

cholesterol biosynthesis, while three are involved in steroid biosynthesis: P450C17 (17 $\alpha$ -hydroxylase/17,20 lyase), P450C21 (21-hydroxylase), and P450CYP19 (aromatase). Transmission of POR gene mutations is autosomal recessive, and the study of several of these mutations has provided greater insight into DSD in association with combined deficits in 21-OH and 17-OH in cases where molecular analysis of CYP21 and CYP17 was normal. In addition to DSD, several patients present craniofacial malformations, suggestive of Antley-Bixler syndrome. The great variability in the phenotype and endocrine findings makes this diagnosis very difficult. This may be due in part to the varying degrees of the enzymatic defects and to the differences in the ability of each mutation to alter enzyme function [27].

### **Leydig Cell Agenesis or Hypoplasia**

This is a rare form of 46,XY DSD, first identified in a patient with female phenotype associated with the 46,XY karyotype. She presented primary amenorrhea and no breast development at puberty, associated with low T at baseline and after hCG stimulation testing. The discrepancy between increased LH and normal FSH levels is generally evocative. This condition is determined by a homozygous or double heterozygous inactivating mutation of the LH receptor gene. Since the identification of these genetic abnormalities, the phenotypic expression has expanded to include conditions that range from ambiguous genitalia to partial forms such as hypospadias or isolated micropenis [28].

### **2.2.2.3 Androgen-Resistance Disorders**

The androgen-resistance disorders are characterized by normal/high T and AMH production, in contrast to undermasculinization. These disorders are classified as 46,XY DSD, which was previously termed male pseudohermaphroditism with normal or high T.

#### **Androgen Insensitivity (AI)**

Androgen insensitivity represents more than 50% of 46,XY DSD in our experience and is caused by a T receptor abnormality. Clinically, a gonad is palpable in the inguinal region in the majority of cases. AI is due to a recessive X-linked mutation that alters the androgen receptor (AR) gene and leads to variable degrees of undervirilization.

Regardless of the initial condition, testes are present and functional, despite cryptorchidism. They secrete T and AMH, and there are thus no Mullerian derivatives, such as uterus, Fallopian tubes or the upper part of the vagina [29]. The AI conditions are classically distinguished by two forms: complete androgen insensitivity (CAI), which is typically monomorphic, and partial androgen insensitivity (PAI), which is conversely far more heterogeneous, with phenotypes varying from Prader scores of I to V (Table 2.1).

CAI leads to the most severe phenotype of a normal female newborn. The diagnosis is often made only in the pubertal period, when primary amenorrhea associated with normal breast development and sparse axillary and pubic hair suggests this diagnosis. This adolescent also presents no acne, which is a further sign of no androgen action on target tissues.

Conversely, PAI is a quite variable condition, with phenotypes expressing all degrees of lost AR function. The range is extremely wide, with the most severe forms characterized by female external genitalia with moderate clitoral hypertrophy to the least severe forms like isolated micropenis [30] or male sterility with no external genital malformation, which is the so-called minimal androgen insensitivity syndrome (MAIS). Between these two extreme conditions, there are all degrees of undervirilization, most frequently with one or both gonads palpable. It is important to note that almost all patients develop gynecomastia in the pubertal period.

The endocrine investigations performed in neonatal PAI patients show normal or high plasma T and AMH, along with a high LH level. Conversely, in CAI patients, the T and LH levels are not always increased. In addition, the LH and T peak level observed around the sixth week of life in normal boys may be absent. The absence of Mullerian derivatives during echography or genitography is an important diagnostic element. An AR gene mutation will confirm this diagnosis. Choosing the sex of rearing at birth is relatively straightforward for CAI patients, who are always raised as females. Conversely, the choice of gender orientation for PAI patients is more difficult and needs to take into account the technical difficulties of virilizing these patients during the pubertal period. It is also important to note the absence of correlation between genotype and phenotype for several AR gene mutations, which may, moreover, express with variable phenotypes within the same family. Since most mutations are transmitted, it is important to screen all women to facilitate prenatal diagnosis.

### 5 $\alpha$ R Deficiency

In the case of 5 $\alpha$ R deficiency, T is not converted to dihydrotestosterone (DHT), which is responsible for external genital virilization. The phenotype is usually female, but it may assume all degrees of undervirilization. Clitoral hypertrophy and the association of hypospadias and micropenis were the most frequently reported findings in a recent study enrolling a large cohort of patients with a 5 $\alpha$ R defect [31]. If the diagnosis was not made in the neonatal period, it is usually made at puberty because of amenorrhea, absence of breast development, striking virilization associated with hirsutism, clitoral hypertrophy, significant muscle development, and a masculinization in behavior. The virilization is due to the presence of an isoenzyme expressed after puberty. The hormonal investigations usually reveal an increased T/DHT ratio >10. However, 5 $\alpha$ R defects have also been identified in patients with a normal T/DHT ratio. The diagnosis of a 5 $\alpha$ R deficit should always be considered for a 46,XY DSD patient with increased T and AMH plasma values, regardless of the T/DHT ratio. The molecular abnormality concerns the gene coding for 5 $\alpha$ R2. This enzyme is expressed in genital skin and prostate during fetal life and transmission is autosomal recessive; conversely, the 5 $\alpha$ R1 enzyme expressed at puberty remains functional in skin. There is high genotype/phenotype variability, as in androgen insensitivity. In severe forms, the gender orientation is often female; conversely, this choice is more difficult in less severe cases. Sex behavior, male identity, pubertal virilization, and preserved fertility in some men with the 5 $\alpha$ R mutation [32] provide arguments for male orientation [1].

### **MALD1 Gene Mutations**

CXorf6 or mastermind-like domain containing 1 (MAMLD1) is a new gene discovered during a study on myotubular myopathy (MTM1 gene). This muscle disease is associated with genital malformations, since the MAMLD1 gene, which is near MTM1, is deleted. MAMLD1 is temporarily expressed in fetal gonad and may thus take part in fetal steroidogenesis. Mutations in this gene are associated with severe forms of hypospadias in a context of DSD [33]; however, this mutation has been also identified in patients with isolated hypospadias and elevated plasma T [34].

### **46,XY DSD and the Environment**

In some cases of 46,XY DSD with elevated plasma T, no genetic abnormality is identified, leading to the diagnosis of “idiopathic” 46,XY DSD. In these cases, it is important to investigate the parental domestic and occupational exposure to environmental endocrine disrupting chemicals (EDCs) in order to identify possible fetal contamination.

Over the past 30 years, several studies have reported the undervirilization of wild animals, as well as an increasing trend in the prevalence of external genital malformations in male newborns. This trend includes isolated hypospadias [35, 36], cryptorchidism [37], and the association of two or more external genital malformations [38]. Several EDCs are known to present anti-androgenic and/or estrogenic activity, which has led to the suspicion that these chemicals may interfere with male sex differentiation during fetal life [39]. Moreover, many epidemiological studies have reinforced the EDC hypothesis by evidencing an elevated prevalence of these malformations in especially polluted areas [40, 41], and others have demonstrated an augmented concentration of EDCs in the mothers’ milk of 46,XY DSD newborns [42]. In addition, animal studies have demonstrated the potentiating effect of an EDC mixture [43]. It is thus probable that the environment plays an important role in this increasing trend of external genital malformations [38–44]. We found increased estrogenic bioactivity in three 46,XY patients who were apparently exposed to EDCs during fetal life [45]. However, a geographic variation in the prevalence of these external genital malformations has also been noted, with a sharp north/south gradient in Europe, which has raised the suspicion of a genetic susceptibility to EDCs [38]. In addition to DSD, the incidence of testis cancer has increased parallel to the decline in male fertility. Based on all this evidence, Skakkebaek et al. hypothesized in 2001 that the increasing trend in external genital malformations in males, the decline in spermatogenesis, and the rise in testis cancer have a common origin: fetal exposure to EDCs, or the so-called testicular dysgenesis syndrome [46].

### **2.2.3 Ovotesticular DSD**

True hermaphroditism is now called ovotesticular DSD, following the ESPE consensus statement of 2006 [1]. This denomination has the advantage of referring directly to the pathology, which is characterized by the coexistence of testicular and ovarian tissue. The clinical phenotype depends on the predominant tissue and may thus express any degree of DSD. It has also been reported that several virilized patients

were diagnosed only in the pubertal period because of gynecomastia and growth stagnation [47]. Diagnosis is based on the histological examination of a gonadal biopsy sample, which generally shows the presence of an ovotestis or ovarian tissue on one side and testicular tissue in the contralateral side. The ovarian tissue is normal; conversely, the testicular tissue is normal in the neonatal period but becomes dysgenetic with age [48]. The karyotype is 46,XX in the majority of these patients, as the SRY gene is found in only 30% of cases, and the 46,XY karyotype is more rarely seen. In much rarer cases, 46,XX/46,XY mosaicism is observed. Female orientation is frequently recommended because of the presence of uterus in 75% of patients and the differentiated ovarian tissue. In addition to DSD management, surgery can preserve the gonadal tissue according to the choice of gender orientation [48].

#### **2.2.4 DSD Caused by Chromosomal Abnormalities**

Mixed gonadal dysgenesis is among the chromosomal abnormalities that cause DSD; the karyotype is 45,X0/46,XY. This genetic condition leads to DSD of variable degrees, frequently with an asymmetry characterized by one palpable gonad in the inguinal or scrotal region and the other gonad, generally a streak, in the abdominal region. The presence of Mullerian derivatives, usually on one side only, is confirmed by genitography. The choice of gender orientation may be male or female, depending on the virilization of the external and internal genitalia and the presence of Mullerian derivatives. When female gender is chosen, a gonadectomy is generally performed to eliminate the high risk of gonadoblastoma. When male gender is chosen, the gonads are surgically lowered and fixed in the scrotum to facilitate screening for gonadoblastoma.

In this classification, we find also the Klinefelter syndromes, whose karyotype is 47,XXY. The phenotype is more usually male and the diagnosis is generally made at adolescence because of pubertal delay or stagnation associated to gynecomastia and low testis volume. Much more rarely, this syndrome is associated with a mild defect of virilization such as micropenis or cryptorchidism [49].

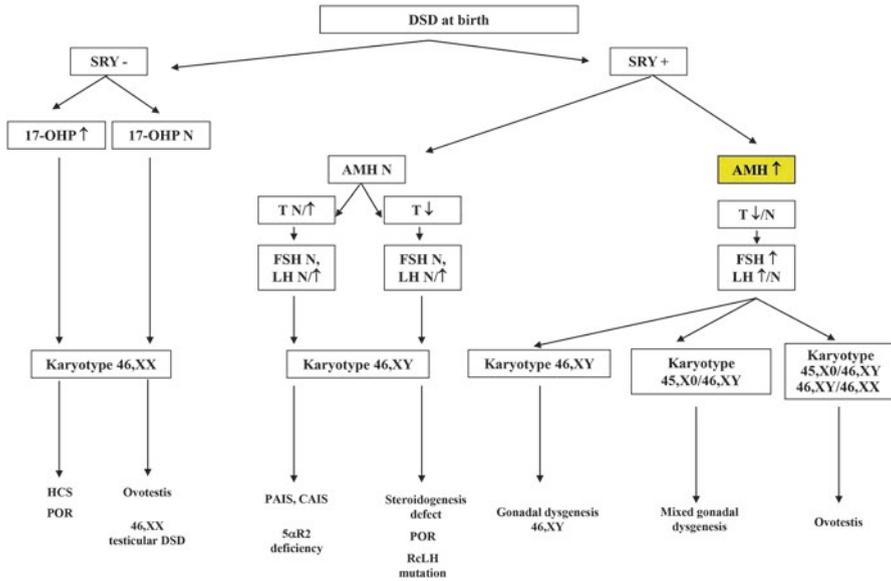
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### **2.3 Elements for Gender Declaration**

The choice of gender orientation should always involve a multidisciplinary team and the decision must be made very carefully. Although the orientation of highly virilized 46,XX DSD is today under discussion, the choice of gender orientation for 46,XY DSD remains an extremely difficult step. The presence of testicular tissue is not an essential factor; conversely, the surgical possibilities and the potential for virilization under treatment at puberty are key factors for orientation:

In testicular dysgenesis: female orientation is standard if vaginoplasty can easily be performed.

In testicular T synthesis abnormality: female orientation is advised, since the possibility of performing a masculinizing genitoplasty is low.



**Fig. 2.3** Management of DSD

In androgen resistance: female orientation is indisputable in newborns with complete androgen resistance; conversely, in partial forms the female orientation should be considered because of the risk of insufficient virilization at puberty.

In 5 $\alpha$ -reductase deficiency: theoretically, the orientation should be toward male gender since pubertal virilization will enable subnormal penile development, normal pubic hair, and male identity.

In the rare ovotestis DSD: female orientation is most often adopted.

**Conclusions**

In conclusion, scrupulous clinical examination and hormonal imaging, genetic, and molecular investigations all lead to the confirmation of DSD diagnosis (Fig. 2.3).

The families should be informed in a calm and balanced manner of the treatment options and their respective difficulties.

A multidisciplinary team should be involved in all diagnostic investigations, treatment, and follow-up.

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# Central Precocious Puberty: From Diagnosis to Treatment

# 3

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## 3.1 Introduction

Precocious puberty (PP) is defined as the onset of clinical signs of puberty before the age of 8 years in girls and 9.5 years in boys. However, the onset of puberty may be subject to the effects of environmental (secular trends, adoption, absence of the father, and possible exposure to estrogenic endocrine-disrupting chemicals), nutritional (body mass index), and constitutional (genetics, ethnicity) factors [1–4], with implications for the definition of precocious puberty. PP may be caused by central or peripheral mechanisms [1].

Premature sexual maturation is a frequent cause for referral. Clinical evaluation is generally sufficient to reassure the patients and their families, but premature sexual maturation may reveal severe conditions and thorough evaluation is therefore required to identify its cause and potential for progression, so that appropriate treatment can be proposed. The clinical expression of precocious puberty is polymorphic. In addition to progressive central PP, with a progressive deterioration of adult height prognosis in the absence of treatment, there are very slowly progressive forms which do not modify predicted final height [5–7]. The heterogeneity of precocious puberty, in terms of its clinical presentation and definition, can be explained by the gradual nature of the transition to puberty. Indeed, the pulsatile secretion of LH begins before the onset of clinical signs of puberty, and an increase in the amplitude of the LH peaks is the key biological sign of pubertal maturation of the gonadotrophic pituitary axis. GnRH stimulation tests indirectly reveal pulsatile endogenous GnRH secretion, as this secretion determines the response

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to exogenous GnRH. The available data indicate that there is no clear boundary between prepubertal and pubertal status, accounting for the frequency of “marginal” forms of precocious puberty.

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### 3.2 Etiologies and Mechanisms Underlying Premature Sexual Development

Central precocious puberty (CPP), which is much more common in girls than in boys [8], results from premature reactivation of the hypothalamo-pituitary-gonadal axis and pulsatile GnRH secretion with a hormonal pattern similar to that of normal puberty. Premature sexual development results from the action of sex steroids or compounds with sex steroid activity on target organs. CPP may be due to hypothalamic lesions, but is idiopathic in most cases, particularly in girls (Table 3.1) [1]. Recent studies have implicated the inactivation of Makorin ring finger 3 (*MKRN3*) genes in “idiopathic” CPP [9, 10]. *MKRN3* is an imprinted gene located on the long arm of chromosome 15, with a potentially inhibitory effect on GnRH secretion. *MKRN3* gene defects have been identified as a cause of paternally transmitted familial CPP, but such defects do not underlie maternally transmitted CPP and are rarely involved in sporadic forms [11].

It is also important to recognize that most cases of premature sexual maturation correspond to benign variants of normal development that can occur throughout childhood. They can mimic precocious puberty but do not lead to long term consequences and are usually benign. This is particularly true in girls below the age of 2–3 where the condition is known as premature thelarche. Similarly in older girls, at least 50% of cases of premature sexual maturation will regress or stop progressing and no treatment is necessary [5, 6]. Although the mechanism underlying these cases of non-progressive precocious puberty is unknown, the gonadotropic axis is not activated. Premature thelarche probably represents an exaggerated form of the physiological early gonadotropin surge that is delayed in girls relative to boys.

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### 3.3 Consequences of Precocious Puberty

Progressive premature sexual maturation can have consequences on growth and psychosocial development. Growth velocity is accelerated as compared to normal values for age and bone age is advanced in most cases. The acceleration of bone maturation can lead to premature fusion of the growth plate and short stature. Several studies have assessed adult height in individuals with a history of precocious puberty. In older published series of untreated patients, mean heights ranged from 151 to 156 cm in boys and 150 to 154 cm in girls, corresponding to a loss of about 20 cm in boys and 12 cm in girls relative to normal adult height [12]. However, these numbers correspond to historical series of patients with severe early onset precocious puberty which are not representative of the majority of patients seen in

**Table 3.1** Clinical characteristics of the various forms of central precocious puberty

Cause	Symptoms and signs	Evaluation
<i>Due to a CNS lesion</i>		
Hypothalamic hamartoma	May be associated with gelastic (laughing attacks), focal or tonic-clonic seizures.	MRI: Mass in the floor of the third ventricle iso-intense to normal tissue without contrast enhancement.
Or other hypothalamic tumors: <ul style="list-style-type: none"> <li>• Glioma involving the hypothalamus and/or the optic chiasm</li> <li>• Astrocytoma</li> <li>• Ependymoma</li> <li>• Pinealoma</li> <li>• Germ cell tumors</li> </ul>	May include headache, visual changes, cognitive changes, symptoms/signs of anterior or posterior pituitary deficiency (e.g., decreased growth velocity, polyuria/polydipsia), fatigue, visual field defects. If CNS tumor (glioma) associated with neurofibromatosis, may have other features of neurofibromatosis (cutaneous neurofibromas, café au lait spots, Lisch nodules, etc.)	MRI: contrast-enhanced mass that may involve the optic pathways (chiasm, nerve, tract) or the hypothalamus (astrocytoma, glioma) or that may involve the hypothalamus and pituitary stalk (germ cell tumor). May have evidence of intracranial hypertension. May have signs of anterior or posterior pituitary deficiency (e.g., hypernatremia). If germ cell tumor: $\beta$ hCG detectable in blood or CSF
Cerebral malformations involving the hypothalamus: <ul style="list-style-type: none"> <li>• Suprasellar arachnoid cyst</li> <li>• Hydrocephalus</li> <li>• Septo optic dysplasia</li> <li>• Myelomeningocele</li> <li>• Ectopic neurohypophysis</li> </ul>	May have neurodevelopmental deficits, macrocrania, visual impairment, nystagmus, obesity, polyuria/polydipsia, decreased growth velocity.	May have signs of anterior or posterior pituitary deficiency (e.g., hypernatremia) or hyperprolactinemia.
Acquired injury: <ul style="list-style-type: none"> <li>• Cranial irradiation</li> <li>• Head trauma</li> <li>• Infections</li> <li>• Perinatal insults</li> </ul>	Relevant history. Symptoms and signs of anterior or posterior pituitary deficiency may be present.	MRI may reveal condition-specific sequelae or may be normal.
<i>Idiopathic – No CNS lesion</i>	$\approx 92\%$ of girls and $\approx 50\%$ of boys. History of familial precocious puberty or adoption may be present.	No hypothalamic abnormality on the head MRI. The anterior pituitary may be enlarged. MKRN 3 gene evaluation if paternally transmitted
<i>Secondary to early exposure to sex steroids</i>		
After cure of any cause of gonadotropin-independent precocious puberty.	Relevant history.	

the clinic today. Height loss due to precocious puberty is inversely correlated with the age at pubertal onset, and currently treated patients tend to have later onset of puberty than those in historical series [12].

Parents often seek treatment in girls because they fear early menarche [13]. However, there are little data to predict the age of menarche following early onset of puberty [14]. In the general population, the time from breast development to menarche is longer for children with an earlier onset of puberty, ranging from a mean of 2.8 years when breast development begins at age 9–1.4 years when breast development begins at age 12 [15].

In the general population, early puberty timing has been shown to be associated with several health outcomes in adult life with higher risks for cardiovascular disease and type 2 diabetes in both women and men [16]. However, there are no long term data on these aspects in case of precocious puberty.

Adverse psychosocial outcomes are also a concern, but the available data specific to patients with precocious puberty have serious limitations [17]. In the general population, a higher proportion of early-maturing adolescents engage in exploratory behaviors (sexual intercourse, legal and illegal substance use) and at an earlier age, than adolescents maturing within the normal age range or later [18, 19]. In addition, the risk for sexual abuse seems to be higher in girls or women with early sexual maturation [20]. However, the relevance of these findings to precocious puberty is unclear, and they should not be used to justify intervention.

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## 3.4 Evaluation of the Child with Premature Sexual Development

The evaluation of patients with premature sexual development should address several questions: (1) Is sexual development really occurring outside the normal temporal range? (2) What is the underlying mechanism and is it associated with a risk of a serious condition, such as an intracranial lesion? (3) Is pubertal development likely to progress, and (4) Would this impair the child's normal physical and psychosocial development?

### 3.4.1 Clinical Diagnosis

Precocious puberty manifests as the progressive appearance of secondary sexual characteristics – breast development, pubic hair and menarche in girls, enlargement of testicular volume (testicular volume greater than 4 ml or testicular length greater than 25 mm) and the penis, and pubic hair development in boys [21, 22] – together with an acceleration of height velocity and bone maturation, which is frequently very advanced (by more than 2 years relative to chronological age). However, a single sign may remain the only sign for long periods, making diagnosis difficult, particularly in girls, in which isolated breast development may precede the appearance of pubic hair or the increase in growth velocity and bone maturation by several

months. However, in some children, the increase in height velocity precedes the appearance of secondary sexual characteristics [23].

*The clinical evaluation* should guide the diagnosis and discussions about the most appropriate management.

*The interview* is used to specify the age at onset and rate of progression of pubertal signs, to investigate neonatal parameters (gestational age, birth measurements) and whether the child was adopted, together with any evidence suggesting a possible central nervous disorder, such as headache, visual disturbances or neurological signs (gelastic attacks), or pituitary deficiency, such as asthenia, polyuria-polydipsia, and the existence of a known chronic disease or history of cerebral radiotherapy. The evaluation also includes the height and pubertal age of parents and siblings, and family history of early or advanced puberty.

*The physical examination* assesses height, and height velocity (growth curve), weight and body mass index, pubertal stage and, in girls, the estrogenization of the vulva, skin lesions suggestive of neurofibromatosis or McCune Albright syndrome, neurological signs (large head circumference with macrocephaly, nystagmus, visual change or visual field defects, neurodevelopmental deficit), symptoms or signs of anterior or posterior pituitary deficiency (low growth velocity, polyuria/polydipsia, fatigue) and to assess the neuropsychological status of the child, which remains the major concern of the child and parents seeking help for early puberty. It is also important to recognize clinically the benign variants of precocious pubertal development, usually involving the isolated and non-progressive development of secondary sexual characteristics (breasts or pubic hair), normal growth velocity or slight increase in growth velocity, and little or no bone age advancement.

*Following this assessment*, watchful waiting or complementary explorations may be chosen as the most appropriate course of action. If watchful waiting is decided upon, then careful re-evaluation of progression is required 3–6 months later, to assess the rate of progression of puberty and any changes in growth.

*Additional testing* is generally recommended in all boys with precocious pubertal development, in girls with precocious Tanner 3 breast stage or higher and in girls with precocious B2 stage and additional criteria, such as increased growth velocity, or symptoms or signs suggestive of central nervous system dysfunction or of peripheral precocious puberty.

These tests include the assessment of bone age (which is usually advanced in patients with progressive precocious puberty), hormonal determinations, pelvic or testicular (if peripheral PP is suspected) ultrasound scans, and brain magnetic resonance imaging (MRI).

### 3.4.2 Biological Diagnosis

The biological diagnosis of precocious puberty is based on the evaluation of sex steroid secretion and its mechanisms. The diagnosis of central precocious puberty is based on pubertal serum gonadotrophin concentrations, with the demonstration of an activation of gonadotropin secretion [24].

**Sex Steroid Determinations** In boys, testosterone is a good marker of testicular maturation, provided it is assessed with a sensitive method. RIA (Radioimmunoassay) is generally used in practice. In girls, estradiol determination is uninformative, because half the girls displaying central precocious puberty have estradiol levels within the normal range of values in prepubescent girls. Very sensitive methods are required, and only RIA methods meet this requirement. The increase in estradiol concentration is also highly variable, due to the fluctuation, and sometimes intermittent secretion of this hormone. Very high estradiol levels are generally indicative of ovarian disease (peripheral PP due to cysts or tumors). Estrogenic impregnation is best assessed by pelvic ultrasound scans, on which the estrogenization of the uterus and ovaries may be visible [25].

**Gonadotrophin Determinations** Basal gonadotropin levels are informative, and are generally significantly higher in children with PP than in prepubertal children [26]. However, basal serum LH concentration is much more sensitive than basal FSH concentration and is the key to diagnosis. Ultrasensitive assays should be used to determine serum LH concentration. Prepubertal LH concentrations are  $<0.1$  IU/L, so LH assays should have a detection limit close to 0.1 IU/L [27–29].

The response to GnRH stimulation is considered the gold standard for the diagnosis of central precocious puberty. Stimulation tests involving a single injection of LHRH analogs can also be used [30, 31]. The major problem is defining the decision threshold. In both sexes, a central cause of precocious puberty is demonstrated an increase in pituitary gonadotropin levels. Indeed, the underlying mechanism of early central puberty is linked to premature activation of the hypothalamic-pituitary-gonadal axis, with the onset of pulsatile LH secretion and an increase in the secretion of pituitary gonadotropins both in basal conditions and after stimulation with LHRH. Before the onset of puberty, the FSH peak is greater than the LH surge. During and after puberty, the LH surge predominates. In cases of central precocious puberty, basal serum LH concentration usually is  $\geq 0.3$  IU/L and serum LH concentration after stimulation is  $\geq 5$  IU/L [1, 32]. FSH is less informative than LH, because FSH levels vary little during pubertal development. However, the stimulated LH/FSH ratio may make it easier to distinguish between progressive precocious puberty (with an LH/FSH ratio  $>0.66$ ) and non-progressive variants not requiring GnRHa therapy.

### 3.4.3 Place of Imaging in the Evaluation of Precocious Puberty

*Pelvic ultrasound scans* can be used to assess the degree of estrogenic impregnation of the internal genitalia in girls, through measurements of size and morphological criteria. A uterine length  $\geq 35$  mm is the first sign of estrogen exposure. Morphological features are also important, as the prepubertal state is marked by a tubular uterus, which becomes more pearl-like in shape during the course of puberty, with a bulging fundus. Measurements of uterine volume increase the reliability of the examination (prepubertal  $\leq 2$  ml). Endometrial thickening on an endometrial ultrasound scan

**Table 3.2** Differentiation between true precocious puberty and slowly progressive forms

		Progressive precocious puberty	Slowly progressive precocious puberty
Clinical	<i>Pubertal stage</i>	Passage from one stage to another in 3–6 months	Spontaneous regression or stabilization of pubertal signs
	<i>Growth velocity</i>	Accelerated: > 6 cm/year	Normal for age
	<i>Bone age</i>	Typically advanced, variable, at least 2 years	Variable, but usually within 1 year of chronological age
	<i>Predicted adult height</i>	Below-target height or decreasing on serial determinations	Within target height range
Pelvic ultrasound scan	<i>Uterus</i>	Length > 34 mm or volume > 2 ml	Length ≤ 34 mm or volume ≤ 2 ml
		Pearl-shaped uterus	Prepubertal, tubular uterus
		Endometrial thickening (endometrial ultrasound scan)	
	<i>Ovaries</i>	Not very informative	Not very informative
Hormonal evaluation	<i>Estradiol (RIA ++)</i>	Not very informative, usually measurable	Not detectable or close to the detection limit
	<i>LH peak after stimulation with GnRH</i>	In the pubertal zone ≥ 5 IU/L	In the prepubertal range
	<i>Basal LH determination</i>	Useful if value is high (≥ 3 IU/L) and frankly in the pubertal range	No definitive value

provides a second line of evidence. Ovary size and the number of follicles are not criteria for the assessment of pubertal development [25, 31, 33]. Testicular ultrasound should be performed if the testicles differ in volume or if peripheral precocious puberty is suspected, to facilitate the detection of Leydig cell tumors, which are generally not palpable.

*Neuroimaging* is essential in the etiological evaluation in progressive central precocious puberty. Magnetic resonance imaging (MRI) is the examination of choice in the study of the brain and of the hypothalamic-pituitary region, for the detection of hypothalamic lesions. The prevalence of such lesions is higher in boys (30–80% of cases) than in girls (8–33%) and is much lower when puberty starts after the age of 6 years in girls, this population accounting for the majority of cases. It has been suggested that an algorithm based on age and estradiol levels could replace MRI, but such an approach has not been clearly validated [34–36].

*At the end of this analysis*, the diagnostic approach should help to determine the progressive or non-progressive nature of pubertal precocity (Table 3.2) and to differentiate between the etiologies of central or peripheral precocious puberty.

Indeed, many girls with idiopathic precocious puberty display very slow progressive puberty, or even regressive puberty, with little change to predicted adult

height and a normal final height close to their parental target height [5, 6]. Therapeutic abstention is the most appropriate approach in most of these cases, because puberty progresses slowly, with menarche occurring, on average, 5.5 years after the onset of clinical signs of puberty, and patient reaching a normal final height relative to parental target height. However, in some cases (about one third of subjects), predicted adult stature may decrease during the progression of puberty, in parallel with the emergence of evident biological signs of estrogenization and a highly progressive form of central PP. Thus, children for whom no treatment is justified at the initial assessment should undergo systematic clinical assessment, at least until the age of 9 years, to facilitate the identification of girls subsequently requiring treatment to block central precocious puberty.

#### 3.4.4 The Normal Variants of Puberty

The distinction between early puberty and normal puberty is not clear-cut. There are several variants of normal puberty, which may pose problems for differential diagnosis, particularly as they have a high prevalence [37–39].

*Premature thelarche* is isolated breast development before the age of 8 years. There are two peaks in the frequency of premature thelarche: the neonatal period, which is marked by gonadotropin activation, this peak potentially lasting for 2–3 years, and the prepubertal period [33]. Premature thelarche differs from early puberty in the absence of any other aspect of sexual development, usually with a lack of scalability of breast development and no acceleration of height velocity or significant advance in bone maturation ( $\geq 2$  years). Uterine ultrasound scans provide a simple means of checking that there is no change in the uterus. No further exploration or treatment is required and the outcome is the persistence of moderate breast development (in two thirds of cases) or regression. However, isolated premature breast development may precede the onset of central precocious puberty, which should not be ignored if patients develop other pubertal signs and an acceleration of height velocity.

*Premature pubarche* is the appearance of pubic hair before the age of 8 years in girls and 9 years in boys. It may be accompanied by clinical signs of hyperandrogenism: acne, axillary hair, accelerated growth rate. It corresponds to adrenal maturation (adrenarche) and is not a differential diagnosis for central precocious puberty. Possible differential diagnoses to be systematically excluded include adrenal tumors and congenital adrenal hyperplasia [40, 41].

**Slowly Progressive Forms of Precocious Puberty** Such forms present clinically as early puberty with the development of secondary sexual characteristics and a moderate advance in bone age. On ultrasound scans, the uterus may show very early estrogen impregnation. However, the response to GnRH is of the prepubertal type. The mechanism underlying these cases of non-progressive precocious puberty is unknown, but the gonadotropic axis is not activated. Studies monitoring these benign variants of precocious puberty have shown that treatment with GnRH agonists is not

appropriate because there tends to be either a total regression of pubertal signs or a slow progression towards puberty [5, 6]. Table 3.2 provides elements guiding differentiation between slowly progressive and progressive forms of central precocious puberty.

### 3.4.5 Psychosocial Aspects

Psychosocial aspects of early puberty are the major concern of patients and families seeking help for early puberty, whereas doctors generally focus on etiological aspects and height prognosis. Psychological assessment usually reveals a normal IQ. Patients tend to be rather solitary, with high scores for isolation, and a tendency to become depressed. They are mostly concerned about their appearance, whereas parents are generally worried about the onset of periods. Little is known about the long-term psychosocial consequences of early puberty or about the psychosocial integration of patients treated for precocious puberty [13, 42].

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## 3.5 Management of Central Precocious Puberty

### 3.5.1 GnRH Agonists

GnRH agonists are generally indicated in progressive central precocious puberty, with the aim to restore genetic growth potential and to stabilize or regress pubertal symptoms. GnRH agonists continuously stimulate the pituitary gonadotrophs, leading to desensitization and decreases in LH release and, to a lesser extent, FSH release [43]. Several GnRH agonists are available in various depot forms and the approval for use of the various formulations varies with countries. Despite nearly 30 years of use of GnRH agonists in precocious puberty, there are still ongoing questions on their optimal use and an international consensus statement has summarized the available information and the areas of uncertainty as of 2007 [17].

GnRH agonist treatments should be followed by experienced clinicians and result in the regression or stabilization of pubertal symptoms, decrease of growth velocity, and bone age advancement [17]. GnRHa-injection dates should be recorded and adherence with the dosing interval monitored. A suppressed LH response to the stimulation by GnRH, GnRH agonist, or after an injection of the depot preparation (which contains a fraction of free GnRH agonist) is indicative of biochemical efficacy of the treatment but is not recommended routinely. Progression of breast or testicular development usually indicates poor compliance, treatment failure, or incorrect diagnosis and requires further evaluation.

There are no randomized controlled trials assessing *long term outcomes* of the treatment of central precocious puberty with GnRH agonists and height outcome have been mostly evaluated. Among approximately 400 girls treated until a mean age of 11 years, the mean adult height was about 160 cm and mean gains over predicted height varied from 3 to 10 cm [12]. Individual height gains were very

variable, but were calculated using predicted height, which is itself poorly reliable. Factors affecting height outcome include initial patient characteristics (lower height if bone age is markedly advanced and shorter predicted height at initiation of treatment) and, in some series, duration of treatment (higher height gains in patients starting treatment at a younger age and with longer durations of treatment). No height gain benefit has been shown in girls treated after the age of 9 years.

*Other outcomes* to consider include bone mineral density, risk of obesity and metabolic disorders, and psychosocial outcomes. Bone mineral density may decrease during GnRH agonist therapy. However, subsequent bone mass accrual is preserved, and peak bone mass does not seem to be negatively affected by treatment [17]. There has been concern that GnRH agonists use may affect BMI. However, childhood obesity is associated with earlier pubertal development in girls, and early sexual maturation is associated with increased prevalence of overweight and obesity. Altogether, the available data indicate that long-term GnRH agonist treatment does not seem to cause or aggravate obesity or have repercussions for body composition, bone mineral density, fertility, and metabolic or cancer comorbidities. General health status is not different as compared to women with normal puberty [44–46]. The development of polycystic ovarian syndrome remains controversial [47–50] and further studies are still required to assess the potential risk of premature ovarian dysfunction. Data concerning psychosocial outcomes are scarce and there is little evidence to show whether treatment with GnRH agonists are associated with improved psychological outcome [13, 46]. Studies of this aspect are required.

Although *tolerance* to GnRH agonist treatment is generally considered good, it may be associated with headaches and menopausal symptoms such as hot flushes. Local complications (3–13 %) such as sterile abscesses may result in a loss of efficacy and anaphylaxis has been described [51].

The *optimal time to stop treatment* has not been established and factors that could influence the decision to stop GnRH agonists include aiming at maximizing height, synchronizing puberty with peers, ameliorating psychological distress, or facilitating care of the developmentally delayed child. However, data only permit analysis of factors that affect adult height. Several variables can be used to decide on when to stop treatment including chronological age, duration of therapy, bone age, height, target height, growth velocity. However these variables are closely interrelated and cannot be considered independently. In addition, retrospective analyses suggest that continuing treatment beyond the age of 11 years is associated with no further gains [52]. Therefore, it is reasonable to consider these parameters and informed parent and patient preferences, with the goal of menarche occurring near the population norms [17]. Pubertal manifestations generally reappear within months of GnRH agonist treatment being stopped, with a mean time to menarche of 16 months [53]. Long-term fertility has not been fully evaluated, but preliminary observations are reassuring [46, 53].

The addition of growth hormone [54] or oxandrolone [55] when growth velocity decreases or if height prognosis appears to be unsatisfactory has been proposed, but data are limited on the efficacy and safety of these drugs in children with precocious puberty.

### 3.5.2 Management of Causal Lesions

When precocious puberty is caused by a hypothalamic lesion (e.g., mass or malformation), management of the causal lesion generally has no effect on the course of pubertal development. Hypothalamic hamartomas should not be treated by surgery for the management of precocious puberty. Precocious puberty associated with the presence of a hypothalamic lesion may progress to gonadotropin deficiency.

#### Conclusion

Knowledge of the different clinical forms of precocious puberty is essential to determine whether there is a tumor (intracranial) or other disease, and the indications for treatment or an abstention from treatment.

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# Management of Peripheral Precocious Puberty in Girls

# 4

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The premature appearance of secondary sexual characteristics like breast development and pubic hair is upsetting and prompts many families to consult endocrinologists. Yet the management of precocious puberty is not always straightforward [1], especially given the unusually wide range of clinical expression: most presentations of precocious puberty are peripheral precocious puberty (PPP).

- Ten to twenty percent of all cases are instances of central precocious puberty (CPP). The course of its development needs to be carefully assessed and a central tumor must be eliminated. In its typical form, CPP progresses rapidly and the accelerated bone maturation leads to early fusion of the bone plates, thus compromising final adult height. Other clinical presentations have been identified and will be discussed further on.
- In 50–60 % of the cases, only one secondary sexual characteristic shows premature development and the diagnosis is premature thelarche (breast development), premature pubarche (appearance of pubic hair). In these cases, the etiology should be sought, and clinical, biological, and radiographic management is devoted to preventing the precocious onset of puberty.

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- The development of secondary sexual characteristics has an ovarian cause, with an autonomous hyperproduction of estrogens causing precocious pseudopuberty independently of gonadotropin activation. In more and more cases, it has become evident that the hyperestrogenism may have an exogenous origin such as environmental chemical pollutants in the air, water, and food chain. These xenoestrogens have a chemical structure that mimics the actions of natural estrogens by stimulating the activity of target tissues.

In all cases, the initial step is to evaluate the level of estrogen secretion, in terms of both its impact on target organs (breasts, growth rate, bone maturation, etc.) and its course over time. A good understanding of the various ways that precocious puberty clinically presents in girls is vital for the decision on therapeutic management, as the optimal treatment is often not evident during the initial evaluation.

## 4.1 Evaluation of the Pubertal Stage

One of the first steps in diagnosis is the estimation of pubertal stage. The Tanner stages have been the reference for many years, as they are very well codified (Table 4.1). In routine clinical practice, this relatively precise staging is complemented by examination of the sesamoid bone of the hand. This marker of bone maturation appears towards 11 years and signals the start of puberty.

The Tanner stages have been the reference for generations of pediatric endocrinologists. In an American study of a very large cohort of girls examined between the ages of 3 and 11 years, most of the girls appeared to enter puberty at a younger age (9.9 years for B2 and 10.5 years for P2). This drop in the age of puberty onset was most evident in black girls. The authors attributed their findings to the impact of environmental factors (chemical pollution). The findings that most of the girls entering puberty at the youngest ages (between 6 and 8 years) do not present with short adult height and that the duration of puberty (tempo) is inversely linked to the age of onset (timing) should be considered as relevant to clinical practice, especially to any decision about therapeutic management.

These works as a whole have done much to elucidate the development of secondary sexual characteristics and the normal course of puberty in girls.

**Table 4.1** Mean ages for normal pubertal development stages in girls, based on Tanner stages for breast development (B) and pubic hair (P)

Pubertal stage	Age (years)
Breast buds (B2)	10.1
Sparse pubic hair growth (P2)	11.2
Darker, coarser pubic hair growth (P3)	12.2
Growth spurt	12.2
Menarche	12.7
Adult pubic hair in type and quantity (P5)	14
Mature breast (B5)	14

The activation of the gonadotropic axis is marked by a peak in LH above 5  $\mu\text{UI/ml}$  and an LH/FSH ratio above 1 during LHRH testing. Conversely, to the assertions of other groups, we do not accept the basal values of LH (whatever the standard used) as a marker of pubertal onset.

The measurement of plasma estradiol by radioimmunology is not a reliable method to evaluate the onset of puberty because of its low specificity and high fluctuations. Only the analysis of the biological activity of estrogens using ultrasensitive methods is able to provide useful information on pubertal onset [2].

Last, pelvic ultrasonography with measurement of the uterus should be systematically performed: onset of puberty shows an increase in ovarian volume ( $>1.5 \text{ cm}^3$ ) and uterine size with length exceeding 3.5 cm. The finding of an increased diameter of the uterine fundus and a uterine vacuity line reflects significant estrogenization.

Clinical, biological, anthropometric, and radiographic evaluations are all helpful in distinguishing normal puberty from precocious puberty, which may have important clinical, psychological, and therapeutic implications.

## 4.2 Clinical Expression of Peripheral Precocious Puberty

Precocious puberty is eight times more frequent in girls than in boys [3]. Premature breast development, pubic hair, and growth acceleration should prompt several questions (Table 4.2), the answers to which will provide clues as to the best adapted treatment strategy.

1. Did puberty clinically begin before 8 years?
2. What has been the progression of the clinical symptoms?
3. Are there biological or radiographic signs of exaggerated maturation?
4. How is predicted adult height affected?
5. What are the psychological consequences?
6. Is the hormonal secretion gonadotropin-dependent or -independent?
7. In the case of central gonadotropin activation, is it due to a tumor or is it idiopathic?

Premature thelarche is the most common clinical expression of PPP. Premature thelarche refers to isolated breast development in girls between 2 and 7 years, which differs from the genital crisis of the newborn whose breast development (associated with strong estrogenization and even milk production) may last for the first 18

**Table 4.2** Clinical forms of peripheral precocious puberty

<i>Peripheral precocious puberty: precocious pseudopuberty</i>
Ovarian autonomy
McCune-Albright syndrome, ovarian cyst
Granulosa cell tumor
Adrenal tumor (feminizing)
Environmental pollution (pesticides)

months of life. This premature breast development is bilateral in half the cases, unilateral, or, less frequently, asymmetric. Volume varies: 60 % at B2, 30 % at B3, and 10 % at B4. The breast is often tender and palpation is sometimes painful. There is no discharge.

In persistent or marked forms of thelarche, the hormonal work-up should be limited to the LHRH test to confirm a predominant FSH response [4]. Bone maturation is rarely accelerated. The progression is characterized by fluctuations over time: spontaneous remission, persistence, and aggravation of breast volume, which should evoke the possibility of puberty onset. In this case, pelvic ultrasound can provide useful information.

When estrogen secretion is patent (simultaneous increase in uterine volume), contamination by products with estrogen-like activity should be considered and sought (soy-rich foods, environmental pesticides, etc.). In its usual form, premature thelarche requires no treatment.

Peripheral precocious or precocious pseudopuberty is not characterized by the premature activation of the gonadotropic axis: it is caused by an abnormally high production of estrogens and, more rarely, androgens because of a “tumor” on the ovary or adrenal glands. It is iso- or heterosexual depending on the whether the excess steroid hormone strengthens or transforms the child’s phenotype.

### **4.2.1 Peripheral Precocious Puberty Caused by Ovarian Autonomy**

The considerable progress in determining the molecular mechanisms of hormone transduction signals has greatly contributed to our understanding of the physiopathology and clinical expression of peripheral precocious puberty caused by ovarian autonomy. This progress may one day culminate in a specific treatment for this rare but incapacitating disorder. Heterosexual precocious pseudopuberty secondary to a virilizing adrenal or ovarian tumor is much rarer.

#### **4.2.1.1 McCune-Albright Syndrome**

McCune-Albright syndrome (MAS) is a sporadic disorder characterized by the classic triad of precocious puberty, fibrous bone dysplasia, and *café-au-lait* spots [5]. Diverse endocrine abnormalities can also be associated: somatotrophic pituitary adenomas, hypothyroid goiter, and adrenal hyperplasia.

#### **Precocious Puberty**

MAS affects girls almost exclusively and is characterized by its extreme precociousness and the gravity of the immediate clinical picture: isolated menstruation as early as the first months or first years of life. The full clinical picture will develop later, with breast enlargement and pubic hair. The often voluminous ovarian cysts discovered by ultrasound are cyclic and are difficult to treat: cystectomy or ovariectomy is often necessary when all other treatments fail. The acceleration of growth velocity is considerable (+2, +3 SD) and constant, on the order of 9–10 cm per year.

Bone maturation is also accelerated and will thus compromise the prognosis for final adult height.

The biological work-up will show very high plasma estradiol associated with dramatically low plasma gonadotropins and no response to GnRH stimulation. This indicates LH/FSH-independent precocious puberty and situates this type of precocious puberty within the context of the ovarian-autonomous syndromes.

### **Café-au-lait Spots**

The *café-au-lait* spots of MAS are hyperpigmented, typically light brown or brown, with irregular borders (“coast of Maine”) that distinguish them from the smooth-bordered spots observed in neurofibromatosis. The spots are usually unilaterally distributed, on the same side as the bone lesions. When associated with precocious puberty, they are an essential diagnostic element.

### **Fibrous Bone Dysplasia**

Fibrous bone dysplasia is the third element in the classic triad. This bone abnormality may remain silent for many years, only to be revealed by a spontaneous fracture or on the occasion of a slight injury. X-rays reveal pseudocysts of the bone cortex that rapidly invade the entire skeleton.

### **Other Endocrine Pathologies**

Other endocrine pathologies are seen to varying degrees: hyperthyroidism, acromegaly, gigantism, hypercorticism, hyperprolactinemia, and hyperparathyroidism. These types of endocrine hyperfunctioning are also caused by autonomous hormonal hyperproduction.

### **Molecular Bases of McCune-Albright Syndrome**

The specific location of the skin lesions and the sporadic character of MAS (no documented cases of hereditary transmission) suggest that this disorder is due to a somatic mutation that occurs early in development. The result is a mosaic distribution of abnormal cells. The appearance and the severity of the bone, skin, and endocrine abnormalities thus depends on the number of cells carrying the mutation.

Moreover, the diverse endocrine hyperfunctioning syndromes observed in the course of MAS have in common the presence and activation of cells that respond to extracellular signaling by activation of the adenylyl cyclase system. The hypothesis of local hyperproduction of cyclic adenosine monophosphate (AMPc) was confirmed several years ago: MAS is caused by a genetic abnormality that causes a constitutive activation of adenylyl cyclase. The evidence of activating  $G\alpha_s$  mutations in various tissues of MAS patients further supports this mechanism.

#### **4.2.1.2 Granulosa Cell Tumors**

Although rare (10% of the ovarian tumors in children), granulosa tumors are expressed in early childhood by strong estrogen secretion that results in marked breast development, accelerated growth velocity, and by menstruation. These ovarian tumors are discovered in a wide variety of circumstances:

- Endocrine disturbances
  - The hormonal activity of the tumor explains why 80–90 % of the girls under 8 years present isosexual precocious pseudopuberty. This pubertal advance induced by the estrogen secretion of the tumor cells is independent of the low levels of GnRH and prepubertal gonadotropins. On clinical examination, breast development is frequently noted but this is variable; metrorrhagia, accelerated growth velocity, and sometimes advanced bone age are also noted. The preoperative plasma estrogen concentration is almost always elevated. Virilization with precocious pubarche, acnea, hirsutism, and rarely clitoromegaly is related to aromatase deficiency inside the tumor.
  - Endocrine symptoms may also be present in cases of ovarian granulosa cells in the post pubertal period. Symptoms are more difficult to detect, such as menometrorrhagia and hyperandrogenism.
  - Precocity of diagnosis and an early recognition of endocrine signs from ovarian granulosa cell tumors significantly improve its prognosis with a lower risk of peritoneal extension.

In most patients, the levels of inhibin and AMH are elevated and return to normal post-surgery. The level of inhibin is correlated with tumor extension and eventual relapse. If the estradiol level is initially high, this too can be useful for follow-up. However, its interest is limited because [1] 30 % of granulosa tumors are nonsecreting [2], its level depends on the surrounding theca cells and is thus variable, and [3] physiological puberty can interfere with measurement.

- During emergency surgery (10 % of the cases) for acute abdominal pain and vomiting that are mistakenly diagnosed as acute appendicitis. These symptoms are due to torsion of the ovary or more rarely tumor rupture.
- An abdominal-pelvic mass that may be quite voluminous. This mass is frequently asymptomatic and it is discovered by the parents. It may also cause compression of the urinary tract (lumbar pain, colic nephritic, urinary infection) or the intestine (constipation, incomplete occlusion syndrome).
- As a fortuitous discovery during surgical intervention for other reasons, such as inguinal hernia repair, or as calcifications or ossifications of abdominal X-rays.
- By discovery of an ovarian cyst during prenatal diagnosis, which is an exceptional situation.

Surgery is the treatment of choice for a granulosa cell tumor. A careful preoperative evaluation is necessary to preserve fertility and future hormonal functioning. Staging includes peritoneal cytology and exploration of the abdominal cavity. Limited tumors can be treated by salpingo ovariectomy, as the rate of relapse is minimal. For bilateral tumor, a conservative treatment of the contralateral ovary should be discussed. In stages with extraovarian extension, chemotherapy is recommended. Follow up includes careful clinical, hormonal, and ultrasonographic evaluation.

Due to its excellent prognosis and the often complete recovery after initial surgery, juvenile ovarian granulosa cell tumors (OGCT) has usually been considered as benign condition. Survival of the patients with stage Ia tumors is around 90 %, the overall

survival in the whole group approximating 85%. However, the minority of the tumors that have spread within the abdomen or recurred after initial therapy have a much poorer prognosis and OGCT should be considered an ovarian cancer of good prognosis rather than a benign condition. It is still not possible to identify those patients who will relapse in the future, and the mechanism of this relapse remains to be elucidated. Nevertheless, molecular biology has identified two markers of prognosis:

- Mutations of the Gs alpha protein – a protein included in the transduction of the FSH signal – have been found in hot spot position 201 in 30% of patients' DNAs [6]. The oncologic stages were significantly different according to the gsp oncogene status. Patients with a hyperactivated G $\alpha$ s exhibited a significantly more advanced tumor ( $P < 0.05$ ). Gsp oncogene is indeed implicated in cell proliferation level and cell invasion capacity.
- Another prognostic factor of OGCT could be the level of the differentiation of the tumoral cell. One of the earliest differentiating genes of granulosa cell in the fetus is FOXL2. FOXL2, a forkhead transcription factor, is a regulatory element of the organogenesis of the mammalian ovary. The patients with no or reduced expression of FOXL2 in their tumor exhibit significantly more advanced oncologic staging. All patients requiring complementary treatment (chemotherapy or complementary surgery) showed reduced FOXL2 expression in the tumor [7]. The extinction of this gene implicated in granulosa cell differentiation is compatible with an uncontrolled proliferation of these cells.

#### 4.2.1.3 Ovarian Cysts

Functional nonneoplastic ovarian cysts and masses are frequent in peripubertal girls and are generally follicular or luteal in origin [8].

Ovarian cysts are mostly unilateral, unilocular, and simple with the size varying between 2 and 5 cm in diameter.

Rapid prepubertal breast development is usual followed by metrorrhagia in some cases. Pelvic US is mandatory.

If the cyst is anechogenic, the patient should be monitored for a period of 3–6 months. If there is any clinical or sonographic change, surgery can be discussed.

#### 4.2.2 Feminizing Tumors of the Adrenal Gland

Feminizing tumors of the adrenal gland are relatively rare causes of precocious pseudopuberty.

#### 4.2.3 Precocious Pseudopuberty and Environmental Pollution

Increasing attention should be given to precocious pseudopuberty secondary to environmental contamination by chemical products such as pesticides, herbicides, and fungicides. Endocrine disruptors with the capacity to mimic estrogens are able to generate estrogen secretion, resulting in simple thelarche to true central PP.

A decline in the age of pubertal onset and a rise in the prevalence of precocious puberty in girls (PP) (pubertal development before 8 years in girls) have been widely documented throughout the world [9, 10]. A better understanding of the “endocrine disrupting” activities of many environmental pollutants, particularly estrogen-mimetic compounds, has prompted the medical and research communities to consider their role in the increased prevalence of PP.

Environmental endocrine disruptors (EEDs) are able to disrupt the endocrine system at various levels, affecting, for example, the hypothalamic-pituitary axis, steroidogenesis, and the binding of steroid nuclear receptors; they therefore theoretically have the ability to interfere with the course of physiological puberty. Most notably, EEDs can mimic estrogen activity, which is why they are called “xenoestrogens” [11]. The acceleration in the incidence of PP cases and greater knowledge of the “endocrine disrupting” activities of EEDs have prompted the scientific community to hypothesize their role in the increased prevalence of pubertal precocity [12, 13].

Endocrine disruptors may be natural or synthetic and are listed below.

Phyto and mycoestrogens

Pesticides

Plastics

Synthetic Estrogens (OCP)

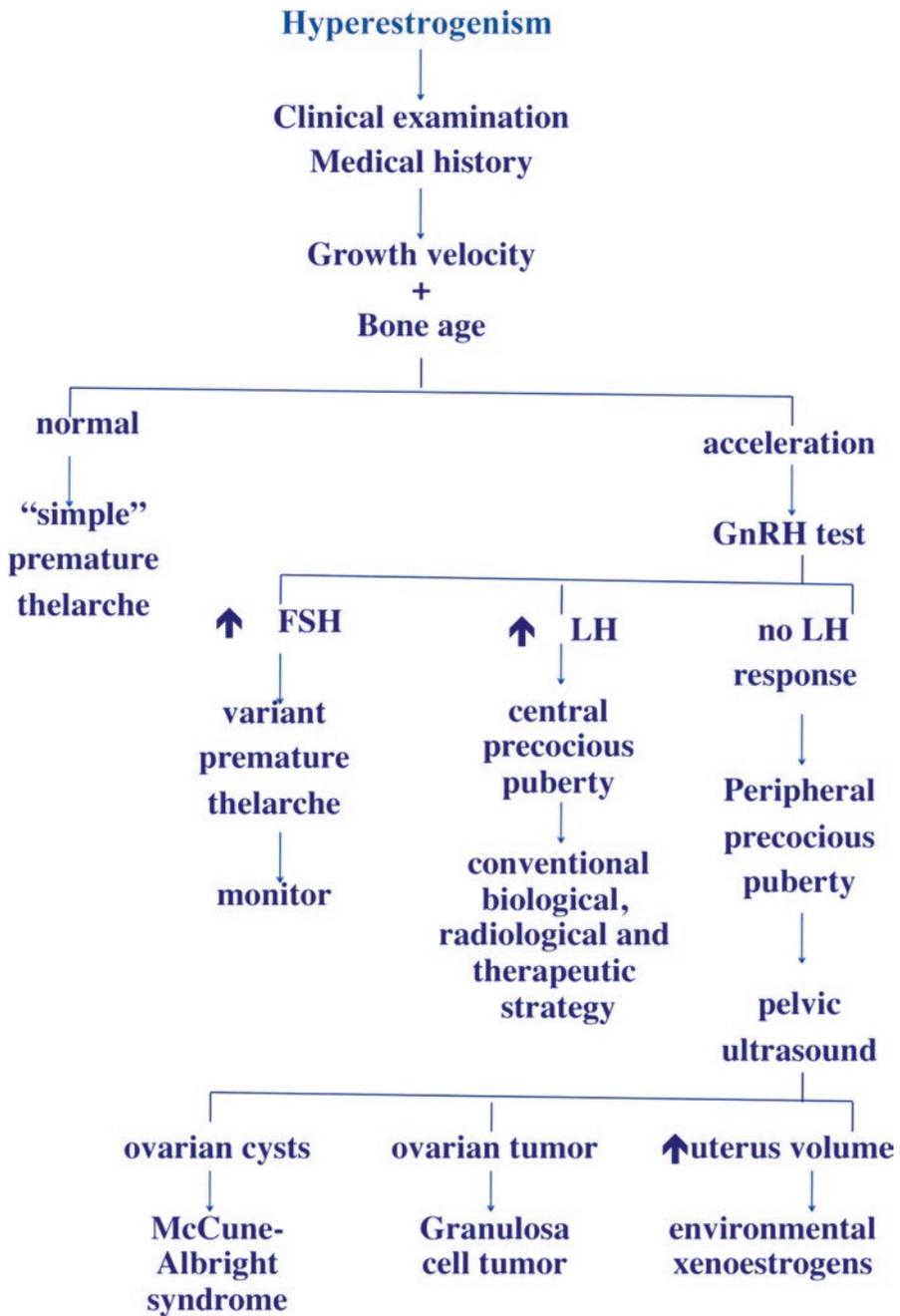
Cosmetics

Dioxin

Over the last 20 years, we have developed an ultrasensitive assay for EED in patient blood.

We have been able to demonstrate the role of EED contamination in a 4-month-old girl with precocious puberty seen in our pediatric endocrinology clinic, as we found very high concentrations of lindane and DDT in the child, her mother, and soil samples taken from their home to confirm our results [14]. In addition, we recently observed that premature thelarche can sometimes be associated with prenatal or postnatal exposure to EEDs, in a study we conducted in a group of young girls in whom we identified an abnormally high ultrasensitive estrogenic activity [15].

Over the last years, we have clearly observed an advance in the age of pubertal onset and it is ongoing. In addition, a rise in the incidence of PP has been reported in France as well as in many countries. Studies conducted in animals and humans support the role of EEDs in peripheral precocious puberty. It is however evident that a central action of EED cannot be excluded. This interaction of EED with the Kiss neurons may also contribute to the increase prevalence of central PP in girls. Beyond their impact on pubertal development, the relationships among the duration of estrogen exposure, the pervasiveness of EEDs, and the rising incidence of breast cancer appear to be generally acknowledged, which underlines—if need be—the importance of this issue for public health.



**Fig. 4.1** Decision tree for peripheral precocious puberty

## Conclusion

In girls, the prevalence of precocious puberty is rapidly increasing all over the world. PPP may be due to ovarian autonomous hyperactivity that should be identified and specifically treated (Fig 4.1). However, the role of EED seems predominant and raises a key question of a health and economic problem.

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Charles Sultan, Laura Gaspari, Nicolas Kalfa,  
and Françoise Paris

Pubertal maturation involves two associated processes: adrenarche due to adrenal androgen production and gonadarche linked to the reactivation of the hypothalamus-pituitary-gonadal axis.

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### 5.1 Definition

Premature pubarche (PP) is characterized by the presence of pubic hair developing in girls younger than 8 years [1].

It is referred to as premature or exaggerated adrenarche and is considered a benign condition of normal development, occurring in 4–10% of prepubertal girls. However, other causes of androgen excess should be considered and ruled out:

- Precocious puberty
- Enzymatic defects of adrenal steroidogenesis (late onset of 21-hydroxylase deficiency)
- Adrenal tumors

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## 5.2 Adrenarche

Adrenarche is the maturation of the adrenal zona reticularis, a physiological process unique to higher primates. In humans it occurs before pubertal onset at a mean age of 6 years, and the result is an increase in adrenal androgens: Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) (Fig. 5.1). Unconjugated DHEA can be converted directly to androgens (to  $\Delta 4$ -androstenedione  $\rightarrow$  testosterone), whereas the conversion of DHEAS to androgens first requires cleavage of the sulfate group [2].

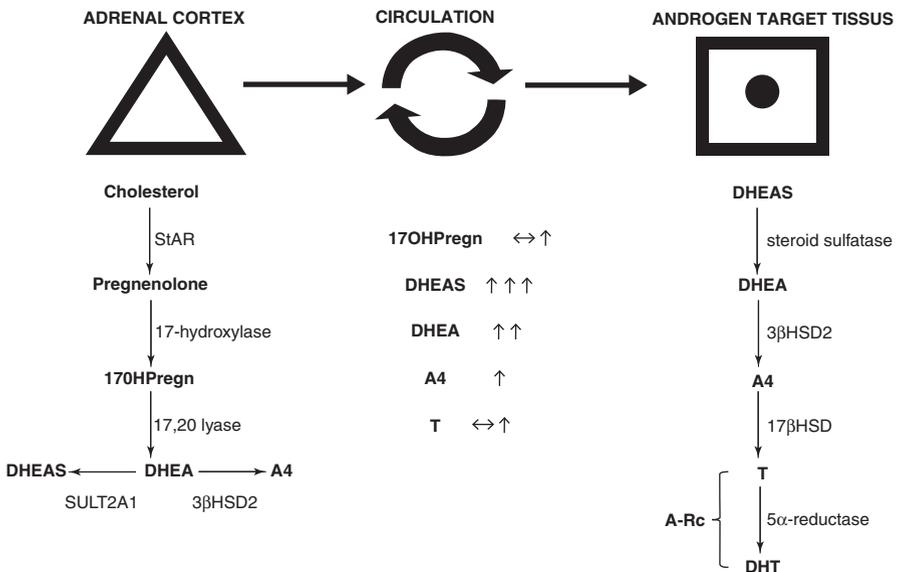
The mechanism of adrenarche onset remains unknown, although the implication of leptin, IGF1, and ACTH has been conjectured. In addition, MC-2 receptor, CYP19, IGF1, and AR gene polymorphisms have been reported to be associated with PP in some cases.

The physiological role of adrenarche in the pubertal maturational process has never been elucidated.

## 5.3 Clinical Features

The consequences of exposure to androgen excess in prepubertal girls are:

- Development of pubic hair
- Development of axillary hair
- Acne
- Oily skin/hair



**Fig. 5.1** Adrenal steroid biosynthesis

- Adult body odor
- Mood swings and/or behavioral changes

In a recent work, R. Voutilanen reported that 63 girls with PP presented the following characteristics: 89 % adult-type body odor, 70 % oily hair, 56 % acne, 48 % pubic hair, 38 % axillary hair, and 12 % acanthosis nigricans. In 20–50 % of these girls, higher than normal childhood weight was observed. Moreover, a bone age advance of 1–2 years was noted. Although some of these girls presented earlier menarche, normal adult target height was reached [3].

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## 5.4 Evaluation of Girls with PP

The diagnosis of PP is based on the exclusion of other causes of pubertal hyperandrogenism. The focus should be on the age at the onset of signs, the tempo of their changes, and recent acceleration of growth. Examination of the girl should include pubertal staging, a search for other signs of hyperandrogenism, and the evaluation of BMI. Laboratory assessment is limited to the measurement of basal serum 17-OH progesterone, DHEAS,  $\Delta$ 4-androstenedione, and testosterone. An adrenocorticotrophic hormone (ACTH) stimulation test must be performed if the basal 17-OH progesterone level is found to be above 2 ng/dL. A bone age evaluation is systematically carried out: in most cases, a 1–2 year advance in bone age is found.

Abdominal MRI should be considered when an adrenal androgen-secreting tumor is suspected.

A definitive algorithm for the evaluation of PP cannot be recommended. We nevertheless propose the following decision tree (REF. *Sultan PAG*), which has usually been helpful in our clinical experience (Fig. 5.1).

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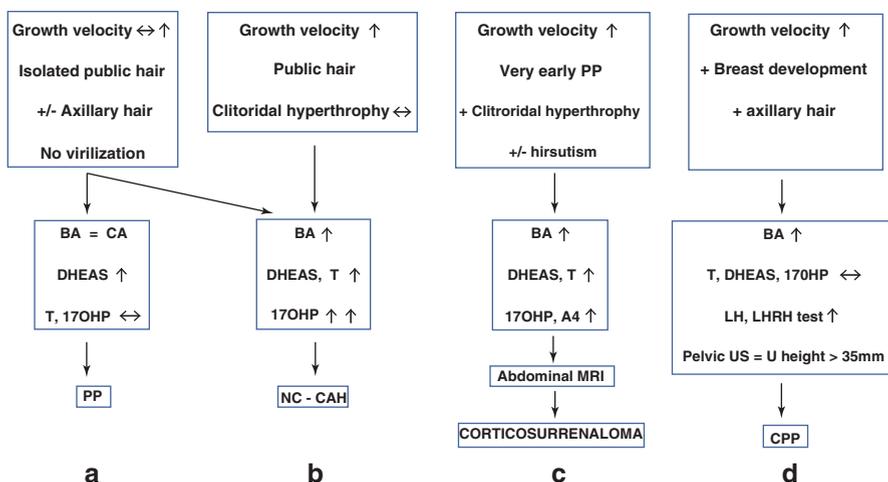
## 5.5 Differential Diagnosis

PP must be distinguished from a number of pathological conditions:

- Precocious puberty
- Non-classical congenital adrenal hyperplasia (NC-CAH), due to 21-OH gene mutation
- Virilizing adrenal tumors
- Exogenous androgen exposure

### 5.5.1 Precocious Puberty

PP may be a marker for the early onset of puberty: idiopathic central precocious puberty may present with pubic and/or axillary hair [4]. Bone age and growth velocity are accelerated and plasma androgens are abnormally elevated for age (Fig. 5.2d).



**Fig. 5.2** Clinical presentation of pre pubertal hyperandrogenism

### 5.5.2 Non-classical Congenital Adrenal Hyperplasia (NC-CAH)

NC-CAH is usually related to 21-OH deficiency [5]. In this condition, NC-CAH has an extremely variable presentation. In prepubertal girls, the presenting signs include: PP, cystic acne, accelerated growth, and advanced bone age (Fig. 5.2b).

NC-CAH has been found in 1–30% of girls with PP. This wide range is likely related to ethnic differences. The biological diagnosis of NC-CAH is suspected when early morning basal 17-OH progesterone is above 200 ng/dL and when an increase in 17-OH progesterone above 1200 ng/dL is found after the ACTH stimulation test [6].

In our experience [7] with a cohort of 40 girls with PP, 24% of them presented a molecular defect within the 21-OH gene.

### 5.5.3 Virilizing Adrenal Tumors

Virilizing tumors are rare but not exceptional (Fig. 5.2c): we recently had the opportunity to manage two very young girls referred to our clinic for PP without exaggerated symptoms of virilization. MRI was performed because abdominal palpation revealed an abdominal mass [8]. In both cases, an adrenocortical carcinoma was diagnosed and surgically removed.

### 5.5.4 Exogenous Androgen Exposure

Exogenous androgen exposure occurs exceptionally through creams or gels containing testosterone.

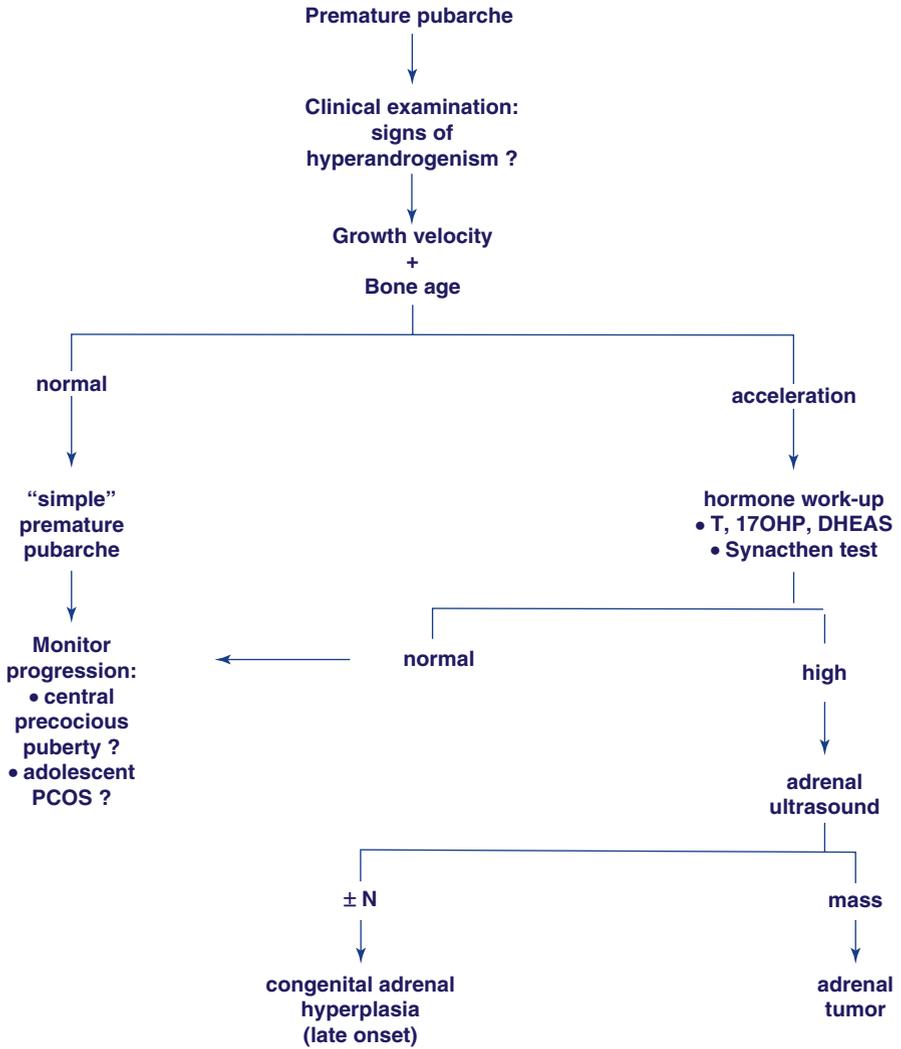


Fig. 5.3 Decision tree for premature pubarche

## 5.6 Management

There are few recommendations for the management of PP [9] (Fig. 5.3):

- If there is unusual weight gain, fasting measures of glycemic and insulin levels are recommended.
- If rapid pubertal progress occurs, laboratory and imaging assessment must be performed.

- In the majority of cases, when clinical and laboratory findings are consistent with idiopathic PP, these girls may be followed at 6-month intervals till menarche.
- The girls should be followed at least yearly after menarche, since they are considered to be at high risk for further adolescent polycystic ovary syndrome (PCOS) essentially for those who had a low birth weight and a prepubertal weight gain [10].

**Table 5.1** Grading of hypothalamic amenorrhea on the basis of the progesterone, clomiphene, and Gn-RH tests, respectively

1	Clomiphene positive with bleeding following
1a	Normal luteal phase
1b	Insufficient luteal phase
1c	Anovulatory cycle
2	Progesterone positive Clomiphene negative
3	Progesterone negative with pituitary response to 100 µg of Gn-RH i.v.
3a	“Adult response”
3b	“Prepubertal response”
3c	No response

From Leyendecker, G., Wildt, L. and Plotz, E.J. et al. [11].

## Conclusions

- PP is a frequent condition in girls, particularly in the Mediterranean area.
- The prevalence of PP is increasing, likely due to the recent higher incidence of obesity among prepubertal girls.
- Clinical and laboratory investigations are useful for distinguishing early adrenal maturation from the first signs of persistent hyperandrogenism.
- Extended longitudinal studies are necessary to confirm the link between early onset of androgen excess, insulin resistance, and PCOS.
- PP is not always a benign condition.

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# Maturation of the Hypothalamic-Pituitary-Ovarian Axis and the Onset of Puberty

Françoise Paris, Laura Gaspari, and Charles Sultan

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## 6.1 Introduction

Puberty is an essential life event, the period in which somatic and sexual maturation and reproductive capacity are achieved [36]. Far from being a punctual event, it is the culmination of a complex series of maturational events that start in utero, are reactivated in the neonatal period, and progress throughout the entire span of childhood [34]. Although the age of puberty onset is in great part genetically determined [17], the downward trends reported by several authors have led to the hypothesis of an interaction between genes and the environment [3, 22, 36]. Puberty has indeed come to be regarded as a biological sensor, and the changes in its timing, which have been detected in recent years in several wildlife species and human populations, suggest that it may serve as a potential biomarker for negative environmental influences on reproduction. Yet this issue is still contested by some, and an evaluation of worldwide epidemiological data is therefore needed to either confirm or invalidate this reported trend. In this short review, we focus on the major neuroendocrine factors involved in puberty onset, as well as its modulation by metabolic signals or epigenetic mechanisms (Fig. 6.1).

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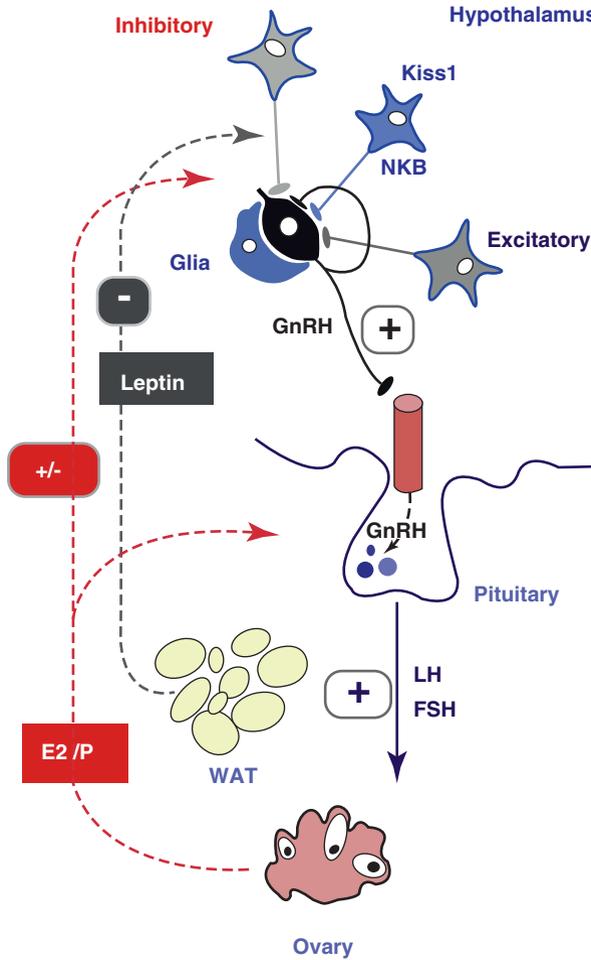
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**Fig. 6.1** Hypothalamic-pituitary-gonadal (HPG) axis and puberty according to [49]. Hypothalamic GnRH neurons receive trans-synaptic inhibitory and excitatory inputs that modulate the pulsatile release of GnRH. This neuropeptide drives the secretion of FSH and LH from the anterior pituitary, which in turn stimulates the secretion of gonadal sex steroids. These steroids have both negative and positive feedback effects for estradiol (E2) or only negative one for testosterone (T). Leptin produced by the white adipose tissue (WAT) is involved in the metabolic regulation of HPG

## 6.2 Neuronal Control of Puberty: Essential Roles of GnRH and Kisspeptin (KP) Neurons

During puberty onset, GnRH pulsatility increases in both amplitude and frequency and stimulates the gonadotroph cells of the anterior pituitary gland. This increases the secretion of FSH and LH, which in turn stimulates the secretion of sex steroids:

testosterone by the Leydig cells of the testis and androstenedione by the thecal cells of the ovarian follicles, which is then converted into estrogen by the aromatase enzyme in granulosa cells [4]. The increase in sex steroids stimulates the somatotroph axis, causing growth acceleration, progressive growth plate maturation, and the development of secondary sexual characteristics, all of which lead to the clinical expression of pubertal development. This key role of GnRH neurons has been clearly demonstrated by the clinical model of Kallmann's hypogonadotropic hypogonadism due to a migration defect of GnRH neurons [43]. Nevertheless, pubertal changes in pulsatile GnRH secretion are induced by modifications in the axodendritic inputs to the GnRH neuronal network [24, 35]. Puberty may thus be the end point in the hierarchical activation/inactivation of excitatory and inhibitory GnRH regulators [35].

Among the neuropeptide regulators of GnRH neurons, kisspeptin (KP) has been recognized as essential for the control of puberty onset [48]. KP is encoded by the *Kiss1* gene and operates through the kisspeptin receptor (KissR), a G protein-coupled receptor also called *Gpr54*. In rodents, two prominent populations of KP neurons have been reported in the arcuate nucleus (ARC) and the rostral periventricular area of the third ventricle (RP3V) of the hypothalamus, whereas in other mammals including primates, KP neurons are significantly expressed in the ARC/infundibular region [38]. In recent years, genetic endocrine disorders have proved useful for gaining a better understanding of physiological processes. With regard to puberty, inactive mutations of the *Kiss1* gene or *Gpr54* gene result in hypogonadotropic hypogonadism [12, 44]. Conversely, activating mutations of either of these genes lead to precocious puberty [46, 47].

Experimental data have also pointed to the key role of KP neurons in puberty onset. First, a rise in hypothalamic expression of the *Kiss1* and *Gpr54* genes has been reported during pubertal transition in rats [30]. Second, KP in mice elicits a better LH response in adults than in prepubertal mice, giving evidence of an increase in the sensitivity to the stimulatory effects of KP on GnRH/LH secretion [21]. The increase in the depolarizing KP action on GnRH neurons from juvenile to adult age reinforces the better *Gpr54* signaling efficiency during the pubertal period [21]. Last, an elevation in the number of KP-positive neurons and their projections toward GnRH neurons has been also demonstrated at the time of puberty [11]. This mechanism is more marked in female than in male mice, leading to the hypothesis of a sexual dimorphism in *Kiss1* gene expression.

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### 6.3 Roles of KP Partners in Pubertal Onset

In addition to the crucial role of KP in the control of puberty onset, other endocrine signals have been reported to modulate GnRH neuron activity by several groups.

Anatomical analyses have demonstrated that a high percentage of KP neurons co-expresses neurokinin B (NKB) and its receptor (NK3R) in the ARC [20, 32, 49]. Since most of these neurons also express dynorphin-A (Dyn), they have

been termed KNDy neurons [25]. As for KP, pubertal disorders underline the role of NKB and its receptor in pubertal regulation. For example, Topoglu reported four human pedigrees with severe congenital gonadotrophin deficiency and pubertal failure that were homozygous for loss-of-function mutation in TAC3 (encoding NKB) or TACR3 (encoding NK3R) [50]. These data, as well as the demonstration of an increase in TAC3 and TACR3 expression during puberty in mice [19], suggest that NKB is a central regulator of human gonadotropic function. Moreover, numerous experimental studies have reported a potent stimulatory action of the

NKB agonist senktide on LH secretion [18]. This stimulatory effect is nevertheless absent in Gpr54 null mice, suggesting that KP signaling may be a link between NKB and GnRH neurons [18]. In support of this hypothesis, experimental data demonstrate the stimulatory action of the NKB agonist senktide on KP neurons, but not directly on GnRH neurons [31]. In parallel, it was demonstrated that KNDy neurons express TACR3 gene, whereas GnRH do not [31]. All these data confirm that NKB activates GnRH neurons indirectly through kisspeptin [32].

Sex steroid levels are also involved in the regulation of pubertal onset. It is well known that sex steroids have both negative and positive feedback effects [4]. This has been corroborated by several clinical reports of central precocious puberty occurring during peripheral pubertal progression [37]. Recent works have underlined that the ARC hypothalamic area may be involved in the negative feedback of estrogens (E), whereas the rostral periventricular region of the hypothalamus (AVPV) is involved in its positive feedback [24]. Comparisons of wild type and ER $\alpha$  knock-out mice revealed that both negative and positive estrogen feedback are mediated by ER $\alpha$  [10]. Nevertheless, ER $\beta$ , but not ER $\alpha$ , is expressed in GnRH neurons, raising a question about the link between E and GnRH neurons [23]. A good candidate for this interaction would again be the KNDy neurons. First, increased Kiss1 gene expression was reported in postmenopausal women and ovariectomized monkeys [41]. Conversely, estrogen replacement in the monkeys markedly reduced Kiss-1 gene expression [41]. Second, the ablation of KNDy neurons prevents the rise of LH in ovariectomized mice [29]. Nevertheless, the positive feedback of E2 on the preovulatory LH surge is not fully understood, although it may involve estrogen-receptive histamine-containing neurons [16, 24].

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## 6.4 Metabolic Control of Puberty

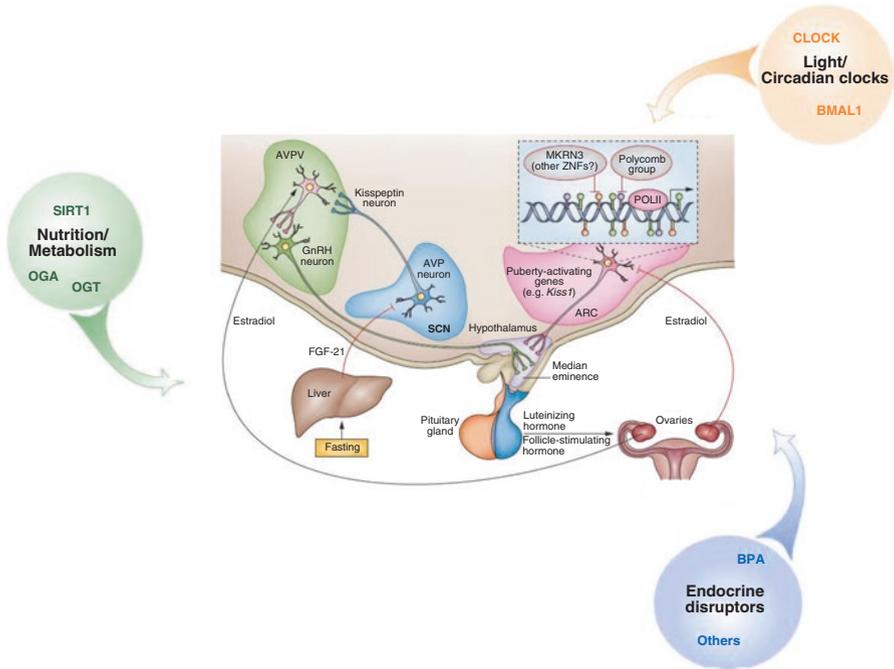
A substantial quantity of data points to the essential role of metabolic factors in the control of puberty. First, the concept of a critical fat mass – that is, the necessity of a minimal weight for normal puberty – emphasizes the role of leptin produced by adipose tissue [8]. In addition, leptin and leptin receptor deficiency in humans are known to be responsible for hypogonadotropic hypogonadism [14, 15], while

overweight and obese prepubertal girls present earlier puberty onset [2]. These data underline the important role of leptin in puberty. However, the normalization of mean leptin concentrations in the condition of negative energy balance failed to restore normal gonadotropin levels in female rats [51]. Moreover, pharmacological studies in humans and rodents with leptin deficiency have clearly demonstrated that leptin alone cannot trigger early puberty [9]. All these data suggest that rather than being a trigger, leptin plays a permissive role in puberty onset [40]. Other impacts of leptin at other levels of the hypothalamic-pituitary-gonadal (HPG) axis have also been considered. This principal central leptin action seems indirectly mediated since leptin receptor (LepR) mRNA was not found in GnRH neurons [39, 42]. It is currently unknown where leptin conducts its reproductive actions. Negative energy balance was reported to induce suppression of Kiss1 mRNA in pubertal rat hypothalamus, whereas administration of KP increased both serum LH levels and hypothalamic Kiss1 mRNA levels [6]. On the other hand, premature onset overweight in female rats increased Kiss1 gene expression [7]. Although these data stress the important role of leptin in GnRH pubertal activation via the KP neurons, one issue remains unresolved: does leptin have a direct or indirect action on the KP neurons? The above-cited works have demonstrated that leptin exerts both a direct and an indirect action on KP neurons. The detection of LepR mRNA in KP neurons of the ARC strengthens the assumption of direct leptin action on these neurons [5]. Nevertheless, not only congenital selective ablation of these LepR from KP neurons is compatible with preserved puberty and fertility [13], but LepR is also expressed in various hypothalamic area [28] underlying an indirect action of leptin via pathways that have yet to be identified.

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## 6.5 Novel Mechanism in the Control of Puberty

As described above, substantial efforts have been made in the past decade to identify the factors involved in the initiation of puberty. This body of work has not only shown the major role of the KP/GPR54 system in pubertal development, but has also indicated that the initiation of puberty requires the involvement of many other genes such as TAC, TACR3, Lep, and LepR. A new concept of pubertal initiation is related to upstream mechanisms involving gene silencing (Fig. 6.2). These upstream mechanisms can also include epigenetic regulation [33]. Kiss1 gene was thus found to be repressed in the ARC before puberty by the polycomb group transcriptional silencing complex (EED and CBX7) [26]. DNA methylation of two key members of this complex lifted this repression at the initiation of puberty, which coincided with an increase in the expression of the Kiss1 gene. Further insight into pubertal regulation was recently provided by several cases of familial central precocious puberty due to MKRN3 mutations [1, 45]. MKRN3 is a maternally expressed gene that encodes the protein MKRN3, whose transcription decreases in mice during peripuberty. These humans as well as experimental data strongly suggest that MKRN3 represses the initiation of puberty during the juvenile pause [1].



**Fig. 6.2** The repressive control of puberty according to [27, 33]. The polycomb group complex and the MKRN3 are the two gene-silencing systems expressed in the ARC. The loss of transcriptional repression identified as a main mechanism underlying the onset of puberty. Different external and internal environmental stimuli (nutrition/metabolism, light/circadian clocks, endocrine disruptors) also modulate the timing and progression of puberty. Abbreviation: *SCN* suprachiasmatic nucleus, *ARC* arcuate nucleus, *AVPV* anteroventral periventricular nucleus, *AVP* vasopressin, *MKRN3* markorin

## Conclusion

Puberty is not a punctual phenomenon. It is the culmination of a general maturation process that starts in fetal life, is reactivated in the neonatal period, and is finally expressed in adolescence. Pubertal timing is shaped by the dynamic interplay between gene load and the environment. Puberty has thus been regarded as a biological sensor, and downward trends in the timing of puberty, as have been detected in recent years in wildlife species and human populations, are considered as potential biomarkers for the suspected deterioration of the hypothalamic-pituitary-gonadal axis due to environmental influences. Our understanding of pubertal regulation has progressed greatly over the past decade, especially with the identification of novel neuropeptide pathways (Kiss, NKB, etc.) and molecular mechanisms (epigenetics, mRNA) involved in its regulation.

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## From Primary Hypergonadotropic Amenorrhea to “POI”: Aetiology and Therapy

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Ovarian function can cease precociously before menarche (primary hypergonadotropic amenorrhea) or can occur after a period of regular ovarian function, leading to the condition defined as “primary ovarian insufficiency.” Various terms have been used to define this deviation from healthy ovarian function including “premature ovarian failure” (POF); “premature ovarian dysfunction” and “occult or incipient ovarian failure” describe lesser forms of partial ovarian failure with raised serum FSH concentration but more or less regular menstrual cycles. The term “primary ovarian insufficiency” (POI), proposed by Albright in 1942 [1] describes a continuum of impaired ovarian function rather than a dichotomous state based on the hypothesis that ovarian follicular activity might resume intermittently, even years after diagnosis, leading to pregnancy in some women. Thus, this term could be considered more appropriate than those used previously [2]. The term “early menopause” is used commonly to refer to ovarian damage in 40–45 year-old women.

The common denominator of POI is amenorrhea lasting 4 or more months accompanied by serum FSH increase to menopausal level (usually over 30–40 IU, minimum three measurements done at least a month apart) and estradiol levels under 50 pg mL. It is estimated to affect approximately 1% of women under 40 years of age, 0.1% of women under 30 years of age, and 0.01% of women under 20 years of age [3] but actually the prevalence is less certain [4]. Progress and improvements in diagnosis and oncological treatments will certainly lead to an increase in the future of the number of recognized cases of POI [5].

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## 7.1 POI Pathophysiology

Although the cause of primary ovarian insufficiency is undefined in most cases, the disorder generally arises from dysfunction or depletion of ovarian follicles [6, 7]. In rare cases of follicular dysfunction, sufficient ovarian follicles are present but they do not function properly. For example, primary ovarian insufficiency can be caused by mutations in *FSHR*, which encodes the FSH receptor [8, 9]. Alternatively, destruction of primordial follicles by toxic agents, autoimmune response, activation of proapoptotic pathways, or accelerated follicular recruitment might result in premature depletion of the pool of primordial follicles.

## 7.2 POI Aetiology

Most cases of POF are idiopathic, with no identifiable etiology. However, diverse etiologies have been associated with POF, in particular genetic aberrations, autoimmune ovarian damage, iatrogenic factors, infectious agents, toxins, and environmental factors [10].

### 7.2.1 Genetic Disorders

The various genetic mechanisms implicated in the pathogenesis of POF include reduced gene dosage and non-specific chromosome effects that impair meiosis. These can lead to premature ovarian failure by causing a decrease in the pool of primordial follicles, increased atresia of the ovarian follicles due to apoptosis, or lack of follicle maturation [10, 11].

Chromosomal abnormalities have been recognized as a cause of POF, but percentages vary widely among reported series. The numerous different karyotype anomalies responsible for precocious ovarian deficiency can be classified as follows.

### 7.2.2 X Chromosome Numerical Defects

The X chromosome has been known to play an essential role in the maintenance of ovarian development and function; therefore, females lacking an X chromosome or those showing an extra X chromosome are predisposed to develop POF [4]. The most frequent X chromosome anomaly is Turner's Syndrome, with X monosomy (45,X) in 50% of cases. In the remaining 50% of cases, the karyotype presents either a two-long-armed X chromosome (isochromosome) or a ring shaped X chromosome or mosaicism (45,X/46,XX). POF in these patients is due to accelerated follicular loss [12]. X monosomy without mosaicism is more typically found in primary amenorrhea, while mosaicism and other forms of association are also associated with POF. Either haploinsufficiency of pivotal genes on the X chromosome

or non-specific meiotic impairment could explain the accelerated atresia of oocytes [4]. 47,XXX: the presence of three X chromosomes can lead to meiotic disturbance and ovarian failure, but the mechanism remains to be defined [13]. Patients presented different symptoms, such as genitourinary tract abnormalities, tall stature, epicanthus, and high incidence of autoimmune diseases [14].

### 7.2.3 X Chromosome Structural Abnormalities and X–Autosome Translocations

Women with structural abnormalities of the X chromosome have primary ovarian insufficiency. The most important of the numerous genes implicated in the mechanisms of POF and located on the X chromosome is FMR1, involved in X fragile syndrome, a pleiotropic Mendelian disorder (when POF is part of the phenotypic spectrum). This dominant, hereditary disease with incomplete penetration is the most frequent cause of hereditary mental retardation with a prevalence of 1:8000 women. The gene is located on Xq27.3 and it has an expandable region composed of repeats of CGG nucleotide in 5'UTR position. A normal subject possesses fewer than 55 repetitions of the CGG triplet. The syndrome depends on amplification of the site of repetition and involves the presence of more than 200 triplets; the clinical features include mental retardation, characteristic facial features with large ears and prominent jaw, joint laxity, and behavioral abnormalities. Pre-mutation is characterized by the presence of an intermediate number of triplets, 55–200, with a 15–20% risk of POF when there are between 80 and 100 triplets. In adult females pre-mutation may be associated with the neurological condition fragile X associated tremor/Ataxia Syndrome (FXTAS) which requires genetic consultation [4, 12, 14–16].

The FMR2 gene is located at Xq28, and the mechanisms generating disease (fragile X and mental retardation syndrome) are similar to those of FMR1. Furthermore, deletions in FMR2 have been described in women with POF [15].

A critical region on the X chromosome is specifically related to ovarian development and function. This region extends from Xq13.3 to Xq27: deletion of the short or long arm of the X chromosome results in either early primary or secondary amenorrhea. Moreover, the site of deletion or translocation can influence POF typology with primary amenorrhea when the Xq13 region is involved or when the Xq25 or Xq26 is involved [4, 14].

Multiple genes on the X chromosome have been identified by X-autosomal translocations, including DIAPH (Xq22), XPNPEP2 (Xq25), DACH2 (Xq21.3), POF1B (Xq21.1), CHM (Xq21.1), PGRMCI (Xq24), COL4A6 (Xq22.3), NXF5 (Xq22.1) [4]. Studies on X-autosome translocation have been significant in evidencing gene influence on autosomes and X-chromosome in the development of POF. The position of the X-autosome translocation is a feasible pathogenetic explanation. In fact, genes transferred to the highly heterochromatic region of the X chromosome tolerate epigenetic effects after a rearrangement which changes their chromatin structure: the result is lesser expression of genes associated with normal ovarian function and fertility [14, 17].

### 7.2.4 Autosomal Rearrangements

Robertsonian and reciprocal autosomal translocation have been observed in sporadic cases of POF, but no specific autosomal regions are preferentially associated with ovarian deficit. It has been suggested that functional changes and interruptions in certain genes critical for ovarian function on acrocentric chromosome due to translocation could be possible causes of POF [14].

### 7.2.5 Single Genes Responsible for Non-syndromic POF

Many genes have emerged as POF candidates, but in non-syndromic POI only a minority have been proven unequivocally causative, with notable differences in frequency among different populations [4].

Single genes associated with POF may be expressed on the X chromosome or autosomal genes. The genes expressed on chromosome X that are most frequently associated with POF are: BMP15 (Xp11.2), AR (Xq12), and FOXO4 (Xq13.1), as well as PGRMCI (Xq22-q24), POF1B (Xq21.2), DACH2 (Xq21.3), and FMRI (Xq27.3). Mutations in various autosomal genes have been associated with POF. However, genetic-linkage studies are difficult to do; the role of many mutations is unknown and they are not a common cause of primary ovarian insufficiency [18].

### 7.2.6 Chromosomes with Autosomal Genes that Are Strong Candidates as a Cause of POF

Chromosome 1: FIGLA (1p13.3), a germ-cell specific transcription factor that plays a crucial role in the formation of the primordial follicle and coordinates expression of zona pellucida genes; TGFBR3 (1p33-p32); GPR3 (1p36-p35); WNT4 (1p36.23-P35.1); MSH4 (1p31)

Chromosome 2: FSHR (2p21-p16); LHR (2p21); Inhibin alpha-INHA (2q35); Inhibin beta B-INHBB (2cen-q13)

Chromosome 5: GDF9 (5q31.1), expressed in oocytes

Chromosome 6: POU5FI (6p21.31); MSH5 (6p21.3); FOXO3 (6q21); CITED2 (6q23.3), gene essential for early embryonic development; ESR1 (6q25.1), coding Estrogen Receptor alpha

Chromosome 7: NOBOX (7q35), an oocyte-specific homeobox gene that plays a critical role in early folliculogenesis; Inhibin beta A-INHBA (7p15-p13)

Chromosome 9: NR5AI (9q33), SF-I (9q33); SOLHLHI (9q34.3)

Chromosome 10: NANOSI (10q26.11); PTEN (10q23.3)

Chromosome 12: Anti-Mullerian hormone receptor type II-AMHR2 (12q13); CDKNIB (12p13.1-p12); KITLG (12q22)

Chromosome 13: SOLHLH2 (13q13.3); FOXOI (13q14.1), an important gene for granulosa cell function and follicle maturation

Chromosome 14: ESR2 (14q23.3), coding Estrogen Receptor 2 (ER beta)

Chromosome 19: NANOS2 (19q13.32); NANOS3 (19p13.13)

Chromosome 20: SALL4 (20q13.2); SPOII (20q13.31)

Chromosome 22: DMCI (22q13.1) [4]

Protein and enzyme deficits implicated in steroid genesis are also responsible for deficiencies in estrogen production, notwithstanding the stimulation of FSH on granulosa cells. The steroidogenic acute regulatory protein (Star) regulates intramitochondrial transport of cholesterol. Mutation of this protein causes congenital adrenal hyperplasia. Mutations of certain enzymes such as aromatase, 17–20 desmolase, and 17alpha hydroxylase desmolase cholesterol cause POF [19].

### 7.2.7 Pleiotropic Single Gene Disorders in POF

The fragile X syndrome described above is the most frequent.

Other pathological syndromes associated with POF are:

- Galactosemia (GALT gene, at 9p13): Ovarian failure is a common long-term complication in girls with this disease. Pathogenesis involves excess galactose toxicity that impairs folliculogenesis, induces resistance to gonadotropins and accelerates follicular atresia. These patients present poor growth, poor intellectual function, and neurological deficit (predominant ataxia) [4, 20].
- Blepharophimosis-ptosis-epicanthus syndrome (BPES) type I (FOXL2 gene, at 3q23): BPES is associated with a characteristic facies (drooping upper eyelids with narrow horizontal eyelid openings, a fold of skin that runs upward and inward from the lower eyelids, and lateral displacement of the inner canthi) and premature ovarian failure. This is a pleiotropic autosomal dominant syndrome with an estimated de novo mutation rate of over 50% [12].
- Polyendocrinopathy-candidiasis-ectodermal dystrophy (AIRE gene, at 21q22.3): The AIRE gene can lead to various multi-system abnormalities such as alopecia, vitiligo, keratopathy, malabsorption, hepatitis, mucocutaneous candidiasis, Addison's disease, hypoparathyroidism, DM1, chronic atrophic gastritis, pernicious anemia, and autoimmune thyroid disorders. POF is common with onset usually in the third decade [4].
- Ovarian leukodystrophy (EIF2B2 gene, at 14q24.3; EIF2B4 gene, at 2p23.3; EIF2B5 gene, at 3q27.1): Ovarian failure may be associated with this genetic disease that is characterized by variable but progressive neurological degeneration [4].
- Carbohydrate-deficient glycoprotein syndrome type I (PMM2 gene, at 16p13): This genetic disorder may include also neurological abnormalities and ovarian failure [4].
- Perrault syndrome (HSD17B4 gene, at 5q21): An extremely rare pleiotropic autosomal recessive disorder characterized by progressive sensorineural deafness, ovarian failure, and sometimes neurological manifestations [4, 12].
- Proximal symphalangism and multiple synostose syndrome (NOG gene, at 17q22): Ankylosis of the proximal interphalangeal joints and POF.

- Bloom syndrome (BLM gene, on 15q26.1): In addition to POF, this disorder is characterized by early aging, short stature, and elevated rates of cancer
- Ataxia telangiectasia (ATM gene, at 11q22–q23): Progressive cerebellar degeneration with telangiectasia, immunodeficiency, recurrent infections, insulin-resistant diabetes, premature aging, high risk for epithelial cancer, radiosensitivity, and POF are characteristic features.
- Werner syndrome (WRN gene, at 8p12): Involves premature aging of the skin, bones, and vasculature with elevated rates of certain cancers (sarcoma) and POF.
- Rothmund-Thomson syndrome (RECQL4 gene, at 8q24.3): Involves small stature, skeletal/dental anomalies, cutaneous rash, cataracts, premature aging, increased risk for cancer, and POF.
- Nijmegen breakage syndrome (NBN/NBS1 gene, at 8q21): Autosomal recessive disorder characterized by microcephaly, facial dysmorphism, growth retardation, immune deficiency, and predisposition to develop cancer [12].

A complete list of syndromes associated with POF is available on line at <http://links.lww.com/COOG/A25>. [21]. However, since these conditions account for only about 1% of cases, genetic investigation is not routine in the absence of anamnestic and clinical findings indicative of POF.

### 7.2.8 Mitochondrial Genes Causing POF

Since the mature oocyte has the greatest number of mitochondria of any human cell perturbations of mitochondrial genes or nuclear gene affecting mitochondria are candidates for POF. Dysregulation of mitochondrial dynamics contributes to excessive oxidative stress and initiation of apoptosis, accelerating follicle depletion. Perturbation of mitochondrial genes is known to be present in various syndromes involving POF, in particular:

- Progressive external ophthalmoplegia (POLG gene, at 15q25) with additional clinical features of proximal myopathy (progressive weakness of the motor muscles of the eyeballs and upper lids), sensory ataxia, and Parkinsonism.
- Perrault syndrome (HARS 2 gene, at 5q31.3; LARS 2 gene, at 3p21.3; CLPP gene, at 19p13.3; C10orf2 gene, at 10q24): ovarian failure is one of the characteristic features of Perrault syndrome in females (as specified above in the paragraph on *pleiotropic single gene disorders in POI*), but the genes specified encode mitochondrial tRNA synthetase.

### 7.2.9 Other Genetic Syndromes

Primary or secondary ovarian failure is associated also with other genetic syndromes, but the causative genes have not been individuated. It is important to remember that only a minority of genes have been proven to be unequivocally, via

functional validation, the cause of non-syndromic POF. The list includes *BPMI5*, *PGRMCI*, and *FMR1* permutation on the X chromosome, *GDF9*, *FIGLA*, *FSHR*, *NOBOX*, *NR5A1*, *NANOS3*, and the recently discovered *STAG3*, *SYCE1*, *MCM8*, *MCM9*, *HFMI* on autosome genes. Note that only 1.5 % of the genome is protein-coding, and non-coding variants must be investigated more thoroughly. Perturbations could affect non-coding RNA (microRNA, long non-coding RNA), disruption or creation of alternative splicing or transcription factor-binding sites, and epigenetic modifications (DNA methylation, chromatin modification) [4].

### 7.2.10 Autoimmunologic Causes

Abnormal self-recognition of the immune system may account for 4–30 % of cases of POF due to the exaggerated autoimmune reaction involved in atretic acceleration, oocyte wastage, and impaired folliculogenesis. The autoimmune attack can be general or partial; it may be reversible and even responsible for the fluctuating course of POI [22]. The presence of lymphocytic oophoritis, demonstration of ovarian or other organ-specific autoantibodies, and associated autoimmune disorders are evidence of autoimmune etiology in a context of general autoimmune dysregulation [10].

There appear to be three different types of autoimmune POI:

- Related to adrenal immunity where the closest association is with autoimmune polyendocrine syndrome autoimmune polyglandular syndromes (APS) type 1 (candidiasis, hypoparathyroidism, hypoadrenalism, and possible comorbidities) and type 2 (hypoadrenalism and hypothyroidism with possible comorbidities)
- Associated with non-adrenal autoimmunity (related to thyroid disease, hypoparathyroidism, hypophysitis, DM1, rheumatoid arthritis, vitiligo, alopecia areata, celiac disease, Sjogren's syndrome, pernicious anemia, myasthenia gravis, SLE, immune thrombocytopenic purpura, glomerulonephritis)
- Isolated idiopathic [23].

The closest association is with adrenal immunity in Autoimmune Polyglandular Syndromes (APS).

Type 1 APS is a rare autosomal recessive disorder characterized by multiple organ-specific autoimmunity secondary to a variety of autoantibodies directed against key intracellular enzymes; it includes oophoritis in 60 % of cases.

Type 2 APS is an autosomal dominant disorder that is associated with gonadal failure in 4 % of patients. Addison's disease is present in both types of APS [10].

Adrenocortical and steroidogenic cell autoantibodies (SCA) are recognized as the best markers of autoimmune POF, but they are also commonly found in POI associated with autoimmune Addison's disease [11, 23]. In fact, more than 60 % of cases of POI associated with APS have positive SCA directed against steroidogenic

cells or enzymes found in adrenal cortex, placenta, ovaries, and testicles, such as StC Ab, anti-CYP21 Ab, P450ScAb, CYP17A1Ab; the same autoantibodies are found in fewer than 10% of cases of POF that do not present adrenal autoimmunity as well as in idiopathic POF. About 15–20% of POI subjects present anti-TPO antibody, 3 $\beta$ -hydroxysteroid dehydrogenase autoantibodies, gonadotropin receptor autoantibodies, zona pellucida autoantibodies, and/or anti-oocyte cytoplasm antibodies without SCA [11, 22, 23]. Circulating 21-hydroxylase antibodies may be immune markers of autoimmune adrenal insufficiency [24].

There have been several reports of antiovarian antibodies in POF, but their specificity and pathognomonic roles are questionable. Clinical or subclinical hypothyroidism is the disorder most commonly associated with POI, with TPO antibodies in 25–60% of patients [22].

Abnormalities in T-lymphocytes, macrophages, and dendritic cells play an important role in autoimmune reactions, and, of specific interest to our topic, in the development of autoimmune lesions in POI, where they support the mechanism of the disease [25]. Autoimmune oophoritis is characterized by mononuclear inflammatory cell infiltrate in the theca cells of growing follicles; early stage follicles do not present lymphocytic inflammation. The infiltrate includes plasma and B and T cells and may also be found in perivascular and perineural regions [26]. Ovarian biopsy might be useful for detection of autoimmune oophoritis, but it is generally not recommended because it is an invasive procedure of uncertain clinical value [11]. Commercially available antiovarian antibody tests give unsatisfactory findings. Certain non-ovarian autoantibodies, in particular autoantibodies to steroid producing cells, may mediate autoimmune damage in POF, but the prognostic value is uncertain and few laboratories carry out the necessary tests [27]. It has been suggested that a significant increase in CD8 density on T cells might be a reliable indicator of immune system involvement in POI [28].

### 7.2.11 Iatrogenic Causes

Even when bilateral ovariectomy is not performed, pelvic surgery can cause early menopause, presumably due to damage to ovarian blood vessels or post surgical local inflammation. However, the risks of pelvic surgery are difficult to quantify and they are generally considered minimal except in subjects with endometriosis, especially in the presence of bilateral endometrial ovarian cysts [12, 29].

Chemotherapy can cause damage by impairing follicle maturation or primordial follicle depletion or both. The effects of chemotherapy on ovarian reserve depend on various factors, in particular patient age, substances used, and duration of treatment [18, 27, 30]. Regarding age, the younger the patient is at the time of chemotherapy, the lower the risk of ovarian failure.

Anthracyclines, alkylating agents, cisplatin and derivatives, and antimetabolites are the most frequently used agents. Anthracyclines (i.e., doxorubicin) are cytotoxic antibiotics whose antineoplastic action is due to interaction with DNA. The mechanism of ovarian damage caused by anthracycline appears to be follicular depletion by apoptosis associated with serious vascular toxicity [30].

**Table 7.1** Risk of POI related to intake of gonadotoxic drugs

POI risk level	Gonadotoxic drugs
High	Busulfan, chlorambucil, chlormethine, cyclophosphamide, ifosfamide, melphalan, procarbazine
Medium	Adriamycin, carboplatin, cisplatin, docetaxel, doxorubicin, paclitaxel
Low	Bleomycin, dactinomycin, 5-Fluoruracil, mercaptopurine, methotrexate, vinblastine, vincristine

Cisplatin and its derivatives cause massive apoptosis of early follicles causing in turn ovarian dysfunction. Alkylating agents (e.g., cyclophosphamide) are the chemotherapeutic agents most frequently associated with gonadal damage (up to 40% of patients risk ovarian failure at childbearing age) because they destroy resting oocytes and possibly also the pregranulosa cells of primordial follicles. Cyclophosphamide triggers follicular atresia with vessel occlusion, focal corticoid fibrosis, and the disappearance of primordial follicles from fibrotic areas [30].

Antimetabolites instead cause less ovarian damage because they act on dividing cells.

Three risk categories have been established for certain chemotherapeutic agents based on their gonadotoxicity: various alkylating agents are classified as high risk; platinum agents, certain anthracycline antibiotics, and taxoids are medium-risk; and vinca plant alkaloids, some anthracycline antibiotics, and antimetabolites are low-risk [18] (Table 7.1).

Oocytes are very sensitive to radiation, and women exposed to total body and/or pelvic/abdominal irradiation are especially likely to suffer irreversible damage to the ovaries. The degree of risk depends on patients' age at the time of exposure, dose, and site of treatment [12, 18, 30].

Ionizing radiation can cause direct DNA damage to ovarian follicles resulting in follicular atrophy and decreased ovarian follicular reserve. Oocyte radiosensitivity presumably varies during the growth phase, and primordial follicles seem more radioresistant than maturing follicles [31]. One mathematical model used suggested that less than 2 Gy would destroy 50% of immature oocytes. Another model used to predict estimated surviving follicles in relation to dose of ionizing radiation showed that the effective sterilizing dose is inversely related to age at time of treatment. Ovarian damage occurs immediately after treatment in 97.5% of cases with the following sterilizing doses or doses of fractionated radiotherapy: 20.3 Gy at birth, 18.4 Gy at 10 years, 16.5 Gy at 20 years, 14.3 Gy at 30 years, and 1 Gy at 50 years [32].

### 7.2.12 Infectious Causes

Oophoritis due to mumps can cause POF, but in the majority of cases, ovarian function returns to normal following recovery [10, 27]. There have been various anecdotal reports of POF following other viral and microbial infections, such as tuberculosis, varicella, cytomegalovirus, malaria, and shigella [10, 12, 33]. HIV

and/or even related antiretroviral therapy may have negative effects on ovarian function and fertility leading to POF [27, 34].

### 7.2.13 Environmental Toxins

People of all ages worldwide, but especially in developing countries, are exposed to increasing amounts of environmental chemicals that can influence fertility. Animal studies and human observations have individuated certain chemical substances that target ovarian cells:

- Phthalates – practically ubiquitous diesters, commonly used as plasticizers in flexible PVC products. They cause large follicle destruction.
- Methoxychlor – a pesticide, widely used against insects that attack fruit and vegetables. Methoxychlor can be metabolized and a variety of its metabolites cause follicle atresia and lack of corpus luteum.
- Dioxin – a product of waste incineration, forest fires, and volcanic eruptions. It has a direct toxic influence on ovarian function.
- Bisphenol-A – a chemical widely used in the production of polycarbonate plastics and epoxy resins. It inhibits follicular growth and induces follicular atresia.
- Polycyclic aromatic hydrocarbons (PAH) – a class of several hundred compounds ubiquitous in the environment as a result of various combustion processes including automobile exhaust and cigarette smoking. Many PAHs and their metabolites are present in ground, surface, and waste water. They are highly lipophilic and extremely bioavailable and thus have easy entry into the food chain. They are toxic for the ovaries causing oxidative stress and reduction in the number of primordial follicles.
- Occupational chemicals, such as butadiene diepoxide, vinylcyclohexane, and bromopropane, are products of various industrial processes. They have been shown to have toxic influence on the ovaries and are able to damage and even destroy primordial and primary follicles, accelerating atretic processes.

The stage of development at which the oocyte-containing follicle is destroyed determines the outcome of xenobiotic damage within the ovary. Temporary infertility can be the result of selective influence of an environmental factor on the ovarian follicle population. The infertility may be reversible after exposure to the negative environmental factor ceases, but when the primordial follicles have been targeted, the stem cell population of germ cells may be destroyed, resulting in irreversible ovarian failure [30, 35].

### 7.2.14 Other Causes of POF

Smoking is the most widely studied toxin responsible for ovarian function. Cigarette smoking has been shown to comport increased risk of idiopathic POF. Tobacco

toxins can have a negative influence on ovarian reserve by increasing apoptosis in primordial germ cells, leading to accelerated follicular atrophy and atresia [10, 27]. Women with epilepsy have been reported to have increased risk of developing POF [10, 36].

*Personal and Family History* There is no specific menstrual history that is characteristic of the development of spontaneous 46,XX primary ovarian insufficiency. In most cases, the condition develops after a normal puberty and established regular menses (secondary amenorrhea), although primary amenorrhea may be present in about 10 % of cases [6, 37]. Occasionally, menses stop abruptly. In some women, menses fail to resume after a pregnancy or after they have stopped taking hormonal contraceptives. Most commonly there is a prodrome of oligomenorrhea, polymenorrhea, or dysfunctional uterine bleeding, usually prior to final cessation of menstruation. Also, we must point out that for young subjects it is inappropriate to attribute amenorrhea to stress without further evaluation. Symptoms of estrogen deficiency (hot flashes and night sweats, sleep disturbance, and dyspareunia related to vaginal dryness) develop in many, but not all, patients. Furthermore, not all patients have profound estrogen deficiency, and vaginal examination often reveals signs suggesting normal estrogen levels.

*Physical examination* may reveal stigmata indicative of Turner's syndrome such as short stature, webbed neck, and high, arched palate or evidence of an associated disorder such as hyperpigmentation or vitiligo (which is associated with autoimmune adrenal insufficiency), thyroid enlargement, galactorrhea, and/or signs of androgen excess.

*Endocrine evaluation* must be done, specifically serum FSH, LH, prolactin, TSH, and estradiol, at the very least and after pregnancy has been ruled out. Similar to the situation in natural menopause, POI is characterized by elevated FSH levels and reduced estrogen production. FSH evaluation should be repeated after 1 month, together with serum estradiol, if the first test shows levels in menopausal range. To date, there is no agreement on the minimum FSH increase necessary to establish a diagnosis of POI [5]. Inhibin A and inhibin B levels are reduced due to the decreased number of follicles and follicle development [2, 38]. Specifically, a decrease in inhibin B production appears to be the first sign of incipient ovarian insufficiency in Turner's syndrome [39]. However, normal levels of inhibin A and inhibin B have been demonstrated in the first stage of autoimmune POI, in agreement with the hypothesis of selective theca cell destruction in auto-immune oophoritis, with initial preservation of granulosa cells [40].

Antimüllerian hormone (AMH) is currently considered the best hormonal marker to estimate ovarian follicle pool. AMH is a dimeric glycoprotein member of the TGF- $\beta$  super family produced in women only by the ovaries. AMH expression is absent in primordial follicles and appears in granulosa cells of primary follicles. The strongest staining of AMH is observed in preantral and small antral follicles, and AMH is present in growing follicles until dominance [41]. The main physiological role of AMH is to inhibit the early stages of follicular development and control recruitment from the primordial pool of follicles [42]. AMH levels have been found to be normal in women with hypogonadotropic amenorrhea, whereas they are very

low or undetectable in women with physiological menopause and premature or primary ovarian insufficiency. The data in the literature confirm that knowledge of serum AMH levels improves menopause prediction and the monitoring of ovarian damage caused by spontaneous events and medical and surgical treatments in young women and adolescents [43]. AMH is considered a biomarker in menopause staging because it declines before FSH levels increase [44]. Recently, AMH was demonstrated to be more sensitive than FSH (80% vs. 28.57%), but the two presented almost equal specificity (78.89% vs. 78.65%) [45]. Caution is required when interpreting a single AMH measurement because biological fluctuations, surgical procedures, medications, and different laboratory methodologies frequently lead to dramatic changes in AMH levels [43].

*Pelvic ultrasonography* helps establish good morphological evaluation and provides some information regarding functioning of the lower genital tract. Antral follicle count (AFC) via trans-vaginal sonography done in the early follicular phase (second to fifth day) is a method for evaluating follicle residue (thus, ovarian reserve) that is more sensitive than FSH levels in normal subjects. There should be 3–8 antral follicles measuring 2–10 mm in each ovary in conditions of normal ovarian function. Some studies consider such counts valid also for young subjects with POI. Serum AMH levels show a strong correlation with antral follicle count [46], but they are more consistently correlated with the clinical degree of follicle pool depletion than inhibin B and AFC in young women with elevated FSH levels [47].

*Genetic investigations* center on specific chromosomal and genetic studies (karyotype, FMR1 gene mutation if there is family history of premature ovarian failure, fragile X syndrome, or mental retardation). When there is no dysmorphism and the family anamnesis is negative for particular pathological conditions, the starting point of genetic workup for women with POF is investigation of FMR1 gene status and karyotype analysis [21]. Specialized centers extend diagnostic testing to include FSHR, BMP15, GDF9, NR5A1, and NOBOX genes.

*Immunological Investigations* When there are symptoms suggestive of adrenal insufficiency (asthenia, apathy, hypoglycemia, vitiligo), it may be necessary to test for adrenocortical and/or steroidogenic antibodies, that are, however, quite rare in idiopathic POI. 17 $\alpha$ -OH antibodies, P450<sub>scc</sub> antibodies, and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) antibodies are considered the main serologic markers for ovarian failure in POF patients with autoimmune Addison's disease, but testing for these antibodies have limited application in routine clinical practice [23, 48]. On the other hand, the presence of 21-OH antibodies in women with idiopathic POF might be an important marker for identifying patients at risk of developing autoimmune adrenal insufficiency [48]. One must always look for thyroid peroxidase antibodies in cases of POF without adrenal autoimmune involvement because thyroiditis is the most prevalent autoimmune endocrine abnormality in POF patients. The search for antiovarian autoantibodies, a possible independent marker of autoimmune ovarian disease, is not done routinely given their questionable specificity in POF [22, 23]. Finally, in cases with positive anamnesis for other autoimmune conditions (vitiligo, hypothyroidism) or patients who present sideropenic anemia,

it is useful to screen for celiac disease and look for anti-gastric parietal cell antibodies to evidence eventual polyendocrine disorders associated with autoimmune atrophic gastritis [49].

*Bone mineral density measurement* is particularly important in establishing bone growth in Turner's syndrome subjects. Over the last decade various methods have been used, in particular:

- X-ray (standard)
- DXA single or dual photon absorptiometry
  - Dual-energy X-ray absorptiometry
  - Three-dimensional volumetric (vBMD) (cm<sup>3</sup>)
- QUS (quantitative ultrasonography)
- pQCT (peripheral quantitative computed tomography)
- HR-pQCT (high resolution peripheral quantitative computed tomography)

Studies using these different methods are commented on under the heading *Bone mineral density* in the THERAPY section 7. 3.10.

*Associated medical condition evaluation* is often a useful complement to diagnosis and can be important in establishing appropriate therapy, in particular substitute therapies in specific syndromes and for high cardiovascular risk patients [50].

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## 7.3 Hormonal Replacement Therapy (HRT) in POI

### 7.3.1 Choice of Therapy

What substitute hormonal therapy to choose depends on:

- Patient's age at the time of ovarian damage (e.g., before full puberal development, including maturation of the genital tract and achievement of peak bone mass, or after years of menstruation)
- Cause of the ovarian damage (genetic, immunologic or neoplastic; hormonal or non-hormonal; etc.)
- Individual congenital and/or acquired risk factors

### 7.3.2 Therapeutic Targets

- In younger subjects therapy aims to correct one or more specific problems, such as restoring an appropriate endocrine milieu for normal growth, enhancing bone mass accrual, promoting uterine growth and maturation, stimulating development of secondary sexual characteristics and/or sexuality, improving cognitive, behavioral, and psychosocial functions, providing cardiovascular protection.
- In subjects who have experienced a period of normal ovarian function the aim is to restore normal hormone milieu.

### 7.3.2.1 Estrogen Replacement Therapy for Puberty Induction in Gonadal Dysgenesis

We note that approximately one third of these patients experience spontaneous puberty, most commonly girls with XX mosaicism. Pubarche is the first feature in most patients and about 20 % present pubarche only after estrogen therapy [51]. Growth failure and altered ovarian function are present in virtually all individuals with Turner's syndrome, and the average adult stature of untreated individuals is about 20 cm shorter than that of their peers. This problem generally begins in utero, continues into infancy and childhood, and is accentuated by the absence of pubertal growth spurt. Growth hormone therapy is now standard care for 90 % of girls with Turner's syndrome [52]. Induction of puberty requires drugs, dosages, times and methods of administration that stimulate normal pubertal patterns and facilitate growth processes. Thus, since the ovaries of prepubertal girls secrete measurable, albeit modest, quantities of estradiol, the current trend is to initiate estrogen therapy in childhood, at very low dose, to achieve E2 levels typical for normal girls at onset of puberty and to add cyclic progesterone therapy 2–4 years after initiation of estrogen therapy or when break-through bleeding occurs. The same protocols are followed for subjects who have suffered irreversible ovarian damage due to other causes.

### 7.3.3 Estrogens Used in HRT

Specific treatment schedules have been established using:

- 17 $\beta$ -Estradiol – transdermal or transcutaneous administration
- Depot 17 $\beta$ -estradiol (as estradiol cypionate), which consists of 67 % estradiol, administered by single monthly intramuscular injection
- 17 $\beta$ -Estradiol – oral administration
- Ethinyl-estradiol (synthetic estrogen) – oral administration

### 7.3.4 Puberty Induction with Transdermal 17 $\beta$ -Estradiol

There are sound theoretical reasons for favoring transdermal administration. Transdermal E2 produces close to normal concentrations of E2, E1, and bioestrogen, and even low doses produce greater reduction in LH/FSH levels than normal doses administered by other methods [53, 54]. The most favorable findings regarding use of transdermal E2 are seen with substitute hormone therapy in normal age menopause subjects, in whom transdermal E2 exerted favorable or neutral effects on serum lipids and hs-CRP, including decrease in serum triglyceride and Lp(a) levels and increase of HDL-2 cholesterol concentration [55]; the risk of venous thromboembolism (VTE) was not increased [56–58]. Physiological transdermal estrogen-based regimens may lead to lower systemic blood pressure and minor

activation of the renin-angiotensin system than oral synthetic estrogen-based therapies in POI subjects under 40 years old [59]. No reduction in IGF1 levels was demonstrated in subjects with hyper- and hypo-hypogonadism [60, 61].

As early as 2001, a group of Swedish investigators recommended increasing the dosages of transdermal 17 $\beta$ -estradiol, adding nocturnal applications to attain levels of estradiol similar to those seen at the onset of spontaneous puberty in normal girls. The starting dose was 0.05–0.07  $\mu\text{g}/\text{kg}$  body weight administered via transdermal matrix patch (25  $\mu\text{g}/24$  h) cut into pieces corresponding to 3.1, 4.2, or 6.2  $\mu\text{g}/24$  h. Dosages were doubled after 4–14 months depending on serum E2 profile. The patch was applied at bedtime and removed the next morning. Progestogen was also administered, beginning within 2 years of the onset of treatment [62]. Subsequently, in 2014, lower starting doses were proposed, except for older girls for whom, when breast development was of high priority, the starting dose ranged from 0.08 to 0.12  $\mu\text{g}/\text{kg}$  body weight [63] (Table 7.2).

The Turner's syndrome Consensus Study Group also proposed a protocol for induction of puberty:

Age (years)	
10–11	Monitor for spontaneous puberty based on Tanner staging and FSH level
12–13	If no spontaneous development and FSH elevated, begin low dose E2 therapy
12.5–15	Gradually increase E2 dose over about 2 years (e.g., 14, 25, 37, 50, 75, 100, 200 g daily via patch) to adult dose
14–16	Begin cyclic progesterone treatment after 2 years of estrogen or when breakthrough bleeding occurs
16–30	Continue full doses at least until age 30 (normally estrogen levels are highest between age 15 and 30 years)

Bondy [52]

Davenport [64] proposed a similar protocol, with scaled doses using portioned patches applied in the evening and removed the following morning, aimed at achieving specific E2 levels:

E2 target level	Patch portion	Dosage equivalent
3–4 pg/ml	Usually 1/6 or 1/8 of 25 $\mu\text{g}$ patch	0.1 $\mu\text{g}/\text{kg}$ for 6 months
6–8 pg/ml		0.2 $\mu\text{g}/\text{kg}$ for 12 months
$\leftrightarrow$ 12 pg/ml	1/2 patch	12.5 $\mu\text{g}/\text{kg}$ for 18 months
$\leftrightarrow$ 25 pg/ml	1 patch	25 $\mu\text{g}/\text{kg}$ for 24 months
$\leftrightarrow$ 37 pg/ml	1 patch	37.5 $\mu\text{g}/\text{kg}$ for 30 months
$\leftrightarrow$ 50 pg/ml	1 patch	50 $\mu\text{g}/\text{kg}$ for 36 months (opportune time to start Progesterone or Progestin therapy, which should be started earlier if breakthrough bleeding occurs)
$\leftrightarrow$ 75 pg/ml	1 patch	75 $\mu\text{g}/\text{kg}$ for 42 months
$\leftrightarrow$ 50–150 pg/ml	1 patch	100 $\mu\text{g}/\text{kg}$ for 48 months (normal adult dose)

**Table 7.2** Ankarberg-Lindgren et al. protocols for treatment of subjects with hyper- and hypo-gonadism

	Age	N° cases	Pathology	Dose	End points
Ankarberg-Lindgren et al. [62]	12.3–18.1	15	Hyper- or hypogonadotropic hypogonadism 8 Turner's syndrome 1 craniopharyngioma 1 hypopituitarism 1 medulloblastoma; PNET 1 ovarian insufficiency 1 androgen insensitivity 1 Mb Glaucher	17 $\beta$ -estradiol 0.08–0.12 $\mu$ g/kg/day in transdermal matrix patch (TD 25 mg/24 h) cut into pieces corresponding to 3.1, 4.2, or 6.2 $\mu$ g/24 h Dose doubled after 4–14 months, depending on serum E2 profile. Application of patch at bedtime and removal the next morning Addition of Progestogen within 2 years of the start of treatment	Achieve spontaneous levels as well as diurnal pattern of serum 17 $\beta$ -estradiol in early puberty
Ankarberg-Lindgren et al. [63]		54 (88 observations)	Hyper- or hypogonadotropic hypogonadism 31 Turner's syndrome 7 hypopituitarism 3 androgen resistance (gonadectomized) 7 primary ovarian failure 6 ovarian failure (chemotherapy)	17 $\beta$ -estradiol 0.05–0.07 $\mu$ g/kg/day as puberty induction starting dose in 29 girls (age range 10.5–16.9 years) 17 $\beta$ -estradiol 0.08–0.12 $\mu$ g/kg in 42 girls (age range 9.9–18 years) 17 $\beta$ -estradiol 0.13–0.15 $\mu$ g/kg in 17 girls (age range 11.7–17 years) Application of patch at bedtime and removal the next morning For older girls, when breast development was high priority, the starting dose was 0.08–0.12 $\mu$ g/kg.	Establishment of <i>Guidelines</i> for the induction of puberty via transdermal E2 patch in a large out-patient setting

### 7.3.5 Puberty Induction with Percutaneous $17\beta$ -Estradiol in Hydroalcoholic Gel

Twenty-three girls ranging in age from 10.7 to 17.7 years (median age 13.6 years) with Turner's syndrome (17,XO/45,X karyotypes and six mosaics) and hypogonadism were enrolled in an uncontrolled, open, multicenter study, carried out between 1992 and 1999. Bone age at onset of treatment was  $> 9$  years. Single-dose sachets containing 0.1 mg estradiol were prepared specifically for the study. The starting dose was 0.1 mg (22.2 pmol/L serum E2 concentrations) in the 1st year, increased to 0.2 mg the 2nd year, 0.5 mg the 3rd year, 1 mg the 4th year, and 1.5 mg (corresponding to 162.2 pmol/L serum E2 concentrations) the 5th year. The starting dose of 0.1 mg of E2 is equal to 0.13 mg of E2V or 0.04 mg of conjugated equine estrogen.

All girls reached at least stage B4P4, and there were no significant differences between GH users and nonusers with regard to height SD score, weight SD score, bone age acceleration, or adult height. The treatment was safe and well accepted [65].

### 7.3.6 Puberty Induction with Depot $17\beta$ -Estradiol Cypionate

Preliminary reports have demonstrated that low doses of estrogen (monthly estradiol cypionate at doses of 1.0–1.5 mg) stimulate statural growth in hypogonadal girls. The association between GH therapy (very low dose) and a monthly intramuscular dose of depot  $17\beta$ -estradiol cypionate, beginning with 0.2 mg, and increasing at 6-month intervals to 0.4, 0.6, and, in 7 of the girls, 0.8 mg achieved better results regarding increased final height. The data support the hypothesis that early (age 12–12.9 years) administration of  $17\beta$ -E2 preserves height without interfering with the effect of GH on the enhancement of height potential. We note that this preparation is not available in Europe [66–68].

### 7.3.7 Puberty Induction with Oral Estrogens

$17\beta$ -Estradiol, ethinyl-estradiol, and conjugated estrogens have been used to induce puberty in subjects with hyper- and hypo-gonadotropic hypogonadism. The metabolic action of these estrogens varies depending on the method of administration. Oral estrogens have a first pass hepatic effect with supra-physiological exposure, reduced production of IGF1, and increases in its binding protein (IGF BP-1); GH production is increased, but its activity is reduced; lipolysis and postprandial oxidation of fatty acids are reduced; triglyceride storage increases (experimental data on rats [69, 70], and postmenopausal women [71]); fat mass increases with tendency to central deposition of fat, and lean mass is reduced due to the reduction in the anabolic action of IGF1 [72]). These effects are, theoretically at least, more remarkable with ethinyl-estradiol, which exercises a stronger influence than natural estradiol on

estrogen-dependent markers such as liver proteins (estrogen-dependent clotting factors, angiotensinogen, VLDL, SHBG, HDL-C) [73]. The doses administered and the individual patient characteristics (including age) condition the clinical effects of the treatment.

Comparison of transdermal E2 (50 µg) and oral E2 (2 mg) therapy in young subjects with hypopituitarism evidences that oral administration achieves a more significant reduction in IGF1 levels and increase in IGFBP1 levels without modifying IGFBP3 levels. However, high-density lipoprotein cholesterol levels increased after 3 months of treatment with oral E2. No differences were found between the two treatments regarding anthropometric measurements; blood pressure; heart rate; glucose, insulin, and C-peptide levels; or the homeostasis model assessment index [61] (Table 7.3).

### 7.3.7.1 Puberty Induction with 17β-Estradiol (E2)

Oral 17β-estradiol is widely used to induce puberty in girls with Turner's syndrome. The drug is composed of metabolized estrone (E1), estriol (E3), and estrone sulfate (E1S), but the pronounced reconversion of these metabolites to estradiol contributes to the maintenance of elevated estradiol levels for up to 12 h after intake [74]. The impact of estradiol on estrogen-dependent markers, such as liver proteins (estrogen-dependent clotting factors, angiotensinogen, VLDL, SHBG, HDL-C), is low with oral E2 and totally absent with E2 transdermal patch. The normally very short half-life of natural oral estradiol is prolonged in micronized formulations (Table 7.4).

There are no substantial negative data that discourage use of oral E2. Neither rates of protein turnover, lipolysis, lipid oxidation rates, nor IGF-levels are adversely affected by either oral (E2) or transdermal estrogen [80]. More recent data confirm that the different routes of 17-E2 delivery do not have different effects on body composition, lipid oxidation, and lipid concentrations in hypogonadal girls with Turner's syndrome [81].

### 7.3.7.2 Puberty Induction with Ethinyl Estradiol (EE2)

This estrogen has a remarkably strong metabolic impact on adult women, and the route of delivery, whether oral, vaginal, or transdermal, does not affect the impact. EE2 is characterized by long half-life, slow metabolism, and long tissue retention, and it is 200–20,000 times more potent than E2. Ample studies show good results for EE2 administration in young subjects. One study in particular (a

**Table 7.3** E2 plasma levels with 25, 50, 100 µg patches in different phases of menstrual cycle

E2 plasma levels with matrix patch		E2 plasma levels during ovulatory menstrual cycle	
Patch delivery	Peak E2 levels	Cycle phase	Normal values (Pg/mL)
25 mcg	30–45 pg/ml	Follicular	10–98
50 mcg	40–80 pg/ml	Ovulatory	170–770
100 mcg	90–140 pg/ml	Luteal	190–340
		Post-menopause	10–38

**Table 7.4** Oral E2 combined with GH therapy in puberty induction

Authors	Age (years)	N°cases	Oral E2 dose	Endpoints
Naeraa et al. [75]	16.3 ± 0.7 (12.8–20.0) BA13.8	10	E2 6–11 µg/kg/day (total dose 600 µg/day)	Morning vs. evening E2 administration; metabolism
Gravholt et al. [76]	16 ± 2	8	Placebo+placebo Placebo+GH Placebo+GH+E2 [0.39±0.12 mg/day (0.25–0.6)]	Body composition; metabolism
Bannink et al. [77]	11.8–15	56	5 µg/kg/day × 2 years 7.5 µg/kg/day 3rd year 10 µg/kg/day >3rd year	Breast and uterus development
Cleemann et al. [78]	22–65 (mean 37)	54 (TS)	E2 2 mg (1–22 day) + NETA 1 mg (13–22)+ Calcium and Vit D	Bone density
Cleemann et al. [79]	12.2–24.9 (16.7 ± 3.3)	41	Mean starting dose: 0.23 ± 0.12 mg (0.10–0.50 mg) Maintenance dose: E2 2 mg (1–22 day) +NETA 1 mg (13–22) + E2 1 mg (23–28)	Uterus and ovary evaluation

randomized, double-blind, placebo-controlled clinical trial on GH and low-dose ethinyl estradiol administered to girls 5–12 years old) showed significantly earlier thelarche (median 11.6 years vs. 12.6 years,  $P$  0.001) and slower tempo of puberty (median, 3.3 years vs. 2.2 years,  $P$  0.003) in the EE2 treated girls than controls [82] (Table 7.5).

### 7.3.7.3 Puberty Induction with Conjugated Equine Estrogens (CEE)

One study focused on establishing the impact of age on the effects of estrogen replacement to influence final height in Turner's syndrome patients receiving GH therapy. Subjects received a daily dose of 0.3 mg conjugated estrogens for 6 months, after which the dose was increased to 0.625 mg daily. After 1 year of this treatment the patients also received 10 mg of MAP for 10 days each month to induce regular menstrual cycles [91]. Another study on the induction of puberty in Turner's syndrome girls compared the results of oral administration of CEE and transdermal E2 patch. Twelve (12) girls with Turner's syndrome naive to estrogen and on GH therapy for at least 6 months were randomly assigned to receive conjugated oral estrogen at a dose of 0.3 mg daily for the first 6 months followed by 0.3 mg or 0.625 mg, on alternating days for the next 6 months, or transdermal E2 0.025-mg patch twice a week for 6 months followed by a 0.0375 mg patch twice a week for the next 6 months, for a total of 1 year. The transdermal E2 patch resulted in faster bone accrual at the spine and increased uterine growth compared with the oral conjugated estrogen. There were no significant differences between the two treatment groups

**Table 7.5** Studies on puberty induction with EE2

Authors	Age (years)	EE dose	GH Therapy	N° cases	End-point
Martinez et al. [83]	8.6–13.3	100 ng/kg BW/day 2.4 µg/day		9	
Ross et al. [84]	5–15	100 ng/kg BW/day 50 ng/kg BW/day 25 ng/kg/day	Yes	39	Leg growth rate
Vanderschueren-Lodeweyckx et al. [85]	5–6.6	25 ng/kg/day	Yes	40	Height increase velocity, bone maturation, breast development
Mauras et al. [86]	7.7±0.5	100 ng/kg BW/day	... No	9	Endogenous Production rate and MCR of GH
Ross et al. [87]	7–9	25 ng/kg/day	Yes		Verbal and non-verbal memory
Johnston et al. [88]	9.1	50–75 ng/kg BW/day (GH after the first year) GH GH + EE	Yes	58	Height increase
Ross et al. [89]	5–12.5	Double placebo EE alone + placebo injection GH +oral placebo EE+ GH	Yes	149	Adult height
Perry et al. [90]	7–13	Year 1: 1–2 µg/day Year 2: 2–4 µg/day Year 3: 6–8/10 µg/day×4 months	yes	92	Height increase velocity, bone maturation Pubertal progression
Quigley et al. [82]	5–12.5	25 ng/kg/day, ages 5–8 years 50 ng/kg/day, ages >8–12 years Escalating EE2 doses after age 12 years, starting at a nominal dosage of 100 ng/kg/day. Reduction by 50 % placebo/EE2 dosages for Breast development < 12 years, Vaginal bleeding <14 years, or Undue advance in bone age.	GH placebo	149	Childhood low-dose EE2 (as early as age 5 years) Followed by Individualized pubertal induction regimen starting after age 12 and escalating to full replacement over 4 years

**Table 7.6** Conjugated estrogens, oral estradiol and transdermal estradiol equivalents [93]

Conjugated estrogens (mg)	Oral estradiol (mg)	Transdermal estradiol ( $\mu\text{g}$ )
0.3–0.45	0.5–1	25–37.5
0.625	1–2	

regarding high-density lipoprotein, low-density lipoprotein, triglycerides, or growth rate over time [92] (Table 7.6).

### 7.3.8 Progesterone and/or Progestin Addition

It is important to add progesterone or a progestin when estrogen therapy is administered to balance the estrogenic stimulation of the endometrium. Moreover, recent findings permit us to hypothesize that progesterone has an effect on human osteoblasts. One meta-analysis showed that postmenopausal women treated with combined hormone replacement therapy presented a 1.7% increase yearly in bone mineral density (BMD) compared to the 1.3% increase yearly in BMD in women using unopposed estrogens; associated administration of progestogen increased BMD by an additional 0.4% yearly [94]. Progesterone is usually added to the treatment schedule either after 2 years of estrogen therapy, when breakthrough bleeding occurs, or when breast Tanner B3 is reached [95].

#### 7.3.8.1 Micronized Progesterone for Oral Administration

Efficient oral delivery of progesterone is achieved by using a micronized form of the hormone [96] in suspension in oil and packaged in a gelatin capsule [97]. The mean plasma levels achieved with oral progesterone doses of  $\geq 100$  mg are at least as high as luteal phase levels. Maximum concentration is reached within 3 h. The concentration remains significantly elevated for up to 12 h and does not return to baseline level until at least 24 h after administration [98]. We note, however, that studies on the endometrium of post-menopausal women undergoing hormonal replacement therapy demonstrated that 200/300 mg per day did not induce uniform endometrial secretory features. There is sufficient evidence that when given orally progesterone is metabolized in the liver to pregnenolone and pregnanediol, but the high concentrations of progesterone metabolites produced during the first liver pass might result in artificially high concentrations of progesterone in serum [99] due to laboratory methodology [100]. In addition, the metabolites produced may cause side effects such as nausea and sleepiness. The half-life for 100–300 mg doses of oral micronized progesterone are:

Single daily dose	Half-life after (h)
100 mg	18.3 $\pm$ 3.5
200 mg	16.8 $\pm$ 2.3
300 mg	16.2 $\pm$ 2.7

With two doses daily (100 mg in the morning + 200 mg in the evening), progesterone plasma concentration remains significantly elevated for 24 h [101].

*Vaginal administration of micronized progesterone* avoids the first-pass metabolism in the gastro-intestinal tract and liver and it produces sustained plasma concentrations [102]. This method does not present any important objective side effects, but it does entail problems of compliance in younger patients who generally do not accept vaginal administration, especially before first intercourse, and in girls and women of any age in some cultures. In addition, this method of administration can have unpleasant effects such as vaginal discharge, vulvar edema, and irritation, as mentioned in numerous reports on the internet but not in the professional literature [103]. This situation has led to the alternative administration of progestins during hormonal replacement therapy.

### 7.3.8.2 Progestins

Four types of active oral synthetic progestins are available: progesterone derivatives, 19-nor progesterone derivatives, 19-nor testosterone derivatives, and spiro-lactone derivative (Drospirenone). All four exercise progestational action and some also have tissue antiestrogenic effects. Depending on their individual chemical structures, they may act as weak androgens or antiandrogens, glucocorticoids or antimineral corticoids (Table 7.7).

**Table 7.7** Endocrine characteristics of principal progestins

Derivatives of progesterone	Androgenic	Progestogenic	Estrogenic	Glucocorticoid	Mineralcorticoid
<i>Derivatives of progesterone</i>					
Dydrogesterone	00	+++	+	0	+
17-OH Progesterone					
Cyproterone acetate	0/-	+++	0	+	+
MAP	+	+++	0	+	+
19-nor progesterone Nomegestrol acetate	---	+++	0	0	0
<i>Derivatives of progesterone</i>					
Estranes (13-methyl gonanes)					
Norethisterone acetate	++	+++	++	+	0
Norethisterone	+++	+++	++	+	+
Gonanes (13-ethyl gonanes)					
Levonorgestrel	+++	+++	0	0/+	0
Desogestrel	+	+++	0	0/+	0
Gestodene	+	+++	0	+	0/-
Dienogest	---	+++	0	0	0

0 = neutral; -, --, --- = antagonist; +, ++, +++, +++++ = agonist [74]

*Dydrogesterone*, included in the group of Pregane derivatives, is a retroprogesterone formed from progesterone by UV light exposure; it is commercially available, does not have clinically relevant androgenic, estrogenic, glucocorticoid, or mineral-corticoid effects, and it resembles progesterone mainly in its progestogenic effects [104]. The metabolite half-life is 17 h, and it has a 75% relative binding affinity for progesterone receptor; the configuration of metabolites remain stable also after oral administration [105]. These are the reasons why dydrogesterone can be considered an ideal oral supplement progestogen in hormone replacement therapy. Other considerations are that it has minimal side effects, as demonstrated in a recent review in the literature [103]; its beneficial effects and safety in hormone replacement therapy have been widely confirmed [106]; and it has been used as support therapy in the second phase of the menstrual cycle in place of micronized progesterone [107, 108].

There are many reasons for choosing non-androgenic progestins in hormone replacement therapy. Androgenic progestins antagonize the favorable cardiovascular effect of estrogens, whereas non-androgenic progestins do not impair, and may even enhance, the beneficial effect of estrogens [109]. Data on progestins in menopausal hormone therapy demonstrated that MPA Medroxyprogesterone acetate, but not oral micronized progesterone or other progestins, increase monocyte cell endothelium adhesion; MPA and the association MPA/E2 have negative effects on the brain, whereas the progesterone/E2 combination has neuroregenerative effects [110] (Table 7.8).

### 7.3.9 Special Considerations in Choosing Hormone Replacement Therapy

Turner’s syndrome patients may present particular cardiovascular anomalies (structural defects and various cardiovascular risk factors, including arterial hypertension, hyperlipidemia, obesity, and diabetes mellitus), autoimmune conditions (such as hypothyroidism, celiac disease) and altered brain structure and function [64, 112, 113]. These subjects present a 4.7% prevalence of metabolic syndrome associated with visceral obesity; fatty liver was observed in Turner’s syndrome patients with metabolic syndrome and insulin resistance [114]. Although insulin resistance in Turner’s syndrome adolescents undergoing GH treatment is comparable to that of obese patients, their overall metabolic risk factors seem to be lower [115].

**Table 7.8** Metabolic effects of Dydrogesterone, MPA, and Norethisterone [111]

Type of effects	Dydrogesterone	MPA	Norethisterone (Norethindrone)
Androgenic	No	Mild	Yes
Estrogenic	No	No	Metabolites
Glucocorticoid	No	Yes	No
HDL cholesterol	No effect	↓ (reduces E effect)	↓↓ androgen effect
Glucose metabolism	No effect	↓ glucose tolerance	↓ glucose tolerance

### 7.3.10 Effects of Hormone Replacement Therapy

*Mammary gland development* to stage B4 within 2 years is the primary criterion for successful puberty induction. Normal breast development up to B5 is generally mimicked, but with a 2-year delay in one series [77]. Another series found incomplete breast development in women with 45,X (54.3 % breast, Tanner Stage 5) and 45,X/46,XY karyotype (50.0 % breast, Tanner Stage 5), whereas 75 % of women with 45,X/46,XX karyotype presented complete breast development [116].

*Uterus development* is generally suboptimal in Turner subjects treated with oral estrogens (Table 7.9).

*Bone Mineral Density* Whatever the cause, ovarian insufficiency with onset in fertile aged women comports reduced bone mass density (BMD). The problem is particularly important when it occurs before the individual reaches peak bone mass. It is well known that the highest individual levels of bone mass are conditioned by genetic and ethnic factors, nutrition and physical activity, as well as

**Table 7.9** Oral estrogen treatment and uterus development in Turner's syndrome

Authors	Estrogen	N° cases (age in years)	Results (uterus)
Paterson et al. [117]	EE	56 (13.5–18)	Incomplete uterus development in 50 %
Doerr et al. [116]	EE CEE E2V COC	75 (15.8–30.8)	Normal uterus sizes in women with karyotype 45,X/46,XX Uterus length <22 SDS in 26 % of TS women with karyotype 45,X Uterus volume <22 SDS in 18 %
Bakalov et al. [118]	E2 (12 %) CEE (32 %) COC (31 %) No ERT (20 %) Other 5 %	86 (28–45)	24.4 % fully developed size and shape of uterus 44.2 % transitional uterus 31.4 % immature “cylindrical shaped” uterus No correlation between age at first exposure to estrogens and uterus size
Nabhan et al. [92]	CEE TD E2	12 (14.0 ± 1.7)	Increased uterine growth after 1 year TD-E2; 66 % in TD-E2 group vs. 0 % in CEE group
Snajderova et al. [119]	E2	57 (18.1–41.5)	Suboptimal uterine development in most patients.
Bannink et al. [77]	E2	56 (19.2)	↓ Uterus volume vs. controls (subnormal uterus dimensions in women aged nearly 20 years)
Cleemann et al. [79]	E2	41 (17 ± 3.3, range 11.2–24.9)	Mean uterus volumes lower than controls
Kim et al. [120]	E2V	19 (13–17)	Better uterine development with 1 mg E2V than 0.5 mg starting dose

sex steroid levels. In Turner's syndrome BMD is present in the cortical bone with osteopenia or osteoporosis, increased risk of fractures, and it is most evident in childhood and after age 45 years [121–124]. The increased risk of fractures is due to the specific characteristics of the bones in Turner's syndrome, e.g., small bones, altered bone geometry, and greater risk of falls and trauma related to hearing impairment [125, 126] as well as to the sedentary lifestyle of many of these subjects.

It has been known for many years that Turner patients have low bone mineralization, documented by standard X-ray examination [127]. Studies on bone fragility in Turner's syndrome using quantitative ultrasonography (QUS), measuring speed of sound, and two dimensional DXA of radius and tibia evidenced low speed of sound (indicating high bone fragility), but the DXA did not evidence lower bone mass density compared to controls [128]. We note that since the two-dimensional nature of DXA scanning gives an area value of BMD, it gives lower measures of BMD in Turner's syndrome patients. Corrected to size, the quantitative DXA findings show normal volumetric BMD in these patients [128].

Subsequent investigations evidenced reduced cortical bone density with normal trabecular bone density in girls with Turner's syndrome, with bone mineral apparent density (BMAD) of the femoral neck together with reduced cortical bone density of the radius (the trabecular bone was spared) [129, 130]. Other studies using the same method of high-resolution peripheral quantitative computed tomography (HR-pQCT) demonstrated higher cortical thickness with lower cortical porosity in radius of Turner's syndrome patients; trabecular integrity was compromised with lower bone volume per tissue volume (BV/TV) (27 % in radius, 22 % in tibia, both  $p < 0.0001$ ), trabecular number (27 % in radius, 12 % in tibia, both  $p < 0.05$ ), and higher trabecular spacing (54 % in radius, 23 % in tibia, both  $p < 0.01$ ) [131]. However, it is an open question. Recently some of the same investigators affirmed that cortical BMD is not decreased in the radius in Turner's syndrome subjects, and that the artificially low cortical bone mineral density is due to the partial volume effect [132]. In addition, a group of 32 TS girls with an average age of 16.7 years (range 12.4–20.2) had lower BMAD ( $\text{g}/\text{cm}^3$ ) at the lumbar spine than an age- and sex-matched population [133].

The altered mechanical strength of the skeleton in Turner's syndrome is probably related to haploinsufficiency due to certain genes located on the X-chromosome; one candidate gene is SHOX, on the distal portion of Xp [134]. Skeletal growth is also impaired by the presence of smaller than normal bones and bones with altered geometry. Recently, Faienza et al. [135] demonstrated for the first time high osteoclastogenic potential both in girls and young women with Turner's syndrome. This seems to be associated with elevated FSH serum levels before hormone replacement therapy and high RANKL levels during the therapy, irrespective of karyotype. A genetic defect related to vitamin D receptor gene polymorphism (genotype BsmI or FokI) could aggravate the hormonal deficit [136] as well as conditions such as celiac disease and thyroid disorders. In summary, various mechanisms

contribute to reduced bone mass in Turner's syndrome [137]. Turner's syndrome related mechanisms are:

- Puberty delay
- Estrogen deficiency which persists throughout life
- Low intake of vitamin D
- Vitamin D receptor gene polymorphisms
- Hormonal factors (i.e., thyroid disorders)
- Autoimmune diseases

### **7.3.10.1 Treatment to Improve Bone Mineral Density in Turner's Syndrome**

A study on longitudinal changes in bone mineral density in Turner's syndrome and related changes in biochemical parameters in 54 TS women (22–65 years, mean 37) after conventional hormonal replacement therapy with 17 $\beta$ -estradiol (E2, 2 mg) for the entire cycle and norethisterone (1 mg), medroxyprogesterone (10 mg), or levonorgestrel (0.25 mg) for 10 days each cycle showed only slight longitudinal changes in bone mass density in the 5.9-year study period. No changes in bone formation markers were seen during the study, but bone resorption markers decreased in Turner's syndrome patients who were all encouraged to maintain a healthy lifestyle and take calcium and vitamin D [78].

Good results were achieved with transdermal E2 administration in a randomized controlled study using conjugated oral estrogen (0.3 mg daily for the first 6 months followed by alternating 0.3 mg and 0.625 mg daily for the second 6 months) versus transdermal E2 (0.025-mg patch twice a week for 6 months followed by 0.0375 mg patch twice a week for the second 6 months) for 1 year [92]. Another study using ECE (0.625 mg + dihydrogesterone 10 mg/day for 11 days) indicated that estrogen therapy may be effective in increasing BMD in young TS patients if initiated before age 18 years [138].

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## **7.4 Hormonal Therapy in POI with Post-menarche Onset**

In POI with post-menarcheal onset, therapy is targeted at recuperation of normal endocrine asset both to treat unpleasant menopause-related symptoms (e.g., vasomotor symptoms and vaginal atrophy) and to prevent the adverse effects of estrogen deficiency. The therapy involves protecting the cardiovascular system, maintaining and optimizing bone mass, sexuality, quality of life, and even preparing patients for assisted reproduction techniques. Subjects with POI are at higher than normal risk for cardiovascular problems, as evidenced by the numerous reports of death risk due to vascular events [139–146], especially women who underwent ovariectomy before age 45 years in a Mayo Clinic Cohort Study on 4780 women [147] and a Nurses' Health Study on 30,000 women [148]. These data have been confirmed in recent meta-analyses [149, 150]. Women ovariectomized before natural menopause are at increased risk of cognitive impairment or dementia [151] and Parkinsonism [152]. In addition, women with premature ovarian failure (or premature menopause)

present early onset vascular endothelial dysfunction, associated with sex steroid deficiency [153, 154]. Circulating EPCs, CIMT, and diastolic function are significantly affected in young women with POF. We note that also healthy women in the transitional phase to menopause [155] and young women in menopause due to surgery [147, 156] present altered endothelial function related to estrogen deficit. The data regarding risk of ischemic stroke in POI patients is controversial: some investigators have reported increased risk [157], others found no changes [158], and others reported increased mean arterial pressure ( $94 \pm 10$  vs.  $86 \pm 5$  mmHg) despite normal endothelial and autonomic modulation of vasculature [159]. Increased risk of venous thromboembolism (VTE) was also reported in these patients [58].

*Natural estrogens* can be administered in oral ( $17\beta$ -estradiol, estradiol valerate, conjugated estrogens) and transdermal (patch, gel, cream) preparations, and synthetic estrogen is also now available for administration in oral and vaginal preparations for use in hormonal replacement therapy. Conjugated estrogens purified from pregnant mare urine have been used in estrogen hormone replacement therapy since 1942, but only recently were the steroidal components of the product fully identified [160]. In Italy, conjugated estrogens are currently available only in 0.3 mg doses combined with 1.5 mg MAP. A synthetic estrogen (EE2) is also available for administration in both oral and vaginal preparations for use in hormonal replacement therapy.

*Transdermal E2* is, from a metabolic point of view, the best choice treatment in adult women with POI. Various investigators have seen that oral estrogens can cause increase in C-reactive protein, decreased IGF-I and IGF binding protein-3 levels, increased SHBG, and triglyceride enrichment of low- and high-density lipoprotein particles [70, 74, 161, 162]. A study comparing administration of oral or transdermal E2 (41 women, age  $49 \pm 6$  years, hysterectomized and oophorectomized) demonstrated statistically significant reduction in tissue factor pathway inhibitor and plasminogen activator inhibitor-1 levels with oral administration without significant changes in D-dimer levels [163]. The metabolic profile of ethinyl estradiol differs from that of natural estrogen primarily with transdermal administration. Ethinyl estradiol is present in many contraceptive preparations, and it has a stronger hepatic impact than natural estradiol on estrogen dependent markers, such as liver proteins, as listed here below [73].

Effects of ethinyl-estradiol on proteins produced in the liver

↑ SHBG

↑ HDL-C

↑ VLDLk

↑ Angiotensinogen

± Modification of certain estrogen-dependent clotting factors

There is growing observational evidence that transdermal E2 therapy may be associated with lower risk of deep vein thrombosis, stroke, and myocardial infarction [164, 165].

In 64 postmenopausal women randomly assigned to receive either oral or transdermal hormonal replacement therapy (HRT), transdermal HRT (estradiol

combined with NETA) exerted favorable or neutral effects on serum lipids and hs-CRP in postmenopausal women, including decrease in serum triglyceride and Lp(a) levels and increased HDL-2 cholesterol concentration. Therapy with the oral combination was associated with a significant decrease in serum LDL cholesterol and a significant increase in hs-CRP, but no change in serum triglyceride levels [55].

The American College of Obstetricians and Gynecologists has published an opinion on adolescent health care suggesting the use of estradiol-17 $\beta$  delivered via transdermal patch with doses of 100  $\mu$ g (or 0.625–1.25 mg/day oral conjugated estrogen or 2 mg/day oral E2) associated with 10 mg oral medroxyprogesterone acetate daily for 12–14 days every 30–60 days (or 200 mg oral micronized progesterone daily for 12–14 days every 30–60 days). The postpubertal patient treatment options proposed include combined hormone contraception [166].

*The addition of natural progesterone or synthetic progestagens* is essential for prevention of endometrial hyperplasia during estrogen replacement therapy regimens. Micronized progesterone seems to be neutral from a metabolic point of view [167]. Oral dydrogesterone in combination with transdermal estradiol showed no elevation in VTE compared to non-HRT users [168] and may be the preferred when considering thromboembolic risk [106]. The synthetic progestogens differ in their affinity with different steroid receptors and may have some negative impact on cardiovascular risk factors [169] (See section on Progesterone and/or Progestin Addition, 7.3.8).

All patients with POI under treatment with hormonal replacement therapy must be made aware that they have a 5–10% possibility of conceiving [170]; thus they must use barrier contraceptive methods, such as a condom or intrauterine devices. *Combined oral contraception* appears to be acceptable for adolescents undergoing hormonal replacement therapy if peak bone mass has been achieved and there are no contraindications. It can also be considered for young women, especially when contraceptive cover is necessary or requested by the patient, especially considering the benefits of hormonal contraception in regard to both prevention and therapy.

### 7.4.1 Bone Mineral Density in POI with Post-menarche Onset

Young women with primary ovarian insufficiency have significantly reduced bone mineral density compared to regularly menstruating women [171–175]. Bone health was the primary endpoint in a few studies. The results of an open-label, randomized, controlled, crossover trial involving 34 women with POF who were randomized to a 4-week treatment with either TD E2 plus vaginal progesterone or 30  $\mu$ g EE plus 1.5 mg NETA (for 1/3 weeks) demonstrate a beneficial effect on bone mass acquisition on the lumbar spine over 12 months of therapy, mediated by increased bone formation and decreased bone resorption [176]. HRT in those with early menopause does appear to reduce the risk of fracture when administered for at least 3 years [177]. The principal studies on HRT in POI with post-menarche onset involve patients 19–40 years old. All patients received the same dose without variations in relation to age or peak bone mass acquisition; the dose was higher than that normally considered standard for HRT in natural menopause (Table 7.10).

**Table 7.10** Studies on HRT in POI with post-menarcheal onset [181]

Investigators	Intervention/comparison	Population	Outcomes	Results
Guttmann et al. [178]	ECE (0.625 mg) for 28 days MPA (5 mg) for the last 14 days	N° 17 young TS	Bone turnover, uterine and cardiac variables	
Panay and Kalu [5]	E2 TD patch (75–100 mg) or estrogen 0.06% twice daily Cyclical progesterone/progestogen (at least 12 days each month)]	Proposals for POI treatment	Short term: maintenance of quality of life Long term: avoidance of sequelae of POI	Need for confirmation from randomized trials
Langrish et al. [59]	pSSR: E2 TD patch (100mcg) for 1 week followed by E2 TD patch (150 mcg) for 3 weeks Cyclical progesterone 200 g twice daily for weeks 3 and 4 or dydrogesterone 10 mg twice daily sHRT: EE (30 mcg) for weeks 1–3 and NETA [0.5 mg] for weeks 1–3	N° 42 POI Age 19–39 years (chemo/radiotherapy, surgery, Turner's syndrome)	Blood pressure and arterial stiffness	
Crofton et al. [176]	pSSR: phonological sex-steroid replacement sHRT: standard hormone replacement therapy	N° 18 POI Age 19–39 years	Bone mass acquisition and turnover	Only with pSSR increase in bone formation markers
O'Donnel et al. [179]	Open-label, randomized, controlled, crossover trial (reported in Langrish 2009): all participants received both treatments (pSSR and sHRT)	N° 34 POF Age 19–40 years (idiopathic, surgery, following cancer treatment, Turner's syndrome)	Uterine volume and ET, uterine blood flow, non-uterine end-points: bone & cardiovascular	
Popat et al. [180]	3-year prospective, double-blind, randomized, placebo-controlled clinical trial. E2 TD patch (100 µg/day) + MAP 10 mg/day (12 d/mo) or Transdermal estradiol (100 µg/d) + oral medroxyprogesterone acetate 10 mg/day (12 d/mo) + testosterone	N° 145 (spontaneous, 46 XX POI and 70 controls)	Change in BMD at the femoral neck measured by DEXA	BMD restored to normal control levels with E2/MAP therapy; no further benefit with T addition

Modified from Gambacciani and Levancini [181]

The optimal dose for patients with POI onset after age 30 years remains to be established. Hormonal replacement therapy regimens must be based on individual patient symptoms and bone mineral density at the time of first observation. HRT must be continued until the patient reaches the age of average normal menopause. Five years ago, a committee of the American College of Obstetricians and Gynecologists proposed bone density monitoring by dual-energy X-ray absorptiometry (DEXA) once a year to document peak bone mass achievement during early- to mid-puberty, and then once every 2 years through late adolescence [166].

Evidence statements regarding eight prospective cohort studies (sample sizes ranging from over 300 to 100,000 women) showed that the risk of any non-vertebral, vertebral, hip and/or wrist fracture was significantly lower for current HRT users than either women not currently using HRT or who had never used HRT [181, 182] (Table 7.11).

### 7.4.2 Cognitive Function in POI

Most available data on cognitive function in POI refer to women over 40 years old, prevalently women who have undergone oophorectomy before menopause. Premenopausal oophorectomy (unilateral and bilateral) increases the risk of cognitive impairment, dementia and/or Parkinsonism [151, 152]. The younger the patient is at the time of oophorectomy the higher the risk of such problems.

### 7.4.3 Psychological Consequences of POI

We emphasize that diagnosis of primary ovarian insufficiency can be emotionally traumatic for the patient and even the family. Women with spontaneous hypergonadotropic hypogonadism have the perception of receiving less social support and have less self-esteem than control women. Specific strategies targeted at alleviating such psychological discomforts could help these women overcome their disappointment and dismay regarding the life altering diagnosis of spontaneous POI [183]. Also parents need assistance in learning how to help their daughters understand and live with the diagnosis in a way that allows for healthy growth and development [184].

**Table 7.11** Estrogen dosages routinely administered for bone protection in HRT

	Oral estradiol (mg)	Conjugated estrogens (mg)	Transdermal estradiol (mcg)	Tibolone (mg)
Standard	2	0.625	50	2.5
Low dose	1	0.45	25	1.25
Ultra-low dose	0.5	0.30	12.5	

Modified from Gambacciani and Levancini [181]

#### 7.4.4 Androgen Deficiency in POI

We know that after menopause both the ovaries and the adrenal glands continue to produce androstenedione and testosterone. Total testosterone concentrations are decreased in women with spontaneous POI or iatrogenic menopause, as pointed out in data from a meta-analysis of 206 articles regarding spontaneous POI and 1358 on iatrogenic menopause [185]. Hypoandrogenism was evidenced in cases with diminished functional ovarian reserve in 140 women under 38 years old, with premature ovarian aging, occult primary ovarian insufficiency, and abnormally low functional ovarian reserve measured by age-specific FSH and/or anti-Müllerian hormone levels and 166 women over 40, with diminished ovarian reserve; the 49 control patients, under 38 years old, demonstrated normal functional ovarian reserve indicated by FSH and/or AMH levels [186].

Regarding dehydroepiandrosterone (DHEA), the limited findings available show that in POI hematic levels are not significantly different from those found in fertile aged normal women, but they are elevated compared to those of postmenopausal women [187, 188]. In a study on 67 women with Hashimoto thyroiditis, only those who tested positive for antithyroid antibody showed comparatively low, but within normal limits, DHEA levels [189].

There is ongoing debate about the repercussions of androgenic deficiency on bone mineral density, sexual desire, quality of life, self-esteem, and mood. Women with POF tend to have a lower sense of sexual wellbeing than normal women; they experience less sexual arousal and thus have more unsatisfactory sex lives, with less frequent sexual intercourse, reduced lubrication and increased genital pain. Although total testosterone and androstenedione levels are lower in women with POF, this is only one of the factors to be considered among the many that may play an important independent role in the various aspects of sexual functioning [190]. The treatment of low/hypoactive sexual desire disorder is multifaceted and should include a combination of both pharmacological treatments able to maximize the biological signals that drive sexual response as well as individualized psychosocial therapies to help patients overcome personal and relational difficulties [191].

#### 7.4.5 Testosterone Replacement Therapy

Administration of testosterone replacement in POI is controversial, and it has been studied only in the context of surgical menopause [192]. Regarding repercussions on bone mineral content, in a double-blind, randomized, placebo-controlled clinical trial involving patients with iatrogenic POI, administration of testosterone did not modify bone mineral density at the femoral neck, evidenced by DEXA. The patients in this study received transdermal testosterone (150 µg/day) and transdermal estradiol (100 µg/day) plus oral medroxyprogesterone acetate (10 mg/day, 12 days/

month) for 3 years [180]. Another trial investigating the efficacy of physiologic testosterone replacement in hormonal replacement therapy with E2 T(100 µg/d)+MAP (10 mg/day) also evaluated quality of life, self-esteem, and depressive symptoms in patients. After 12 months of treatment, there were no differences between treatment groups regarding changes in quality of life, self-esteem, and/or mood symptoms [193].

#### 7.4.6 DHEA Therapy

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are the most abundant steroid hormones in humans. DHEA is produced mainly by the adrenal cortex and to a limited extent by the ovaries, and is rapidly sulfated by sulfotransferases into DHEAS [194]. Large amounts of DHEA and DHEAS are produced during fetal development; the production falls sharply after birth and remains low for several years; during adrenarche (age 6–8 years) synthesis resumes and high levels are maintained throughout adult life, providing substrates for conversion into potent androgens and estrogens in peripheral tissues [195–197]. An age-dependent decline in DHEA and DHEAS levels begins by the third decade and progresses with advancing age, by around 2–5 % per year, such that by menopause the DHEA level has decreased by 60 %.

“DHEA and its unconjugated and sulfated metabolites participate in various physiological and pathophysiological processes, including management of GnRH cyclic release, regulation of glandular and neurotransmitter secretions, maintenance of glucose homeostasis [...] and insulin insensitivity [...], control of skeletal muscle and smooth muscle activities including vasoregulation, promotion of tolerance to ischemia and other neuroprotective effects.” [198] The biological actions of DHEA(S) in the brain involve neuroprotection, neurite growth, neurogenesis and neuronal survival, apoptosis, catecholamine synthesis and secretion, as well as anti-oxidant, anti-inflammatory, and antiglucocorticoid effects. In addition, DHEA affects neurosteroidogenesis and endorphin synthesis/release [199]. DHEA is also a precursor of estrogen receptor beta (ERβ) ligands [200].

Several studies have explored the role of DHEA in sexual function in women, and replacement therapy with DHEA in women with androgen insufficiency and sexual dysfunction appears to have some beneficial effects on sexual desire, arousal, activity, interest, fantasy and drive, and relationships, but further studies are necessary [201].

The role of DHEA/DHEAS in POI therapy is intriguing. The basic question is whether or not DHEA supplementation is useful in POI. This is a relatively recent therapeutic addition to the management of female infertility, in particular for women with diminished ovarian reserve. The mechanism of action of DHEA on the ovaries is not clear. DHEA has been found to be a prehormone of the follicular fluid testosterone during the administration of gonadotropins for ovulation induction which

leads to the formation of estradiol. Androgen has been proposed to be a metabolic precursor for steroid production and acts as a ligand for androgen receptors, thus influencing follicular growth in ovaries. Another hypothesis is that the beneficial effect of DHEA may be due to an increase in insulin-like growth factor-I. In fact studies on rhesus monkeys showed that androgen treatment (testosterone and dihydrotestosterone) can promote IGF 1 and IGF 1 receptor gene expression in the primate ovary [202]. Finally, some investigators have postulated that DHEA has a beneficial effect on oocyte quality and mitochondrial function [203].

The effectiveness and safety of DHEA and testosterone as adjuncts to androgen replacement therapy in women undergoing assisted reproduction have been investigated. Treatment with DHEA and testosterone appeared to improve the live birth/ongoing pregnancy rate and clinical pregnancy rate in women identified as poor responders [204]. However, when studies with a high risk of bias in sensitivity analyses are excluded the effects of DHEA and T treatment are no longer statistically significant, also given the variable lengths of treatment. In addition there is scarce proof of the safety of DHEA and testosterone treatment, and evidence to date is limited by generally small sample sizes and inadequately reported study methodologies.

A Task Force appointed by the Endocrine Society, American Congress of Obstetricians and Gynecologists (ACOG), American Society for Reproductive Medicine (ASRM), European Society of Endocrinology (ESE), and International Menopause Society (IMS) commissioned two systematic reviews of published data, meta-analyses, and trials on DHEA and T replacement therapy. The Endocrine Society continues its strong recommendation against the general use of testosterone in treating infertility, sexual dysfunction (except for a specific diagnosis of hypoactive sexual desire, and only for 3–6 months), cognitive, cardiovascular, metabolic and bone health, or general well-being as well as against the routine use of DHEA for women due to limited data concerning its effectiveness and safety in normal women or those with adrenal insufficiency. The Task Force recommends against routine prescription of testosterone or dehydroepiandrosterone for women with low androgen levels due to hypopituitarism, adrenal insufficiency, surgical menopause, pharmacological glucocorticoid administration, or other conditions associated with low androgen levels because the data supporting improvement in signs and symptoms with these therapies are limited and there are to date no long-term studies of risk [205]. Another two recent meta-analyses also confirm the need for further investigations to clarify the effects of DHEA exposure in assisted reproduction technology [206, 207].

On the other hand, all the scientific societies recommend use of hormonal therapy or oral contraception until the median age of natural menopause with periodic reassessment for all women with POI. A fundamental part of that therapy is psychological support to help patients address the issues associated with early menopause, including loss of fertility, changes in self image, and sexual dysfunction [208]. Early initiation of HRT is, however, always necessary to optimize metabolic parameter and enhance bone, mass [209].

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# Functional Hypothalamic Amenorrhea as Stress Induced Defensive System

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## 8.1 Introduction

Usually amenorrhea defines the absence or abnormal cessation of the menstrual cyclicity [1], and it is defined as primary or secondary amenorrhea depending from the occurrence of amenorrhea before or after menarche, respectively. Most of the causes of primary and secondary amenorrhea are similar. Timing of the evaluation of primary amenorrhea recognizes the trend to earlier age at menarche and is therefore indicated when there has been a failure to menstruate by age 15 in the presence of normal secondary sexual development (two standard deviations above the mean of 13 years), or within 5 years after breast development if that occurs before age 10 [2].

Failure to initiate breast development by age 13 (two standard deviations above the mean of 10 years) also requires investigation [2]. In women with regular menstrual cycles, a delay of menses for as little as 1 week may require the exclusion of pregnancy; secondary amenorrhea lasting 3 months, and oligomenorrhea involving less than 9 cycles a year require investigation.

The prevalence of amenorrhea not due to pregnancy, lactation, or menopause is approximately 3–4% [3, 4]. Although the list of potential causes of amenorrhea is long, the majority of cases can be restricted to four conditions: polycystic ovary syndrome, hypothalamic amenorrhea, hyperprolactinemia, and ovarian failure. Other causes can involve diseases that are typical for internal medicine or endocrinological diseases such as diabetes, adrenal gland or thyroid diseases and where amenorrhea is not really related to a typical reproductive problem but is related to an unbalanced endocrine control of organs or systems that can induce an abnormal control of the hypothalamus-pituitary-ovarian axis.

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Primary amenorrhea is not so common, in our Centre for Gynecological Endocrinology only 8–10 patients per annum are diagnosed a primary amenorrhea, while higher number of patients is diagnosed with secondary amenorrhea [5–7]. The World Health Organization (WHO) has logically recognized specific groups of amenorrheic patients: WHO group I: no evidence of endogenous estrogen production, normal or low FSH (Follicle Stimulating Hormone) levels, normal prolactin levels, and no evidence of a lesion in the hypothalamic-pituitary region; WHO group II: evidence of estrogen production and normal levels of prolactin and FSH; WHO group III: elevated serum FSH levels indicating gonadal failure [8, 9].

Whatever are the causes, the patients affected by functional hypothalamic amenorrhea (FHA) belong to the WHO group II, that is, they show evidence of low estrogen production, FSH secretion and PRL levels normal or within the upper limit of normality.

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## 8.2 Functional Hypothalamic Amenorrhea (FHA)

Though functional hypothalamic amenorrhea (FHA) is classified in the WHO group I, it is considered as a hypogonadotropic hypogonadism related to a severe change of the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus [10–12]. The disturbances of the hypothalamic-pituitary-ovarian axis in FHA may be very broad and includes from low to the complete absence of LH pulses in presence of normal level of FSH [12]. Because of this, there are low or very low estradiol plasma levels due to a reduced estradiol production in the ovary. The disturbed hypothalamic-pituitary-ovarian axis in FHA cases is associated typically with stress, weight loss, and/or excessive physical exercise and is one of the most common causes of secondary amenorrhea [12]. Depending on the triggering factor, there are three typologies of FHA: weight loss related, stress related, and exercise related [13]. However, it is relevant to state that though the trigger might be one of these three, usually all of them result to be tightly interconnected and regardless of the specific trigger, a complex state of hypoestrogenism, other endocrinological aberrations, and metabolic abnormalities due to FHA may affect the whole body homeostasis [14].

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## 8.3 Epidemiology and Diagnosis

Classically, secondary amenorrhea is defined when there is at least an interval of 3 months of absence of menstruation, and usually occurs in approximately 3–5 % of women after menarche. FHA can be recognized in 20–35 % of secondary amenorrhea cases and in 3 % of FHA cases of primary amenorrhea that is in those cases where menarche does not occur at the right time but later than expected and no other cause is recognized as causal factor for such situation of delayed menarche [15]. Hypothalamic amenorrhea is very frequent among athlete women. In fact, it has been estimated [16] that approximately 50 % of women who exercise regularly

experience menstrual disorders and approximately 30% of them experience amenorrhea. Several of them show the triad of distorted eating, amenorrhea, and osteoporosis, first described in 1997 and is known as female athlete triad [17].

Functional hypothalamic amenorrhea can be differentiated from the other forms of primary or secondary amenorrhea on the basis of the anamnesis as well as from the assessment of low or very low gonadotropins and estradiol plasma levels [12]. In patients with FHA, the GnRH stimulation test shows the LH and FSH response, thus distinguishing FHA from pituitary diseases, where hypogonadism is also characteristic [13, 15]. Once the hypothalamic origin has been found, it is important to exclude eventual rare genetic and organic diseases such as Kallman syndrome (characterized by anosmia, specific mutations), Prader-Willi syndrome (with characteristic hyperorexia, obesity, retardation), and other rare syndromes with idiopathic hypogonadotropic hypogonadism [12, 15, 18]. Obviously, features such as delayed puberty, primary amenorrhea, and the presence of additional symptoms (anosmia, mental retardation, extreme obesity, facial dysmorphism, and malabsorption) are suggestive of congenital diseases [15, 18].

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## 8.4 Neuroendocrine Dysfunctions of FHA

As it can be argued from what is reported in the previous section, FHA [19–21] is a model of reproductive dysfunction characterized by the fact that there is no organic disease to trigger it. Indeed, FHA is classically characterized by a hypoestrogenic condition as a result of several neuroendocrine aberrations, which occur after a relatively long period of exposure to a repetitive and/or chronic stressor(s) that negatively affect the neuroendocrine hypothalamic activity [10, 22] as well as the release of several hypophyseal hormones [23]. In these patients, the reproductive axis is severely impaired and both the opioid and dopaminergic systems are involved as potential mediators of stress-related amenorrhea in humans [24, 25]. As demonstrated in experimental animals, the EOPs exert an inhibitory effect on the episodic release of both GnRH and LH also in humans [26]. Naloxone infusion is able to induce the increase of LH plasma levels only during the late follicular and luteal phases of the menstrual cycle but not during the early follicular phase [27, 28], and such response was recorded also in postmenopausal women only after hormonal replacement therapy [29]. All these reports sustain the fact that estrogens modulate the opioidergic blockade and when naltrexone cloridrate is administered in FHA, LH plasma levels start to increase within few weeks [30]. In the last two decades, it has been demonstrated that also dopaminergic and serotonergic pathways are deeply involved in the mechanisms that link stress-induced amenorrhea and reproductive function in FHA [24, 31, 32]. In addition, most of the patients that suffer really for a FHA show an elevation of the cortisol plasma levels close to the upper limits of normality, while in other amenorrheic conditions (i.e., hyperprolactinemic or hyperandrogenic amenorrhea) cortisol shows normal levels [33].

At the basis of the mechanism of stress, there is the perfect combination and overlapping of various independent situations that occur whenever the stressant

condition(s) is long lasting or chronic. Psychological, metabolic, and physical stressors are the relevant ones that negatively impact on the brain and on the neuro-vegetative systems as well as on the neuroendocrine pathways. The central structure for the reproductive control is the hypothalamus where GnRH is synthesized and released. GnRH secretion is under a specific control of various other hypothalamic nuclei that are all around the paraventricular and supraoptic nuclei where GnRH is secreted. These other nuclei control satiety, glucose, water, and salt levels, and they also receive inputs from the cortex and from other subcortical areas of the brain deeply connected on whatever refers to information coming from the environment in terms of sounds, sights, metabolism, and danger. Practically the hypothalamus is informed on whatever is going on inside and outside the human body. Whatever event changes the perfect equilibrium of all these elements, it results to be able to activate a reaction by the hypothalamus so that to counteract such adverse environmental change and to predispose a defensive condition. Such hypothalamic activation parallel the occurrence of stressant situations, more frequent is the stress, more repetitive activation of the hypothalamic reaction leads to the significant changes that are observed under chronic stressant situations. The block that occurs on the reproductive axis is mainly exerted by the significant reduction of the amount of GnRH secreted by the hypothalamic nuclei (supraoptic and paraventricular) that reduce the gonadotropin episodic discharge with the concomitant reduction of mean plasma concentrations of LH, FSH, and of ovarian steroids.

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## 8.5 Stress as Defensive System

From what is described above, it appears evident that hypothalamus acts as a “computer” that takes care of all inputs and defines specific outputs to monitor/modulate/regulate a lot of other biological functions such as sleep, glucose control, heart function, feeding, reproduction, and many others. This happens also in males, but it is incredibly active and sensitive in females. Nowadays, weight control, physical activity, and a healthy choice of our food are primary elements of our everyday life. These are relevant issues since our western civilization is becoming even more overweight up to obese! Indeed, at least in Italy, progression of obesity has been up to 25% in 25 years and the higher occurrence of obese-related diseases, such as diabetes, hypertension, stroke, has stimulated a part of the population to keep weight and feeding under control. Within this last decade, a higher amount of women started to exercise more than 20 years ago and a certain percentage of them realized that they could move from simple physical activity to a more serious training. It is clear that physical activity is absolutely a healthy element in our everyday life, but the excess of training can frequently occur and this can induce negative effects of the reproductive ability of young girls as well of adult women [34]. These negative effects have been identified in a lower fertility rate due to a higher anovulatory condition. Moreover, it is relevant to note that the pregnancy rate of patients that attend IVF programs are incredibly lower for those women that train too much or have an

agonistic training [35–37] and that the more is the training the higher is the infertility risk (3.2 fold than controls) [37].

Abnormal or irregular menstrual cyclicity is always possible and it is considered normal to have an irregular event per year but those that train or perform an intense physical activity usually experience such irregular cycles more frequently and it has been demonstrated to be correlated to the reduced BMI [38]. The causal factors of this are clear: the beginning of the follicular phase is slowed by the physically induced stress on the hypothalamic areas, these triggers a difficulty in gaining an optimal ovulation so that the following luteal phase results to be insufficient in terms of progesterone released. All this induces an abnormal menstrual cycle in terms of length and/or frequency. Such mechanism can replicate frequently (one cycle after the other) or rarely, but in case the physical stress is furthermore enriched by psychological factors (i.e., working or familiar problems, school failure, etc.); it might evolve to a menstrual cycle blockade. It is important to observe that in such conditions, even though a pregnancy starts, there are higher chances that it stops prematurely within the first 2–4 weeks. The reason of this relies on the fact that a lower estrogen secretion and later a reduced progesterone secretion do not prepare adequately the endometrial tissue to the embryo implantation. An inadequate endometrial tissue that is too thin (below 8–9 mm) is frequently unable to support the embryo implantation.

It is important to consider that physical activity is a positive activity but while it is important to reduce weight in overweight or obese women, for other women, usually with normal BMI, it represents a sort of “running away” from life problems and everyday life. If this is the case, stress of physical activity becomes a sort of “drug” that initially cures the stress cumulated, but after some time it becomes an additional stress on the stressant situations that are still pressing those women. For some time, biology tries to face the adverse conditions (everyday life and physical activity), but if the training becomes too much (i.e., too much hours of sport/gym every week), the neuroendocrine blockade is quite certain.

Not all these events are a real disease, they all belong to a sort of biological attempt to protect those women. Something dated back more or less 30 thousand years ago. For that time, reproduction was probably the less important function since a human being (or a primate) could perfectly survive without reproduction, and the survival of the single human being could not be guaranteed if reproduction hits its goal (i.e., the occurrence of a pregnancy) especially during seasons/periods during which no food was available or a strenuous energy consumption was ongoing (migration), as well as psychological stressors (fear to die). For those times, a pregnancy in the wrong moment might mean an incredibly higher risk to die. To avoid this and to avoid losing that single human being, the hypothalamus developed the ability to block the reproductive axis in reversible way to avoid ovarian function (i.e., ovulation), with the chance to restart it only when the external conditions might be improved and more adequate.

Life in this twenty-first century is not like that many thousand years ago, but there are always many stressful events that can trigger our hypothalamus and

homeostatic system(s) and induce a blockade of reproduction. Although this has to be considered an incredibly well designed defensive system, unfortunately, it might be activated in peculiar period such as during pubertal development, inducing a delayed menarche/puberty. Indeed, when intensive sport training or recreational activity is started at a prepubertal age, it is not so rare to observe a significant delay in the occurrence of pubertal maturation up to 2–4 years [3, 38]. The meaning of this observation is obvious since it indicates that if energy consumption and physical stressors are too much, growth and reproductive axis maturation might not occur in the same time. In fact, most of the girls who suffer from this condition recover into normal development and/or menstrual cyclicity as soon as training is significantly reduced or suspended.

The presence of the right amount of fat is fundamental for pubertal development since it is considered as a specific and obliged reserve of energy that the body might need to have stored in case a pregnancy occurs within the very first ovarian cycles [38, 39]. Nowadays, this reserve of fat is important just for the beginning of the menstrual cyclicity (i.e., menarche) since pregnancy during pubertal age is not so common in Western countries (more frequent in underdeveloped countries), but it is important to consider this biological aspect every time we study a girl who wants to start or has just started any kind of physical activity. Physical activity has not to be strenuous or stressful to avoid any conflict with the maturation/activation of her reproductive axis. A delay in such event might induce reduced bone mass peak, osteopenia, and then reduced bone mass density during fertile life, exposing the girl to damages of the skeleton and of the muscle activity. Hypoestrogenism during adolescence, with no menarche and/or menstrual cyclicity and with a sub-normal development of sex-steroid-dependent tissues (fat distribution, breast development), might be responsible also for an abnormal or conflicting self-image.

What has to be pointed out is that our biology is for sure a perfect system that controls our homeostasis, but we are always forgetting that nowadays, this biology is still working as 30 thousand years ago. Since in those times food was scarce, our biology developed systems/pathways that permitted a fast and optimal way to store the energetic elements derived from food (i.e., glucose, lipids) and a not so fast system to download energetic substances once stored (i.e., lipids from fat cells). Doing so our biology was sure to accumulate at least a minimum of the energy introduced as food, putting the basis of the fact that in the presence of relevant amount of food (as in our times), our homeostatic system would have stored the great part of that energy as fat, inducing overweight up to obesity. It is well clear that we all have to take care of the right amount and quality of food. Even though today we can access to whatever kind of food, we always have to keep feeding under control. That is why the amount of overweight/obese people is a growing social problem and a health issue in all Western countries.

The right amount of food is crucial, not too much and not too low. Quality is essential since it permits a good maintenance and better performances of whatever organ/homeostatic system of our biology. Excess in restriction or excess in feeding induces only abnormal behavior(s) of our homeostatic systems, that in general create the predisposition to reproductive and health concerns.

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## Amenorrhoea and Anorexia Nervosa in Adolescent Girls

# 9

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Anorexia nervosa (AN) is characterized by an intense fear of becoming fat despite an obvious thinness and extreme behaviours for weight loss, such as food restriction with or without self-induced vomiting or use of laxatives. The result is a massive weight loss and pathological thinness. The 12-month prevalence of AN among young females is approximately 0.4%. The presence of AN dramatically affects quality of life both of people with AN and their relatives, and people with eating disorders have particularly high utilization rate of health services [29]. AN is a multifactorial disorder currently conceptualized with biopsychosocial model. There is a strong genetic component, since the heritability is about 60%. These genetic factors are likely to predispose to vulnerability via endophenotype such as perfectionist traits, lack of cognitive flexibility, facilitating the secretion of opioids during fasting, etc. [23, 29]. These factors will be expressed in a specific environmental context and will lead to emergence of disorders, adolescence being a period of particular vulnerability. The main environmental factors favouring the emergence of disorders include idealization of thinness and performance of our western societies, a focused education on the ideal of thinness or food rigidity and the presence of trauma including early trauma. Management of AN is difficult and few treatments have shown efficacy. Cares are usually multidisciplinary and aims to restore normal weight, a suitable and relaxed behaviour with food, improve social and interpersonal relationships, as well as self-perception of patients. Despite these supports, approximately 30% of patients will not cure from their disease. This creates an excess mortality in AN. Mortality increases over time, and is estimated to be 5% per decade of illness [25]. Thus, AN is the psychiatric disorder with the highest mortality rates. From people who will cure, the recover process will take several months to years. In this short review we will discuss three points: what are the features of amenorrhoea in AN, why amenorrhoea has been removed as a criteria of AN and the therapeutics issues of amenorrhoea in AN.

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## 9.1 Features of Amenorrhoea in AN

Amenorrhoea in AN is related to a functional hypothalamic disorder (FHA) which is a condition characterized by the absence of menses due to the suppression of the hypothalamic-pituitary-ovarian axis, in which no anatomical or organic disease is identified. The decreased pulses of gonadotropins and the lack of LH surges lead to the absence of follicular development, anovulation and low estradiol levels. Variable neuroendocrine patterns of LH secretion can be seen [9, 24] including a lower mean frequency of LH pulses, the complete absence of LH pulsatility, as well as a normal-appearing secretion pattern and higher mean frequency of LH pulses. Serum concentrations of FSH often exceed those of LH, similar to the pattern in prepubertal girls in the early stage and as the disease is more advanced both are very low. The wide spectrum of hypothalamic-pituitary disturbances may reflect different stages of the disease and/or genetic susceptibility. In patients with anorexia nervosa, secretion of gonadotropin releasing hormone (GnRH) is also reduced which leads to anovulation and causes a functional hypothalamic amenorrhoea. Several factors are involved in the GnRH dysfunction in AN who share a lot of common characteristics of women with FHA without AN criteria (Table 9.1). The disturbed hypothalamic-pituitary-ovarian axis in FHA cases is usually associated typically with weight loss, stress and/or excessive physical exercise and is one of the most common causes of secondary amenorrhoea. According to the eliciting factor, there are three classes of FHA: weight loss related, stress related and exercise related. The precise mechanisms underlying the pathophysiology of FHA are complex and unclear. Attention should be paid to such substances as kisspeptin, neuropeptide Y (NPY), ghrelin, leptin, corticotropin-releasing hormone (CRH),  $\beta$ -endorphin and allopregnanolone. An energy deficit (which can occur independently of body weight) appears to be the critical factor in both weight-loss and exercise-induced forms of hypothalamic amenorrhoea.

Some of the behavioural features of AN have a direct impact on amenorrhoea:

- Weight loss of between 10 and 15% of normal weight disrupts the menstrual cycle in most women [15]. The value of oestrogen is reduced according to BMI and in patients where the BMI are lower to 15 kg/m<sup>2</sup>, plasma estradiol was not

**Table 9.1** Common characteristics in FHA and AN

Characteristics	Functional Hypothalamic Amenorrhoea (FHA)	Anorexia nervosa (AN)
Body weight	Normal or low	Very low to normal
Body fat	Normal or low	Very low to normal
Intake of calories or fat	Normal or low	Low
Strenuous exercise	Frequent	Hyperactivity
Eating disorders	Variable	Restriction
Leptin	Low	Very low to normal
Emotional stress	Variable	Variable

detected [13]. However, amenorrhoea may precede weight loss in up to 20% of women with AN. Although a minority of women with AN maintains some menstrual activity even at significantly low weights, this should not falsely reassure clinicians or patients that weight restoration is not necessary. Weight gain usually restores normal menstrual cycles. The time course and amount of weight required for resumption of menses has varied among different studies but a goal of attaining 90% of ideal body weight in order to restore normal menses is often described or the weight at which menstruation ceased [26]. Nevertheless, amenorrhoea persists in about 10–30% of patients with AN despite weight gain, because of ongoing abnormal eating behaviours (binge eating and purging), exercise, or stress.

- Nutritional deficiencies that are not associated with weight loss or hyperactivity may lead also to FHA [5]. In contrast to their menstruating counterparts, the women with amenorrhoea severely restricted their fat consumption and had lower body fat mass.
- Patients with AN experience high levels of stress. Increased CRH secretion results in an increased secretion of adrenocorticotrophin from the pituitary and cortisol from the adrenal glands, and these phenomena are linked to a reduced GnRH drive. Elevated serum and also cerebrospinal cortisol concentrations have been reported in FHA [27]. For patients distressed by persistent amenorrhoea, eventual recovery of menstrual periods may occur following psychotherapy [3].

This amenorrhoea has some important consequence on the prognosis of AN. Patients suffering from AN exhibit impaired bone remodelling, which is characterized by a decrease in bone formation and a concomitant increase in bone resorption [19–21]. Oestrogen plays a crucial role on bone mass acquisition and on its maintenance. The effects of oestrogen on bone metabolism have been described as inhibitory for the resorption process, although direct effects on osteoblastic activity have been also described, resulting a reduction of bone turnover [2]. The demonstrated effects of oestrogen on bone metabolism associated with very low values had highly oriented clinicians to claim that the oestrogen deficiency is the major cause of bone loss in AN patients. However, osteopenia is much more severe in AN patients compared to other amenorrhoeic-deficient populations [11]. This suggested that osteopenia/osteoporosis genesis in AN is multifactorial and that other endocrine factors such as IGF-1, cortisol or sclerostin acting on the alteration of the bone cell activity [19–21]. By the way, amenorrhoea has clinical utility because it alerts clinicians to potential deficits in bone mineral density (BMD).

Amenorrhoea and level of oestrogen might also impact the phenotype of anorexia also at a psychological level. Behavioural abnormalities as well as depression and anxiety driven by underlying cognitive processes are a prominent feature of AN and suggested directions for developing innovative therapeutic programmes. Oestrogens, acting through oestrogens receptor- $\beta$ , is anxiolytic in animals [18] and levels of anxiety change across the estrus cycle. Oestrogen may also be involved with perception of body shape, and may account for greater body shape concerns in females than males [22]. Finally, a study suggests that AN people who were in amenorrhoea

or had irregular menses showed significant cognitive deficits across a broad range of many cognitive domains [6].

All this aspects suggest than amenorrhoea is a critical issue in AN. Nevertheless, question of this utility as a marker of AN is controversial.

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## 9.2 Amenorrhoea a Criteria of AN ?

Until the last revision, one of necessary criteria for the diagnosis of AN was amenorrhoea. The fourth edition of the *Diagnostic and Statistical Manual of Mental Disorder* (DSM-IV) specifies the diagnostic criterion for amenorrhoea in AN as “the absence of at least three consecutive menstrual cycles (a woman is considered to have amenorrhoea if her periods occur only following hormone)”. In the updated edition fifth edition (DSM-5) as well as in the upcoming International classification of disease (ICD-11), amenorrhoea has been removed from diagnostic criteria for AN. Several arguments have been advanced for this removal. The first is the lack of specificity of amenorrhoea regarding AN. Indeed, in comparison of groups of patients with AN according to DSM IV criteria and of patients who meet all the criteria with the exception of the amenorrhoea, there are no differences in behavioural phenotype or on any aspects of the psychopathological and neuropsychological functioning between [28]. Moreover, in a latent class analysis amenorrhoea is not linked to AN but distributed in all lower weight classes [4]. We currently do not have markers of anorexia specific enough beings used for diagnostic purposes, but some adipocytokines such as leptin or ghrelin seems to have a higher specificity towards anorexia than amenorrhoea [23]. Another argument is the clinical utility of amenorrhoea as a marker of malnutrition. Amenorrhoea is no more predictive of physical complications and somatic consequences (including bone remodelling) of AN than current BMI and lowest BMI lifetime [1]. On a biological level, the most predictive somatic marker of complications related to under nutrition is the prealbumin [8]. Also, the requirement of amenorrhoea is irrelevant in some subgroups of patients with AN such as women under contraceptive medication, men or menopausal women. Finally, a recurrent critical made to DSM-IV diagnosis was the proportion of patients not responding to a specified disorder (AN, bulimia nervosa). Without amenorrhoea but with all the others features of the disease, AN patients were switched to a category called Eating Disorders Not Otherwise Specified (EDNOS). Up to 60 % of eating disorders were classified in this category [7]. This heterogeneous category initially designed to include residual disorders, was actually a wastebasket class of no interest to identify a clinical description and guide treatment. By relaxing the criteria of AN and bulimia nervosa, and including a new category the binge eating disorders as a separate diagnosis, as well as a number of smaller sub-diagnoses, the DSM-5 aims to reduce numbers of EDNOS. All these arguments therefore logically led to moving amenorrhoea from a mandatory diagnosis criterion to an associated features supporting diagnosis.

### 9.3 Therapeutics Implications

The weight restoration with a return of menstruation is considered the prerequisite to an improvement of the disease. The global aim of care is to restore normal weight, a suitable and relaxed behaviour with food, improve social and interpersonal relationships, as well as self-perception of patients. Given alterations in gonadal function usually observed in patients suffering from AN, it has been proposed to compensate low oestrogens levels by introduction of substitution therapy. Several studies hypothesised that replacement might be effective in increasing BMD. However, randomized clinical trials using different molecules (Premarin, Provera, ethinyl estradiol, norethindrone) [10, 12, 16] showed no significant benefits on BMD. However, the response to the treatment may depend on various factors. Klibanski et al. [16] reported that patients with the lowest percent ideal body weight had the most significant improvement in spinal BMD during oestrogen administration, suggesting that oestrogen replacement therapy may be beneficial in preventing bone loss in young women with extremely low body weight. It was also probable that the lack of significant BMD gain may be attributed to the dose which is usually too low to have a favourable impact on BMD [17]. A too short duration of prescription as well as a poor compliance might also be in play [14]. Klibanski et al. [16] reported a marked improvement in spinal BMD in patients who spontaneously resumed menses during the study, suggesting that resumption of normal menstrual function has a different effect on bone mass than oestrogen replacement. It was probable that oestrogen treatment alone cannot correct the multiple factors (nutritional or other hormonal variable) contributing to bone loss [16]. From these results, hypoeostrogenia may be considered only as a contributing factor to the bone alteration in AN patients, and it may be implicated principally in the reduction of the volumetric BMD, while malnutrition may account for reduced bone size [14]. For a better efficacy, hormonal replacement therapy should be considered soon after diagnosis.

Other studies regarding impact oestrogen in AN are scarce. One study has examined if physiologic oestrogen replacement would ameliorate anxiety and improves eating attitudes [22]. Seventy-two adolescents from 13 to 18 years old were randomized to transdermal estradiol with cyclic progesterone or placebo patches and pills for 18 months. Among the 37 who completed the study, oestrogen replacement improved trait-anxiety (the tendency to experience anxiety), but did not impact eating attitudes or body shape perception. No studies have assessed impact of substitution on cognitive function.

In summary, even if amenorrhoea has been removed from criteria of the disorders, it is an important feature of the disease the clinician has to take into account. The recovery of menstrual function in adolescent patients with AN should be a major treatment goal to prevent severe long-term somatic and psychiatric consequences.

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# The Long-Term Cardiovascular Risks Associated with Amenorrhea

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Amenorrhea is the absence or abnormal cessation of the menses. Primary and secondary amenorrhea describe the occurrence of amenorrhea before and after menarche, respectively. The majority of the causes of primary and secondary amenorrhea are similar. Timing of the evaluation of primary amenorrhea recognizes the trend to earlier age at menarche and is therefore indicated when there has been a failure to menstruate by age 15 in the presence of normal secondary sexual development (two standard deviations above the mean of 13 years), or within 5 years after breast development if that occurs before age 10. Failure to initiate breast development by age 13 (two standard deviations above the mean of 10 years) also requires investigation. In women with regular menstrual cycles, a delay of menses for as little as 1 week may require the exclusion of pregnancy; secondary amenorrhea lasting 3 months and oligomenorrhea involving less than nine cycles a year require investigation. The prevalence of amenorrhea not due to pregnancy, lactation, or menopause is approximately 3–4%. Although the list of potential causes of amenorrhea is long, the majority of cases are accounted for by four conditions: polycystic ovary syndrome, hypothalamic amenorrhea, hyperprolactinemia, and ovarian failure. Secondary amenorrhea, which is defined as 3 months absence of menstruation, occurs in approximately 3–5% of adult women [1, 2].

According to the American Society of Reproductive Medicine, Functional Hypothalamic Amenorrhea (FHA) is one of the most common causes of secondary amenorrhea; therefore, it is responsible for 20–35% of secondary amenorrhea cases and approximately 3% of cases of primary amenorrhea. There are three types of FHA: weight loss-related, stress-related, and exercise-related amenorrhea therefore, DeSouza et al. estimated that approximately 50% of women who exercise regularly

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experience subtle menstrual disorders and approximately 30% of women have amenorrhea, for that reason the incidence of FHA is higher in athlete women. The complex of distorted eating, amenorrhea, and osteoporosis was first described in 1997 and is known as female athlete triad. FHA results from the aberrations in pulsatile gonadotropin-releasing hormone (GnRH) secretion, which in turn causes impairment of the gonadotropins (follicle-stimulating hormone and luteinizing hormone). The final consequences of these clinical conditions are complex hormonal changes manifested by profound hypoestrogenism leading to several short and long-term health implications [3].

As it is known, hypoestrogenism can interfere with the cardiovascular system function in many ways, this is the reason why Cardiovascular disease (CVD) is the leading cause of death in women in developed countries and, interestingly, proportionally more women die from CVD than men. Coronary and peripheral vessels contain estrogen receptors that permit estradiol to play a regulatory role in vascular function. Estrogen excites the synthesis of nitric oxide (NO) through both genomic and nongenomic effects, leading to the augmented production of endothelial-derived NO, causing vasodilatation [4]. Estradiol exerts a positive, cardioprotective effect through its influence on the endothelial, myocardial, and vascular function and metabolic parameters [5]. In contrast, hypoestrogenism can lead to endothelial dysfunction, an impaired bioactivity of nitric oxide, perturbation in autonomic function, activation of the rennin-angiotensin system, and lipid profile changes [6]. These physiological and pathological phenomena are reflected in clinical studies. Several investigators have demonstrated a correlation between FHA and endothelial dysfunction. It was clearly shown that the flow-mediated dilation of the brachial artery, which is a precise predictor of coronary endothelial dysfunction, is impaired in women with FHA. It is suggested that the decrease of endothelial NO bioavailability is caused by chronic estrogen deficiency. Moreover, some authors have proved the protective effect of exogenous estrogens in young women against impaired vessels' dilatation. Rickenlund et al. documented significantly increased brachial artery dilatation after 9 months of treatment with low-dose combined contraceptives (30 µg of ethinyl estradiol and 150 µg of levonorgestrel): from  $1.42 \pm 0.98\%$  before treatment to  $4.88 \pm 2.20\%$  during treatment [7].

A Women's Ischemia Syndrome Evaluation (WISE) study found a significant association between premenopausal angiographic coronary artery disease (CAD) and hypothalamic hypogonadism [8]. O'Donnell et al. recently showed that young athletes with chronic hypoestrogenemia displayed an impaired peripheral vascular function that was combined with lower resting blood pressures and heart rate and reduced ischemic responses to occlusion challenge compared to ovulatory women. Impaired cardiovascular function in hypothalamic amenorrhea is believed to be linked mainly to hypoestrogenism, but it is also aggravated by negative energy balance and metabolic disturbances [9]. Patients with FHA are characterized by an impaired lipid profile and are at risk of glucose metabolism abnormalities. Women with exercise-related amenorrhea present higher serum total cholesterol, LDL cholesterol, apolipoprotein B and triglyceride concentrations than healthy individuals [10]. On the other hand, premenopausal women with hypoestrogenism of

hypothalamic origin present an increased frequency of diabetes mellitus. Moreover, Ahmed et al. showed that coronary artery disease (detected in coronarography) has an increased prevalence and extent among women with diabetes and hypothalamic hypoestrogenism in comparison to women with diabetes alone. These observations substantiate the importance of cyclic ovarian function as an indicator of cardiovascular health. However, the influence of hypoestrogenism in young women of hypothalamic origin on cardiovascular health requires further studies. Especially the issue of the long-term consequences of FHA on CVD risk needs to be cleared to possibly minimize the risk of cardiovascular events in this group of women [11].

Despite the large number of studies investigating the effects of menopause on cardiovascular outcomes and endothelial function, currently, little is known about the cardiovascular effects of Premature Ovarian Failure (POF) in young women. Flow mediated vasodilatation (FMD) of the brachial artery is the most common method in the assessment of endothelial function and measures the changes in arterial diameter in response to increased blood flow by stimulating endothelial nitric oxide production. It has been shown previously that endothelial function assessed by FMD is impaired in patients with POF; in particular it has been found that FMD was significantly lower in women with POF. New findings suggested that Circulating Endothelial Progenitor cells (EPC) originate from the bone marrow and play an important role in vascular homeostasis for both repair and regeneration of damaged endothelium. This line of progenitor cells has recently been recommended as a novel biomarker of endothelial function showing a close relationship with FMD. Circulating EPCs may have an important contribution to estrogen-induced cardiac protection but they have not been studied in POF previously. Also decreased EPCs may lead to accelerated vascular remodeling like increased carotid intima media thickness (CIMT) due to chronic impairment of endothelial maintenance [12]. Remarkably, polycystic ovary syndrome (PCOS) patients who have chronic anovulation and hyperandrogenism have been shown to have decreased EPC counts and increased CIMT. In this view, Kalantaridou et al. evaluated endothelial dysfunction in a patient group including 18 patients with POF compared to age-matched premenopausal women investigating the relationship between POF and EPC, endothelial function, carotid intima media thickness (CIMT), and left ventricular diastolic function [13, 14].

This group of research found lower FMD in patients with POF compared to healthy age-matched controls. Furthermore, there was a significant correlation between estradiol and FMD, consistent with previous studies where low levels of estradiol were associated with endothelial dysfunction. The association between EPCs and endothelial function has been evaluated in different previous studies, suggesting that EPCs may play a critical role in maintaining endothelial function in mature blood vessels in addition to mature endothelial cells. In this study, it has been demonstrated for the first time a lower circulating number of EPCs in patients with POF. It has been also found that there is a significant correlation between estradiol level and EPC which may be one of the mechanisms impairing endothelial function. In this view, Kalantaridou and colleagues demonstrated that hormone replacement restored endothelial function within 6 months of treatment among patients with POF.

The same group investigated the endothelium-dependent and -independent vascular function by measuring flow-mediated dilation (FMD) and nitrate-mediated dilation in the brachial artery in patients affected by PCOS. Women with PCOS showed significant endothelial dysfunction at an early age (i.e., early 20s) and largely independent of obesity, but in this study, there were no modifications on the hormonal status of the patients and any therapy was administered. This evidence suggested that women with PCOS are at increased risk for early onset cardiovascular disease and may gain particular benefit from measures to improve endothelial function, as well as women with POF have high risk of premature death from cardiovascular causes, therefore, early recognition or diagnosis may provide clinical significance. However, whether these findings might translate into clinical benefit requires further prospective studies [13, 14].

Many reports associate cardiovascular disease and mortality with age of menopause (within the healthy age range, premature, or following oophorectomy). The relation between late menopause and a reduced cardiovascular disease (CVD) risk is well established. Observational studies have shown a reduced risk of cardiovascular disease, fewer calcifications in the aorta, and less extensive atherosclerosis in women with later menopause, moreover, age at menopause is associated with carotid atherosclerosis as assessed with ultrasonography [15, 16].

Menopausal state has been added to the Framingham risk function for estimating absolute cardiovascular disease risk in women. The postmenopausal use of estradiol seems to reduce progression of CVD although studies about postmenopausal hormone therapy and cardiovascular risk are inconclusive [17]. The risk of cardiovascular mortality was significantly higher for women with natural early compared with late menopause (age-adjusted hazard ratio [HR] 0.98, 95% CI 0.97–0.99,  $p=0.01$  for every year menopause is delayed) in a cohort of 12,000 postmenopausal women. A meta-analysis showed the association between postmenopausal hormonal status and cardiovascular disease was pronounced for women with an artificial menopause (age-adjusted HR 2.62, 95% CI 2.05–3.35) following bilateral oophorectomy. Investigators of the same cohort showed that a long overall survival occurred in women with late menopause [18, 19]. A small but significant inverse correlation between age of natural menopause and all-cause mortality was established in both a 37-year follow-up study in almost 20,000 women from Norway and a 20-year follow-up in 68,000 women from the USA [20, 21]. Increased cardiovascular mortality was also noted after bilateral oophorectomy before 45 years of age (HR 1.67, 95% CI 1.16–2.40,  $p=0.006$ ) with increased overall mortality in women who did not have estrogen therapy until they were aged 45 years or more (1.84, 1.27–2.68,  $p=0.001$ ) [22, 23]. A 24-year follow-up of more than 16,000 women who had a hysterectomy with or without bilateral oophorectomy (from a cohort of 30,000 women in the Nurses' Health Study) had the following long-term health outcomes, after adjustment for the use of estrogens: an HR of 1.12 (95% CI 1.03–1.21) for total mortality, 1.17 (1.02–1.35) for coronary heart disease, 1.26 (1.02–1.56) for lung cancer, and 1.14 (0.98–1.33) for stroke [24]. Knauff and colleagues showed small changes in the lipid profile (e.g., raised triglyceride and marginally lowered HDL cholesterol concentrations) in women presenting with ovarian

insufficiency [25]. Some data show a cardioprotective effect of estrogen treatment. Steroid replacement results in increased carotid pulsatility index, decreased blood pressure, improved renal function, and lowered activation of the renin-angiotensin system. Although direct evidence of the beneficial effects of estrogen replacement on primary outcomes is limited, hormone replacement therapy is indicated, despite the hazards of this therapy after chemotherapy in young women who have experienced thromboembolic events such as pulmonary embolism or deep vein thrombosis, and in women with breast cancer [26, 27].

Despite the encouraging results of several findings, further studies are needed to exactly elucidate both pathophysiological mechanisms affecting cardiovascular system and new therapies aiming to prevent premature atherosclerotic process of the early and later impact of amenorrhea and subsequent hormonal withdrawal in young women, during menopausal transition and in postmenopausal period.

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## 11.1 Introductory Remarks

Puberty is the period of life that leads to adulthood through complicated and sometimes painful physiological and psychological changes. The hypothalamic-pituitary-gonadal axis undergoes an active phase during foetal and neonatal development and then enters a resting phase that lasts for the rest of childhood until puberty. Puberty begins with an activation of the hypothalamic-pituitary-gonadal system. It occurs today earlier than a century and even earlier than 20 years ago. Delayed puberty may have a dramatic impact on the mental and social development of an adolescent. In the literature, different definitions for “delayed puberty” can be found and there are no guidelines indicating when in the absence of pubertal signs an investigation should be started. Usually, in girls, a first evaluation should be done not later than at the age of 13. However, the initiation of a first evaluation has to be earlier in some cases: it depends on the psychosocial pressure exerted on a child by her personal delay when it is compared to the pubertal development of the pair group of schoolmates and friends. One aspect that quite often worries the patient and her parents the most is the impact of delayed puberty might have on later fertility.

This chapter intends to give a short overview for non-specialized endocrinologists over normal puberty, the practically most important forms of delayed puberty and the measures to be taken.

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## 11.2 Normal Puberty

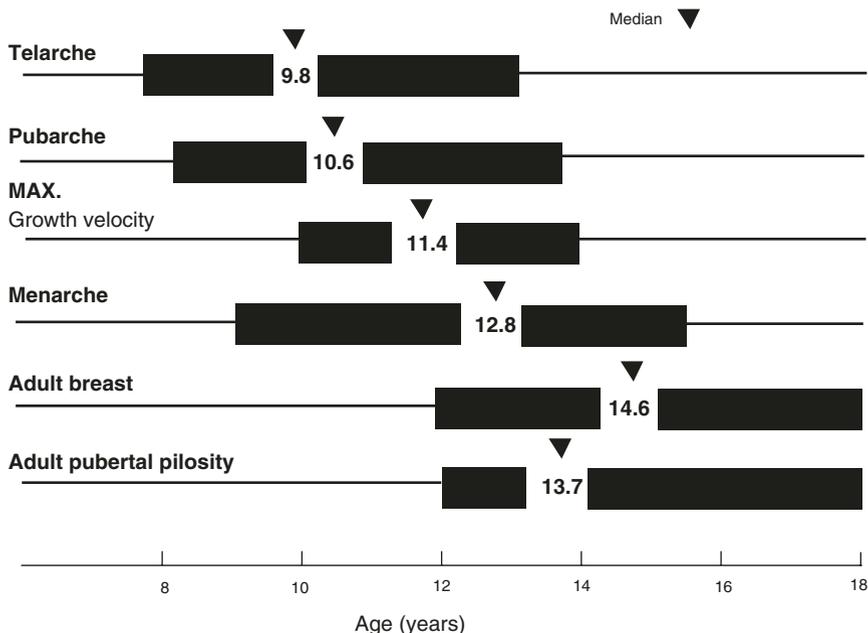
### 11.2.1 Clinical Aspects

The clinical onset of external pubertal development is announced by the appearance of secondary sex characteristics. In females, these signs are the appearance of breast buds and of pubic and axillary hair.

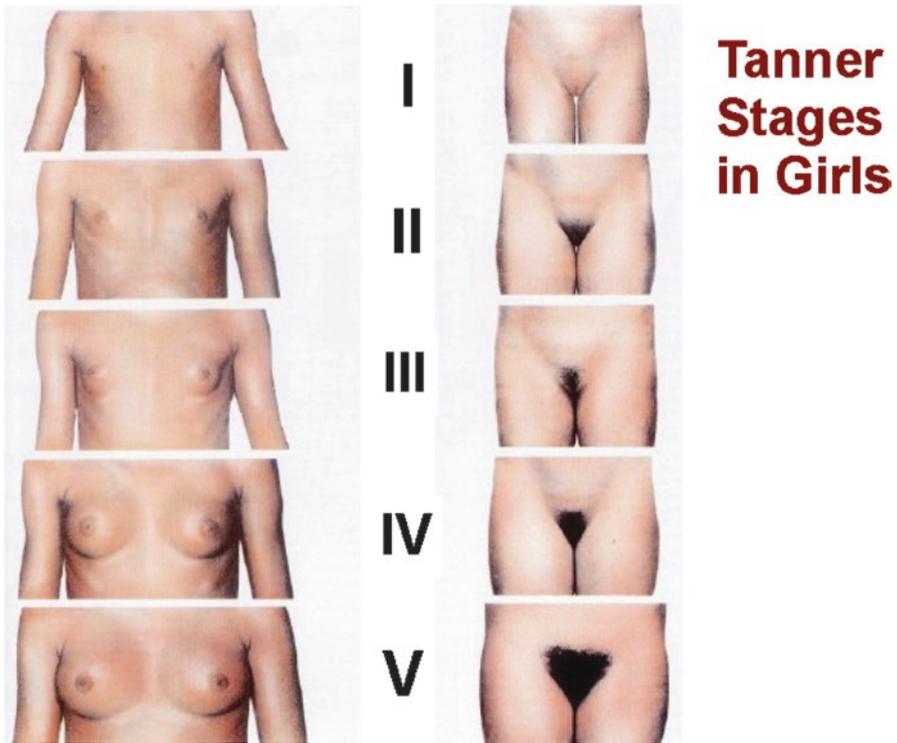
The development of secondary sex characteristics is rated into five stages according to Tanner's criteria [1–5]. They evolve progressively over several years until adulthood is reached and are designated as Tanner stages B1 through B5 for breast development and PH1 through PH6 for pubic hair growth (Figs. 11.1 and 11.2). A detailed description of the Tanner stages can be found in standard textbooks of paediatrics and paediatric or gynaecological endocrinology [1–3].

In girls, pubertal growth spurt occurs during Tanner stages 2 and 3. In girls, it occurs 2 years earlier than in boys. Girls do not show the same slowing down of growth velocity as boys before puberty and increase their growth velocity to 6 cm/year during the first year of puberty, and to 8 cm/year on average during the second year [6].

**Menarche** A variety of environmental and genetic factors are involved in the regulation of menarche. The first menstrual period (menarche) occurs at an average age of 13.4 years, according to the longitudinal data obtained by Largo et al. [4].



**Fig. 11.1** The sequence of events during puberty in girls. Breast bud appearance is usually before pubic hair growth; in the meantime growth velocity increases reaching the peak at Stage 4 of puberty. At this time menarche may appear [4]



**Fig. 11.2** Breast development in girls. The mammary gland grows from a breast bud that can be palpated under the nipple (Tanner stage B2) to a fully developed female breast (Tanner stage B4 or B5) over a period of 3.6 years, on average [4]

Menarche occurs generally at Tanner stage 4. The mean age at menarche is highly correlated within families, between monozygotic twins, and within ethnic groups [7]. Twin analyses suggest that 53–74% of the variation in age of menarche may be attributed to genetic effects [7].

## 11.2.2 Endocrinological Aspects of Normal Puberty

### 11.2.2.1 Adrenarche

Endocrinologically, the first signal for puberty is given by the adrenals (adrenarche). The onset of DHEA-S production from the adrenal zona reticularis leads to the phenomenon of adrenarche.

During infancy and early childhood, adrenal androgens (androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone-sulphate) are secreted in small amounts. Their secretion increases gradually with age. This increase in androgen levels is responsible for the appearance of body odour, pubic hair and axillary hair. Therefore, pubic hair develops independently of the activation of the hypothalamic-pituitary-gonadal pathways.

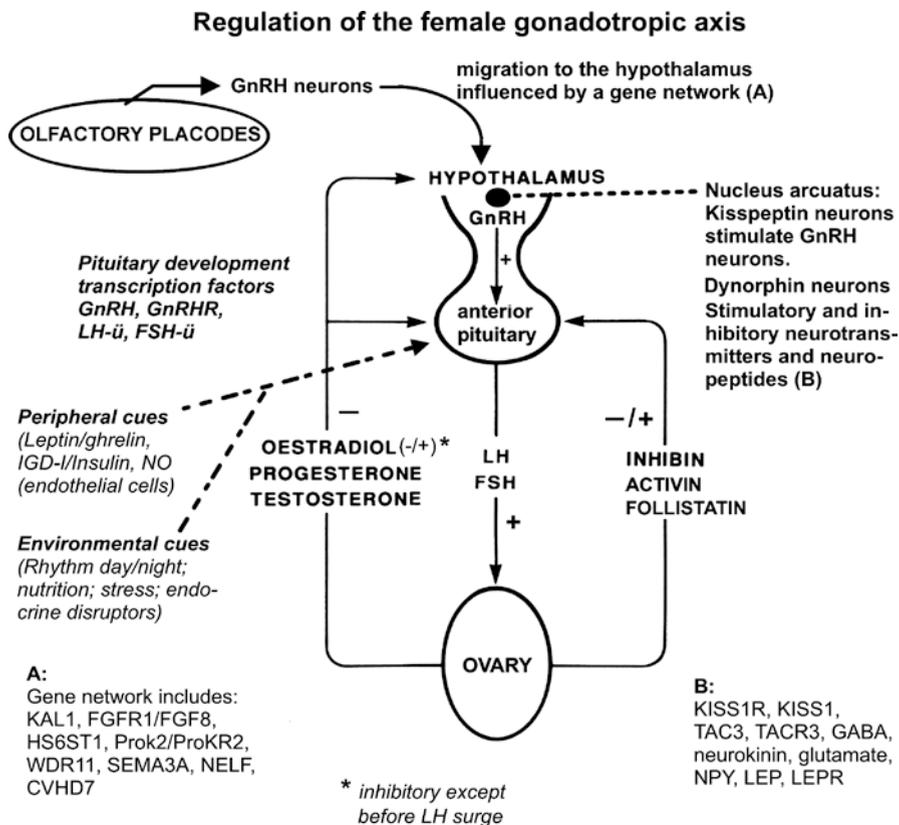
Adrenarche is marked by the growth of the zona reticularis [8] and a parallel increase in the adrenal androgen levels. This phenomenon is only seen in the human beings and in some old world primates, such as the chimpanzee [9]. Plasma concentrations of the adrenal androgens increase, whereas those of cortisol remain stable, suggesting that factors other than corticotropin are involved. Hormones postulated for this role are the yet undefined androgen-stimulating factor, Corticotrophin Releasing Hormone (CRH) and more recently hormones related to body mass, such as insulin and leptin [10–13]. Although the temporal relation between adrenarche and the onset of puberty suggests that adrenal androgens might have a regulatory influence on the timing of puberty, it is now accepted that the two events are independent processes.

### 11.2.2.2 Regulation of the Hypothalamo-Hypophyseal-Gonadal Axis

*Gonadotropin releasing-hormone* Gonadotropin releasing-hormone (GnRH), a decapeptide secreted by approximately 1000 neurons located in the basal forebrain and extending from the olfactory bulbs to the mediobasal hypothalamus, is responsible for the gonadotropin secretion by the pituitary gland. GnRH stimulates the release of LH and FSH from the pituitary which in turn stimulate the gonads. LH and FSH have negative feedback effects on the hypothalamus, whereas testosterone (T) and Androstenedione (A) produced by the testis, and Estradiol (E2) produced by the ovary, inhibit both the hypothalamus and the pituitary gland. Inhibin, activin, and follistatin have also feedback effects at both levels. GnRH secretion by the hypothalamus is under the control of a great amount of central and peripheral signals: excitatory amino acids and other neurotransmitters such GABA, gonadal sex steroids, adrenal and thyroid hormones, the GH-IGF-IGFBP axis, nutrition and related hormones such as leptin and insulin (Fig. 11.3).

Two types of GnRH neurons have been identified to date, GnRH neuron I and II. GnRH neurons II have no known function in humans and are not involved in reproductive function, as inferred from Kallmann's syndrome patients in whom GnRH neurons I only are affected. GnRH neurons I originate in the embryonic period and exhibit an endogenous secretion very early in development. After birth, their activity is "turned-off" by the low circulating levels of androgens/oestrogens released by the gonads, by means of a negative feedback mechanism. At puberty, the reactivation of this "gonadostat" is independent of the effect exerted by the steroids and is related to a reduced sensitivity to their action [14–17].

*Transcriptional factors* Recently three *transcriptional factors*, Oct-2, TTF-1, and EAP-1, have been identified as potential regulators of the cell network, which controls the GnRH secretion ("Upstream control"). They regulate the expression of genes involved in cell function and cell-cell communication (for details, see [18–21]). In the mammalian, hypothalamic lesions that induce sexual precocity activate both Oct-2 and TGF  $\beta$  expression in astrocytes near the lesion site [18], suggesting that TGF  $\beta$  is one of Oct-2 target.



**Fig. 11.3** Principals of the development and the regulation of the female gonadotropic axis (see text)

The second candidate is TTF-1 (thyroid transcriptional factor-1), another homeobox gene. After birth, it remains expressed in selected neuronal and glial population of the hypothalamus. At the onset of puberty, TTF-1 enhances GnRH and *erbB2* and *KiSS-1* gene transcription but inhibits preproenkephalin promoter activity [19].

The third candidate is EAP-1, earlier known as C14ORF4. Like TTF-1, EAP-1 transactivates the promoter of genes involved in facilitating the advent of puberty while suppressing the expression of genes inhibitory to the pubertal process. Knocking down hypothalamic EAP-1 expression causes delayed puberty and disrupted oestrous cyclicity, both in rats and monkeys [20, 21].

***KiSS-1/GPR54* system** Kisspeptin/metastin (*KiSS-1*) is a 53-amino-acid-peptide, earlier known as a suppressor of tumour metastases [22, 23]. The proteolytic cleavage of the primary *KiSS-1* protein product originates the decapeptide kisspeptin-10 (*KiSS-10*), whose target is GPR54 receptor. GPR54-containing cells are diffusely distributed [24, 25]. Kiss neurons are important for the gonadal axis. They are located in

discrete neuronal subsets of the preoptic area and the nucleus arcuatus [24, 26]. These cells include GnRH neurons and the anterior pituitary [27, 28].

The KiSS-1/GPR54 system has been recognized recently as the director of central functional network and peripheral signals. Genetic, physiological and clinical data strongly indicate that the KiSS-1/GPR system is an essential gatekeeper of GnRH function, and not just one more element in the cascade of signals controlling the gonadotropic axis. It allows the integration of central and peripheral inputs and plays therefore a decisive role in the control of reproductive function [29].

Both in rats and in primates, a marked increase in KiSS-1 and GPR54 mRNA levels coincide with the onset of puberty [24, 30]. Moreover, the sensitivity of GnRH system to kisspeptin is dramatically enhanced in adult versus juvenile mice [42]. Thus, the developmental activation of the GnRH axis by KiSS-1 at puberty reflects a dual phenomenon involving, not only the increase of kisspeptin tone, but also the enhancement of its efficiency to activate GnRH neurons, probably through post-transcriptional changes in GPR54 signalling [31].

Hypothalamic KiSS-1 system also plays an essential role in relaying the negative feedback input of sex steroids onto GnRH neurons. In male and female rats, bilateral gonadectomy evoked a consistent increase in KiSS-1 mRNA at the hypothalamus. Recent studies added further elements to the role of kisspeptin in the feedback control of gonadotropins by showing that negative regulation of hypothalamic KiSS-1 gene expression by oestrogen appears to be restricted to the nucleus arcuatus (Arc), known to be pivotal for negative feedback of sex steroids. In contrast, at the anteroventral periventricular nucleus (AVPN), KiSS-1 mRNA decreased after gonadectomy and increased after sex steroid replacement [32, 33]. AVPN is involved in mediating the positive feedback effects of oestrogen upon GnRH and LH surges. Therefore, via positive regulation of GnRH secretion, KiSS-1 neurons might be involved also in generation of the pre-ovulatory gonadotropin surge.

New strong evidence indicates that hypothalamic KiSS-1 may participate also in delivering information regarding the nutritional status of the organism to GnRH-neurons. Kiss-1 may therefore contribute to the link between energy stores and fertility [34, 35]. It has been shown that the permissive actions of leptin on the reproductive axis are mediated through modulation of GnRH secretion. Because GnRH neurons do not express leptin receptors, [35] kiss peptins might explain badly understood metabolic processes, signalled onto GnRH neurons via peripheral hormones such as leptin. However, several key aspects of the physiology of this system still remain open [36].

In conclusion, KiSS-1 system is an essential downstream element in the negative and (probably) positive feedback loops controlling gonadotropin secretion [37]. In addition, it may participate in the signalling to GnRH neurons of peripheral inputs from hormones such as leptin [38].

*Leptin* Leptin is a 16-kDa peptide secreted by adipocytes. It is supposed to signal to the brain the critical amount of fat stores necessary for LHRH secretion, which in turn activates the hypothalamic-pituitary-gonadal axis [38]. Leptin was recently

shown to suppress neuropeptide Y (NPY) expression in the nucleus arcuatus. NPY stimulates appetite, has an inhibitory effect on the gonadotropin axis and is involved with the inhibition of puberty in conditions of food restriction. Therefore, it has been hypothesized that leptin might exert its effects by acting on NPY. Under favourable nutritional conditions, the rise in leptin levels would suppress NPY, and in turn release the inhibitory effect of NPY neurons on the GnRH-LH/FSH axis, allowing the initiation of puberty [39].

On the other hand, there might be a direct peripheral negative effect of leptin on gonadal function through inhibition of the steroidogenic enzymes [39, 40].

In humans and animals, leptin blood concentrations rise with the onset of puberty. In adolescents of both sexes, the gradual rise in serum leptin levels before puberty together with a decline in circulating levels of soluble leptin receptor suggest that these changes may serve as one of the signals to the central nervous system that metabolic conditions are adequate to support pubertal development and trigger puberty [41].

No gender differences were detected in the relationship between leptin serum levels and fat mass in pre-pubertal and early pubertal subjects. In contrast, at Tanner stages IV and V, the serum hormone concentrations decrease in males and increase in females. In addition, a significant negative correlation between circulating concentrations of testosterone and leptin was described in males only [38].

Finally, it has been shown that normal leptin levels are necessary for the maintenance of menstrual cycles and normal reproductive function in adolescents of both sexes.

In conclusion, leptin seems to exert a positive central effect on the hypothalamic-pituitary-gonadal axis and a negative peripheral one on the gonads. Leptin might signal that the metabolic conditions are adequate to support pubertal development and trigger puberty.

*Inhibin, activin, and follistatin* Inhibin and follistatin inhibit, and activin stimulates the expression, biosynthesis, and secretion of FSH [42–44]. They are synthesized mainly in the gonads. Inhibin, follistatin and activin are all three involved in the modulation of the hypophyseal-gonadal axis function. Inhibin and follistatin are both negative regulators of FSH secretion.

Inhibin, a heterodimeric glycoprotein, belongs to the TGF- $\beta$  super family produced by ovarian granulosa cells. It is composed of an alpha and one or two beta subunits. These form two different products, inhibin A and B, respectively. FSH stimulates the synthesis and secretion of inhibins by the gonads, which in turn are involved in the feedback regulation of FSH secretion. In girls, inhibin A concentrations increase between stage 2 and 3 of puberty, remain constant throughout stages 4 and 5, and correlate positively with bone age, inhibin B and oestradiol serum levels [45, 46]. Inhibin B blood concentrations increase further similarly to inhibin A levels, reaching a plateau at 12–18 years. They correlate with oestradiol [45, 46] and FSH serum levels [48].

Blood concentrations of follistatin decrease slightly from stage 1–4 and 5 of puberty in girls [46]. Blood levels of activin A were shown to remain unmodified from stage 1–3 of puberty in females [48].

In conclusion, at puberty the concentrations of the two negative regulators of FSH secretion, inhibin and follistatin, change in opposite directions [46], whereas the blood levels of a positive regulator, activin A, increase, at least in females. All together, these alterations in serum concentrations of FSH-regulatory peptides lead to an increase in FSH secretion.

*Melatonin* The marked increase in LH amplitude at night observed in early puberty occurs at the same time of melatonin secretion. On the other hand, precocious puberty associated with pineal tumours and due to ectopic secretion of gonadotropins is independent of melatonin [14]. The role of melatonin in puberty is questioned.

*Other hormones* Growth hormone (GH), insulin, insulin-like growth factor (IGF)-I, and its major binding protein, IGFBP-3, normally rise at puberty [49]. The increase in growth hormone and IGF-I concentrations is probably responsible for most of the metabolic changes observed during puberty, including insulin-resistance, increased beta-cell response to glucose, and growth spurt. GH, and not androgens, may directly affect insulin sensitivity regulating the glucose-insulin homeostasis at the time of puberty [50]. Adiponectin, an adipocytokine with antidiabetic and antiatherogenic effects, were recently shown to progressively decline in parallel with pubertal development in boys [51]. It is inversely related to serum testosterone and dehydroepiandrosterone sulphate levels [51].

### 11.2.3 Pubertal Maturation of the Hypothalamo-Hypophyseal-Gonadal Axis (Fig. 11.5)

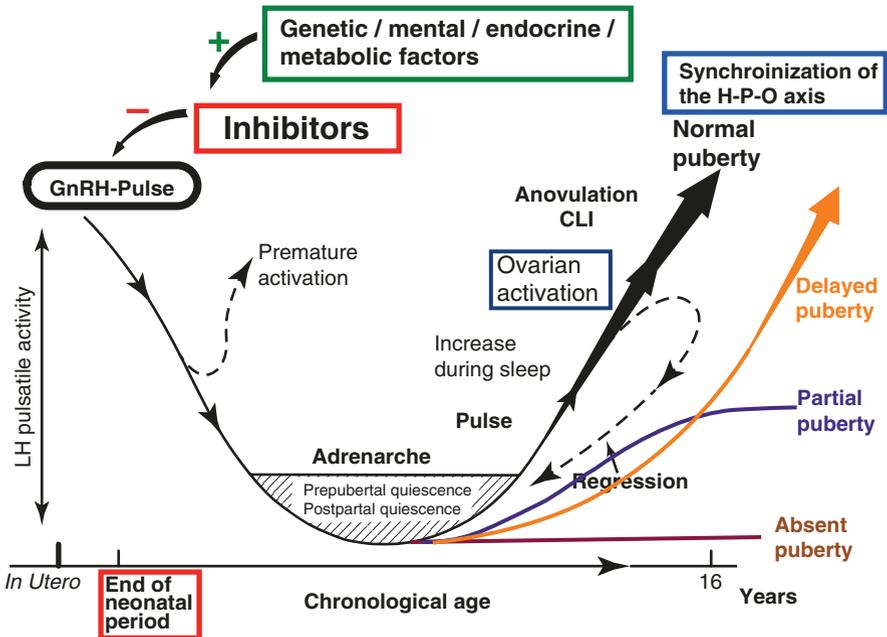
The hypothalamic-pituitary-gonadal axis undergoes an active phase during foetal and neonatal development and then enters a resting phase that lasts for the rest of childhood until puberty.

Puberty begins with an activation of the hypothalamic-pituitary-gonadal system. Changes in GnRH pulsatility during puberty are reflected by the peripheral LH- and FSH-Levels. Qualitative and quantitative changes in LH secretion resulting from pulsatile GnRH secretion, occur approximately 2 years before the appearance of secondary sexual characteristics. At puberty, LH pulsatile secretion is characterized by a 28-fold increase in the pulse amplitude, whereas pulse frequency increases only 1.8-fold. During prepubertal years, both LH and FSH secretions are preponderant during night-time. In the peripubertal period the secretion of gonadotropins increases during sleep, and stimulation with exogenous GnRH shows an enhanced release of LH from the pituitary gland that may be useful in differentiating a pubertal from a pre-pubertal response. Throughout puberty then, gonadotropin pulses further increase becoming apparent during daytime also.

Several studies have been published suggesting that the mechanisms underlying the onset of puberty are different in girls and boys, and different modes of transmission of induction of puberty in boys and girls were revealed [52]. Among other differences, in girls, FSH levels increase during the early stages, and LH levels during the later stages of puberty with a 100-fold increase in hormone concentrations. In contrast, in boys, FSH levels rise progressively through puberty with an increase in amplitude only, whereas LH levels increase in early puberty reaching a plateau shortly [14, 17].

### 11.2.4 Acceleration of Puberty

Puberty occurs today earlier than a century and even earlier than half a century or 20 years ago. In Tanner’s original report [1, 5] white girls had a mean age at onset of breast development and pubic hair of 11.2 and 11.7 years, respectively. The normal mean age at onset of pubertal characteristics in young girls has been revised in 1997 in a considerable population of 17,000 girls evaluated in a cross-sectional study [53]. It has been shown to vary with race, ethnicity, geographical location, and environmental and nutritional conditions.



**Fig. 11.4** Normal and delayed maturation of the hypothalamo-hypophyseal-gonadal axis (H-P-O-axis). Onset of puberty implies the regression of the inhibitory factors blocking the gonadal axis in childhood. Maturation and activation of the ovarian axis can be interrupted at each stage and may even regress to prepubertal quiescence

Compared to Tanner's original report, pubertal development appears to begin up to 1 year in advance in white and up to 2 years in African-American girls. Breast stage 2 is reported to occur in white girls at  $9.96 \pm 1.82$  years (mean  $\pm$  SD) with upper and lower limits of 7 and 13 years, and in African-American at  $8.87 \pm 1.93$  years with limits between 6 and 13 years. Pubic hair would occur at  $10.51 \pm 1.67$  and  $8.78 \pm 2.00$  year in white and African-American girls, respectively [54]. In the US white girls puberty would begin by 10 years of age on average, and African American between 8 and 9 years [1, 5, 54, 55].

The age of menarche has been shown to decrease significantly since the nineteenth century. With respect to the first data published by Tanner [5] and Largo [4] 50 and 30 years ago, respectively, it continues to decrease. Menarche seems to occur earlier in white British girls (13.5 years) in 2004 [56] than in 1962 [5] and is reported to occur at  $12.88 \pm 1.2$  years in white and at  $12.16 \pm 1.21$  year in African-American girls [54]. In 2006, a large German survey found the median age at menarche to be 12.8 years [57], suggesting that the secular trend to an earlier menarche is continuing.

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## 11.3 Delayed Puberty

### 11.3.1 Definition

Puberty is the period of life that leads to adulthood through complicated and sometimes painful physiological and psychological changes. Delayed puberty may have a dramatic impact on the mental and social development of an adolescent.

In the literature, different definitions for "delayed puberty" can be found.

The classical endocrinological definition and the current paediatric definition are identical for girls, but slightly different for boys:

*Endocrinological definition* (Grumbach and Styne [42])

Delayed puberty is defined as the absence of signs of puberty in healthy girls at age 13 years and in healthy boys at the age 13.5 years (2 SD above the mean age at start of puberty).

*Paediatric definition:* Delayed puberty is defined as the absence of signs of sexual maturation by an age more than 2–2.5 SD values above the mean of the population (traditionally breast development by 13 years in girls and testicular development by 14 years in boys) (Marshall and Tanner [1]; Lee [2]; Brämswig and Dübbers [55]).

### 11.3.2 Incidence

Delayed puberty is a rare condition, occurring in only approximately 2.5% of the population. [1, 2, 42, 55]. The relative incidence of the different forms of hypogonadism in delayed puberty is shown on Table 11.1. In the series of Reindollar et al. [58], hypogonadotropic hypogonadism is found in 31%, hypergonadotropic

**Table 11.1** Relative incidence of observed hypogonadism in delayed puberty [58]

<i>Hypogonadotropic Hypogonadismus</i>	31 %
Idiopathic	10 %
GnRH-deficiency	7 %
Anorexia	3 %
Other endocrinopathies	4 %
Organic	13 %
<i>Hypergonadotropic Hypogonadismus</i>	43 %
Abnormal karyotype	26 %
Normal karyotype	17 %
<i>Eugonadotropic hypogonadism<sup>a</sup></i>	26 %
Rokitansky-Kuster and similar	17 %
Testicular feminization	1 %

<sup>a</sup>Primary amenorrhea in presence of partial or complete development of secondary sex characteristics

hypogonadism in 43% and eugonadotropic hypogonadism leading to primary amenorrhea in presence of partial or complete development of secondary sex characteristics in 26%.

### 11.3.3 When and How to Investigate?

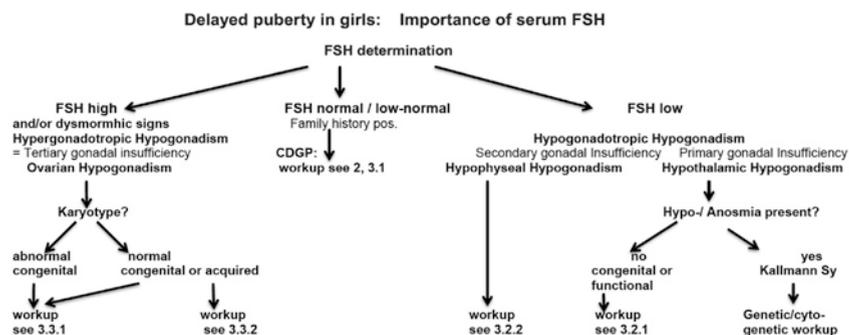
There are no guidelines indicating when in the absence of pubertal signs an investigation should be started. Following both definitions listed above, in girls, a first evaluation should be done not later than at the age of 13.

Important is empathetic counselling to counteract the mostly deep anxiety due to the fact of being different from other girls at the same age. The child and the parents have to be fully and accurately informed and reassured that an underlying pathological process is rare and that the delay of the onset of puberty is mostly due to a benign, often familiar, deviation from the normal time course.

In most recommendations, a precise diagnostic evaluation is recommended in girls with persisting absence of the onset of puberty at the age of 14.5 years (mean + 3 standard deviations). However, a further evaluation is recommended earlier if a girl without onset of puberty starts to suffer because she becomes socially isolated among her classmates because of her physical retardation. Therefore, acceleration of puberty has to be taken into account for the decision when to start clinical evaluation in absence of pubertal signs.

In conclusion, investigation has to be started earlier than it has been recommended 20 years ago. It depends on the psychosocial pressure exerted on a child by the pubertal development of the pair group of schoolmates and friends.

Figure 11.5 presents a simplified flow chart of the assessment of delayed female puberty. It describes schematically the process for the investigation of adolescent girls presenting with lack of spontaneous pubertal development. Shaded boxes show the major differential diagnoses of constitutional delay of growth and puberty,



**Fig. 11.5** The determination of serum FSH, together with family history and clinical signs, allows in a simple way a first preliminary classification of girls suffering from delayed puberty. Workup numbers relate to the chapters of this review

hypogonadotropic hypogonadism, and hypergonadotropic hypogonadism. However, no clinical algorithm can fully meet the requirements of all individual cases. Thus, adapted clinical decision-making is important at each stage.

### 11.3.3.1 Hormone Measurements

#### Gonadotropins

- Basal levels of FSH and LH are low in patients with HH or constitutionally delayed puberty and elevated in hypergonadotropic hypogonadism.
- Levels of FSH and LH remain low after one GnRH injection in hypothalamic and in hypophyseal hypogonadism.
- Levels of LH and FSH increase in hypothalamic hypogonadism with intact pituitary function (but not in hypophyseal hypogonadism) after repeated pulsatile administration of GnRH (0.1 mg GnRH per injection).
- When 0.1 mg of GnRH is injected, pubertal onset is characterized by LH/FSH >1.

#### Oestradiol

In girls, at pubertal onset, oestradiol levels are >40 ng/ml (<10 ng/ml before puberty).

#### Inhibin B and Anti-Müllerian Hormone (AMH)

The distinction between constitutional delay of growth and puberty (CDGP) and idiopathic hypothalamic or hypophyseal hypogonadism (IHH) is still a difficult clinical issue. Harrington and Palmer conclude that basal inhibin B may offer a simple, discriminatory test if results from recent studies are replicated: very low levels indicate a high likelihood of IHH [59]. However, current literature does not allow today for recommendation of any diagnostic test for routine clinical use. This applies, too, to the clinical use of AMH in the investigation of delayed puberty [60].

### **Other Hormones to be Checked**

Pituitary deficits should be evaluated by measuring IGF-I, T4, TSH and cortisol.

#### **11.3.3.2 Bone Age**

A bone age <11 years in girls with growth failure is encountered in constitutionally delayed puberty.

Bone ages >11 years in girls require further investigation to eliminate hypogonadism.

#### **11.3.3.3 Pelvic Abdominal Ultrasonography**

In case of hypergonadotropic hypogonadism, gonads may be small or absent. At the onset of puberty, the ovaries develop follicular cysts long before menarche. Multicystic ovaries with more than six cysts are a normal phenomenon and are already observed in the early stages of puberty [61, 62]. At that stage, these normal cysts should not be confounded with an early expression of a later PSO-syndrome. If ovarian volume is >2 ml and the uterus >35 mm, puberty is imminent [63].

The uterine volume increases at first without, and then with, a visible layer of uterine mucosa. This mucosa layer is induced by the slowly increasing oestrogen secretion.

#### **11.3.3.4 Karyotype**

Independent of dysmorphic features suggestive of Turner syndrome, a karyotype should be performed in hypergonadotropic hypogonadism if the patient's history (e.g., chemotherapy, X-ray treatment) cannot explain the gonadal pathology.

#### **11.3.3.5 Brain Magnetic Resonance Imaging (MRI)**

In presence of unexplained low levels of LH and FSH, organic pituitary or hypothalamic disease should be eliminated. MRI is the most efficient imaging examination. Agenesis of the olfactory bulbs is typical for Kallmann syndrome. Measurement of the pituitary and pituitary stalk is fundamental.

An elaborate discussion of the investigational process is presented by the specialized literature [55, 59, 64, 65].

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## **11.4 Impact on Fertility of the Different Forms of Delayed Puberty**

### **11.4.1 Constitutional Delay of Growth and Puberty**

Constitutional delay of growth and puberty (CDGP) is the most common cause of delayed puberty in girls with 30% of cases, as it is in boys [66]. CDGP is defined as a delay of growth occurring in otherwise healthy adolescents with stature reduced

for chronological age, but generally appropriate for bone age and stage of pubertal development, both of which are usually delayed. It is more frequent in boys than in girls with a 10:1 ratio and is the most common cause of delayed puberty (80–90%).

In most cases delayed puberty is not due to any underlying pathology, but instead represents an extreme end of the normal spectrum of pubertal timing, a developmental pattern referred to as constitutional delay of growth and maturation [66]. The characteristically retarded linear growth occurs during the early years of life and is followed by regular growth paralleling the normal growth curve throughout the rest of prepubertal years. Pubertal growth spurt is attenuated and occurs after the usual expected time. In girls, exclusive maternal inheritance seems to be the major mode of inheritance whereas for boys the mode of inheritance is almost equally maternal, paternal or bilineal [52]. The majority of cases (70–80%) are familial. Sedlmyer & Palmert classified family histories of pubertal timing among primary relatives in 95 of 122 of the CD and in 25 of 45 of the functional hypogonadotropic hypogonadism (FHH) cases. Analysis revealed at least a tendency to pubertal delay in 77% of the CDGP and in 64% of the FHH families and a diagnosis of delay in 38% of the CDGP and 44% of the FHH families. Both parents contributed to the positive family histories. The rates of positive family histories among the CDPD and FHH groups were approximately twice those seen among the other subjects in our case series [66]. Bone mineral density can be compromised by the low serum steroid concentrations measured [67, 68]. Specifically, the attainment of peak bone mass may be impaired, although recent data do not indicate significant changes in volumetric bone mineral density in young men with previous CDGP compared with appropriate controls [69].

The sleep-related increase in LH concentrations that characterizes the onset of puberty, is normally present in CDGP children. As a consequence of inadequate production of gonadal steroids, acute provocative tests may show a GH response wrongly consistent with partial GH deficiency [70]. Pre-treatment with oestrogens in girls results as expected in the normalization of the GH responses. The LH response to the LH-RH analogue leuprolide acetate is intermediate between that of hypogonadal patients and normal pubertal children, and is therefore useful in differentiating CDGP from hypogonadotropic hypogonadism. Recently, a critical appraisal of available diagnostic tests has been published [59].

Supportive care is essential. Although no specific treatment is required, the psychosocial problems faced by CDGP children may force physicians to substitute [55, 64, 71]. In girls, oestrogen therapy is recommended only after statural considerations have been carefully taken into account. Ethinylestradiol should be avoided. The administration of oestrogen, even in small amounts, leads to progressive skeletal maturation, and ultimately to epiphyseal fusion. The use of anabolic steroids or growth hormone to stimulate growth is highly controversial [72–75] and is not recommended in most reviews [66].

The inheritance patter of CDGP has been recently analysed by Winter et al. [52]. In girls, exclusive maternal inheritance seems to be the major mode of inheritance.

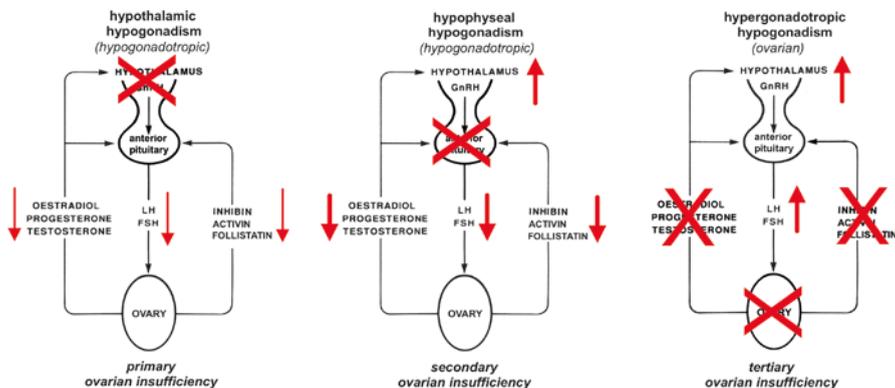
**Impact on fertility** There are no published data suggesting that compared to children with normal puberty, fertility may be decreased in adulthood in individuals who had lived a constitutionally delayed puberty.

### 11.4.2 Other Forms of Hypogonadotropic Delay of Growth and Puberty

Table 11.1 lists the most important causes of delayed puberty other than constitutional delay of puberty and growth. These causes are usually grouped in four categories:

- Delayed puberty due to congenital hypothalamic hypogonadotropic hypogonadism
- Delayed puberty due to functional hypothalamic hypogonadotropic hypogonadism
- Delayed puberty due to hypophyseal hypogonadotropic hypogonadism
- Delayed puberty due to congenital or acquired hypergonadotropic hypogonadism

The characteristic endocrine pattern for hypothalamic hypogonadotropic, hypophyseal hypogonadotropic and hypergonadotropic hypogonadism is presented on Fig. 11.6.



**Fig. 11.6** Schematic presentation of the classical endocrine defects in primary, secondary and tertiary ovarian insufficiency

### 11.4.2.1 Hypothalamic Hypogonadotropic Hypogonadism (Primary Ovarian Insufficiency)

#### Delay of Puberty in Organic Hypothalamic Hypogonadotropic Hypogonadism (HH) (Table 11.2a)

In girls, HH is proposed when plasma gonadotropins are normal or low, with lack of pubertal signs at 13 years of age. Puberty is absent or partial (congenital and early acquired forms), or arrested in an intermediate stage (acquired forms), depending on the appearance of the pathology in relation to the onset of puberty. Serum gonadotropin levels are low or inappropriately low-normal, sex steroids are low.

#### Isolated GnRH Deficiency

Congenital isolated hypothalamic hypogonadism (CHH) is clinically characterized by a partial or complete lack of puberty and a primary infertility due to a deficient GnRH-induced gonadotropin secretion, in the absence of anatomical abnormalities in the hypothalamic and pituitary region, and by normal basal and reserve testing of the remaining pituitary hormones.

Biologically, CHH is defined by low or normal serum levels of LH and FSH in the setting of low sex steroids. All other hypophyseal functions are normal as is the

**Table 11.2a** Classical causes of Hypothalamic Hypogonadism (primary ovarian insufficiency) [55, 64, 65, 77, 80]

<i>Congenital (permanent) hypothalamic hypogonadism</i>
Isolated GnRH deficiency
With anosmia (Kallmann syndrome)
Without anosmia
Associated with a syndrome such as
Prader-Willi
Laurence–Moon
Bardet–Biedl
etc.
<i>Acquired hypothalamic hypogonadism</i>
CNS tumours (cranyopharangeoma, germinoma etc.)
Metastases from non-CNS-tumours
Infections of the central nervous system
Systemic infections, such as tuberculosis, syphilis, Trypanosomiasis
Infiltrating processes and storage diseases such as haemochromatosis (Thalassaemia major!), histiocytosis, granulomas, sarcoidosis, M. Wilson
Multiple sclerosis
Head injury
Stroke, rupture of aneurysm
Cerebral surgery
Chemotherapy/radiotherapy

imaging of the hypothalamo–pituitary region. Patients with CHH typically present in adolescence or early adulthood with delayed onset of puberty, primary amenorrhea, poorly developed sexual characteristics, and/or infertility.

There exist two clinical variants of congenital GnRH deficiency: the form without anosmia and the GnRH deficiency with anosmia. When CHH is associated with anosmia or hyposmia it is termed Kallmann syndrome [76–78]. The Kallmann syndrome is the classic example of congenital hypothalamic hypogonadism. The first description of the so-called Kallmann syndrome has been published by de Morsier [79]. It is due to an impairment of the normal migration of the GnRH neurons from the region of the olfactory nerve to the ventral hypothalamus through the cribriform plate (Fig. 11.4). The clinical features of Kallmann syndrome are variable, with X-linked and autosomal-dominant and -recessive causes and variable penetrance described. Renal anomalies and syncynesia may exist. Its prevalence is 1/8000–1/10,000 in men and 1:50,000 in women. MRI confirms aplasia or hypoplasia of the olfactory bulbs.

Most cases of Kallmann syndrome seem to be sporadic as a consequence of mutations in at least two autosomal genes.

Mostly, genetic mutations are responsible for CHH [55, 64, 80, 81]. Mutations in the KAL1 gene on the short arm of the X chromosome (Xp22.3) are responsible for the X-chromosomal recessive form, while mutations in the FGFR1 (fibroblast growth factor receptor 1) gene on the short arm of chromosome 8 (8p11.2–p11.1) are responsible for the autosomal dominant form (e12). At present, in human, the only type of hypothalamic hypogonadism attributed to a single gene defect is the alteration of GPR54 [76] where KiSS-1 is involved (see above). Subjects with mutations in the human leptin receptor gene have no pubertal development. Table 11.2b lists the classical genetic mutations leading to permanent hypothalamic hypogonadism, with and without anosmia. Gene mutations with normosmic congenital hypogonadal hypogonadism are shown on Table 11.3. In all six listed

**Table 11.2b** Gene mutations leading to congenital permanent hypogonadal hypogonadism [55, 64, 76, 80–83]

Migration disorder of the GnRH neurons (Kallmann syndrome) due to mutations in (e12):
The KAL1 gene (chromosome Xp22.3)
The fibroblast growth factor receptor 1 (FGFR-1) gene (chromosome 8p11.2–p11.1)
The prokineticin 2 gene (e13)
The prokineticin 2 receptor gene
Nasal embryogenic LHRH factor (NELF)
Disturbances of GnRH secretion without anosmia or hyposmia (e19)
Mutations of the GnRH receptor gene
Mutations of the leptin gene
Mutations of the leptin receptor gene
Mutations of the G-coupled protein receptor 54 gene (GPR54) (e18)

mutations, heredity is recessive. This explains why expression of the anomaly is rare although the frequency of the abnormal gene GnRH1 in Europe is quite high (1/50) [80].

Additional developmental anomalies can occur with CHH including unilateral renal agenesis, synkinesia (mirror movements), cleft lip and/or palate, sensorineural hearing loss, dental agenesis, and skeletal malformations [81]. In some forms of CHH, additional defects are observed. These specific phenotypes are known as syndromes with CHH and additional abnormalities such as coloboma, heart defect, atresia of nasal choanae, retarded growth/development or genital abnormalities. The best known of these clinical syndromes are the Prader-Willi, the Laurence-Moon and the Bardet-Biedl syndrome. CHH and ear abnormalities up to deafness is known as the CHARGE syndrome [82, 83].

### Delay of Puberty in Functional Hypogonadal Hypogonadism (Primary Ovarian Insufficiency)

It has been estimated that 10–20% of all women suffer at least once in their life from functional hypothalamic disorders, mostly stress [17]. If such a functional disorder occurs before the normal age of puberty, puberty may be delayed.

Transient hypogonadal hypogonadism is seen in systemic conditions such as anorexia starting before or around puberty, excessive exercise (athletic triad) in

**Table 11.3** Genes responsible, frequency and phenotype in normosmic hypothalamic hypogonadism. Heredity is in all six listed mutations *recessive* [80]

Gene responsible of hypogonadism	Frequency of gene	Phenotype
GnRH1	Europe: 1/50 USA: 1/310	Complete HH
GnRH-R	40% of cases of familial normosmic HH Sporadic mutations: 6–17% of cases of hypogonadism	Complete HH
KiSS-1	Rare, no sporadic mutations described	Severe gonadotropic deficiency, absence of puberty
KiSS-R	Rare, sporadic or familial idiopathic HH: 26 cases from 9 different families described	Severe gonadotropic deficiency, absence of puberty
TAC3 neurokinin B	Rare, no sporadic mutations described	2 of the 4 sisters with TAC3 mutations had spontaneous pregnancies, another has regular cycles and the fourth had an early miscarriage
TAC3-R	Rare, no sporadic mutations but rare variant described in sporadic cases	6 of the 7 males and 4 of the 5 females demonstrated evidence for reversibility of their hypogonadism

pubertal girls, in severe chronic diseases of any origin, in malnutrition and in emotional deprivation [66, 84–88]. Sedlmeyer et al. [66] and other groups [84, 85] listed over 25 different underlying chronic diseases in their analysis of children investigated for functional delayed puberty. Among them, in addition to eating disorders and intense exercise, endocrine diseases (GH deficiency, hyperprolactinemia (see above), hypothyroidism), diabetes mellitus, cystic fibrosis, Crohn's disease, celiac disease, severe asthma, nephrotic syndrome, rheumatoid arthritis, systemic lupus erythematoses, sickle cell disease and thalassemia major, congenital heart disease, focal segmental glomerulosclerosis, glycogen storage disease type 1A, several oncological diseases (Hodgkin's disease, leukaemia etc.), CNS disorders (particularly seizure disorders) and poor nutrition.

Acquisition of fat mass is involved in pubertal development. During starvation, in stress-induced amenorrhea with weight loss, in subjects with anorexia nervosa, and in strenuously exercising athletes, leptin and E2 levels fall concomitantly. By limiting the apposition of adipose tissue, chronic diseases affect the development of puberty and fertility by the same mechanism relayed through the hypothalamus, apart from the specific impact of their molecular alteration. As the effect of the drugs used to treat chronic diseases (e.g., corticosteroids) are undistinguishable from the chronic disease itself, pharmacological side effects have to be considered, too [85].

As long as these conditions persist, the onset of puberty remains blocked or its normal continuation stays arrested. In severe cases, a functional regression to prepuberty equivalent with the prepubertal quiescence of the ovarian axis may occur (see Fig. 11.4).

#### **11.4.2.2 Delay of Puberty in Hypophyseal Hypogonadotropic Hypogonadism (Secondary Ovarian Insufficiency)**

Congenital or permanent hypophyseal hypogonadism is rare (Table 11.4). Intracranial tumour is a common cause of acquired hypogonadism in adolescence. Among these, craniopharyngeoma, a typical CNS tumour in adolescents, may lead to destructions in the hypothalamo-hypophyseal region [89]. If the pituitary stalk is compressed which is not rare in extrapituitary tumours such as craniopharyngeomas or metastases from non-CNS-tumours, other hypothalamo-hypophyseal axes in addition to the gonadal axis are affected. Neurosurgery for craniopharyngeoma is mostly followed by radiotherapy. In some other tumours, too, surgical resection may be complemented with radiotherapy and/or chemotherapy leading to secondary damage [90, 91].

In presence of an adenoma of the pituitary including makroprolactinoma, hypogonadotropic hypogonadism can result from the compression of pituitary tissue. In the case of prolactinoma or Cushing's disease, delayed puberty may be secondary to the inhibition of GnRH secretion by the hormones secreted by the endocrine active hypophyseal adenoma, even it is small.

**Table 11.4** Classical causes of hypophyseal hypogonadotropic hypogonadism (secondary ovarian insufficiency)

<i>A. Congenital or permanent hypophyseal hypogonadism</i>
Classical congenital forms are:
Isolated LH and FSH deficiency (“idiopathic isolated gonadotropin deficiency”)
Panhypopituitarism (complete or partial)
Congenital (genetic, “idiopathic”)
Associated with a lesion of the midline/Rathke’s pouch
Syndromes, such as CHARGE syndrome: combined pituitary hormone deficiency (coloboma, heart defect, atresia of nasal choanae, retarded growth/development, genital abnormalities, and ear abnormalities/deafness)
<i>B. Acquired hypophyseal hypogonadotropic hypogonadism</i>
Panhypopituitarism (partial or complete)
CNS tumours, such as craniopharyngioma, hamartoma, germinoma etc.
Metastases from non-CNS-tumours
Prolactinomas
Non-prolactin secreting pituitary adenomas
Hypophysitis
Infections, such as tuberculosis, syphilis, trypanosomiasis
Sarcoidosis
Eosinophilic granuloma
Haemochromatosis (Thalassaemia major!)
Multiple sclerosis
Trauma
Chemotherapy/radiation therapy

A rare cause of hypophyseal hypogonadotropic hypogonadism is the empty sella syndrome. Primary ES occurs when CSF enters the sella through a rent in the sellar diaphragm that may or may not be associated with increased intracranial pressure. Secondary ES is a result of an injury to the pituitary itself or the consequence of surgical or radiation treatment. The incidence of ES in children varies greatly depending on the population surveyed, ranging from 1.2% (children without endocrine symptoms) to 68% (children with known endocrinopathy) in the survey of Lenz and Root [92].

In adenomas of the pituitary, in empty sella and in craniopharyngeoma, clinically, visual disturbance or headaches may accompany pubertal arrest. It is essential that all patients with intra- or extrahypophyseal tumours undergo a complete evaluation of anterior and posterior pituitary function.

### 11.4.2.3 Impact on Fertility

*Hypogonadotropic hypogonadism due to congenital hypothalamic disorders* have very rarely and only in very light partial forms the chance to get later spontaneously

pregnant. However, with the adequate treatment, the possibility to live later a normal pregnancy is excellent even in complete forms of hypothalamic hypogonadism (see below).

*Hypogonadotropic hypogonadism resulting from hyperprolactinaemia* can be treated medically by dopamin agonists [93]. Because the normalization of prolactin secretion by dopamin agonists allows not only the onset of normal pubertal development but also the uptake of a normal fertility, adolescents have to be informed that in case of intercourse without the desire of a child they need an adequate and efficient contraception.

*In non-prolactin-secreting adenomas of the pituitary and in most other CNS tumours*, surgical intervention is the usual first line treatment [94–96], followed frequently by radiotherapy or chemotherapy. These treatments per se may lead in survivors to permanent hypogonadism [90, 91]. Later spontaneous fertility depends on the destructions left by the tumour itself or by its treatments. As long as the ovaries are intact and have not suffered by chemotherapy or radiotherapy, the chances to become pregnant through ovulation induction remain intact.

*In women, where the delay of puberty has been due to functional hypothalamic hypogonadism*, the successful treatment of the underlying disease decides on later fertility. Particularly, in women with eating disorders, a complete remission is the *conditio sine qua non* if normalization of fertility is intended. However, the few longitudinal studies on later fertility show that the risk of a subnormal fertility pattern remains increased, as it has been observed in the “*Avon Longitudinal Study of Parents and Children Fertility and prenatal attitudes towards pregnancy in women with eating disorders*” [97]. In this study, Singleton and live births were included across four groups of women suffering from lifetime eating disorders:

- Lifetime anorexia nervosa (AN;  $n = 171$ )
- Lifetime bulimia nervosa (BN;  $n = 199$ )
- Lifetime anorexia nervosa and bulimia nervosa (AN+BN;  $n = 82$ )
- General population ( $n = 10,636$ ).

The results show that women with AN (OR 1.6, 95 % CI 1.1–2.5;  $P < 0.021$ ) and women with AN+BN (OR 1.9, 95 % CI 1.1–3.4;  $P < 0.020$ ) were more likely to have seen a doctor for lifetime fertility problems than women from the general population. Furthermore, women with AN+BN were also more likely to take >6 months to conceive (OR 1.9, 95 % CI 1.0–3.5;  $P < 0.04$ ) and to have conceived the current pregnancy with fertility treatment.

All eating disorders groups experienced more frequently negative feelings upon discovering their pregnancy. Negative feelings remained still higher in the AN+BN group at 18 weeks of gestation. Finally, in spite of the longer time the AB women needed to get pregnant, unplanned pregnancies were more common in the AN group

compared with the general population. This points to the persistence of an increased ambivalence against pregnancy in women with eating disorders. These last two findings have been confirmed by a second study [98, 99].

Women with lifetime AN had a higher prevalence of twin births compared with those without the disorder (3.5 versus 1%), as did women with BN and women with AN+BN, albeit to a lesser extent [99]. All eating disorders taken together were associated with increased odds of having twins (OR 2.7, 95% CI 1.0–7.9;  $P=0.06$ ). These associations persisted after adjustment for potential confounding factors such as lifetime AN, OR 2.7, 95% CI 1.0–8.0, lifetime BN, OR 2.7 (95% CI 1.1–6.4) and lifetime AN+BN, OR 3.9, (95% CI 1.3–11.1). Interestingly enough, women with other lifetime psychiatric disorders had similar odds as women without psychiatric disorders.

#### **11.4.2.4 Profertile Measures: Ovulation Induction in Hypogonadotropic Hypogonadism (Primary and Secondary Ovarian Insufficiency)**

In the absence of the uptake of normal menstrual cycles, as it is the case in all forms of delayed puberty with permanent hypogonadal hypogonadism, ovulation induction should be used to induce pregnancy. It has to be stressed that the administered hormones have to be considered and handled as a substitution. Therefore, the lowest efficient dose of GnRH/pulse or of HMG resp. FSH/LH per day has to be used to obtain a monofollicular response of the ovary.

To decide on the optimal treatment in hypogonadal hypogonadism, the grading system described by Leyendecker (Table 11.5) is recommended. Finally, it has to be remembered that the administration of pure FSH does not make sense in the absence of endogenous LH secretion.

Considering the risk/benefit ratio, in primary ovarian insufficiency the best results are obtained by the pulsatile administration of GnRH (Table 11.6). The pregnancy rate within 1 year is identical to the one of a healthy fertile couple of the same age with a normal fertility (Fig. 11.8). The incidence of hyperstimulation and the number of multiple pregnancies are close to normal. The success rate in patients <35 years is above 90% [15–17, 100, 101]. However, pregnancy rate is age dependent: Fig. 11.8 lists the results in women aged <35 years, Table 11.7 the results in women with a mean age >35 years (own data). The same age-dependency has been shown for the pregnancy rate with HMG treatment in hypogonadotropic women [102]. The cumulative pregnancy rate has been after six treatment cycles 97% in women <35 years and 63% in women >35 years.

Therefore, in women with hypothalamic hypogonadism, first line treatment of infertility is the pulsatile administration of GnRH, with one exception: in partial hypothalamic insufficiency (Table 11.6), the more economic ovulation induction by oral Clomiphene may be used first although its success rate is lower (Fig. 11.7).

Table 11.7 shows the results of ovulation induction by pulsatile GnRH (i.v.) in 17 patients with hypothalamic amenorrhea grade 3c needing a higher dosage of GnRH per pulse (15–20 GnRH  $\mu\text{g}/\text{pulse}$ ). In spite of the severity of the hypothalamic deficiency and a mean age >35 years, the pregnancy rate was 53%.

**Table 11.5** Grading of hypothalamic amenorrhea on the basis of the progesterone, clomiphene, and Gn-RH tests, respectively [156]

1	Clomiphene positive with bleeding following
1a	Normal luteal phase
1b	Insufficient luteal phase
1c	Anovulatory cycle
2	Progesterone positive Clomiphene negative
3	Progesterone negative with pituitary response to 100 µg of Gn-RH i.v.
3a	“Adult response”
3b	“Prepubertal response”
3c	No response

**Table 11.6** Ovulation induction in hypothalamic and hypophyseal hypogonadism

Wish for a child positive	Grading (see Table 11.3)	Doses
<i>In presence of a potentially normal pituitary function:</i>		
Pulsatile GnRH [15–17, 100, 101]	Grade 1 or 2	5 µg GnRH/pulse every 90 min in the follicular phase 5 µg GnRH/pulse every 4 h in the luteal phase (or HCG)
Pulsatile GnRH [15–17, 100, 101]	Grade 3	10–20 µg GnRH/pulse every 90 min in the follicular phase 10–20 µg GnRH/pulse every 4 h in the luteal phase (or HCG)
<i>In presence of normal pituitary and hypothalamic structures:</i>		
Clomiphene [17]	Grade 1	50–100 mg/day day 5–9 after progestin-induced bleeding
Naltrexon [103]	Grade 1–3	25 mg/day in the evening day 1–3, then 50 mg/day in the evening (until pregnancy is confirmed, then stop)
<i>In presence of an absent or deficient gonatropin secretion:</i>		
HMG or FHS/LH		Begin low-dose (37.5–50 IU/day), individual increase of the dose
First line treatment in pituitary hypo-gonadism (secondary ovarian insufficiency)		Ovulation induction: 5000–10,000 IU HCG
Second line treatment in hypothalamic hypo-gonadism (primary ovarian insufficiency)		

In presence of a congenital defect or an acquired lesion of the pituitary, ovulation induction by HMG or FSH/LH has to be used (Table 11.6). To avoid hyperstimulation and multiple pregnancies in ovulation induction by gonadotropins, the classical

**Table 11.7** Results of ovulation induction by pulsatile GnRH (i.v.) in hypothalamic amenorrhea grade 3c (15–20 GnRH  $\mu\text{g}/\text{pulse}$ , own data)

49 treatment cycles in 17 patients (1 Kallmann syndrome, 16 IHH.), mean age 37.2 years (range 32–39 years)

Spermiograms have been normal in all male partners. All patients had been treated without success by Clomiphene and/or by HMG during a period of at least 1 year.

*Results per cycles:*

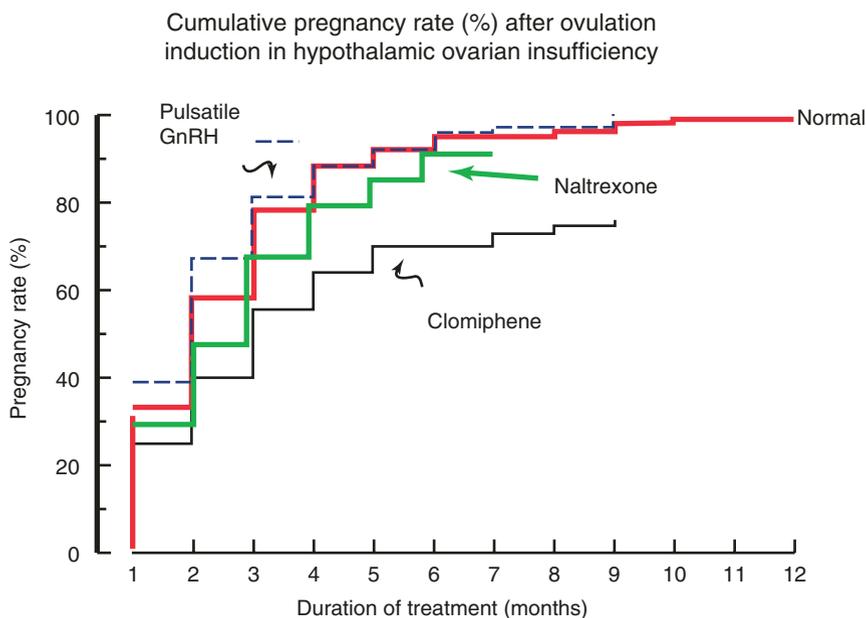
Ovulatory cycles	40 cycles (83%)
Clinical pregnancies	10 cycles (21%)
Biochemical pregnancies	6 cycles (13%)
Clinical abortion	1 cycle (2.1%)
“Take home babies”	9 cycles (18%)

53% of all patient became pregnant.

No patient developed a hyperstimulation syndrome

No multiple pregnancies have been seen.

Complications: Two cycles were interrupted due to two superficial phlebitis

**Fig. 11.7** Cumulative pregnancy rate (%) after ovulation induction in hypothalamic ovarian insufficiency by Clomiphene, pulsatile GnRH and Naltrexon compared to normal fertility within 12 months

Doses used:

– Clomiphene citrate 50–100 mg/day from day 5–9 after a progestin-induced bleeding.

– Pulsatile GnRH s.c.: 5–10  $\mu\text{g}/\text{pulse}$  every 90 min in the follicular phase and every 4 h in the luteal phase.

– Naltrexone 25 mg/day in the evening day 1–3, then 50 mg/day in the evening (until pregnancy is confirmed, then stop) (Adapted from Beier et al. [155])

rules have to be observed, meaning administration of the lowest efficient dose and regular supervision by serum oestradiol determination and sonographic control of follicular growth.

Naltrexone, an opioid antagonist, has been used in functional hypothalamic hypogonadism if there was a suspected deregulation of the endogenous opioid system [103]. However, success rate is inferior to pulsatile GnRH (Fig. 11.7); 20–30 % of all patients do not respond sufficiently or at all to Naltrexone. Side effects (mainly nausea, restlessness, sleep problems) are frequent in the first days of treatment.

### 11.4.3 Delay of Puberty in Hypergonadotropic Hypogonadism

(Table 11.8)

#### 11.4.3.1 Congenital Forms

##### Gonadal Dysgenesis: Phenotypic Female Variants

The term “gonadal dysgenesis” is generally used to describe a variety of clinical conditions. Their common denominator is an abnormal development of the foetal gonads. Gonadal dysgenesis includes Turner syndrome (45/X0, mosaics), mixed gonadal dysgenesis (45X/46, XY), female 46/XY dysgenesis (“pure gonadal dysgenesis, XY type”, Swyer’s syndrome) and the combination of a normal female sex chromosome constitution and hypergonadotropic hypogonadism (female 46/XX; “pure gonadal dysgenesis, XX type” or “XX gonadal dysgenesis”).

##### Turner Syndrome

*Definition, incidence* [104] The most frequent disorder in the category of gonadal dysgenesis is Turner syndrome and its variants. Turner syndrome (TS) affects approximately one in 2500–3000 live-born females; in abortions the incidence is 1:270. Girls and women confront the clinician with a challenging array of genetic,

**Table 11.8** Delay of puberty in hypergonadotropic hypogonadism

<i>Congenital forms</i>
Turner syndrome
Disorders of sexual development (gonadal dysgenesis and androgen resistance)
Mutations in LH or FSH receptor
Galactosemia
<i>Acquired forms</i>
Chemotherapy/radiotherapy
Endocrine disruptors
Ovarian torsion
Gonadectomy
Autoimmune disease

developmental, endocrine, cardiovascular, psychosocial, and reproductive issues. Intelligence is usually normal, but it may be limited in some cases.

*Karyotype* All individuals with suspected TS (see below) should have a karyotype performed. A standard 30-cell peripheral blood karyotype is recommended. It identifies at least 10 % mosaicism with 95 % confidence [104]. If there is a strong clinical suspicion of TS, despite a normal blood karyotype, a second tissue, such as skin, may be examined.

TS is a chromosomally determined disorder where complete ovarian agenesis is present with deletion of one X chromosome (karyotype 45 X0). Incomplete forms may be seen in patients with mosaicism (X0/XX, XO/XXX, X0/XX/XXX, 45X0/46XF etc.), in which case the patient may be chromatin positive.

Mosaics may present with a Y chromosome (45X0/46XY). The presence of Y chromosome material is associated with an approximately 12 % risk of a gonadoblastoma which may transform into malignant germ cell neoplasms. Therefore, prophylactic laparoscopic gonadectomy is recommended [105]. The patient and/or her parents should be informed of the finding of Y chromosome material with the utmost sensitivity regarding gender identity issues to minimize psychological harm.

*Clinical presentation* The phenotype may be oligosymptomatic, dominated by short stature. The diagnosis of TS should be considered in any female with unexplained growth failure or pubertal delay or any constellation of the following clinical findings: oedema of the hands or feet, nuchal folds, left-sided cardiac anomalies, especially coarctation of the aorta or hypoplastic left heart, low hairline, low-set ears, small mandible, short stature with growth velocity less than the 10th percentile for age, markedly elevated levels of FSH, cubitus valgus, nail hypoplasia, hyperconvex uplifted nails, multiple pigmented naevi, characteristic facies, short fourth metacarpal, high arched palate or chronic otitis media.

*Absent or delayed puberty* Absent pubertal development is one of the most common clinical features of TS, although up to 30 % or more of girls with TS (mosaicisms included) will undergo some spontaneous pubertal development. Ultimately, over 90 % of individuals with TS will have gonadal failure. Therefore, the critical importance of oestrogen treatment for feminization and for bone health during the adult years has to be emphasized. Today, it is accepted that induction of puberty should not be delayed from the age of 12 years until the age of 15 years as recommended earlier to promote statural growth by GnRH treatment first: the positive effect of GnRH administered today to most patients to promote adult height is not hindered by a prudent start of oestrogen substitution at the age of 12 [104].

*Impact on fertility* Most women with TS are infertile. Only 2–5 %, including forms with mosaicism, achieve spontaneous pregnancy [104]. In a review from 1993 [106], the literature contained reports of 28 spontaneous pregnancies in 16 women

with 45, X karyotype and 106 pregnancies in 48 women with mosaicism. Miscarriages, stillbirths and malformations were common. Since then, more spontaneous pregnancies have been known.

Today, various assisted reproductive techniques, particularly egg or embryo donation, are now legally available in many countries for achieving pregnancy. Recent studies show that using these techniques, women with TS become pregnant as easily as women with other types of infertility and carry their pregnancies to term without an increased miscarriage rate [104] if the uterus has been prepared adequately. Oestrogen/progestogen-substitution should be started at least 1 year before assisted reproduction. To be ready for donation, the thickness of the endometrium should reach 7 mm. TS women do have an increased rate of maternal complications, in part because of their small size. Only one embryo should be transferred to avoid the additional risks of a multiple pregnancy.

Reports of fatal aortic dissection during pregnancy and the postpartum period have raised concern about the safety of pregnancy in TS. Therefore, spontaneous or assisted pregnancy in TS should be undertaken only after thorough cardiac evaluation. It is recommended to consider a history of surgically repaired cardiovascular defect, the presence of BAV, or current evidence of aortic dilatation or systemic hypertension as relative contraindications to pregnancy. The possibility of prenatal genetic testing has to be offered.

Women with TS who have spontaneous menstrual cycles and ovulate normally should not postpone the timing of pregnancies without good reason because of the increased risk of premature ovarian failure. They have to be informed about the possibility of oocyte or embryo cryopreservation [107, 108], although these techniques are far from being an established method in TS.

### Other Forms of Gonadal Dysgenesis

Mixed gonadal dysgenesis (45X/46, XY), female 46/XY gonadal dysgenesis (“pure” gonadal dysgenesis “XY type”, also called “XY females”) and “pure” gonadal dysgenesis (“XX type” or “XX gonadal dysgenesis”) are rare. They have no Turner stigmata. Complete and partial forms are observed. Pure 46/XX is at least in part due to ODGI gene mutation, inherited as an autosomal recessive, female-limited disorder [109]. SRY Mutations in XY females may lead to complete 46,XY gonadal dysgenesis or “pure” gonadal dysgenesis (also called “XY females”). These individuals suffer rapid and early degeneration of their gonads (gonadal dysgenesis), which are present in the adult as streak gonads consisting mainly of fibrous tissue and variable amounts of ovarian stroma. The external genitalia in these subjects are completely female, and Müllerian structures are normal. The frequency of SRY mutations in XY females (“pure” gonadal dysgenesis) seems to be higher than current estimates [110]. By contrast, subjects with 46,XY partial gonadal dysgenesis have ambiguous genitalia, a mix of Müllerian and Wolffian structures, and dysgenic gonads. These gonads usually consist of disorganized seminiferous tubules admixed with ovarian stroma. Again, the presence of Y chromosome material is associated with an increased risk of germ cell neoplasms so that prophylactic gonadectomy is recommended [105].

There are no practical guidelines for the clinical handling of these patients and no statistics about their later fertility available. It seems therefore logical to counsel and to treat them in analogy to the Turner Syndrome, after a full clinical and laboratory work-up including a full karyotype.

*Impact on fertility* There are no data pointing to the possibility of a spontaneous pregnancy. However, as long as a normal uterus is present, fertility can be reached through egg or embryo donation as clinical data confirm, describing pregnancies in pure 46/XX and pure 46/XY gonadal dysgenesis following ovum donation. Pregnancy rate per transfer was normal [111].

### Noonan Syndrome

Noonan syndrome (NS) is a rare autosomal dominant disorder characterized by a phenotype [112] including short stature, facial dysmorphism and congenital heart defects.

Karyotype is normal. Noonan syndrome affects both sexes. In both sexes, there is a delay in pubertal development.

Other associated features are webbed neck, chest deformity, mild intellectual deficit, cryptorchidism, poor feeding in infancy, bleeding tendency and lymphatic dysplasias. The incidence of Noonan syndrome has been estimated to be between 1 in 1000 and 1 in 2500 live births [113]. In about 50 % of cases, NS is caused by mutations in the PTPN11 gene on chromosome 12, in a small proportion of patients by mutations in the KRAS gene. The aetiology of NS in individuals without mutations in PTPN11 or KRAS (together almost 50 % of cases) is still unknown.

NS should be considered in all foetuses with polyhydramnion, pleural effusions, oedema and increased nuchal fluid with a normal karyotype. With special care and counselling, the majority of children with NS will grow up and function normally in the adult world.

*Impact on fertility* Fertility appears to be normal in Noonan females but has been reported to be decreased in males, although male transmission of the disorder to the next generation is not uncommon.

### Androgen Insensitivity Syndrome, Testicular Feminization (“Hairless Women”)

Androgen resistance is a broad continuum reaching from male infertility to testicular feminization. Complete testicular feminization is a highly distinctive disorder where genotypic males (46/XY) are phenotypically female.

Androgen insensitivity syndrome (AIS) is a genetic condition carried on the X chromosome. Although it is inherited in an X-linked, recessive fashion in 70 %, up to 30 % of mutations are sporadic de novo mutations [114]. The estimated prevalence of AIS is between 1 in 20,000 and 1 in 99,000 genetic males. If one examines phenotypic females with inguinal hernias, the prevalence is noted to be 0.8–2.4 %.

In androgen insensitivity syndrome, there is a no activity at the androgen receptor leading to a tissue resistance to androgens. Wolffian structures are poorly (although partially) developed. Since AMH (anti-Müllerian hormone, or Müllerian inhibiting substance) is produced normally by the testes, there is a complete involution of the Müllerian ducts.

*Clinical presentation* [114] The typical presentation is that of primary amenorrhea in a phenotypic female adolescent. However, in an infant or child, the presentation may be of an inguinal hernia in a phenotypic female. Recent data shows a 1.1% incidence rate of complete AIS in a child with a premenarcheal inguinal hernia, while 80–90% of girls with complete AIS eventually develop an inguinal hernia

The external genitalia are female, breasts are developed. The “vagina” ends in a blind pouch; its average length is 2.5–3.0 cm. The testes are found in the labia majora, the inguinal canals or intra-abdominally, and should be prophylactically removed after puberty because of the risk of malignancy. Gonadectomy has to be followed by oestrogen substitution. Rates of dysgerminoma and gonadoblastoma in XY gonadal dysgenesis can rise as high as 15–30%, but might be lower in AIS. Tumours prior to puberty are rare.

At the time of puberty (usually not delayed), testosterone levels increase in presence of abnormally high LH. Female secondary sex characteristics develop as a result of increased oestrogen levels. Sexual hair is scanty or absent because of the androgen resistance. The final diagnosis is given by the XY-karyotype.

*Differential diagnosis* The Mayer-Rokitansky-Küster-Hausler (MRKH) syndrome or Müllerian Agenesis is part of the differential diagnosis. MRKH is a more common cause of primary amenorrhea. Its incidence rate is 1 in 5000 [119]. MRKH, too, has a normal breast development and an underdeveloped vagina, but it has normal axillary and pubic hair and a normal female karyotype (46/XX).

*Impact on fertility* To enable sexual intercourse, there are different options for vaginal creation (dilatation, surgical creation). Today, there is no reasonable way to treat infertility.

### **Mutations in LH or FSH Receptor**

Today, several different homozygous or compound heterozygous inactivating mutations of the LH receptor known. Inactivating mutations of LH receptors can be a very rare cause of delayed puberty and primary hypergonadotropic amenorrhea or premature ovarian failure [115–118]. Clinically, these patients are characterized by female external genitalia, spontaneous breast and pubic hair development at puberty, and normal or late menarche followed by oligo-amenorrhea and infertility. Oestradiol and progesterone levels are normal for the early to midfollicular phase, but do not reach ovulatory or luteal phase levels, confirming lack of ovulation. Notably, serum LH levels are high in patients with LH receptor mutations, whereas follicle-stimulating hormone levels are normal or only slightly increased. Pelvic

ultrasound has demonstrated a small or normal uterus and normal or enlarged ovaries with cysts [118].

Mutations of the LH receptor gene may cause primary or secondary amenorrhea and infertility in sisters of male pseudohermaphrodites [115]. Clinically, they present a phenotype of hypergonadotropic hypogonadism (“LH resistance”).

On the other hand, only few mutations of FSH receptor were discovered so far. Mutations of the FSH-receptor gene [116, 117, 120–122] cause ovarian dysgenesis leading to delayed puberty, primary or secondary hypergonadotropic amenorrhea and small ovaries with variable development of female secondary sex characteristics and infertility.

*Impact on fertility* In the different syndromes of inactivating mutations of gonadotropin receptors, no pregnancies have been described. In contrast to the clinically similar women harbouring inactivating mutations in luteinizing hormone (LH) beta subunit that may be treated with hCG (human chorionic gonadotropin) or LH, those with mutations in LH receptor are resistant.

### **Galactosemia**

Galactosemia is a rare inborn error of metabolism that results from impaired activity of any of the three enzymes of the Leloir pathway: galactokinase (GALK, EC 2.7.1.6), galactose-1-phosphate uridylyltransferase (GALT, EC 2.7.7.12), or UDP-galactose 4'-epimerase (GALE, EC 5.1.3.2). Classic galactosemia (OMIM 230400), the most common clinically severe form of the disorder, results from profound impairment of GALT. Classic galactosemia impacts about 1/60,000 live births, although prevalence differs substantially among populations [123].

Sequelae include cognitive and/or behavioural impairment in close to half of all patients, speech difficulties in at least half of all patients, low bone mineral density in many patients, ataxia or tremor in some patients, absent or delayed puberty and primary or premature ovarian insufficiency (POI) in at least 80% of all girls and women. Neonatal diagnosis with immediate dietary galactose restriction prevents or resolves the acute symptoms of classic galactosemia. However, despite pre-symptomatic diagnosis and strict lifelong dietary intervention, a majority of patients go on to experience a constellation of troubling long-term complications

*Impact on fertility* [124] In imaging studies of girls with POI examined at pubertal age or later, the ovaries are invariably abnormal and usually described as hypoplastic or “streak-like.” Histological examination most often shows few if any follicles; in the cases where follicles have been observed they did not appear to have matured beyond the primordial stage. The streak-like transformation of the ovaries may be related to accumulated galactose ootoxicity over time. One study of a small cohort of galactosemic women looked at spontaneous fertility outcomes (Gubbels et al. 2008) and reported that galactosemia patients with a diagnosis of POI may be more likely to conceive than women who have POI due to other causes. In a study, [124] 22 galactosemic women were followed. Nine women have tried to conceive, of which four were successful. Three mothers were diagnosed with POF before the

first pregnancy and/or in between pregnancies. In the literature, 50 pregnancy reports were found. The genotype and GALT-activity do not seem to predict the chance of becoming pregnant, whereas the occurrence of spontaneous menarche might. For those women with galactosemia who do achieve pregnancy, evidence from studies of small cohorts suggests that there are no adverse effects on the galactosemic mother or her infant.

Options to preserve endogenous fertility in galactosemia need to be explored further. For postpubertal women, freezing embryos after in vitro fertilization is an option.

### 11.4.3.2 Acquired Forms

#### Delayed Puberty and Reduced Fertility After Cancer Treatment

Childhood cancer is relatively rare, with an incidence of around 110 cases per million children per year [125, 126]. The Belgian Society of Pediatric Haematology and Oncology (unpublished date, in [108]) estimated childhood cancers are to occur in approximately 13 of 100,000 children under 15 years of age, with 45% being cases of leukaemia and lymphoma, 20% craniospinal tumours, 8% neuroblastomas, 8% soft tissue tumours, 7% nephroblastomas, 3% retinoblastomas and 9% other rare tumours. The majority of children diagnosed with cancer are expected to be cured and become long-term survivors. The remarkable success in improving childhood cancer survival is exemplified by the 5-year survival rate for all leukaemias approaching 80% (Office for National Statistics, 2004, UK) [125]. In 2010, in the USA, a total of 10,700 children and adolescents under the age of 14 were diagnosed with cancer. More than 80% will survive the disease. For some common paediatric cancers such as Wilms' tumour, Hodgkin's disease (HD), and B-cell non-Hodgkin lymphoma (B-NHL) cure rates approach 90% [127]. As such, it has been estimated that at the start of the twenty-first century, one in 1000 young adults in their third decade is a survivor of childhood cancer [126].

The results from the large and important *Childhood Cancer Survivor Study* (CCSS; [128]) show the natural outcome, without fertility preservation measures, of treatment for cancer diagnosed during childhood or adolescence on ovarian function and reproductive outcomes. The frequency of acute ovarian failure, premature menopause, live birth, stillbirth, spontaneous and therapeutic abortion and birth defects in the participants have been reviewed in the CCSS. Acute ovarian failure (AOF) occurred in 6.3% of eligible survivors. Exposure of the ovaries to high-dose radiation (especially over 10 Gy), alkylating agents and procarbazine, at older ages, were significant risk factors for AOF. Premature nonsurgical menopause (PM) occurred in 8% of participants versus 0.8% of siblings (rate ratio = 13.21; 95% CI, 3.26–53.51;  $P = .001$ ). Risk factors for premature menopause included attained age, exposure to increasing doses of radiation to the ovaries, increasing alkylating agent score, and a diagnosis of Hodgkin's lymphoma. 1915 female survivors reported 4029 pregnancies. Offspring of women who received uterine radiation doses of more than 5 Gy were more likely to be small for gestational age (birth weight < 10

percentile for gestational age; 18.2% v 7.8%; odds ratio=4.0; 95% CI, 1.6–9.8;  $P=.003$ ). The CCSS did not reveal any differences in the proportion of offspring with simple malformations, cytogenetic syndromes, or single-gene defects.

The CCSS demonstrates that women treated with pelvic irradiation and/or increasing alkylating agent doses were at risk for acute ovarian failure, premature menopause, and small-for-gestational-age offspring. There was no evidence for an increased risk of congenital malformations.

Based on these data, although problems with fertility do not become apparent until after puberty, it is clear that many treatments for childhood cancer can lead to infertility and subfertility in later life. Having survived cancer as a child, it can be very difficult for many patients to accept that they cannot produce their own children because of the treatment they received during childhood.

How can this outcome after cancer treatment be improved? Fertility preservation is becoming increasingly important to improve the quality of life in cancer survivors. Despite guidelines suggesting that discussion of fertility preservation should be done prior to starting cancer therapies, there is a lack of implementation in this area. The need for fertility preservation has to be weighed against morbidity and mortality associated with cancer. Thorough psychological counselling is required. Recommendations should be individualized and should not violate the ethical principles.

Particularly in young girls and in adolescents, there is a need for a multidisciplinary collaboration between oncologists, paediatrician and reproductive specialists to improve awareness and availability.

### **Effect of Chemotherapy and Radiotherapy**

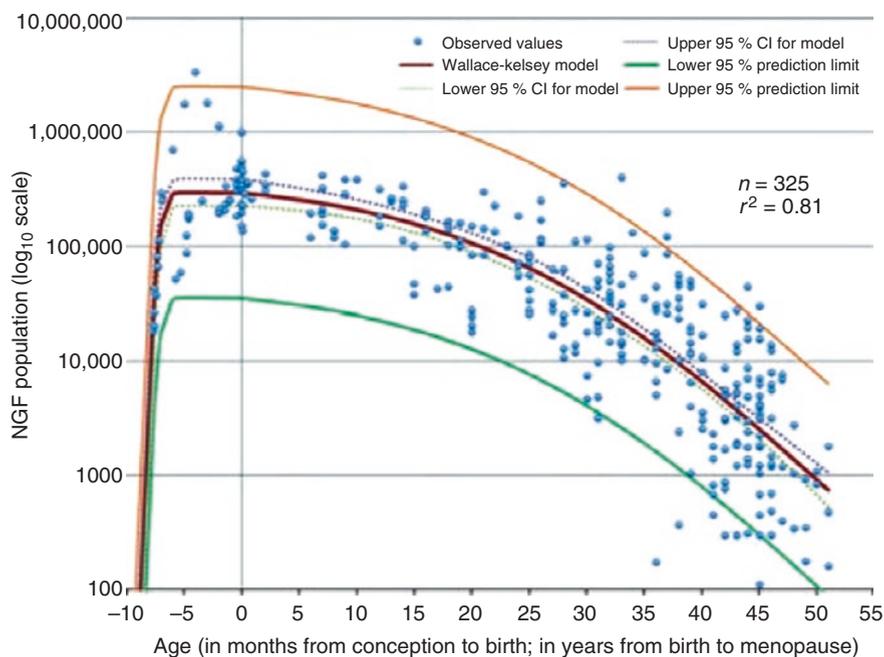
Children that undergo treatment for cancer are at risk of suffering from primary amenorrhea, delayed puberty, hormonal dysfunction and subfertility. Both chemotherapy and radiotherapy have a major impact on the gonadal axis and its hormonal and reproductive potential.

The oocyte has been in an arrested stage of meiosis since before birth and remains so until the onset of puberty, and this does not advance until ovulation. Chemotherapy, radiotherapy and surgery can all have adverse effects on reproduction. It is the effects on the nongrowing stockpile of primordial follicles that is of particular importance for future reproductive potential (Fig. 11.8) [129]. The mechanism by which chemotherapy causes loss of primordial follicles is, however, poorly understood. At present, it is impossible to predict the functional life span of the chemotherapeutically damaged ovary and the reproductive potential of patients with cancer.

It is important to emphasize that there is no evidence to suggest that the prepubertal female (or male) reproductive tract is protected from the adverse effects of cancer therapies. The danger of pelvic radiotherapy is significantly greater than the risk of all kinds of chemotherapy. The susceptibility of the prepubertal uterus to radiotherapy is also clearly demonstrated [108, 129].

### **Chemotherapy**

Chemotherapeutic drugs act by interrupting vital cell processes and arresting the normal cellular proliferation cycle. They cause DNA abnormalities as well as



**Fig. 11.8** The number of nongrowing follicles in the ovary is increasing as a function of time. Inversely, the number of potentially fertile promordial follicle available for recruitment is decreasing already before birth

oxidative damage in somatic and germ cells. Persistent unrepaired DNA double-strand breaks activate apoptotic death in oocytes. Genetic effects on the oocyte result in aneuploidy and early embryonic mortality.

In contrast to the effects on male fertility, females are, in general, less susceptible to the gonadotoxic effects of chemotherapy [130]. The clinical impact of chemotherapeutic drugs on the ovary is variable, ranging from no effect to complete ovarian atrophy. The degree of damage is dependent upon the type of the chemotherapeutic agent used, dose given, age of the patient and her baseline ovarian reserve. The prepubertal ovary is less susceptible to damage by chemotherapeutic agents. Compared to older women, early postpubertal adolescents have a higher ovarian reserve and are less susceptible to premature ovarian failure (POF). Fibrosis of stromal blood vessels adds to the ovarian damage.

Depending on the chemotherapy used, the clinical manifestation of the follicular loss ranges from a complete amenorrhea to POF and to varying degree of infertility (Table 11.9) [131]. Some paediatric chemotherapy regimens such as MOPP (mustagen, oncovin, procarbazine, and prednisone) for Hodgkin's disease, and high-dose cyclophosphamide and busulfan for bone marrow transplantation, cause sterility in a significant number of patients. Other regimens such as high dose cyclophosphamide for B-NHL and Ewing sarcoma are associated with a significant risk for fertility impairment [131]. Because no systematic comprehensive data exists on the exact

rates of fertility impairment associated with current paediatric oncology therapeutic regimens, the following grading of the damaging potential is derived from adult data [125, 132]:

Alkylating agents such as cyclophosphamide and procarbazine are high risk drugs with the highest age-adjusted odds ratio of ovarian failure rates.

Platinum-based compounds such as cisplatin cause DNA damage. They carry a medium risk of amenorrhea.

Anthracycline antibiotics such as doxorubicin (DXR) induce oxidative stress. The amenorrhea and fertility risk is medium to low with this group of drugs. DXR administration in female mice caused dominant lethal mutations and aneuploidy in maturing/preovulatory oocytes.

Vinca alkaloids do not seem to increase the risk of ovarian failure though animal experiments show a high rate of oocyte aneuploidy.

Anti-metabolites like methotrexate and 5-fluorouracil do not seem to affect the ovary based on the limited current data available. Methotrexate is commonly used to treat the ectopic pregnancy without any effect on subsequent fertility.

Taxanes: The data available for taxanes are controversial. Some studies show increased risk of ovarian failure, others suggest that there is no increased risk.

Biological targeted therapies (herceptin, tamoxifen, rituximab) are designed to interfere with specific receptors or molecules expressed by tumours (herceptin or tamoxifen), or act via the immune system (rituximab). Fertility risk data for these drugs are limited. Since they target specific cells, it is believed that the risk should be low.

The risk of POF with polyagent adjuvant chemotherapy has been reported to range from 53 to 89%. The risk of POF is related to the patient's age, treatment protocol and type of malignancy [125]. Restoration of menstruation after CRA is possible. Again this is influenced by age and duration of follow-up and has been estimated at 39–55% in younger women (<40 years) and 0–11% in older patients (>40 years) [133]. However, women who maintain normal menses throughout

**Table 11.9** Gonadotoxic chemotherapy agents

Alkylating agents
Cyclophosphamide
Ifosfamide
Nitrosureas, e.g., carmustine and lamustine
Chlorambucil
Melphalan
Busulphan
Vinca-alkaloids
Vinblastine
Antimetabolites
Cytarabine
Others
Cisplatin
Procarbazine

**Fig. 11.9** Comparison of the ovarian toxicity exerted by the main groups of cytotoxic drugs with the damaging effect of pelvic radiotherapy. The number of *arrows* indicates their relative potency. No particular risk is known for substances marked by a *horizontal arrow* (Modified from Meirov et al. [131])

#### Toxicity of different oncological chemotherapies on ovarian reserve

Pelvic radiotherapy	↑↑↑↑↑
Alkylating agents	↑↑↑
Platinum agents	↑↑
Taxanes	↑↑
Plant alkaloids	↑
Anthracyclines	→
Anti-metabolites	→

chemotherapy remain at risk for developing POF. This is evident from the high rates of POF seen in adolescents receiving alkylating agents for cancer [134]. There are no reports of uterine damage after chemotherapy [135] (Fig. 11.9).

#### Effect of Radiotherapy

Unlike chemotherapy, radiotherapy affects both the ovary and the uterus [129].

Human oocyte is sensitive to radiation, with an estimated median lethal dose (LD) of <2 Gy. Damage to the ovary by radiotherapy is dependent on the age of the patient and dose of the ovarian exposure. The effective sterilizing dose (ESD) is the dose of fractionated radiotherapy (Gy) at which POF occurs immediately after treatment in 97.5 % of patients. The degree of damage depends on the radiation dose and field, fractionation schedule, and the patient's age. A radiation dose of 2 Gy is estimated to damage 50 % of ovarian follicles irreversibly; doses ranging from 5 to 20 Gy cause complete loss of ovarian function resulting in sterility [136]. The number of primordial follicles present at the time of treatment and the dose of radiation received by the ovaries determines the fertility "window." In females, it has been shown that for a given dose of radiation, the younger the patient at the time of treatment, the later the onset of premature menopause, and inversely, the higher ESD to cause permanent ovarian failure [131, 132, 136, 137]: ESD is 20.3 Gy at birth, 18.4 Gy at 10 years, 16.5 Gy at 20 years, and 14.3–16 Gy at 30 years, with only 6 Gy being required in women over 40. With total body irradiation, the location of the radiation field impacts the degree of ovarian damage (10–15.75 Gy) observed to result in ovarian failure in 90 % of patients in long-term follow-up. After abdominal radiation, ovarian failure rates may be as high as 97 %. [131].

After abdominal, pelvic, or TBI, the uterus is at risk of damage in a dose- and age-dependent manner [135]. Uterine growth and uterine blood flow start at puberty and are completed almost 7 years after menarche. Exposure to radiation leads to reduced vascularity, damage to myometrium leading to fibrosis and hormone dependent endometrial insufficiency. These uterine damages result in adverse reproductive outcomes such as increased rates of infertility, miscarriage, preterm labour, intra-uterine growth retardation and low birth weight.

Uterine function may be impaired after radiation doses of 14–30 Gy as a consequence of disruption to the uterine vasculature and musculature elasticity. In adults, an exposure to TBI of 12 Gy is associated with significant uterine damage. In childhood, radiation doses of >25 Gy directly to the uterus appears to induce irreversible damage. However, even lower dose of irradiation, as in total body irradiation, have been reported to cause impaired growth and blood flow. There is no agreement on the dose of radiation to the uterus, above which a pregnancy would not be sustainable.

Efforts to improve uterine function have been tried. However, there was no significant difference noted with regard to uterine volume, endometrial thickness, or uterine artery blood flow after high dose oestrogen replacement.

*Preventive measure* Ovarian transposition has been recommended to get the ovary out of the radiation field (laparoscopic “ovarian suspension” or “oophorectomy”; [132]).

### **Estimation of the Damage Induced by Chemotherapy and Radiotherapy by Biological Markers and By Sonography**

In a prospective cohort study on girls treated for cancer, AMH, inhibin B, and FSH have been measured before, during, and after completion of treatment [138]. The aim of our study was to evaluate these biochemical measures as potential markers of early gonadotoxicity in young girls treated for cancer. As a result, AMH has been shown to be a clinically useful marker of damage to the ovarian reserve in girls receiving treatment for cancer. AMH is detectable in girls of all ages and falls rapidly during cancer treatment in both prepubertal and pubertal girls [138, 139]. Both the fall during treatment and recovery thereafter varied with risk of gonadotoxicity. In medium/low risk patients, AMH recovery is highly significant and reaches pre-treatment levels [139]. The value of FSH and Inhibin B as markers of ovarian activity is limited by very low/undetectable concentrations before puberty and the need for measurement in the early follicular phase thereafter [129, 138].

The number of small antral follicles within the ovary (AFC) counted by transvaginal ultrasound, too, provide substantially more accurate indicators than FSH (or inhibin B) of what is known as the ovarian reserve. AFC allows to mean the number of primordial follicles remaining in the ovary. Both AMH and AFC show very good correlation with oocyte yield following superovulation needed for mature oocyte preservation, with a very high correlation between the two [129]. Today, this elegant method is not yet validated for abdominal transvesical ultrasound in prepubertal children.

The assessment of ovarian reserve in the prepubertal girl who has been successfully treated for cancer remains difficult. Larsen et al. evaluated ovarian function in 100 childhood cancer survivors and 21 controls of similar age [140]. Survivors with spontaneous menstrual cycles ( $n=70$ ) were found to have smaller ovarian volume per ovary compared with controls (median, 4.8 cm<sup>3</sup> vs. 6.8 cm<sup>3</sup>;  $P<.001$ ) and a lower number of antral follicles (AFC) per ovary (median, 7.5 AFC vs. 11 AFC;  $P<.001$ ). A regression analysis was performed to predict the total AFC number per ovary, which demonstrated a reduced number in women who were treated with ovarian irradiation (beta=-.40;  $P<.001$ ),

alkylating chemotherapy ( $\beta = -.25$ ;  $P = .01$ ), older age at diagnosis ( $\beta = -.25$ ;  $P = .01$ ), and longer time period off treatment ( $\beta = -.19$ ;  $P = .044$ ) [140].

## Fertility Preservation in Young Girls and in Adolescents

### Oncological Indications

In general, fertility preservation before cancer treatment is strongly recommended if the chance of losing fertility is over 30% with cancer therapy. In adult patients that have a partner, cryopreservation of embryos remains the most reliable method to preserve fertility in women <35 years of age. Another efficient option is vitrification of mature unfertilized oocytes. Today, an extensive database is available for adult cancer patients [107, 141].

Children and adolescents represent a special patient group. Jadoul et al. published in 2010 an important review of ovarian cryopreservation in adults and, more specifically, in children using the PubMed databases and added their own experience with cryopreservation in children [108]. They conclude that ovarian cortex cryopreservation is feasible and as safe as comparable operative procedures in children. However, the absence of consensus on the indications for fertility preservation, as well as the optimal timing and quantity of ovarian cortex for cryopreservation, should be taken into consideration when discussing fertility issues with girls at risk of POF and their parents.

Cryopreservation of embryos and vitrification of mature unfertilized oocytes require both hormonal stimulation and delay of treatment; thus, they cannot be offered to prepubertal patients and to adolescents that must receive urgent treatment. Therefore, cryopreservation of gonadal tissue is the only option today to be offered to young prepubertal girls with malignancies that require gonadotoxic therapy [108, 129], although no births have yet resulted from freeze-thawing of prepubertal ovarian cortex.

Wallace et al. recommended the following risk assessment for fertility preservation in girls [146]:

- Intrinsic factors
  - Health status of patient
  - Consent (patient/parent)
  - Assessment of ovarian reserve in girls/young women
- Extrinsic factors
  - Nature of predicted treatment (high/medium/low/uncertain risk)
  - Time available
  - Expertise available

A series of women having undergone ovarian cryopreservation has shown that these patients did not necessarily need reimplantation of the ovarian tissue to get pregnant. Of the 36 women treated by cytostatic agents (20% by cyclophosphamide because of generalized Lupus erythematoses), 11 had died at the time of last follow-up, but 5 experienced spontaneous pregnancies, with none to date having requested reimplantation of their stored ovarian tissue [142].

Is there a lower limit of age? Michaeli et al. [143] consider that ovary cryopreservation can be safely offered even to younger girls since the potential benefits are currently more evident and the risks of anaesthesia appear not to be increased, although they earlier recommended a lower limit of 3 years of age. They believe that there is an ethical obligation of clinicians to offer fertility preservation and to discuss fertility issues with cancer patients or their parents, in order to provide the opportunity for future parenthood.

Postpubertal girls from puberty to the age of 18 may be candidates for mature oocyte cryopreservation following ovarian stimulation.

The exact procedures for these modern techniques of fertility preservation including the benefits and risks of later autotransplantation of ovarian tissue as well as pregnancy outcome in high-risk survivors of cancer are summarized in specialized reviews [107, 108, 129, 132, 135, 138–140, 143].

### Non-Oncological Indications

Non-oncological indications for fertility preservation procedures include haematological or autoimmune diseases, as well as certain genetic conditions such as Fragile-X and Turner syndrome, which predispose women to premature ovarian failure. In addition, repeated surgery due to ovarian cysts or ovarian torsion may result in decreased ovarian reserve [108].

### Ethical Considerations

There is a need for extreme sensitivity when broaching the topic of fertility preservation. This also may be an option for adolescents who are peripubertal, but still premenarchal. In vitro maturation (IVM) and ovarian tissue conservation (OTC) can also be offered. In prepubertal girls, OTC is currently the only way to cryopreserve gametes. Careful counselling and informed consent is especially recommended. In children, OTC implies the removal of an entire ovary because of its small size. Prepubertal girls who do not have any other options have to be informed about a high risk for POF when significant loss of ovarian follicles in the remaining ovary is anticipated with cancer therapy.

Parents have to be given full information of the invasive process needing a total anaesthesia, the associated risks and the success rates. The patient and her parents should understand the still experimental nature and the potential risks of cancer cell transmission.

Wallace et al. recommend applying the Edinburgh selection criteria to young girls and adolescents. The Edinburgh selection criteria accurately identify the few girls and young women who will develop premature ovarian insufficiency. They have been validated recently for their use for selection of patients for ovarian tissue cryopreservation [144]:

- Age younger than 35 years
- No previous chemotherapy or radiotherapy if aged 15 years or older at diagnosis, but mild, non-gonadotoxic chemotherapy acceptable if younger than 15 years

- A realistic chance of surviving for 5 years
- A high risk of premature ovarian insufficiency (>50%)
- Informed consent (from parents and, where possible, patient)
- Negative serology results for HIV, syphilis, and hepatitis B
- Not pregnant and no existing children

It has always to be kept in mind that these procedures are in part still experimental and not yet clinical routine. As Wallace and Barr stated recently, it is not likely to be feasible or indeed ethical to perform a randomized study in a well-characterized group of young women to test laparoscopic collection of ovarian cortex versus either dummy laparoscopy or indeed no intervention. In their opinion, and in mine too, it is highly unlikely that IRBs would pass such a study, or indeed that such a randomized study would be able to recruit sufficient patients [145]. Therefore, all treatments for fertility preservation in young girls and adolescents should be done in accredited centres, documented and the data pooled so that a true and accurate description of the success of ovarian cryopreservation for young women at risk of a premature menopause can be provided.

### **Acquired Delayed Puberty and Reduced Fertility Due to Other Causes**

#### **Ovarian Torsion and Gonadectomy**

Usually, ovarian torsion and gonadectomy are listed in this category. However, unilateral loss of an ovary by torsion or gonadectomy has no impact on the age of puberty and on later fertility.

The loss of both ovaries implies a timely induction of puberty by the slowly progressive administration of oestradiol and, in a second time, oestradiol combined with a progestatif. Fertility can be reached only by egg or embryo donation if these methods are legal in the country where the patient is living.

#### **Autoimmune Diseases**

In young girls and in adolescents, endocrine and non-endocrine autoimmune diseases involving the ovary and provoking a progressive destruction of the ovarian tissue may cause exceptionally a delayed puberty. The more common physiopathology leading to a delayed puberty in presence of an autoimmune disease involves functional hypothalamic hypogonadism (see above).

However, there are reports showing an early progressive destruction of the ovarian tissue by autoimmune processes, mainly in young girls and adolescents suffering from generalized lupus erythematoses [142, 146]. Later infertility can be prevented by applying the same methods of cryopreservation of ovarian tissue recommended for young cancer patients (see above).

#### **Endocrine Disruptors**

There have been speculations about a potential responsibility of endocrine disruptors in causing delayed puberty. However, ES have been shown mostly to advance

and less to delay puberty. The potential of EDs to cause precocious puberty has first been noticed in the early 1990s and has been confirmed in numerous animal studies. Subsequently, the use of some of these substances has been prohibited in products used by humans and domestic animals.

In the last years, several environmental endocrine disruptors (EDs) such as phytoestrogens, topical and natural oestrogens, pesticides, industrial chemicals and phthalates have been identified as possible agents affecting pubertal development in humans in different ways [148, 149]. EDs exert their effects through different mechanisms: by binding to the relevant hormone receptors; by direct action on cell signalling pathways or on the central nervous system and the neuroendocrine system, by suppression of hormone synthesis or through their toxic effects on the relevant organs. ES may influence puberty through their oestrogenic, antioestrogenic, androgenic, antiandrogenic effects or through their direct effects on the gonadotropin-releasing hormone (GnRH). EDs may affect puberty by inhibiting the synthesis of endogenous hormones such as testosterone, 17 beta-oestradiol and adrenal steroids via competitive inhibition of P450 steroidogenic enzymes (C17,20-lyase, aromatase). Other environmental chemicals may impair neuroendocrine functions through their effect on the central nervous system and the hypothalamic-hypophyseal-gonadal (HHG) axis. These include pesticides such as thiram, molinate, metam sodium, chlordimeform, amitraz, triazoles, dichloroacetic acid, atrazine, propazine, simazine, methanol and linuron. Depending on their mechanism of action, EDs may lead to precocious puberty, to delayed puberty, or to sexual differentiation disorders.

However, in humans, most known ES exposures lead to early pubarche and menarche in girls.

Delayed puberty has been reported by exposure to imidazole group fungicides, ketoconazole and fadrozole in the peripubertal period in animals. In a study on rats, atrazine caused delayed puberty by suppressing luteinizing hormone (LH) and prolactin levels. Another pesticide, prochloraz, suppresses oestrogen and androgen synthesis via inhibition of aromatase and 17,20-lyase [148].

In humans, Den Hond et al. [150] observed a reduction in testicular volume in boys and a delay in telarche in girls exposed to a substance with dioxin-like activity, but failed to note a change in pubertal development, or a change in the age of pubarche or menarche. Another study confirmed the relation between prenatal PCDD/F exposure and later initiation of breast development [151].

The heavy metal lead, one of the major environmental pollutants, has also been found to affect puberty [149, 152, 153]. Menarche and pubarche were delayed in girls with high serum lead levels. In addition, blood lead concentration was inversely and significantly associated with IQ. In the linear model, each increase of 10 µg per deciliter in the lifetime average blood lead concentration was associated with a 4.6-point decrease in IQ ( $P=0.004$ ). When estimated in a nonlinear model with the full sample, IQ declined by 7.4 points as lifetime average blood lead concentrations increased from 1 to 10 µg per deciliter [154].

In conclusion, there are several alarming reports showing that ES and the heavy metal lead influence pubertal development. The only way to stop this unacceptable poisoning of animals and of humans is the strict observance and enforcement of the already existing legal prescriptions and the targeted formulations of new laws protecting the environment against endocrine disruptors.

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# Premature Ovarian Failure in Adolescence and Young Adults: From Diagnosis to Therapy and Follow-up for Fertility Preservation

# 12

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Primary ovarian failure (POF) is a subclass of ovarian dysfunction in which the cause is within the ovary. POF is a primary ovarian defect characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles before the age of 40 years (secondary amenorrhea). It is an heterogeneous disorder affecting approximately 1% of women <40 years, 1:10,000 women by age 20 and 1:1000 women by age 30 [1] (Table 12.1).

Various terms have been suggested to define deviation from healthy ovarian function, including premature menopause (PM) or premature ovarian insufficiency (POI). Many authors regard ovarian insufficiency as more accurate than ovarian failure, because ovarian insufficiency can be used to describe a wide range of impaired ovarian function. The term also suggests that ovarian follicular activity might intermittently recover, even years after diagnosis and lead to pregnancy in about 5–10% of these women [2].

Follicle depletion or dysfunction in adolescent or young women may be caused by many different factors. POF might also result from genetic defects, chemotherapy, radiotherapy, or surgery. A common cause of POF in adolescent is gonadal dysgenesis, with or without Turner syndrome. When ovarian failure presents as a primary amenorrhea and no associated comorbidities, approximately 50% are found to have abnormal karyotypes. However, most causes of spontaneous POF present as secondary amenorrhea and among younger women aged 30 years or younger with secondary amenorrhea, 13% also have been noted to have an abnormal karyotype. Thus, in most cases the diagnosis will be 46, XX spontaneous POF, meaning the karyotype is normal [2]. In 90% of cases an unknown mechanism leads to premature exhaustion of the resting pool of primordial follicles and no etiology for spontaneous POF will be

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**Table 12.1** Etiology of Premature Ovarian Failure (POF)

Primary POF	Genetic aberrations	X-linked (monosomy, trisomy, deletions, translocations, fragile X)
		Autosomal dominant (FSH receptor gene polymorphism, inhibin B mutation, etc.)
	Enzyme deficiency	Metabolic
	Autoimmune disease	
Secondary POF	Surgical	Bilateral oophorectomy
		Hysterectomy without oophorectomy/uterine artery embolization
	Chemotherapy or radiotherapy	
	Infections	

identified even after a thorough evaluation. Approximately 4% of women with 46, XX spontaneous POF will have steroidogenic cell autoimmunity as the mechanism of POF [3]. Approximately 6% of women with 46, XX spontaneous POF will have pre-mutations in the FMR1 gene. This is the gene responsible for fragile X syndrome, the most common cause of familial mental retardation. The risk of having an FMR1 pre-mutation is higher if there is a family history of POF, therefore, in these patients it is crucial to take a family history, and women who have relatives with spontaneous POF should be referred for genetic counseling. Moreover, approximately 6% of women with familial POF will have a pre-mutation in the FMR1 gene as compared with 2% of women with isolated POF. Women found to have a pre-mutation in the FMR1 gene are at risk of having a child with mental retardation, should they be one of the 5–10% who conceive [4]. There are other rare genetic causes of familial POF for which routine genetic testing in sporadic cases is now not clinically indicated, such as mutations involving FSHR (FSH receptor), GALT (galactose-1-phosphate uridylyltransferase associated with galactosemia), FOXL2 (a forkhead transcription factor associated with the blepharophimosis/ptosis/epicanthus inversus syndrome), INHA (inhibin alpha gene), EIF2B (a family of genes associated with central nervous system leukodystrophy and ovarian failure), BMP15 (bone morphogenetic protein 15), and AIRE (autoimmune regulator gene associated with the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome) [5] (Table 12.2).

POF can be explained by different autoimmune mechanisms including a general immune dysregulation such as polyglandular syndrome (i.e., hypothyroidism, adrenal insufficiency, and hypoparathyroidism 14–27%), dry-eye syndrome, myasthenia gravis (2%), rheumatoid arthritis, diabetes mellitus (2%), or systemic lupus erythematosus. It might also arise from an inflammatory autoimmune process against ovarian-specific germ line antigens or regulatory factors, making the disorder more complex than initially thought. The prevalence of these diseases in women with POF is higher than in the general population, suggesting that there may be an autoimmune component in these women that is not well understood. Autoimmunity in ovarian failure can be divided into two categories: ovarian failure associated with autoimmune adrenal insufficiency and ovarian failure associated with other autoimmune

**Table 12.2** Genes implicated in Premature Ovarian Failure (POF)

Categories	Chromosomes	Gene
Identified mutations	X	FMR1, 2, BMP15
	Autosomal genes	FOXL2, FSHR, LHR, FSH $\beta$ , LH $\beta$ , inhibin A, GALT, AIRE, NOGGIN, POLG
Unidentified mutations	X	AT2, c-kit, sox 3
	Autosomal genes	MIS
Candidate genes	X	DIAPH2, DFFRX, XPNPEP2, 2FX, FSHPRH1, XIST
	Autosomal genes	WT1, ATM

disease. It is only when POI is associated with adrenal autoimmunity or insufficiency that true autoimmune oophoritis can be demonstrated. Approximately 2–10% of POF is associated with adrenal failure or autoimmunity and would thus be expected to result from autoimmune oophoritis. Circulating steroidogenic cell antibodies have been recorded in these autoimmune cases, therefore, steroid cell and/or enzyme antibody markers are present in 60–87% of women with secondary amenorrhea and adrenal autoimmunity and/or Addison's disease; therefore, it has been suggested that antibodies discriminate best between autoimmune POF and POF of other etiologies. However, ovarian antibody assays have a poor specificity and testing is therefore not recommended in women with primary ovarian insufficiency [6]. Although POF can happen as a result of ovarian surgery, oophorectomy, or exposure to viral or environmental toxic agents such as smoking, the most common cause of acquired disorder is chemotherapy or radiotherapy for cancer treatment. Chemotherapy-induced ovarian damage can arise through impairment of follicular maturation or primary follicle depletion, or both. Moreover, oocytes are very sensitive to radiation and age of the patient, and extent, type, and schedule of irradiation are key prognostic factors for development of primary ovarian failure. One dose is more destructive to the oocytes than are fractionated doses, and pelvic and abdominal irradiation has the highest risk, although scatter radiation can cause substantial damage even when the ovaries are not within the radiation field [7].

In the absence of symptoms, a change in the regular menstrual bleeding pattern is the main presenting symptom with POF. The absence of the menses in a 15-year-old girl (primary amenorrhea) or the cessation of menses for 4 months or more (secondary amenorrhea) can point towards the diagnosis; however, there is no consensus on criteria to identify primary ovarian failure in adolescence and delay in diagnosis is common. Some adolescent females report hot flushes and night sweats or vaginal symptoms like dryness or dyspareunia, low libido, low energy levels, sleep disturbance, lack of concentration, stiffness, skin/hair changes, and mood swings, however the most frequent symptoms of POF is primary or secondary amenorrhea. Among patients with amenorrhea, the incidence of POF ranges from 2 to 10%. Abnormal bleeding patterns also include oligomenorrhea (bleeding that occurs less frequently than every 35 days), non-structural causes of abnormal uterine bleeding (ovulatory dysfunction, iatrogenic, or not classified), or polymenorrhea (bleeding that occurs

more often than every 21 years). Because irregular menstrual cycles are both common during early adolescence and an initial symptom of early POF, diagnosis can be difficult in this population. Although less than 10% of women presenting abnormal menses will ultimately be found to have POF, the condition has such detrimental consequences on bone health that early diagnosis of this condition is important [8]. Therefore in young female it is important to evaluate amenorrhea or change from regular to irregular menses for three or more consecutive months in the absence of hormonal preparations such as oral contraceptives (OCs). Differential diagnosis is based on the exclusion for all potential causes of primary and secondary amenorrhea including pregnancy, polycystic ovary syndrome, hypothalamic amenorrhea, thyroid abnormalities, and hyperprolactinemia. In a woman aged less than 40 years, the diagnosis of POF is usually confirmed by the combination of the triad of oligo/amenorrhea for at least 4–6 months, sex steroids deficiency, and two recording of serum concentrations of follicle-stimulating hormone (FSH) of more than 30–40 IU/L at least 1 month apart. If the result indicates that FSH is elevated, a diagnosis of POF can be established. Estradiol levels of less than 50 pg/ml indicate hypoestrogenism. However FSH is not an ideal diagnostic tool because it rises only in the later stages of follicle depletion, has marked cycle- to-cycle variability, and it is poor at predicting reproductive status. In this view, there has been interest in more direct markers of ovarian reserve such as anti-Mullerian hormone (AMH), which closely follows the reduction in follicle number over time in healthy women and falls to very low levels prior to menopause. In assessment of amenorrhea, AMH or transvaginal ultrasound scan will exclude polycystic ovarian syndrome as a cause. In POF, the antral follicle count is very low, and seeing this as a related marker of ovarian function may help to understand the diagnosis. However, even in POF, the intermittent ovarian function means that follicular activity is seen in the majority of women [9, 10].

In the absence of an obvious cause of POF (oophorectomy, chemotherapy, or pelvic radiotherapy), young women should be offered investigation in etiology, however, in the majority of cases no cause is found. Even if causation is established, the management, including fertility options, remains unchanged. E genetic etiology will be identified in approximately 5% and autoimmune in up to 30% [10]. Genetic counseling is nowadays recommended for several reason when a genetic form of POF is suspected or identified and a karyotype should be offered to women with POF in the onset of amenorrhea or oligomenorrhea before age 25. A karyotype is also indicated in women of any age in whom Turner's syndrome mosaicism is suspected, as well as e FMR1 (fragile x) permutation testing. Overall the permutation is found in 4–5% of women with POF, among those with a family history of POF, 14% have a positive result, in this view a full family history is important as many cases of spontaneous POF appear to be inherited, estimates vary from 4 to 31%. Thirty percent of the cases of POF are estimated to be owing to autoimmunity. Presumed autoimmune etiology may be inferred from a family history of autoimmune disease or a positive anti-thyroid antibody result, which is found in approximately 24% of women affected by POF. If a POF patient appears to be a sporadic and not hereditary case, the risk of other female relatives developing POF will probably be equal to the risk in the general population [11].

For adolescents with POF, the objective of treatment is to replace the hormones that the ovary would be producing before the age of menopause (51 years), making the treatment specifically different from hormonal therapy for menopause that focuses on the treatment of menopausal symptoms. The aims of hormonal therapy extend beyond simply symptom relief to levels that support bone, cardiovascular (CV), and sexual health. Regardless of the etiology, patients with POF are estrogen deficient. Thus, young women with POF may need higher doses of estrogen than menopausal women to ensure adequate replacement and optimal bone health. In girls with absent or incomplete breast development, estrogen therapy should be initiated and increased slowly before administration of graduated progesterone dosages until breast development is complete to prevent tubular breast formation. For those patients who have not initiated or completed pubertal growth and sexual maturity, consultation with a specialist in growth and development and hormonal therapy in children is recommended. Once pubertal development is complete, ongoing hormonal therapy will be necessary for long-term health. Hormonal support involves daily therapy with the goal of maintenance of normal ovarian functioning levels of estradiol (E2). There are no controlled studies regarding the ideal hormone replacement strategy for young women with spontaneous POF. There is room for individualization. Most women do well using a 100- $\mu$ g E2 transdermal patch, which averts the first-pass effect on the liver and can be considered full-dose physiologic replacement for young women. On average this achieves a serum E2 level of 100 pg/mL, near the normal mean E2 level for normally cycling women of 104 pg/mL. Oral E2 can be given to women who prefer this route of administration, however, oral E2 increases the potential for thromboembolism relative to transdermal estradiol due to the first-pass effect on the liver. Typically, about twice as much E as is required for postmenopausal women is needed to alleviate the symptoms (100- $\mu$ g E2 patch, 2 mg daily dose of oral micronized E2, or 1.25 mg of conjugated equine E). Cyclic medroxyprogesterone acetate (MPA; 10 mg/day) or oral micronized P (200 mg/day) for 12 days each month should be given to induce regular monthly withdrawal bleeding. Progestin withdrawal less frequently than each month has been associated with the development of endometrial hyperplasia in older women. A case can be made for providing a 10-mg dose of MPA or equivalent to fully induce a secretory pattern [12, 13]. Lower doses may reduce side effects but require monitoring. Still some patients may prefer a less frequent progestin withdrawal with appropriate monitoring. Oral contraceptives contain higher doses of estrogen than are necessary for hormonal therapy; therefore, they are not recommended as first-line hormonal therapy.

Estrogens are important in the process of bone remodeling. Consequently, estrogen deficiency results in an imbalance between osteoclast and osteoblast activity and a progressive loss of trabecular bone. Osteoporosis can be diagnosed by the assessment of bone density, with a reasonable ability to predict fracture risk. Decreased bone density can be noted within 6 months of the start of severe ovarian suppression by gonadotrophin-releasing hormone agonists given for steroid-dependent disease. Increased bone fragility leads to a 50% lifetime risk of fracture and a 15% risk of hip fracture—a major cause of disability and death—in white women with a normal age of menopause. There are no reports that suggest the chance of

fracture is increased with POF; however, because these women are at increased risk for osteoporosis, they should also be advised regarding calcium intake (1200–1500 mg/dl), daily weight bearing exercise, and the need to take daily multivitamin to avoid vitamin D deficiency.

Data have documented androgen deficiency in patients affected by POF, but whether exogenous androgen is indicative is uncertain. The benefits and risks of androgen therapy have not been established and data are controversial and androgen replacement therapy has only been studied in the context of surgical menopause. On the other hand, presumably androgen replacement would be more beneficial for young women with very low levels of circulating androgen than for postmenopausal women with relative androgen deficiency. In the absence of evidence, it seems reasonable to give a trial of testosterone, in the form of patch or gel, if low libido or lack of energy persists despite adequate estrogen replacement [14].

A 5–10 % background pregnancy rate is seen in spontaneous POF. It is important to counsel patients about the small chance of spontaneous conception, and the need to use contraception if pregnancy is not desired. The COC (combined oral contraceptive) may be a good choice for these women because HRT (hormone replacement therapy) is not contraceptive. It is not possible to predict which patients will conceive, although positive prognostic factors include short duration of amenorrhea, autoimmune etiology, and ovarian activity seen on ultrasound scan. Ultrasound monitoring might benefit conception rates by improving timing of intercourse; however, monitoring has not been examined in a controlled manner. In our own experience, monitoring ovarian activity can help patients accept their diagnosis and move ahead with alternate family planning options if no follicle activity is observed or pregnancy achieved after an agreed upon timeframe [15]. Pregnancy can occur in young women despite very low AMH. Sadly, no intervention has been found to improve conception rates: ovarian stimulation, FSH suppression, and the use of steroids in autoimmune POF are all ineffective. The only treatment option is in vitro fertilization with donor oocytes. Prior to embryo transfer, the endometrium is thickened using high-dose HRT. Treatment has a high success rate (which can reach 50 % per cycle) dependent on the age of the donor. The psychological and ethical implications must be carefully considered, and counseling is mandatory for both the recipient and donor. Fertility-preservation options can be suggested to some patients with cancer and those at risk of early menopause, such as those with familial cases of POF. Early diagnosis of familial POF should provide the opportunity to predict the likelihood of early menopause, and allow other reproductive choices to be made, such as freezing embryos or having children earlier [16] (Table 12.3).

Menopause condition adversely affects cognitive function, and surgical menopause has been associated with distinct impairment of cognitive function and memory. In 100 women diagnosed with POF, substantial changes in social anxiety, depression, and self-esteem have been described. Increased risks of minor cognitive impairment or dementia, depressive and anxiety symptoms, parkinsonism, and increased mortality from neurological and mental diseases have been noted in a cohort of over 2000 women who had had unilateral or bilateral oophorectomy, in

**Table 12.3** Diagnosis, initial evaluation, and management of Premature Ovarian Failure (POF)

<i>Definition</i>
At least 3–4 months of amenorrhea in association with menopausal levels of serum FSH concentration on two occasions
<i>Initial assessment and investigations</i>
Good history, including family history
<i>Tests</i>
Serum FSH, LH, prolactin, TSH, estradiol; if FSH in menopausal range, repeat. AMH? Inhibin B?
<i>Further investigations</i>
Chromosomal and genetic studies: karyotype, FMR1 gene mutation if family history of POF, fragile X syndrome or mental retardation
Autoantibodies: autoimmune screen for polyendocrinopathy, thyroid antibodies, anti-adrenal antibodies? ovarian antibodies
DEXA: estimation of bone mineral density
Pelvic ultrasonography
<i>Management</i>
Provide counseling, information and emotional support
Provide HRT/COC up to age 50 years and then yearly review as required
Provide calcium and vitamin supplements and encourage weight-bearing exercise
Provide contraception if required and advice on fertility issues
<i>FSH</i> follicle-stimulating hormone, <i>LH</i> luteinizing hormone, <i>TSH</i> thyroid-stimulating hormone, <i>AMH</i> anti-Mullerian hormone, <i>FMR1</i> gene responsible for fragile X syndrome, <i>DEXA</i> dual-energy X-ray absorptiometry, <i>HRT</i> hormone replacement therapy, <i>COC</i> combined oral contraceptive

this view it is crucial to treat adolescent and young women affected by POF in order to prevent long-term risk of cognitive impairment [17].

When POF is diagnosed in the adolescent female, the patient and her family are often unprepared for such news with its implications for compromised fertility and impaired self-image and the need for long-term hormonal therapy. It is best to inform the patient and family by having a direct conversation. Adolescents may demonstrate myriad emotions ranging from apathy or denial to remorse or sadness, and these emotions may be different from those of their parents or guardians. Psychological counseling also should be offered because impaired self-esteem and emotional distress have been reported after diagnosis of primary ovarian insufficiency. Because many patients will use the Internet to learn more about their diagnoses, referral to appropriate sources for support is an efficient means to enhance patient care. A greater understanding of female reproductive biology and the physiologic effect of POF enables health care providers to offer counseling for these young women. Once POF is diagnosed, patients should be evaluated at least annually. Physicians should address the special needs of this population and counsel family members and patients on the risk of comorbidities associated with primary ovarian insufficiency and the condition's potential for genetic inheritance [18].

Concluding, as POF has cumulative negative effects over time, it is important for clinicians to make a timely diagnosis and bring appropriate strategies for symptoms

management, emotional support, and risk reduction. Therapies in patients affected by POF need to be individualized and depend on the endpoints of treatment in different moments of women's life. The approach to POF patients should be multidisciplinary and the management of these patients includes counseling and emotional support, planning fertility and a hormonal replacement strategy up to at least age of natural menopause, therefore, long-term deprivation of estrogen has serious implications for female health for bone density, cardiovascular and neurological systems, wellbeing, and sexual health in particular.

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## Abbreviations

EC	Emergency contraception
IUD	Copper bearing intrauterine device
LH	Luteinising hormone
LNG	Levonorgestrel
PK	Pharmacokinetic
POP	Progestogen only pill
PRM	Progesterone receptor modulator
UPA	Ulipristal acetate

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## 13.1 Introduction

Emergency contraception (EC) is defined as any drug or device that is used after sexual intercourse to prevent unwanted pregnancy. Abortion is defined as termination of an established pregnancy, i.e., of an embryo that has already implanted in the uterus. The World Health Organization (WHO) recently published a report on sexual health, human rights and the law, that specified that the evidence shows that for adolescents increased access to EC protects from negative health outcomes, and does not increase the risk of unwanted pregnancies, or sexually transmitted infections [1]. The WHO list of essential medicines includes EC and also includes the medical abortion drugs mifepristone and misoprostol [2].

The methods of EC that are in current use include the copper bearing intrauterine device (IUD), and the oral methods of levonorgestrel (LNG) and ulipristal acetate

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(UPA). Mifepristone is also used for oral EC, but it is only available in China, Russia, Armenia, Ukraine and Vietnam [3].

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## 13.2 Efficacy Assumptions

There has never been a randomized placebo-controlled trial of EC. The efficacy of EC in clinical trials is therefore calculated from the number of observed pregnancies compared to the number of expected pregnancies (in the absence of EC), based on the conception probability at the time in the menstrual cycle when intercourse occurred [4]. In most clinical trials, the expected pregnancy rates in the absence of EC have been low, in the region of 5%. In the clinical trials of oral LNG compared to UPA for EC, the observed pregnancy rate was 1–2% [5]. On this basis, we might conclude that oral EC prevents one half to two thirds of pregnancies that might otherwise have occurred. However, these calculations are based on a number of assumptions. Firstly, we assume that we can accurately determine the phase in the menstrual cycle when intercourse took place. In clinical studies, as many as 30% of women who stated that were sure of the date of their last menstrual period and believed that they were in the first half of the cycle when they took EC, had actually already ovulated (based upon subsequent urine or serum hormone analyses) [6, 7]. In addition, we know that many women have had more than one episode of unprotected sexual intercourse when they request EC [6]. Efficacy calculations also assume that sperm have entered the reproductive tract. However, in a study of women presenting for EC that assessed the presence of sperm on vaginal smears, there was no sperm present in one third of cases [8]. We do know however, that oral EC methods can delay or inhibit ovulation, so on this basis alone they have to be better than nothing [9].

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## 13.3 Effectiveness, Acceptability and Availability

The IUD is by far the most effective method of EC. In a systematic review of more than 40 studies involving over 7000 women, the observed pregnancy rate was one in 1000 [10]. The IUD can also be used over a wider time period than oral EC. It can be inserted up to 5 days after the earliest estimated date of ovulation. It also can be retained as an ongoing method of highly effective, long acting reversible contraception. But the IUD is not popular. In the UK, where women can access the IUD for EC at no financial cost to themselves, only 2–5% of women seeking EC choose the IUD [11, 12].

There have been two comparative randomised controlled trials (RCTs) conducted to date of UPA versus LNG, with over 3400 women treated [5, 13]. In the meta-analysis of these trials, use of UPA was associated with almost halving of the risk of pregnancy for women treated within 120 h of unprotected sex. For women who were treated within 24 h of intercourse, the difference was more marked, with women receiving UPA having approximately one third the risk of pregnancy, compared to women receiving LNG [5]. Both LNG and UPA are well tolerated and

associated with a similar side effect profile [5]. The European Medicines Authority (EMA) recently reviewed the safety and efficacy data pertaining to UPA and recommended that UPA should be available throughout Europe without the need for a prescription [14]. The EMA's decision on UPA also resulted in LNG becoming available without prescription in many of those countries (e.g., Italy, Germany), where LNG had formerly required a prescription.

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### 13.4 Mechanism of Action and Effect on Pregnancy

The IUD has both pre- and post-fertilization effects. Copper ions are toxic to sperm and oocytes. The IUD also induces a local inflammatory reaction in the endometrium that may prevent implantation [15]. It should not be inserted for EC beyond 5 days after the earliest date of ovulation as this could potentially disrupt an early pregnancy. Generally, the IUD should be removed if possible, in women who become pregnant with an IUD in situ, given the higher risk of adverse outcome such as septic abortion [16].

It is generally accepted that both LNG and UPA exert their contraceptive effect as EC through their ability to delay ovulation by up to 5 days (presumed lifespan of sperm in the reproductive tract). However, once the Luteinising Hormone (LH) surge (ovulatory trigger) has started, then LNG is no longer able to delay ovulation, although UPA still remains able to delay ovulation until the LH peak [9]. There is good evidence that LNG does not prevent implantation and is not abortifacient. Bio medical studies have demonstrated that the EC dose of LNG when given after ovulation, does not affect the development of a secretory endometrium at either histological level, molecular level nor at electron microscopic level [17]. Furthermore, an *in vitro* model of blastocyst implantation showed that the addition of LNG was unable to prevent implantation [18]. LNG use is not associated with a higher risk of miscarriage, ectopic or pregnancy complications and there is no adverse effect on children conceived after exposure to LNG for EC [19, 20].

There is also evidence that the EC dose of UPA does not exert significant effects on the endometrium [21]. Although immediate post-ovulatory administration of the EC dose of UPA has been associated with a reduction in endometrial thickness, the thickness remains within the normal range and histological assessment showed that secretory development was unaffected [21]. Furthermore, serum progesterone levels remained in the normal range for the luteal phase, suggesting that there would be sufficient luteal support for an implanting embryo. *In vitro* studies have also shown that UPA does not prevent implantation of blastocysts [22]. Safety data regarding the effects of UPA on pregnancy are inevitably limited since use of UPA for EC has been limited. Also, pregnancy rates after use are low, most women chose to terminate an unintended pregnancy after UPA, and the outcome of pregnancies that continue to birth are unlikely to be reported unless there is a complication. However, the available data from over one million women who used UPA in the clinical trials and post marketing surveillance have been consistent with no increased risk of miscarriage or ectopic or congenital abnormality in babies after exposure to UPA [23].

**Table 13.1** Risk factors for failure of EC (LNG or UPA) from Glasier et al. [24]

	Odds ratio [95 % CI]	<i>P</i> value
Cycle day of intercourse <sup>a</sup>	4.4 [2.3–8.2]	<i>P</i> <0.0001
Further UPI <sup>a</sup>	4.6 [2.2–9.0]	<i>P</i> <0.0002
BMI Obese vs. normal	3.6 [1.96–6.53]	<i>P</i> <0.0001

<sup>a</sup>No treatment group effect

**Table 13.2** Risk of pregnancy in women with obese BMI vs. normal BMI and EC treatment (LNG or UPA) from Glasier et al. [24]

BMI group	LNG OR (95 % CI)	UPA OR (95 % CI)
Obese vs. normal	4.41 (2.05–9.44) <sup>a</sup>	2.62 (0.89–7.00)

<sup>a</sup>*P*=0.0002

### 13.5 Factors Predictive of Failure of EC

In a secondary analysis of the meta-analysis of the RCTs of UPA and LNG, three factors were significant for failure of EC: (i) cycle day that intercourse occurred, (ii) further episodes of sex in the same cycle after EC and (iii) obesity [24] (Table 13.1).

Cycle day when sex occurs and further sex after EC have previously been established as risk factors [25], however, obesity (Body mass index  $\geq 30$  kg/m<sup>2</sup>) as a risk for failure was a new finding.

Further statistical analysis of this dataset showed that there was a treatment effect with women with a BMI  $\geq 30$  kg/m<sup>2</sup> having an almost fourfold increased risk of EC failure compared to women of normal BMI receiving LNG [24]. In contrast, women with a BMI  $\geq 30$  kg/m<sup>2</sup> who received UPA had a doubling risk of pregnancy compared to women of normal BMI receiving UPA compared to women of normal BMI receiving LNG (Table 13.2).

Additional analysis showed that the limit of efficacy (when the observed pregnancy rate is no less than the expected rate in the absence of EC) was reached with LNG at a BMI of 26 kg/m<sup>2</sup>, yet this limit of efficacy was not reached with UPA until a BMI of 35 kg/m<sup>2</sup>. Similar analyses were conducted for weight alone, and showed that the limit of efficacy with LNG and UPA was reached at weights of 70 and 88 kg respectively. However, the findings came from secondary analysis of studies that were not designed to address the issue of BMI and efficacy of EC. In addition, as for most clinical trials that have strict inclusion/exclusion criteria, the numbers of women with BMI  $\geq 30$  kg/m<sup>2</sup> were low as were the number of pregnancies in this group. Also, in a substantial proportion of all participants, the height and weight had been self-reported, which introduces inaccuracies in BMI determination [5, 13].

Previous studies of LNG for EC conducted by the WHO had failed to show an impact of BMI upon pregnancy rate [26]. The EMA reviewed the data from the clinical trials, together with data from unpublished studies of LNG for EC and

concluded that the data were not sufficiently robust to conclude that obesity was associated with a decrease in efficacy of EC, and recommended that women with obesity could continue to use either UPA or LNG [27]. Nevertheless, the uncertainty remains. Also, there is some pharmacokinetic (PK) data that might some support the notion that efficacy of LNG for EC may be adversely impacted upon by obesity. Specifically a PK study of low dose combined contraceptive pill containing LNG showed that in women with obesity, LNG takes a longer time to achieve steady state levels than in women of normal BMI [28]. Whilst it is very unlikely that there will be a definitive RCT of women with/without obesity with efficacy of EC as an end-point, ongoing studies examining the PK of UPA in women with/without obesity will provide useful information to inform this debate.

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### 13.6 Can EC Prevent Abortion?

The evidence from both population studies and clinical trials of advance provision of EC (supply to keep at home in case of need) would indicate that use of EC does not reduce unintended pregnancy rates or abortion rates at population level. Indeed, a Cochrane review of six RCTs of over 6300 women (with one or more supplies of EC at home) compared to standard access, failed to demonstrate any reduction in pregnancy rates at 3, 6 or 12 months [29].

In Norway, availability of LNG for EC from the pharmacy without prescription was associated with 30-fold increase in sales over 10 years, but the abortion rate remained unchanged over this time [30]. Similarly, in Great Britain, abortion rates continued to increase up to 2008, some 7 years after availability of LNG for EC from the pharmacy [31]. However, abortion rates are influenced by many factors and demographic changes in society with earlier age of coitarche, later age at first birth, relationship break ups and divorce may mean that women now spend a greater proportion of their reproductive lives at risk of unintended pregnancy. Also, we know from the clinical trials of advance provision of EC that women do not use EC for every episode of unprotected sex [29].

Studies conducted in UK, France and Denmark amongst women requesting abortion have shown that 12% of women have used EC in the conception cycle to try to prevent the pregnancy [32–34]. Furthermore, a survey of women requesting abortion in the UK showed that although availability of LNG EC at ‘no cost’ from the pharmacy did not affect this percentage, in spite of most respondents being aware that EC was free at the pharmacy [32].

Research has also shown that women often fail to recognize that they are at risk of pregnancy, neglect the risk or are deterred from using EC due to stigma, or misconceptions about the method [33, 34]. It has also been suggested that the increase in EC use may be amongst women who are least at risk of an unintended pregnancy, but are anxious about that risk [30]. In addition, we do know that many women may have more episode(s) of sex in the same cycle after using EC, and that further sex increases the risk of pregnancy [25].

### 13.7 Establishing Effective Contraception After EC

Given the higher risk of pregnancy if further sex occurs in the same cycle after EC, it is important that women start an effective method of ongoing contraception as soon as possible. The term ‘quick start’ is used to denote starting a method of contraception at any point in the menstrual cycle, without waiting for the next menses. If women attend a family planning (FP) service for EC, then they can be provided with ongoing contraception at this same visit. A study from the UK suggested that 23% of women attending a FP service for EC chose to start effective contraception, usually the combined oral contraceptive pill [11]. Of course, women are increasingly choosing to access EC from the pharmacy (in countries where this is available without prescription), and most pharmacists cannot provide hormonal contraception without a prescription. A pilot study from the UK examined two pharmacy-based interventions designed to facilitate access to effective contraception after EC [35]. It randomised 12 pharmacies to provide women with either (i) standard care, i.e., pharmacist provides EC as usual (LNG), or (ii) pharmacist supplied a 1 month supply of the progestogen only pill (POP) with EC – so that women could start the POP immediately and have up to 1 month to arrange an appointment with their usual care provider for effective ongoing contraception or (iii) women had a rapid access pathway to the local FP for contraception upon presentation of their empty EC box. Women in this study consented to be contacted by the researcher 8 weeks later to determine what method of contraception they were using. Although, it was a small study with just under 60 participants in each of the arms of the study, there was a significantly higher proportion of women in the POP (52%) and rapid access arm (52%) using effective contraception than in the standard care arm alone (16%). This suggests that provision of a POP or rapid access to a FP could prove to be an effective measure to increase the uptake of effective contraception after EC from the pharmacy. In addition, the POP is a very safe method of contraception and could be provided more easily than a combined oral contraceptive pill. Larger robust studies in this area are therefore needed.

### 13.8 Contraception After Ulipristal Acetate for Contraception

UPA is a progesterone receptor modulator (PRM), and PRMs may interfere with the action of progestogen containing contraception. There is concern therefore that UPA may affect the efficacy of UPA or vice versa. Guidelines from the Faculty of Sexual and Reproductive Healthcare UK advise that women can commence hormonal contraception after UPA, but should rely on additional barrier contraception or abstain for 14 days if the method is a COC [36]. This is based upon an estimated 7 days to clear UPA (based upon its half life of 32 h) plus a further ‘theoretical’ 7 days to induce ovarian quiescence with a COC.

A recent placebo-controlled RCT that examined the effects of quick starting a COC after UPA in healthy volunteers with a follicle of at least 13 mm in size (equivalent to mid follicular phase), showed that there was no difference between UPA and placebo in either the proportion of women who achieved ovarian quiescence on

**Table 13.3** Strategies to improve effectiveness of EC

Increase use of IUD for EC
No further sex in same cycle after oral EC
Commence effective ongoing contraception
Develop a more effective oral EC

a COC nor the number of days to achieve quiescence [37]. This study design was such that it could not determine whether the COC might impact upon the ability of UPA to delay ovulation. A further RCT study has examined the effects of starting a progestogen only pill (POP) containing desogestrel, the day after the UPA or placebo [38]. This study demonstrated that although UPA did not affect the contraceptive actions of this POP (ovarian suppression and cervical mucus), that the POP did affect the ability of UPA to delay ovulation [38]. In view of this, guidelines advise that women should not start a hormonal method of contraception for at least five days after using UPA for EC [39].

### 13.9 A More Effective Oral EC

Prostaglandins play a critical role at the time of follicle rupture and the cyclo-oxygenase-2 inhibitor meloxicam inhibits prostaglandin synthesis. A pilot RCT comparing the effect of the EC dose of LNG plus meloxicam versus LNG and placebo on follicle rupture when administered to healthy female volunteers in the pre-ovulatory phase of the cycle demonstrated that there was no ovulation at 5 days (i.e., lifespan of spermatozoa in the female reproductive tract) in 39% of participants receiving LNG and meloxicam vs. 16% of those receiving LNG and placebo [40]. This suggests that perhaps a combination of meloxicam and LNG might increase the efficacy of LNG for EC. It is also possible that meloxicam combined with UPA might increase the efficacy of UPA further. Large robust trials are now required.

A Cochrane review demonstrated that mid doses of mifepristone (25–50 mg) are more effective than LNG [25]. Studies have also shown that higher doses of mifepristone (200 mg) have profound effects on endometrial secretory development that may prevent implantation [41, 42]. Further research into the development of more effective oral ECs is therefore warranted.

#### Conclusion

So what strategies can we adopt in order to maximize the chances that EC can prevent unintended pregnancy? (Table 13.3). Given the fact that the IUD is the most effective method of EC it seems only logical to promote increased use of the IUD for EC. With increasing proportions of women choosing to access EC from the pharmacy, developing close links between IUD providers (FP clinics/Gynaecology) and pharmacies will therefore be required.

However, if an IUD for EC is not acceptable to a woman or available, then UPA is more effective than LNG. In addition, there should be no further episodes

of unprotected sex after oral EC and women should establish an effective ongoing method of contraception as soon as possible.

More research is required to explore the efficacy of the approach of providing a temporary supply of the POP with oral EC from the pharmacy.

We also need more research into the possible impact of obesity on efficacy of oral EC and we need to have a more effective oral EC; that also prevents implantation.

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Matan Elami-Suzin and Joseph G. Schenker

## Key Points

- There is much difference in the incidence of adolescent pregnancy between various nations, but in recent years, the overall incidence has decreased.
- Adolescent pregnancy is a medical and social problem.
- Adolescents vary in their ability to implement various types of contraception.
- Sexually active adolescents are more likely to seek contraception if they perceive pregnancy as a negative outcome, have long-term educational goals, are older, experience a pregnancy scare or actual pregnancy, or have family, friends, and/or a clinician who encourage the use of contraception.
- Barriers that may impede adolescent access to contraceptive services include lack of access to confidential services and concerns about side effects such as weight gain, loss of bone density, and increased risk of thromboembolism or pelvic inflammatory disease.
- Before initiating contraception, it is important to review the adolescent's history for absolute or relative contraindications, discuss the risks and benefits, and obtain consent.
- Contraception counseling should include education about the anticipated adverse effects, the need to use condoms to protect against sexually transmitted infections, the availability of and indications for emergency contraception, and strategies to increase adherence.

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## 14.1 Adolescent Pregnancy [1–3]

Adolescent pregnancy is defined as a pregnancy in an adolescent girl, usually within the ages of 13–19.

About 16 million girls aged 15–19 and some one million girls under 15 give birth every year. Ninety-five per cent of these births occur in low- and middle-income countries. The proportion of births that take place during adolescence is about 2% in China, 18% in Latin America and the Caribbean and more than 50% in sub-Saharan Africa. In low- and middle-income countries, almost 10% of girls become mothers by age 16 years, with the highest rates in sub-Saharan Africa and south-central and south-eastern Asia.

In South Asia, the Middle East and North Africa, age at marriage has traditionally been low in kinship-based societies. In such cases, most girls married soon after menarche, fertility is high, and consequently many children were born from adolescent mothers.

In developing countries, declining age at menarche and increased schooling have prolonged the period of adolescence. Together with a growing independence from parents and families, this has led to premarital sexual relations and increasing numbers of adolescent pregnancies.

Children age 12–14 years old are more likely than other adolescents to have unplanned sexual intercourse.

The risk factors for adolescent pregnancy are: school drop-out, absence of future plans, low self-esteem, alcohol and drug abuse, lack of knowledge as to sexuality, and inappropriate use of contraceptive methods.

In sub-Saharan Africa, South Asia and Latin America, unsafe abortion is a frequent phenomenon and a cause of illness and even death, especially among young girls. In the USA, Europe and sub-Saharan Africa, high frequencies of physical abuse are reported in pregnant adolescents. In a number of African countries, female genital mutilation in young girls is a traditional practice, with possible consequences for the course of labor.

### 14.1.1 Complications During Pregnancy

Complications during pregnancy and childbirth are the second cause of death for 15–19-year-old girls globally.

In Latin America, the risk of maternal death is four times higher among adolescents younger than 16 years than among women in their twenties. Although some of this risk can be attributed to factors other than young age – e.g., giving birth for the first time, lack of access to care, or socioeconomic status – there appears to be an independent effect of young maternal age on pregnancy risk to the mother. Prenatal care tends to be inadequate among adolescent mothers which shows the importance of prenatal visits to decrease complications of pregnancy in this age group.

Young adolescents may still grow during pregnancy. Comparison of measurements of maternal height in adolescents and older mothers indicate that the mean

height of the adolescents was significantly smaller than the height of older pregnant women.

Anemia frequently occurs in pregnant women. In a number of studies, anemia appears to be higher in pregnant adolescents than in older pregnant women. The cause is often nutritional deficiency and this can be treated adequately during antenatal care, if such care is available.

Infectious diseases like malaria, hookworm and HIV infection play an important role. In developing countries, malaria parasitemia occurs frequently during pregnancy, especially in primigravida. Adolescents are at increased risk of contracting HIV infection. Modern antiretroviral combination therapy during pregnancy considerably reduces mother-to-child transmission.

Reviewing the literature, there is no evidence that the incidence of hypertensive disease in adolescent pregnancies is higher than the incidence in adult women of the same parity; and there is also no indication of a difference between countries.

Rates of ectopic pregnancy, pre-eclampsia, eclampsia, preterm labor, premature rupture of membrane and cesarean section were significantly higher among adolescents <15 years of age; the risk then decreased steadily with age and became comparable with the control group after 16 years of age.

**Labor** As a result of insufficient prenatal care, the global incidence of premature births and low birth weight is higher among teenage mothers. Risks for medical complications are greater for girls 14 years of age and younger. An underdeveloped pelvis can lead to difficulties in childbirth. Young women under 20 face a higher risk of obstructed labor, which if cesarean section is not available can cause an obstetric fistula, a tear in the birth canal that creates leakage of urine and/or feces. At least two million of the world's poorest women live with fistulas. Higher incidence of postpartum hemorrhage and mental disorders, such as depression, were observed in adolescent pregnancy.

### 14.1.2 Adolescent Pregnancy—Effect on Newborn

Stillbirths and death in the first week of life are 50% higher among newborn to mothers younger than 20 years than among babies born to mothers 20–29 years old. Deaths during the first month of life are 50–100% more frequent if the mother is an adolescent versus older, and the younger the mother, the higher the risk.

The rates of preterm birth, low birth weight and asphyxia are higher among the children of adolescents, all of which increase the chance of death and of future health problems for the baby. Approximately 14% of infants born to adolescents 17 years or younger are preterm versus 6% for women 25–29 years of age. Young adolescent mothers (14 years and younger) are more likely than other age groups to give birth to underweight infants, and this is more pronounced in black adolescents.

Pregnant adolescents are more likely to smoke and use alcohol than are older women, which can cause many problems for the child after birth.

### 14.1.3 Social Aspects

Teenage mothers are at risk for long-term problems in many major areas of life, including school failure, poverty, and physical or mental illness. Teenage pregnancy is usually a crisis for the pregnant girl and her family. Common reactions include anger, guilt, and denial. If the father is young and involved, similar reactions can occur in his family.

Pregnant teenagers require special understanding, medical care, and education, particularly about nutrition, infections, substance abuse, and complications of pregnancy. Pregnant teens can have many different emotional reactions: some may not want their babies, others may view the creation of a child as an achievement and not recognize the serious responsibilities. Depression is common among pregnant teens, some teenage become overwhelmed by guilt, anxiety, and fears about the future.

Rates of ectopic pregnancy, pre-eclampsia, eclampsia, preterm labor, premature rupture of membrane and cesarean section were significantly higher among adolescents <15 years of age; the risk then decreased steadily with age and became comparable with the control group after 16 years of age.

Rates of adolescent childbearing have dropped significantly in most countries and regions in the past two decade.

Teenage girls account for 14 % of the estimated 20 million unsafe abortions performed each year, which result in some 68,000 deaths.

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## 14.2 Adolescent Contraception

As a means of preventing teenage pregnancy and reducing the amount of induced abortions, adolescents may be offered a variety of contraceptive methods. Choosing a contraceptive method in adolescents may not be an easy task. The features of an ideal contraceptive method would be that it is: cheap; highly effective; have no side effects; independent of intercourse; does not require a regular action on the part of the user; is acceptable to all cultures and religions, and; is easily distributed and administered by non-healthcare personnel.

There is no one method that fits all of these criteria, and one must choose from a variety of available methods – each with its own advantages and disadvantages.

Adolescents may vary in their ability to initiate, and continue using, various types of contraception. Thus, prescribing “The Pill” for an adolescent patient – a contraceptive method that requires planning and forethought to prevent possible pregnancy – may not necessarily be the best option for them.

The intention of the present chapter is to demonstrate on ways of initiating the use of contraceptives by adolescents, overcoming barriers and solving problems regarding its use.

The female adolescent is more likely to seek contraception if she [4] perceives pregnancy as a negative outcome, is older, has future educational goals, is afraid of becoming pregnant, and has family, friends or physician who encourage the use of contraception.

### 14.3 Barriers for Contraception Use

As clinicians treating adolescents, we should strive to identify and overcome various barriers that prevent use of contraceptives. Female teens perceive cost, lack of information, and lack of access to confidential healthcare services, as significant barriers. Other barriers include concerns regarding potential pelvic examination and fear from side effects.

A prospective cohort study, which included 1404 urban female teens, has shown that removal of major barriers appears to be associated with use of more effective methods of contraception and decreased rates of pregnancy [5]. About 50% of participants had experienced a past pregnancy; minors had parental consent to participate. Participants were educated about reversible contraception, with an emphasis on the benefits of long-acting reversible contraception. They were provided with their choice of reversible contraception at no cost, and followed for 2–3 years. Nearly 75% chose an intrauterine device (IUD) or implant. Younger adolescents tended to choose the implant and older ones the IUD. Compared with sexually active United States teenagers in 2008, study participants had lower rates of pregnancy (34.0 versus 158.5 per 1000), birth (19.4 versus 94.0 per 1000), and abortion (9.7 versus 41.5 per 1000).

**Confidentiality** The adolescent's need for privacy should be recognized and respected. Health providers are required to develop and maintain a stable relationship with the adolescent, independently from the relationship with her parents. She should be given the chance to ask questions and tell her concerns freely, and to obtain gynecologic and sexual information directly from the clinician. The adolescent's age is a factor in whether she will choose an option of total confidentiality. A young teenager may ask her mother to stay with her through the entire office visit, especially if her mother is aware of her sexual behavior and is the one who desires the teenager to use a contraceptive method. Nevertheless, encouraging some independent time with the adolescent is important to obtain a more detailed history about other possible high-risk behaviors.

**Pelvic Examination** Many teenagers are afraid to consult healthcare providers, fearing that pelvic examination would have to be carried in order for them to receive contraception [6]. The physical exam of a female adolescent who seek contraception should perform general physical examination, including inspection of the external genitalia, but internal pelvic examination is only needed for insertion of an intrauterine device [7]. No specific findings on pelvic examination would be a contraindication for the initiation or restitution of oral contraceptives (OCs), contraceptive patch, vaginal ring, and progesterone implant or progesterone injections. Speculum and bimanual examination may be necessary to test for sexually transmitted infections (STI) if urine or vaginal swab tests are not available.

**Legal Issues** A minor's right to access contraceptive services in a health care setting without parental involvement varies among different countries, and the clinicians treating adolescents should be familiar with local law, protocols, and regulations.

**Concerns Regarding Side Effects** Adolescents have specific fears and concerns regarding use of contraception.

- **Weight Gain** – Many teenagers fear of gaining weight with the use of hormonal contraception. However, they should be reassured that available evidence does not support this regarding OCs. In the case of depot medroxyprogesterone acetate (DMPA), weight gain might be a concomitant event, but no causal relationship has been established. Factors other than DMPA may contribute to weight gain.
- **Bone Density** – Ultra-low dose estrogen (20 mcg) OCs and DMPA might interfere with achieving peak bone mass in teenagers and young adults. Rare cases of osteoporosis, including fractures, have been reported in patients taking DMPA, and the prescribing information for DMPA includes a boxed warning that DMPA should be used as a long-term birth control method (e.g., longer than 2 years) only if other birth control methods are inadequate [8]. It is vital to share information with the adolescent regarding the potential effects of DMPA on bone density. There is a general convention that DMPA should be avoided early puberty, but there is lack of evidence to limit its use in older adolescents. In fact, some experts argue that for adolescents in whom DMPA is the only acceptable contraceptive option, the benefit of pregnancy prevention outweighs the potential risk to bone health.
- The Society for Adolescent Health and Medicine has published a position paper regarding the boxed warning for DMPA [9]. This paper suggest that:
  - With adequate explanation of benefits and potential risks, *DMPA may continue to be prescribed to adolescent girls* who need contraception.
  - Decisions regarding bone density monitoring of adolescents using DMPA for contraception should be individualized; the decisions should be made by the clinician in concert with the adolescent, and potentially the adolescent's guardian.
  - *Duration of use of DMPA need not be restricted to 2 years.*
  - *Adolescents using DMPA should be encouraged to take 1300 mg of elemental calcium and 400 international units vitamin D and to exercise each day.*
  - *Estrogen supplementation should be considered* for girls who are doing well on DMPA and have osteopenia or are at risk for osteopenia and who have no contraindications to estrogen.
- **Thromboembolism** – Probably one of the most feared negative consequence of combined estrogen-progestin hormonal contraception is venous thromboembolism (VTE). Use of combined OCs increases VTE risk by two to fourfold compared to non-users. *However, this should be put readily in perspective, because the absolute risk is still very low (increases from 2–3:10,000 non-users to 8–12:10,000 current users) and much lower than the VTE risk during pregnancy and postpartum period (60–80:10,000).* It is, indeed, safer to prevent an unwanted pregnancy than terminating or continuing carrying it !

Although reducing the estrogen dose had improved the safety and side-effects profile of OCs, it did not eliminate completely the increased risk of VTE [10–12]. There are several factors which influence the risk:

- Progestin type: Several studies and meta-analyses have reported a greater VTE risk with some of the third-generation OCs (desogestrel and gestodene, but not norgestimate) when compared with levonorgestrel, a second-generation progestin. In addition, two anti-androgenic progestins (drospirenone and cyproterone acetate) have been associated with a similar increase in VTE risk. However, the absolute excess risk is small, and is considered medically acceptable given the many benefits of combined hormonal contraceptives.

In 2011, the European Medicines Agency also concluded that drospirenone-containing birth control pills carry a higher risk of VTE, but noted the overall risk of blood clot from any birth control method remains small and stopped short of advising women to stop taking pills containing drospirenone [13].

Acquired resistance to activated protein C has been suggested as one mechanism by which the third-generation OCs could predispose to VTE [14, 15]. In addition, third-generation and second-generation progestins have different effects on the anticoagulant pathway [16, 17]. It has been suggested that second-generation progestins, such as levonorgestrel, are more effective than third-generation progestins, such as desogestrel, in counteracting the thrombotic effects of estrogen [18].

- Obesity – Among OC users in two case-control studies, obese women had a 2 [19] to 24-fold [20] higher risk of VTE than nonobese women. In a third case-control study of 454 consecutive patients with a first episode of objectively diagnosed deep vein thrombosis (DVT), the risk of thrombosis among those with both obesity and OC use was increased tenfold compared with women with neither risk factor; the increase in risk was lower (2.3–4.6-fold) with either risk factor alone [21].
- Age – In a pooled analysis of two case-control studies, the risk of VTE rose sharply after age 39 years among women taking estrogen-progestin OCs. The estimated incidence in women over age 39 years, when compared with adolescents, was 100 versus 25 cases of VTE per 100,000 person-years, respectively [22]. *According to this analysis, the risk of VTE in adolescents who use OCs is actually the same as for non-users.*

*Transdermal contraceptive patch* – Data are conflicting on whether the risk of VTE is higher in users of the transdermal contraceptive patch (Ortho Evra) than users of OCs.

*Oral and injectable progestin-only preparations for contraception do not appear to be associated with an increased risk of thromboembolism.*

In order to prevent thromboembolism, it is important to take a comprehensive and thorough history from the adolescent regarding personal and family history of VTE, for risk-stratification purposes.

- Pelvic inflammatory disease (PID) – The relationship between oral contraceptives (OCs) and pelvic inflammatory disease (PID) is complex. Whereas some studies indicate that the use of OCs increases the risk of PID, others suggest that OCs increase the risk for cervicitis and endometritis, but not salpingitis, thereby decreasing the severity of PID [23–26]. No association was found between DMPA and PID [27].

- *IUD itself does not elevate PID risk of Pelvic inflammatory disease!*  
However, the use of an IUD may increase the risk of STIs, which, in turn, increases the risk of PID [28].
- Birth defects and infertility – While many teenagers are worried about these future consequences, there is no solid evidence to support these associations [29, 30]. It is extremely important to actively refute such common misconceptions.

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## 14.4 Emergency Contraception (EC)

Emergency contraception (also known as postcoital contraception and the morning-after pill) refers to the use of drugs or a device as an emergency measure to prevent pregnancy. As adolescents are particularly prone to have unprotected intercourse, they are potential candidates for EC. It is intended for occasional or back-up use, not as a primary contraceptive method for routine use.

Health professionals should inform all adolescents (females and males) about use of emergency contraception and the available options. This discussion should include the advantages and disadvantages of each method, including efficacy and side effects.

In most developed countries today, some form of orally given EC is available as over-the-counter drug. Health professionals should inform all adolescents (females and males) about use of emergency contraception and the available options.

### 14.4.1 Mechanism of Action of EC

It is very important to stress that EC is not an abortifacient (i.e., does not act by interrupting an ongoing pregnancy). Direct laboratory evidence overwhelmingly supports the hypothesis that oral emergency contraceptives work primarily by delaying ovulation [31, 32]. Copper intrauterine contraception inhibits fertilization by affecting sperm viability and function [33], and of course provide long-term contraception afterwards.

Both oral and intrauterine EC are *only effective before a pregnancy has implanted*.

### 14.4.2 Available EC Options

Various EC and their reported efficacies are summarized in Table 14.1 [34].

Drugs that induce liver enzymes have the potential to decrease the contraceptive efficacy of levonorgestrel and ulipristal. A guideline from the Royal College of Obstetricians and Gynecologists recommends that women on liver enzyme-inducing drugs (e.g., many anti-epileptic and anti-retroviral drugs) or who have stopped using them within 28 days and require emergency contraception be offered a copper IUD, as its efficacy is not affected by these drugs [35]. For adolescents who are ineligible or who do not want to use an IUD, they suggest a single 3 mg dose of levonorgestrel, although there is no evidence to support this approach.

**Table 14.1** Options for emergency contraception with reported efficacies

Method	Dose	Timing of use after unprotected intercourse (better to use as soon as possible)	Reported efficacy
Levonorgestrel	0.75 mg given twice, 12 h apart <i>or</i> 1.5 mg given as a single dose	Up to 3 days (72 h)	59–94 % of pregnancies prevented
Estrogen plus progesterone (Yuzpe regimen)	100–120 micrograms ethinyl estradiol plus 500–600 mcg levonorgestrel in each dose, given twice, 12 h apart	Up to 5 days (120 h)	47–89 % of pregnancies prevented
Mifepristone	Single 600 mg dose	Up to 5 days (120 h)	99–100 %
Copper intrauterine device	Inserted within 120 h after intercourse	Up to 5 days (120 h)	At least 99 %
Ulipristal	Single oral dose of 30 mg	Up to 5 days (120 h)	98–99 %

Experts recommend that ulipristal acetate be avoided in women using enzyme-inducing drugs or who have taken them within the last 28 days. Patients should also be advised to avoid ulipristal if they are currently taking drugs that increase gastric pH (e.g., antacids, histamine H<sub>2</sub> antagonists, and proton pump inhibitors).

### 14.4.3 Contraindications

Although the Centers for Disease Control and Prevention (CDC) and the World Health Organization's (WHO) Medical Eligibility Criteria for Contraceptive Use applies contraindications to daily use of hormonal contraceptives in some women based on their medical history, these contraindications do *not* apply to adolescents and women seeking emergency contraception. In particular, cardiovascular disease, thrombophilic disorders, migraine, liver disease, and breastfeeding are considered conditions where the advantages of using the method generally outweigh the theoretical or proven risks [36].

The medical eligibility guidelines do not include information about ulipristal. Contraindications to its use are available in the package insert and include suspected pregnancy, poorly controlled asthma, and hepatic dysfunction.

Contraindications to copper IUD use include severe uterine distortion, active pelvic infection, copper allergy, and suspected pregnancy.

### 14.4.4 Efficacy of EC in Overweight and Obese Adolescents

Some data suggest levonorgestrel and ulipristal emergency contraception may be less effective in overweight or obese women. A 2015 study reported that levonorgestrel emergency contraception was less effective at preventing pregnancy as weight and

body mass index increased [37]. The women in the 80 kg group had a pregnancy risk of 6%, which was similar to the pregnancy risk as if no contraceptive had been used. At least one pharmaceutical package insert for levonorgestrel emergency contraception warns of reduced contraceptive efficacy in women weighing  $\geq 75$  kg. However, the European Medicines Association concluded that the available data were too limited and not robust enough to be certain the contraceptive efficacy of levonorgestrel or ulipristal emergency contraception is reduced with increased bodyweight and that the benefits of taking these medications outweigh any risks [38].

We counsel overweight and obese adolescents of potentially reduced or absent efficacy of levonorgestrel emergency contraception as BMI increases above  $30 \text{ kg/m}^2$  or at weight  $\geq 75$  kg, and offer them a copper-releasing IUD as first-line therapy to prevent pregnancy. There is no evidence of impaired contraceptive efficacy for obese women who rely on the copper IUD for contraception. If the IUD is not an option, ulipristal is more likely to be effective than levonorgestrel.

#### 14.4.5 Resuming or Initiating Hormonal Contraception

The duration of effectiveness of emergency contraception has not been determined. Thus it is not clear when regular contraception should be resumed or initiated within the menstrual cycle of emergency contraceptive use [39]. The safest approach is to advise adolescents using emergency contraception pills that a risk of pregnancy still exists if they have unprotected sexual intercourse after the emergency contraceptive pills have been taken; therefore, they should immediately initiate an effective contraceptive method.

For those taking levonorgestrel or combined estrogen-progestin emergency contraception, any regular contraceptive method can be started immediately after the use of the emergency contraceptive [36]. Backup methods of contraception (e.g., condom or diaphragm) or abstinence are required during the first 7 days of use. Although efficacy is not compromised by immediate initiation, it is prudent to avoid starting longer-acting hormonal methods (e.g., depot-medroxyprogesterone acetate, etonogestrel implant, levonorgestrel-releasing intrauterine devices) until it is certain that pregnancy has not occurred. However, this concern should be balanced against risk of unintended pregnancy if initiation of one of these methods is delayed.

For those taking ulipristal for emergency contraception, there is theoretical concern that starting a hormonal method after taking ulipristal could make either the ulipristal or the hormonal method less effective by competitive binding to the progestin receptors. In 2015, the US Food and Drug Administration amended the package insert and stated that progesterone-containing contraceptives should not be used with ulipristal or for 5 days following ulipristal use [40]. The ulipristal package insert recommends using a barrier method for the remainder of the cycle to avoid any potential drug interactions with a hormonal contraceptive. Some experts advise waiting 7 days after ulipristal to start a hormonal method. A guideline from the United Kingdom recommends that additional precautions be taken for 14 days after

taking ulipristal (9 days if using or starting the progestogen-only pill, 16 days for estradiol valerate/dienogest pill) [41].

#### 14.4.6 Repeated Emergency Contraception

There is no contraindication to giving a second dose of hormonal emergency contraception if a second episode of unprotected intercourse occurs any time after the first dose was administered. In contrast, there is no support for use of ulipristal more than once per cycle or if there has been another episode of unprotected sexual intercourse outside the treatment window (>120 h) [36].

#### 14.4.7 Adolescents Who Are Using EC as a Primary Method

This is also commonly encountered situation. Hormonal emergency contraception is less effective, contains higher hormone levels per dose, and causes more menstrual irregularities than ongoing use of combined or progestin-only oral contraceptives [36]. The key to proper use of EC is education by the healthcare provider.

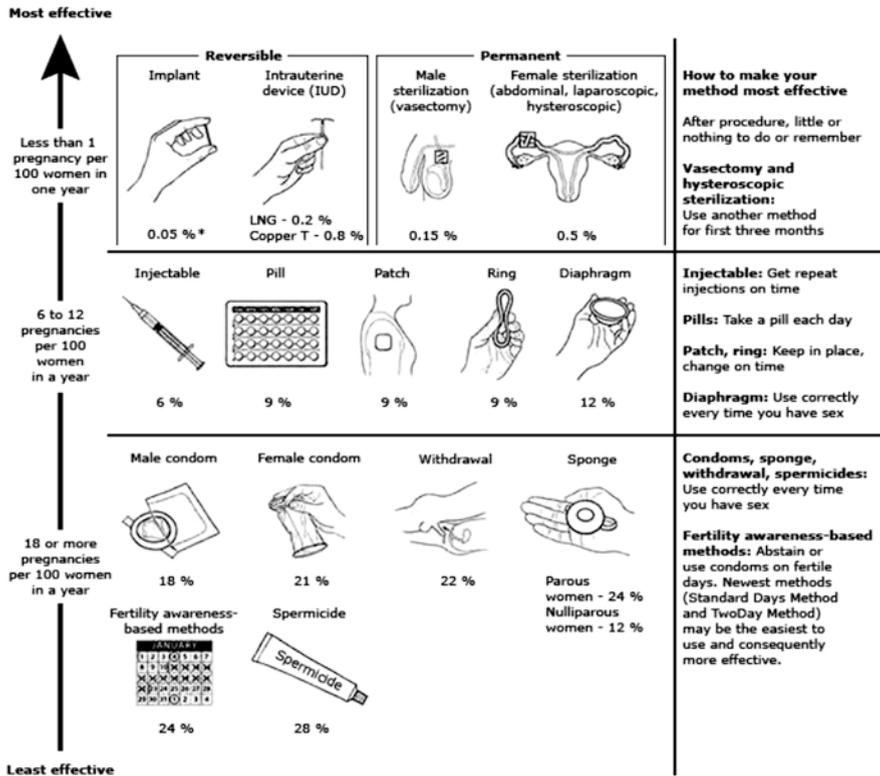
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### 14.5 “Getting Started” with Contraception

When discussing contraceptive use with the adolescent, several points must be stressed:

1. The selection of the contraceptive method is based on effectiveness, frequency of use, and personal convenience (Fig. 14.1). Recently, the long-acting reversible contraceptives (LARC), including the etonogestrel implant and intrauterine devices (copper and levonorgestrel) have been recommended by both the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP), as the most effective means for prevention of an unintended pregnancy [42].
2. Adverse effects
3. Tips on increasing adherence and to promote use
4. Protection against STIs (i.e., use of condoms)
5. Availability and indications for emergency contraception

There are many sexually active teenagers who are worried about becoming pregnant, but also not sure if to start using contraception, and what kind of. The encounter with the teen should be focused on her thoughts and concerns, reviewing stories and beliefs about contraceptives that she heard from friends or family members, and stressing the importance of dual protection (i.e., both against pregnancy and STIs). Teens that are not sexually active yet should be educated about the use of condoms and emergency contraception, in case their status changes.



**Fig. 14.1** Comparing effectiveness of contraceptive methods. Condoms should always be used to reduce the risk of sexually transmitted infections. Other methods of contraception: Lactational amenorrhea method - LAM is a highly effective, temporary method of contraception. Emergency contraception - Emergency contraceptive pills or a copper IUD after unprotected intercourse substantially reduces risk of pregnancy. LNG levonorgestrel. \* The percentages indicate the number out of every 100 women who experienced an unintended pregnancy within the first year of typical use of each contraceptive method. World Health Organization (WHO) et al. [76], Trussell [77] (Reproduced from: U.S. Selected Practice Recommendations for Contraceptive Use, 2013; Adapted from the World Health Organization Selected Practice Recommendations for Contraceptive Use, 2nd Edition. MMWR Morb Mortal Wkly Rep 2013; 62:1.)

The healthcare provider must also discuss with the adolescent potential advantages and adverse effects:

- **Benefits** – Advantages of combined estrogen-progestin hormonal contraception include improved bone density and protection against ovarian cancer, endometrial cancer, salpingitis, ectopic pregnancy, benign breast disease, dysmenorrhea, acne, and iron deficiency [43]. The same benefits, with the exception of improved bone density, are provided by DMPA. The adolescent may also view the change in menstruation pattern (i.e., fewer periods or amenorrhea), as a potential advantage.

Neither the progestin implant nor the IUDs has been associated with decreased bone density, which might also be regarded as a potential advantage.

- *Adverse effects* – The combined estrogen-progestin hormonal contraception (OCs, transdermal patch and vaginal ring), as well as DMPA, etonorgestrel implant and intra-uterine progestin systems, can cause breakthrough bleeding and/or amenorrhea. These can frighten or upset the adolescent, and she would likely discontinue the use of the method, unless she was educated in advance about these potential problems and their treatment [44].

The transdermal patch might cause local contact dermatitis (mild or severe). Also, partial or complete detachment of the patch from the skin was reported more commonly in adolescents (up to 35 %) [45, 46] than in adults (<5 %) [47, 48]. No detachments were reported when the patch was worn on the arm. The likely explanation may be inadequate care in application and increased activity among teenagers compared with adults.

Although it is not necessary to obtain written informed consent before the initiation of hormonal contraception in adolescents, the use of a structured informed consent form can ensure that the risks and benefits are adequately discussed.

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## 14.6 Long Acting Reversible Contraception (LARC)

### 14.6.1 The Intra-uterine Device (IUD)

Usually, most physicians are reluctant to insert an IUD to an adolescent, and even to nulliparous adult patient. This reluctance stems from several reasons, including: [49] history of negative publicity; disinformation about the risk of ectopic pregnancy, infection and infertility; misconceptions about the mechanism of action of the IUD; lack of provider training; and fears of litigation.

In the 1970s, the IUD was used by 10% of women in the United States using contraception. When the Dalkon Shield (a common IUD used in that era) was linked to pelvic inflammatory disease, utilization of all IUDs fell and litigation rose.

One probable explanation for the increased infectious morbidity in the 1970s is the construction of the IUD tail, which were multifilament and braided and acted like “ropes” that allowed bacteria to ascend upward from the lower genital tract. Coupled with the fact that no test had yet been developed to detect asymptomatic Chlamydia infection, many women developed severe pelvic infections.

Current IUDs, which have monofilament tail strings, have not been associated with an elevated infection risk, beyond the first 2–3 weeks after insertion. In 2005, the US Food and Drug Administration (FDA) approved liberalized package labeling for the copper T380A (Paragard), removing any proscription against its use in nulliparous women or in those with more than one sexual partner. Initial studies with the newest levonorgestrel releasing IUD (LNg14 IUD; Jaydess\*, Skyla\*) include nulliparous women. Based on the results of these trials, the product label contains no recommendation against use in nulliparous women. Although the LNg20 IUD

(Mirena\*) package label contains a recommendation for its use in parous women, the LNG20 has been studied in nulliparous women and found to be safe. The American College of Obstetrics and Gynecologists (ACOG) states that IUDs are safe and appropriate for most women, including nulliparous women and adolescents, and that use of IUDs should be encouraged as a first-line approach to pregnancy prevention [42, 50]. The IUD is an attractive option for adolescents and adolescent mothers who desire long-term, uninterrupted contraception. It lasts 3–10 years depending on the type of device. Parous adolescents may be better candidates for the IUD than nulliparous adolescents because higher expulsion rates have been reported in nulliparous adolescents. However the 3- and 5-year devices have been successfully used in nulliparous adolescents. This has been shown in a large study based on medical records of 1177 female teenagers age 13–24 years [51]. In this study the first-attempt success rate was 95.8% for nulliparous women and 96.7% for parous women. The first-attempt success rate was 95.5% ( $n=169$ ) for women aged 13–17 years compared with 96.3% ( $n=963$ ) for women aged 18–24 years.

The World Health Organization suggests: [52]

- The IUD is an unacceptable risk in a woman with pelvic inflammatory disease (PID) or purulent cervicitis currently or in the past 3 months.
- The risks outweigh the advantages of inserting an IUD in a woman with multiple partners or a partner with multiple partners.
- There is no restriction on IUD placement when there is a past history of PID and no current sexually transmitted infection.

#### 14.6.1.1 Mechanisms of Action

There are several possible mechanisms of action for IUD [53, 54], including changes in cervical mucus that inhibit sperm transport (e.g., increased copper concentration, thickening, glandular atrophy or decidualization); chronic inflammatory changes of the endometrium and fallopian tubes, which have spermicidal effects and inhibit fertilization and implantation; thinning and glandular atrophy of the endometrium, which inhibits implantation; and direct toxic effects on the oocyte. It is important to stress that *the IUD is not an abortifacient* (defined as interruption of an implanted pregnancy) [53].

#### 14.6.1.2 Absolute and Relative Contraindications

- Absolute contraindications for any IUD placement include: possible or confirmed pregnancy; severe distortion of the uterine cavity (such as by fibroids or anatomic anomalies) that precludes IUD insertion; acute, recent (within 3 months) or recurrent uterine infection (includes sexually transmitted, postpartum and post-abortion infections); untreated cervicitis; active genital actinomycoses.
- Absolute contraindications for copper IU include Wilson's disease and known copper allergy.
- Absolute contraindications for levonorgestrel IUD include: known allergy to levonorgestrel; acute liver disease or liver tumor; and known or suspected carcinoma of the breast.

- Relative contraindications for any IUD placement include: patients at high risk for STIs (multiple sexual partners, past infections); previous IUD problem (perforation, significant cramps); unresolved abnormal uterine bleeding; known severe vasovagal reactions.
- Relative contraindications for copper IUD include current anemia or menorrhagia-associated anemia.
- Relative contraindications for levonorgestrel IUD include past intolerance to progestin and patient unwilling to accept a change in menstrual pattern (hypomenorrhea or amenorrhea).

The risks and benefits of IUD for an adolescent must be determined on a case-by-case basis.

#### 14.6.1.3 Quick Guide for IUD Insertion

- Screen every adolescent for chlamydia and gonorrhea before insertion. If not screened and proven negative we usually give 1 g azithromycin orally at the day of insertion.
- The IUD may be inserted at any time during the cycle if pregnancy can be excluded. A pregnancy test should be obtained if pregnancy cannot be excluded clinically, and a negative result documented before insertion.
- As NSAIDs or topical anesthetics have not been found helpful to ease insertion pain, we do not use them. We also do not routinely use paracervical block, as the procedure itself is painful. Paracervical block is an option for patients at risk of *greater than average* insertional pain, such as those requiring cervical dilation or with a history of prior painful IUD insertion, and it can also be useful for patients at risk for a vasovagal reaction.
- Each IUD type has a unique insertion procedure. The provider should be familiar with device instructions and never use force when measuring the uterine cavity or passing the IUD through the cervical canal, as this increases the risk of perforation.
- We use misoprostol 400 mcg vaginally or sublingually before IUD insertion to *nulliparous* adolescents or adolescents with *previous failed insertion*.
- Although not necessary, we find it more relaxing to insert the IUD under abdominal ultrasound guidance. If the insertion was not performed under ultrasound guidance we confirm proper placement with ultrasound immediately after insertion.
- One can insert the IUD immediately after abortion or at 4–6 weeks post-partum. The copper IUD may be placed immediately following a vaginal or cesarean delivery.
- Back-up contraception is not needed after insertion of a CuT380A, but should be provided for 7 days if a levonorgestrel IUD is inserted more than 7 days from the start of menstrual bleeding.

#### 14.6.1.4 Complications

- *Uterine perforation* – This occurs in about 1:1000 interval IUD insertions. The risk factors include cervical stenosis, inexperienced provider, postpartum period, breastfeeding and fixed or retroverted uterus. If perforation by the sound or the

IUD is suspected, the adolescent must be monitored for tachycardia, hypotension, syncope, or an acute abdomen. Even though these rarely occur after perforation, they warrant a transfer to hospital for further management. If perforation is suspected in a stable patient (which usually is the case), IUD removal can be attempted by placing gentle traction on the string. If the string is not visible, the location of the IUD can usually be determined by ultrasound examination. Depending on the location, the IUD can then be retrieved using a hysteroscope or laparoscope. Removing the IUD under direct vision helps to prevent injury to pelvic organs that could be snagged by the device. Laparotomy is required if laparoscopic or hysteroscopic removal is difficult, if perforation of intraabdominal organs is suspected or with ongoing intraperitoneal hemorrhage. Immediate intervention is not essential if the patient is stable. A uterine perforation caused by a sound or an IUD is not a contraindication for future vaginal delivery.

- *Vasovagal response* – Sometimes, the IUD insertion causes transient syncope or presyncope, hypotension, bradycardia, and/or nausea. These typically resolve by stopping the procedure, placing the adolescent in a recumbent position, and giving supportive care.
- *Postinsertion patient counseling*

We teach the adolescent to feel the IUD strings and to verify periodically and especially after the menses that the IUD is retained. We guide her, that if she doesn't feel the strings, she should schedule an appointment and use a back-up contraceptive method until her healthcare provider confirms whether or not the IUD is in place. Even with absent problems we schedule a follow-up visit in 1–3 months, because it was shown to increase continuation of the method.

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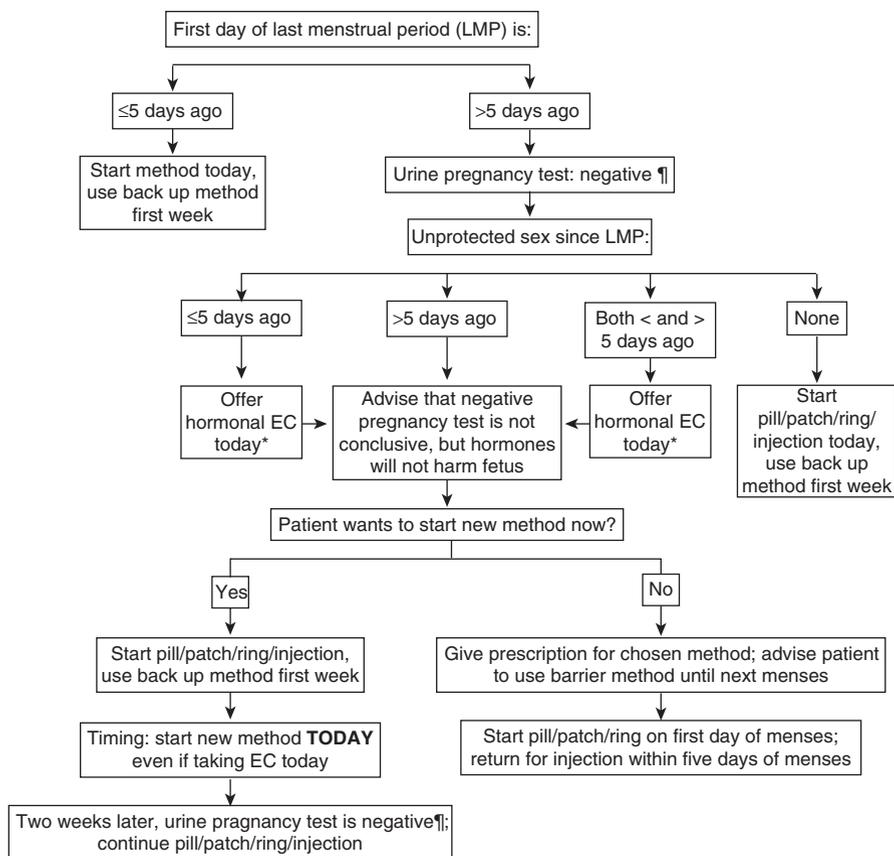
## 14.7 Contraceptive Progestin Implant

These were also suggested (together with the IUD) as a first-line contraceptive method in adolescents [42, 43]. The contraceptive implant is potentially an attractive option for adolescents and adolescent mothers who desire long-term contraception. It provides pregnancy protection within 24 h of insertion, and fertility returns quickly after its removal. Unexpected and prolonged bleeding can be a problem and can trigger request for premature removal. One should reliably exclude pregnancy before implant insertion. Therefore the “Quick-Start” method is *not recommended* for the implant.

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## 14.8 Depo Medroxy Progesterone Acetate (DMPA) Injections

Typically, the first injection is given during the menstrual period to ensure absence of pregnancy. Alternatively, it can be given using the Quick-Start method (Fig. 14.2) [55]. If DMPA is initiated more than 7 days after the first day of the menstrual period and she has had unprotected intercourse during the cycle, clinicians and patients must recognize that there is a small chance of preimplantation or early pregnancy despite a negative pregnancy test prior to the injection. We suggest that



**Fig. 14.2** The “Quick-Start” algorithm for initiation of short acting reversible contraception (pill, patch, vaginal ring and DMPA injection). EC emergency contraception. \* Because hormonal EC is not 100% effective, check urine pregnancy test 2 weeks after EC use. If pregnancy test is positive, provide options counseling (Reproduced with permission from: RHEDI/The Center for Reproductive Health Education In Family Medicine, Montefiore Medical Center, New York City. Copyright © 2007 RHEDI)

in these cases to receive emergency contraception if intercourse occurred within the previous 120 h, and we advise to use back-up contraception for 7 days after DMPA injection since ovulation may occur within 24 h of the initial injection, and we counsel them to repeat the pregnancy test in 2–3 weeks.

### 14.8.1 Switching from Another Contraceptive Method to DMPA

A common scenario is an adolescent girl not happy from one contraceptive method and asking another one. Many mistakes can be made during this changing period, both by the patients and by the providers. Table 14.2 is designed to provide short and clear guidelines regarding safe switch between various methods.

**Table 14.2** Switching between various contraceptive methods

	Pill	Patch	Ring	DMPA injection	Implant	Hormonal IUD	Copper IUD
Pill	No gap: take first pill of new pack the day after taking any pill in old pack	Start patch 1 day before stopping pill	No gap: insert ring the day after taking any pill in pack	First shot 7 days before stopping pill	Insert implant 4 days before stopping pill	Insert hormone IUD 7 days before stopping pill	Can insert copper IUD up to 5 days after stopping the pill
Patch	Start pill 1 day before stopping patch		No gap: insert ring and remove patch on the same day	First shot 7 days before stopping patch	Insert implant 4 days before stopping patch	Insert hormone IUD 7 days before stopping patch	Can insert copper IUD up to 5 days after stopping patch
Ring	Start pill 1 day before stopping ring	Start patch 2 days before stopping ring		First shot 7 days before stopping ring	Insert implant 4 days before stopping ring	Insert hormone IUD 7 days before stopping ring	Can insert copper IUD up to 5 days after stopping ring
DMPA injection	Can take first pill up to 15 weeks after the last shot	Can start patch up to 15 weeks after the last shot	Can insert ring up to 15 weeks after the last shot		Can insert implant up to 15 weeks after the last shot	Can insert hormone IUD up to 15 weeks after the last shot	Can insert copper IUD up to 16 weeks after the last shot
Implant	Start pill 7 days before implant is removed	Start patch 7 days before implant is removed	Start ring 7 days before implant is removed	First shot 7 days before implant is removed		Insert hormone IUD 7 days before implant is removed	Can insert copper IUD up to 5 days after implant is removed
Hormonal IUD	Start pill 7 days before IUD is removed	Start patch 7 days before IUD is removed	Start ring 7 days before IUD is removed	First shot 7 days before IUD is removed	Insert implant 4 days before IUD is removed		Can insert copper IUD right after hormone IUD is removed
Copper IUD	Start pill 7 days before IUD is removed	Start patch 7 days before IUD is removed	Start ring 7 days before IUD is removed	First shot 7 days before IUD is removed	Insert implant 4 days before IUD is removed	Insert hormone IUD right after copper IUD is removed and use back-up method for 7 days	

### 14.8.1.1 What to Do if She's Late for Injection?

Adolescents who are late for injection shouldn't be turned away solely because of that. After a 150 mg injection, ovulation does not occur for at least 14 weeks. Therefore, a 2-week "grace period" (repeat injection without pregnancy testing) is appropriate for adolescent women receiving injections every 3 months (13 weeks). For adolescents more than 2 weeks late for their injection (>15 weeks from the last injection), we suggest a pregnancy test before administering DMPA and back-up contraception (or abstinence) for 7 days.

As a result of a study showing that contraceptive efficacy is maintained with a "grace period" as long as 4 weeks [56], the World Health Organization (WHO) adopted a longer grace period in its updated guidelines. A limitation of these data is that more than one third of the women in the study were lactating, placing them at low baseline risk for conception. Therefore, in high-resource countries, we continue to encourage women to return on time and allow a 2-week grace period, and require documentation of a negative pregnancy test in women who present more than 2 weeks (>15 weeks from their last injection).

### 14.8.2 Pros and Cons of DMPA Injections

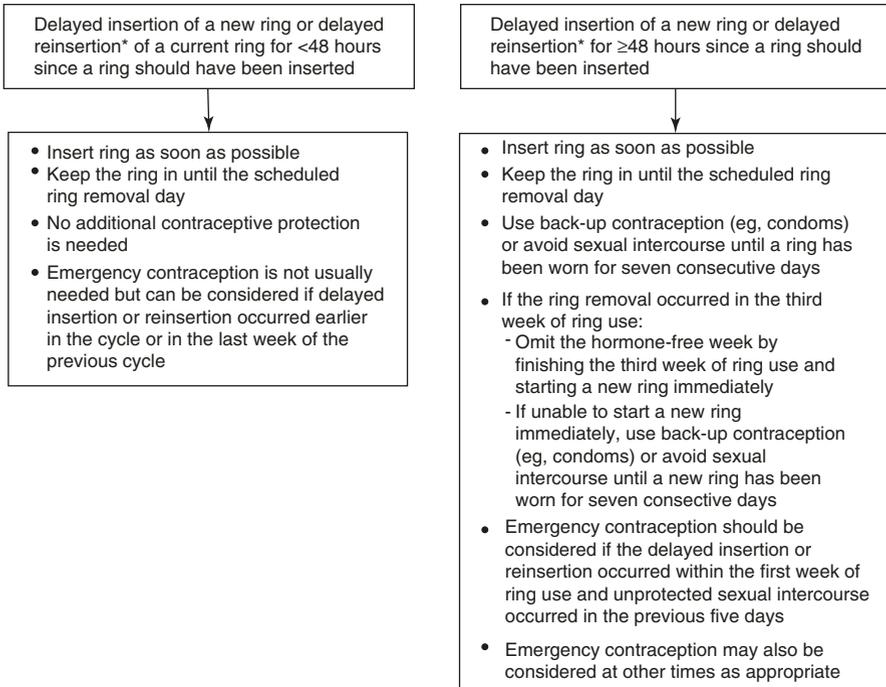
- *Advantages* of DMPA injections include: reversibility, privacy, lasts 12 weeks, effective in obese adolescents, reduces risk of ectopic pregnancy, menses decreases or ceases, reduces dysmenorrhea and other menstrual symptoms, few drug interactions, less seizures in epileptics, less pain crises in adolescents with sickle cell anemia, reduction in endometriosis related pelvic pain, reduction in abnormal uterine bleeding associated with leiomyomas, possible reduction in the incidence of PID.
- *Disadvantages* of DMPA injections include: increase in unscheduled bleeding, possible increase in weight gain, possible induction of depression, a decrease in bone density that is *usually reversible*, small risk of severe allergic reaction, a delay in return to fertility, repeated injection is needed every 12 weeks.

A thorough proactive counseling about possible side effects of DMPA, and especially menstrual irregularities and the need for repeat injections is vital for adolescents choosing this method. It was shown that women who are well informed are much more likely to become satisfied users with high continuation rates [44, 57–61].

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## 14.9 Vaginal Contraceptive Ring

For some adolescents this is a very attractive option because it needs to be changed only every 3 weeks. However, some adolescents do not feel comfortable in insertion of things into their vaginas. Those who use tampons might be better candidates for this method. The vaginal ring can be initiated according to the Quick Start method (Fig. 14.2).



**Fig. 14.3** Management of delayed insertion or reinsertion of the contraceptive vaginal ring. \* If removal takes place but the woman is unsure of how long the ring has been removed, consider the ring to have been removed for  $\geq 48$  h since a ring should have been inserted or reinserted (Reproduced from: US Selected Practice Recommendations for Contraceptive Use, 2013; Adapted from the World Health Organization Selected Practice Recommendations for Contraceptive Use, 2nd Ed. MMWR Morb Mortal Wkly Rep 2013; 62:1)

If the ring remains in place more than 3 but  $\leq 5$  weeks, it is removed and a new one is inserted after a 1-week ring-free interval; if the ring is left in place for  $>5$  weeks, back-up contraception is recommended until a new ring has been in place for 7 days. The ring remains effective when left in place for up to 5 weeks, as the ring contains sufficient steroids to maintain stable blood concentrations for this period of time [62]. Thus, inhibition of ovulation is sufficiently maintained if an adolescent forgets to remove the ring after the usual 3-week use period. In 2013, the US Selected Practice Recommendations for Contraceptive Use published an algorithm for counseling patients who delay insertion or reinsertion of the ring (Fig. 14.3) [63].

Adolescents who desire fewer days of withdrawal bleeding and are willing to tolerate some spotting can safely use an extended ring regimen whereby the ring is changed every 3 weeks, omitting the hormone-free week for up to 1 year [64–68]. Extended use is effective and does not worsen bothersome side effects, except for unscheduled bleeding.

Unplanned removal or expulsion of the contraceptive ring can occur. The ring should not be removed during intercourse. If it accidentally falls out, it may be

rinsed with cool or warm (not hot) water and reinserted into the vagina within 3 h. From our own experience, many teenagers do not feel “clean” with the vaginal ring and have a need to wash it frequently. Frequent washing decreases effectiveness and may cause intermittent bleeding or spotting.

If the ring is out of the vagina for more than 3 consecutive hours, subsequent steps depend on the week of the cycle that the ring is out:

- During the first 2 weeks of the cycle, the ring is reinserted as soon as possible. Pregnancy may not be prevented if the ring is out for more than 3 h during this time, so an additional form of contraception, such as condoms with spermicide, is used until the reinserted ring has been in place for 7 continuous days. The ring should subsequently be removed according to the original schedule, after which the adolescent can expect to have her normal period.
- During week three of the cycle, the adolescent discards the ring. She then chooses one of two different restart options:
  - Option one – Insert a new ring and begin a new 3-week cycle. Back-up contraception or abstinence is recommended until the new ring has been used continuously for 7 days. The adolescent may not have a regular period until she reaches her next ring-free week, but she may have vaginal spotting or bleeding prior to that point.
  - Option two – Insert a new ring and begin a new 3-week cycle. Back-up contraception or abstinence is recommended until the new ring has been used continuously for 7 days. The adolescent may not have a regular period until she reaches her next ring-free week, but she may have vaginal spotting or bleeding prior to that point.

These steps are consistent with recommendations from the manufacturer. In contrast, the CDC recommendations are more liberal and state that back-up contraception is not needed if reinsertion of the current ring occurs in less than 48 h (see Fig. 14.3).

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## 14.10 Transdermal Patch

The fact that the transdermal patch does not require daily attention makes it an attractive option for adolescents. However, the need for weekly change also may promote decreased adherence. The transdermal patch can be initiated according to the Quick Start method (see Figure 14.2).

The patch is applied to the buttock, abdomen, upper arm, or upper torso (but not the breast, as it might cause breast tenderness due to high local estrogen concentration). A different site is used each time a new patch is applied. The patch is changed once a week for 3 weeks (21 total days), followed by 1 week that is patch-free. It should always be changed/applied on the same day of the week. Reminder systems are useful to ensure appropriate weekly changes. The consequences of failing to change the transdermal contraceptive patch at the appropriate time should be addressed with the adolescent.

- *Delay in beginning the first patch in a cycle* — When a new patch cycle is delayed beyond the scheduled start day, users are instructed to apply a patch as soon as they remember and use back-up contraception (or avoid sex) for at least 1 week. The day they apply the new patch becomes the new patch change day.  
*Delay in beginning the second or third patch in a cycle* — There is a 2-day (48 h) period of continued release of adequate contraceptive steroid levels when the patch is left on for two extra days. If users change the patch within this window, the patch change day remains the same and there is no need for back-up contraception.  
After this 2-day (48 h) time period, failure to replace the second or third patch in a cycle increases the risk for contraceptive failure. Therefore, users will need to use back-up contraception (or avoid sex) for 7 days and, in some instances, use emergency contraception, if this occurs. The day the patient remembers to apply the patch becomes the new change day.
- *Delay in removing the third patch in a cycle* — Forgetting to remove the third patch on time carries less risk than forgetting to remove the first or second patch. The user is instructed to remove the patch when she remembers; the patch change day is not altered.

These recommendations are similar to those of the 2013 US Selected Practice Recommendations for Contraceptive Use, which included the following algorithm for counseling patients who delay application of the patch (Fig. 14.4) [63]. Strategies to promote weekly changes should be reviewed with adolescents using the transdermal patch. These include using a wall, computer, or cell phone calendar; a cell phone weekly alarm; or a sticker designating the change day on the bathroom mirror.

If a patch becomes partially or completely detached for less than 24 h, it should be reapplied at the same location (if it has not lost its stickiness: ancillary adhesives or tape should *not* be used), or replaced with a new patch immediately. If detachment lasts longer than 24 h, a new patch should be applied, and this day of the week becomes the new patch change day. An additional method of contraception (e.g., condoms, spermicides) should be used for the first 7 days of this cycle or the patient should avoid sex.

In various trials, 1.8% of transdermal patches required replacement for complete detachment and 2.9% became partially detached [47]. Living in a warm, humid climate did not increase the risk of detachment. The quality of adherence was illustrated in a study in which 30 women were subjected to various conditions over several 7-day time periods during transdermal patch use [47]. The conditions included normal activity, use of a sauna, immersion in a whirlpool bath, use of a treadmill followed by showering, cool water immersion, and a combination of these activities. Only one patch became detached during the 87 cycles that were evaluated, suggesting that skin adherence is not adversely affected by a vigorous, athletic lifestyle.

## 14.11 Oral Contraceptives (OCs)

Especially in the adolescent age group there are various adherence problems with OCs:

1. Not refilling the prescription
2. Forgetting to take the pill
3. Starting next pack late
4. Using pills inconsistently and sporadically
5. Not using a back-up method when needed

One study has shown that up to 33 % of adolescents missed a pill in the previous 3 months [29]. Therefore, the adolescent should be provided clear verbal and written instructions. The clinician should ascertain that she has sufficient reading skills to interpret labels and instructions.

To optimize the adolescent's adherence to OCs, we suggest giving her only three things to remember:

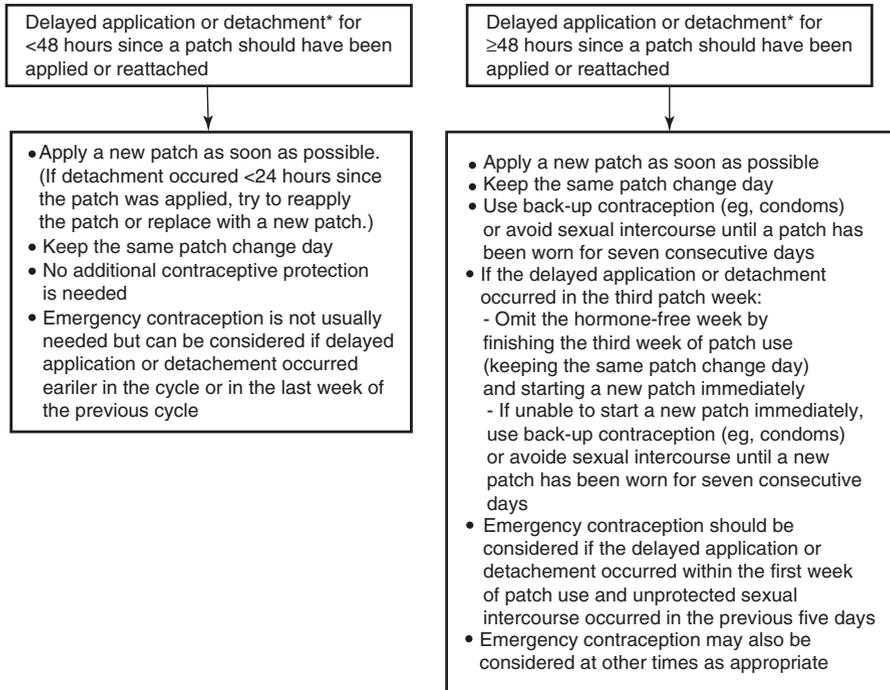
1. When to start the pill
2. Take the pill every day at the same time, especially when doing something else regularly, like teeth brushing
3. Call the clinic if there are any questions

OCs can be started at any time. In adolescents, they are typically started on the first day of the next menstrual period or the Sunday after the onset of the menstrual period ("Sunday start method"). The rationale for this delayed start date is to make sure that the adolescent is not pregnant. However, as many as 25 % of adolescents who seek OCs from family planning clinics never take the first pill [69, 70].

To address this issue, a "same day" or "Quick Start" method is now the preferred approach. The Quick Start method requires increased attention to the adolescent's self-report of sexual activity since her last menstrual period, the accuracy of the pregnancy test in the context of her sexual history, and the use of emergency contraception when applicable (see Fig. 14.2). It's also, in our opinion, important to give teenage girls the regimens with less hormone free interval (i.e., 24/4 preparations instead of 21/7) because these preparations allow the adolescent to simply take a pill every day and to start the next pack immediately after finishing the previous one. This makes things easy on her and she will be less likely to forget.

### 14.11.1 Extended-Cycle or Continuous Pill Use

The desire to avoid monthly periods may be related to participation in athletic events or summer camps, or to the general discomfort and "hassle" of monthly periods. A schedule that involves continuous pill use for 84 days followed by a week of



**Fig. 14.4** Management of delayed application or detachment of the contraceptive patch. \* If detachment takes place but the woman is unsure when the detachment occurred, consider the patch to have been detached for  $\geq 48$  h since a patch should have been applied or reattached (Reproduced from: US Selected Practice Recommendations for Contraceptive Use, 2013; Adapted from the World Health Organization Selected Practice Recommendations for Contraceptive Use, 2nd Ed. MMWR Morb Mortal Wkly Rep 2013; 62:1)

pill-free days may help to increase adherence in adolescents who wish to avoid a monthly period. In addition, an oral contraceptive that provides continuous, year-round contraception has been approved by the FDA. It contains levonorgestrel 90 mcg and ethinyl estradiol 20 mcg.

Until studies are conducted in adolescents, information on extended-cycle and continuous, year-round pills must be drawn from studies and clinical experience in adults. One-half to two-thirds of adult women report a decrease in breakthrough spotting or bleeding (BTB) in the second half compared with the first half of the extended or year-long regimen. To manage BTB, a 3-day, hormone-free interval was more beneficial than continuous pills [71].

A systematic review of continuous use of oral contraceptives in adult women found that other menstrual symptoms, including headaches, genital irritation, tiredness, bloating, and dysmenorrhea also improved with extended-cycle pill use [72].

## 14.12 Condoms

We always stress the benefit of dual-protection against both pregnancy and STIs. Girls planning to use condoms should receive them before leaving the office or clinic, if possible, to ensure availability and promote adherence.

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## 14.13 Continuation Rate of Various Contraceptive Methods Among Adolescents

A systematic review of hormonal and intrauterine methods of contraception in young women found limited data about continuation rates [73]. Nevertheless, “zooming in” on the CHOICE project, the 12-month continuation rates among 1099 urban adolescents (age 14–19 years of age) who were provided with contraception at no cost were as follows: [74]

- Levonorgestrel intrauterine system ( $n=330$ ) – 81 %
- Copper intrauterine device ( $n=55$ ) – 76 %
- Etonogestrel implant ( $n=378$ ) – 82 %
- Depot medroxyprogesterone acetate ( $n=112$ ) – 47 %
- Oral contraceptives ( $n=146$ ) – 47 %
- Contraceptive patch ( $n=21$ ) – 41 %
- Vaginal ring ( $n=57$ ) – 31 %

Continuation rates were greater among adolescents who used long-acting reversible contraception (i.e., levonorgestrel intrauterine system, copper intrauterine device, or etonogestrel implant) than other methods (84.7 versus 44 %). This study strengthens further, that *LARC should be first line contraception methods in this age group if we truly want to prevent unintended pregnancy.*

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## 14.14 Providing Contraception to Adolescents with Chronic Illness

Hormonal contraception for the chronically ill adolescent can be a challenge. Estrogen-based hormonal contraceptives, as an example, should not be offered to an adolescent with significant valvular disease who is not being treated with an anticoagulant. Although DMPA is useful in such cases, its effect on fluid retention should be evaluated and monitored.

Similarly, estrogen-based hormonal contraceptives should not be offered to an adolescent with antiphospholipid antibody abnormalities, uncontrollable hypertension, and vascular involvement. DMPA is useful in such cases; there have been no reports of systemic lupus erythematosus flares with long-term DMPA use.

In addition, multiphasic or ultra-low dose oral estrogen-progestin contraceptives are not recommended in adolescents taking many anticonvulsants because anticonvulsants other than valproic acid increase the clearance of sex steroids. DMPA is a good alternative because progesterone levels are high enough to be unaffected by increased P-450 activity.

Other medications that alter the effectiveness of estrogen-based hormonal contraceptives by increasing clearance of sex steroids include rifampin, griseofulvin, and certain antiretroviral agents. Herbal medication such as St. John's Wort also can increase clearance of sex steroids. There are inadequate data to support any significant drug interaction between common antibiotics and estrogen-based hormonal contraceptives.

It is important to review the choice of hormonal contraceptives in the context of the chronic illness, potential increase in estrogen-related complications, and drug interactions during the counseling process. The risk of pregnancy needs to be weighed against the risk of a medical complication from hormonal contraception. In addition, emergency contraception should be discussed with adolescents whose current treatment may be teratogenic to the fetus or in whom pregnancy would severely compromise health [75].

Detailed guidelines for contraceptive methods in patients with medical issues were recently updated but the World Health Organization and are available online at [http://www.who.int/reproductivehealth/publications/family\\_planning/MEC-5/en/](http://www.who.int/reproductivehealth/publications/family_planning/MEC-5/en/)

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# Index

## A

### Abortion

- definition, 189
- emergency contraception, 193
- Turner syndrome, 157

### Adolescent pregnancy.

*See also* Emergency contraception (EC)

- anemia, 201
  - complications, 200–201
  - contraceptive method
    - confidentiality, 203
    - features, 202
    - legal issues, 203
    - pelvic examination, 203
    - side effects, 204–206
  - definition, 200
  - effect on newborn, 201
  - infectious diseases, 201
  - labor, 201
  - social aspects, 202
  - stillbirths and death, 201
- Adrenal gland, feminizing tumors of, 45
- Adrenal steroid biosynthesis, 50, 51
- Adrenarche, 50, 135–136
- AIS. *See* Androgen insensitivity syndrome (AIS)
- Allopregnanolone (AP), 4–5
- 17-Alpha-hydroxylase, 16
- Ambiguous genitalia. *See* Disorders of sex differentiation (DSD)
- Amenorrhea
  - in anorexia nervosa
    - behavioural features, 120–121
    - characteristics, 120
    - DSM-5, 122
    - gonadotropin releasing hormone secretion, 120
    - oestrogen effects, 121–122

- causes, 111
  - definition, 111
  - description, 127
  - hypothalamic, 112–113
  - long-term cardiovascular risks, 127–131
  - prevalence, 111, 127
  - primary and secondary, 111, 112
- Androgenic progestins, 89
- Androgen insensitivity (AI), 17–18
- Androgen insensitivity syndrome (AIS)
  - clinical presentation, 161
  - differential diagnosis, 161
  - impact on fertility, 161
  - prevalence, 160
- Androgen-resistance disorders, 46, XY DSD
  - androgen insensitivity, 17–18
  - 5aR deficiency, 18
  - environmental endocrine disrupting
    - chemical exposure, 19
  - MALD1 gene mutations, 19
- Anorexia nervosa (AN)
  - in adolescent girls
    - amenorrhoea in, 120–122
    - characterization, 119
    - mortality rates, 119
    - prevalence, 119
    - therapeutics implications, 123
  - in women with lifetime AN, 153–154
- Anteroventral periventricular nucleus (AVPV), 6
- Anthracycline antibiotics, 166
- Anti-metabolites, 166
- Anti-Mullerian hormone (AMH), 11, 17, 18, 44, 77–78, 144, 168, 184, 186
- 5aR deficiency, 18
- Ataxia telangiectasia, 72
- Autoimmune diseases
  - delayed puberty and reduced fertility, 171
  - non-oncological indications, 170

**B**

- β-endorphin (β-END), 5–6
- 3-Beta-hydroxysteroid dehydrogenase, 16
- 17-Beta-hydroxysteroid reductase, 16
- Biological targeted therapies, 166
- Blepharophimosis-ptosis-epicanthus syndrome (BPES) type I, 71
- Bloom syndrome, 72

**C**

- Café-au-lait spots, 43
- Carbohydrate-deficient glycoprotein syndrome type I, 71
- Cardiovascular disease (CVD), 128
  - mortality, 130
  - postmenopausal estradiol use, 130
- Central nervous system (CNS), sexual differentiation in
  - 17-β-estradiol, 3
  - dehydroepiandrosterone, 4
  - estrogens, 2–4
  - testosterone levels, 3
- Central precocious puberty (CPP).
  - See also* Precocious puberty (PP)
  - clinical characteristics, 26, 27
  - management
    - GnRH agonist treatment, 33–34
    - hypothalamic lesion, 35
  - progression, 39
- CHARGE syndrome, 150
- Chemotherapy-induced ovarian damage, 183
- Childhood Cancer Survivor Study (CCSS), 163, 164
- Chromobox homolog 2 gene mutation, 16
- Circulating endothelial progenitor cells (EPC), 129
- Combined estrogen-progestin hormonal contraception
  - advantages, 210–211
  - adverse effects, 211
  - venous thromboembolism, 204
- Combined oral contraceptives (COCs), 186, 194, 195. *See also* Oral contraceptives (OCs)
- Complete androgen insensitivity (CAI), 17, 18
- Condoms, 223
- Congenital isolated hypothalamic hypogonadism (CHH), 148–150
- Conjugated equine estrogens, 85, 87
- Constitutional delay of growth and puberty (CDGP), 145–147

Contraceptive methods, adolescents.

- See also* Emergency contraception (EC)
- with chronic illness, 223–224
- condoms, 223
- confidentiality, 203
- continuation rates, 223
- effectiveness, 209, 210
- features, 202
- legal issues, 203
- pelvic examination, 203
- side effects
  - birth defects and infertility, 206
  - bone density, 204
  - depot medroxyprogesterone acetate, 204
  - venous thromboembolism, 204–205
  - weight gain, 204
- Contraceptive progestin implant, 214
- Copper bearing intrauterine device. *See* Intrauterine device (IUD)
- CXorf6, 19
- Cyclophosphamide, 75, 165, 166, 169

**D**

- DAX1 gene, 15–16
- Delayed puberty, 133
  - after cancer treatment
    - chemotherapy, 164–167
    - childhood cancer, 163, 164, 168
    - damage estimation, by biological markers and sonography, 168–169
    - radiotherapy effects, 167–168
    - reduced fertility, 163–164
  - autoimmune diseases, 171
  - bone age, 145
  - brain MRI, 145
  - causes, 147, 148
  - constitutional delay of growth and puberty, 145–147
  - definition, 142
  - diagnostic evaluation, 143
  - empathetic counselling, 143
  - endocrine disruptors, 171–173
  - in females, assessment of, 143, 144
  - fertility preservation
    - ethical considerations, 170–171
    - non-oncological indications, 170
    - oncological indications, 169–170
    - ovarian cortex cryopreservation, 169
    - risk assessment, 169

- functional hypogonadal hypogonadism, 150–151
  - heavy metal lead, 172–173
  - hormone measurements
    - anti-Müllerian hormone, 144
    - gonadotropins, 144
    - inhibin B, 144
    - oestradiol levels, 144
    - pituitary deficits, 145
  - hypergonadotropic hypogonadism
    - androgen insensitivity syndrome, 160–161
    - FSH receptor mutations, 161–162
    - galactosemia, 162–163
    - gonadal dysgenesis, 157, 159–160
    - LH receptor mutations, 161–162
    - Noonan syndrome, 160
    - Turner syndrome, 157–159
  - hypophyseal hypogonadotropic hypogonadism, 151–152
  - impact on fertility, 152–154
  - incidence, 142–143
  - karyotype, 145
  - organic hypothalamic hypogonadotropic hypogonadism, 148–150
  - ovarian torsion and gonadectomy, 171
  - pelvic abdominal ultrasonography, 145
  - profertile measures, 154–157
  - Depot 17 $\beta$ -estradiol cypionate, 83
  - Depot medroxyprogesterone acetate (DMPA), 204, 210, 211
  - injections
    - advantages, 217
    - disadvantages, 217
    - initiation, 214, 215
    - switching between contraceptive methods, 215, 216
    - 2-week grace period, 217
  - Disorders of sex differentiation (DSD)
    - causes
      - chromosomal abnormalities, 20
      - ovotesticular DSD, 19–20
      - 46,XX DSD, 12–14
      - 46,XY DSD, 14–19
    - clinical diagnosis, 9–11
    - fetal ultrasonography, 12
    - genetic investigations, 11
    - genitography, 12
    - hormonal investigations, 11–12
    - management, 21
    - Prader scores, 11
    - urogenital endoscopy, 12
  - Doxorubicin (DXR), 166
  - Dydrogesterone, 89, 94
- E**
- Early menopause, 67
  - Eating disorders not otherwise specified (EDNOS), 122
  - Edinburgh selection criteria, 170
  - Effective sterilizing dose (ESD), 167
  - Emergency contraception (EC)
    - acceptability and availability, 190–191
    - contraindications, 207
    - definition, 189
    - education by healthcare provider, 209
    - effectiveness, 190–191, 195
    - effect on pregnancy, 191
    - efficacy assumptions, 190
    - failure factors, 192–193
    - family planning service, 194
    - long acting reversible contraception, 211–214
    - mechanism of action, 191, 206
    - meloxicam, 195
    - methods, 189–191
    - options, 206, 207
    - overweight and obese adolescents, 207–208
    - pharmacy-based interventions, 194
    - progestogen only pill, 194
    - resuming/initiating hormonal contraception, 208–209
    - risk factors, 192
    - ulipristal usage, 208–209
    - unintended pregnancy/abortion rates, 193
  - Empty sella syndrome, 152
  - Endocrine disrupting chemicals, 19
  - Endocrine disruptors (ED), delayed puberty, 171–173
  - Endogenous opioid system, gender response of, 5
  - Environmental endocrine disruptors (EEDs), 46, 172
  - Estradiol-induced organizational changes, 6
  - Estrogens, 2–3
  - Ethinyl estradiol (EE2), 84–86
  - Exogenous androgen exposure, 52
  - Exogenous hyperandrogenism, 14
  - External genitalia, malformations of. *See* Disorders of sex differentiation (DSD)

**F**

- Familial primary ovarian failure, 182, 186
- Fertility preservation, in young girls and adolescents
- ethical considerations, 170–171
  - non-oncological indications, 170
  - oncological indications, 169–170
  - ovarian cortex cryopreservation, 169
  - risk assessment, 169
- Fetal hyperandrogenism, 12–14
- FHA. *See* Functional hypothalamic amenorrhea (FHA)
- Fibrous bone dysplasia, 43
- Flow mediated vasodilatation (FMD), 129, 130
- 5-Fluorouracil, 166
- FMR1 gene, 69, 78, 182
- Follicle-stimulating hormone (FSH), 11, 30, 67, 77–78, 112, 139–141, 144, 145, 161–162, 184
- Follistatin, 139–140
- FOXL2, 45
- Functional hypogonadal hypogonadism, 150–151
- Functional hypothalamic amenorrhea (FHA). *See also* Amenorrhea
- in athlete women, 128
  - epidemiology and diagnosis, 112–113
  - fat reserve, 116
  - hypothalamic-pituitary-ovarian axis disturbances, 112
  - neuroendocrine dysfunctions, 113–114
  - physical activity, 114–116
  - types, 127

**G**

- Galactosemia, 71, 162–163
- Genomic actions, of neurosteroids, 2
- Gonadal determination
- abnormalities, 14
  - gene influence, 1
- Gonadal dysgenesis
- description, 157
  - estrogen replacement therapy, for puberty induction, 80
  - forms, 159–160
  - mixed, 20
  - phenotypic female variants, 157
  - Turner syndrome (*see* Turner syndrome (TS))
- 46,XY DSD
- chromobox homolog 2 gene mutation, 16

- DAX1 gene, 15–16
  - NR0B1 gene, 16
  - renal dysfunction, 15
  - SFI gene mutation, 15
  - Sox9 gene abnormalities, 15
  - SRY gene abnormalities, 15
  - WT1 gene abnormalities, 15
- Gonadectomy, 4, 5, 20, 138, 161, 171
- Gonadotoxic chemotherapy agents, 166
- Gonadotropin-releasing hormone (GnRH) neurons, 149
- hypothalamic KiSS-1 system, 138
  - kisspeptin, 58
  - and neurokinin B, 59, 60
  - role, 58
  - types, 136
- Gonadotropin-releasing hormone (GnRH) stimulation tests, 25–26
- Gpr54 genes, 59
- Granulosa cell tumors, 43–45
- endocrine disturbances, 44
  - estrogen secretion, 43
  - inhibin level, 44
  - ovarian, 44–45
  - surgical treatment, 44

**H**

- Heterologous (XY) sex chromosomes, 1
- Homologous (XX) sex chromosomes, 1
- Hormonal replacement therapy, POI
- bone mineral density, 90–91
  - choice of, 79
  - considerations, 89
  - estrogens used, 80
  - mammary gland development, 90
  - oral micronized progesterone, 87–88
  - progestins, 88–89
  - puberty induction (*see* Puberty, induction)
  - uterus development, 90
- Hormonal therapy, POF, 185
- Hormone measurements, delayed puberty
- anti-Müllerian hormone, 144
  - gonadotropins, 144
  - inhibin B, 144
  - oestradiol levels, 144
  - pituitary deficits, 145
- Hypergonadotropic hypogonadism, delayed puberty
- androgen insensitivity syndrome, 160–161
  - FSH receptor mutations, 161–162
  - galactosemia, 162–163
  - gonadal dysgenesis, 157, 159–160

LH receptor mutations, 161–162  
 Noonan syndrome, 160  
 Turner syndrome, 157–159

Hypoestrogenism, 128–129

Hypogonadotropic hypogonadism  
 congenital hypothalamic disorders,  
 152–153  
 hyperprolactinaemia, 153  
 hypophyseal, 151–154  
 hypothalamic, 148–150  
 Leyendecker grading system,  
 154, 155  
 ovulation induction, 154–157

Hypophyseal hypogonadotropic  
 hypogonadism, 151–154

Hypoplasia, 17

Hypothalamic amenorrhea, 112–113  
 grading of, 54

Hypothalamic hypogonadotropic  
 hypogonadism, 148–150

Hypothalamo-hypophyseal-gonadal axis  
 pubertal maturation, 140–141, 144  
 regulation  
 activin, 139–140  
 adiponectin, 140  
 EAP-1, 137  
 gonadotropin releasing-hormone,  
 136, 137  
 growth hormone, 140  
 inhibin and follistatin, 139–140  
 insulin-like growth factor-I, 140  
 KiSS-1/GPR54 system, 137–138  
 leptin, 138–139  
 melatonin, 140  
 Oct-2, 136  
 thyroid transcriptional factor-1, 137

**I**

Inhibin A, 77, 139, 182

Intrauterine device (IUD).  
*See also* Emergency contraception  
 (EC)  
 absolute contraindications, 212  
 acceptability and availability, 190  
 complications, 213–214  
 effectiveness, 190  
 effect on pregnancy, 191  
 insertion guide, 213  
 LNG20, 211–212  
 mechanism of action, 191, 212  
 monofilament tail strings, 211  
 postinsertion patient counseling, 214  
 relative contraindications, 213  
 uterine perforation, 213–214

vasovagal response, 214  
 WHO suggestions, 212

In vitro maturation (IVM), 170

**K**

Kallmann syndrome, 145, 149  
 Kiss1 gene, 59–61, 137, 138  
 Kisspeptin (KP) neurons, 59  
 Klinefelter syndromes, 20

**L**

Leptin receptor (LepR) mRNA, 61  
 Levonorgestrel (LNG), 195, 205–208. *See also*  
 Emergency contraception (EC)  
 availability, 191  
 effectiveness, 190  
 effect on pregnancy, 191  
 mechanism of action, 191

Leydig cell agenesis, 17

Long acting reversible contraception (LARC)  
 contraceptive progestin implant, 214  
 DMPA injections, 214–217  
 intra-uterine device, 211–214  
 vaginal contraceptive ring, 217–219

**M**

Makorin ring finger 3 (*MKRN3*), 26, 61  
 MALD1 gene mutations, 19  
 MAS. *See* McCune-Albright syndrome (MAS)  
 Mastermind-like domain containing 1  
 (MAMLD1), 19  
 Mayer-Rokitansky-Küster-Hauser (MRKH)  
 syndrome, 161  
 McCune-Albright syndrome (MAS)  
 café-au-lait spots, 43  
 endocrine pathologies, 43  
 fibrous bone dysplasia, 43  
 molecular bases, 43  
 precocious puberty, 42–43  
 Medial basal hypothalamus (MBH), 6, 7  
 Medroxyprogesterone acetate (MPA), 89  
 Meloxicam, 195  
 Methotrexate, 166  
 Mifepristone, 189, 190, 195  
 Minimal androgen insensitivity syndrome  
 (MAIS), 18  
 Misoprostol, 189, 213  
 Mitochondrial genes, premature ovarian  
 failure, 72  
 Mixed gonadal dysgenesis, 20  
 Molecular bases, of MAS, 43  
 Mullerian derivatives, 20

**N**

- Naltrexone, 157
- Neurokinin B (NKB) agonist senktide, 59, 60
- Neurosteroids, 2, 4, 5
- Nijmegen breakage syndromee, 72
- Non-androgenic progestins, 89
- Non-classical congenital adrenal hyperplasia (NC-CAH), 52
- Non-genomic actions, of neurosteroids, 2
- Non-syndromic POF, 70
- Noonan syndrome (NS), 160
- Normal puberty
  - acceleration, 141–142
  - clinical aspects, 134–135
  - endocrinological aspects
    - adrenarche, 135–136
    - hypothalamo-hypophyseal-gonadal axis (see Hypothalamo-hypophyseal-gonadal axis)
  - menarche, 134–135
  - Tanner stages, 134
- Normosmic congenital hypogonadal hypodonadism, 149, 150
- NR0B1 gene, 16

**O**

- Oral contraceptives (OCs). *See also* Emergency contraception (EC)
  - adherence problems, in adolescents, 221–222
  - extended-cycle/continuous pill use, 221–222
  - and pelvic inflammatory disease, 205
  - ultra-low dose estrogen, 204
  - VTE risk, 204–205
- Oral estrogens, 83–84
- Oral micronized progesterone, 87–88
- Orchidectomy (OCX), 5
- Ovarian cortex cryopreservation, 169
- Ovarian cysts, 45
- Ovarian granulosa cell tumors (OGCT), 44–45
- Ovarian leukodystrophy, 71
- Ovarian tissue conservation (OTC), 170
- Ovarian tissue cryopreservation, patient selection for, 170–171
- Ovarian torsion, 171
- Ovarian transposition, 168
- Ovariectomy (OVX), 5
- Ovotesticular DSD, 19–20
- Ovulation induction, in hypogonadotropic hypogonadism, 154–157

**P**

- Partial androgen insensitivity (PAI), 17, 18
- Pelvic inflammatory disease (PID)
  - IUD, 206
  - oral contraceptives, 205
- Percutaneous 17 $\beta$ -estradiol, in hydroalcoholic gel, 83
- Peripheral precocious puberty (PPP)
  - adrenal gland, feminizing tumors of, 45
  - clinical forms, 41
  - decision tree, 47, 48
  - ovarian autonomy
    - granulosa cell tumors, 43–45
    - McCune-Albright syndrome, 42–43
    - ovarian cysts, 45
  - premature thelarche, 41–42
  - pseudopuberty and environmental pollution, 45–46
  - pubertal stage, evaluation of, 40–41
- Perrault syndrome, 71
- Platinum-based compounds, 166
- Pleiotropic single gene disorders, 71–72
- POI. *See* Primary ovarian insufficiency (POI)
- Polycystic ovary syndrome (PCOS), 54, 129, 130
- Polyendocrinopathy-candidiasis-ectodermal dystrophy, 71
- Postcoital contraception. *See* Emergency contraception (EC)
- P450-oxydoreductase (POR) deficit, 16–17
- PP. *See* Precocious puberty (PP)
- Prader scores, 11
- Precocious puberty (PP)
  - biological diagnosis, 29–30
  - clinical diagnosis, 28–29
  - clinical expression, 25
  - consequences, 26, 28
  - definition, 25
  - gonadotrophin determinations, 30
  - magnetic resonance imaging, 31
  - neuroimaging, 31
  - pelvic ultrasound scans, 30–31
  - premature pubarche, 51, 52
  - progressive vs. slowly progressive forms, 31
  - psychosocial aspects, 33
  - sex steroid determinations, 30
  - therapeutic abstention, 32
- Premature menopause (PM), 92, 163, 164, 167, 171, 181
- Premature ovarian failure (POF), 67
  - in adolescence and young adults
    - abnormal bleeding patterns, 183–184

- androgen deficiency, 186
- antral follicle count, 184
- autoimmune mechanisms, 182–183
  - causes, 181
- cognitive impairment, 186–187
- combined oral contraceptives, 186
- description, 181
- diagnosis, 184, 186, 187
- estrogen deficiency, 185
- etiology, 181, 182
- fertility-preservation options, 186
- follicle-stimulating hormone, 184
- genes implicated, 182, 183
- genetic counseling, 184
- hormonal therapy, 185
- initial evaluation, and management, 186–188
- pregnancy rate, 186
- psychological counseling, 187
- symptoms, 183
- anti-Mullerian hormone, 77–78, 184
- autoimmunologic causes, 73–74
- bone mineral density measurement, 79
- cardiovascular effects of, 129
- chromosomes with autosomal genes, 70–71
- endocrine evaluation, 77
- environmental toxins, 76
- genetic investigations, 78
- genetic syndromes, 72–73
- iatrogenic causes, 74–75
- immunological investigations, 78–79
- infectious causes, 75–76
- LH receptors, 161
- menstrual bleeding pattern, 183
- mitochondrial genes, 72
- pelvic ultrasonography, 78
- personal and family history, 77
- physical examination, 77
- pleiotropic single gene disorders, 71–72
- with polyagent adjuvant chemotherapy, 166
- single genes associated with, 70
- smoking, 76–77
- Premature pubarche (PP), 32
  - clinical features, 50–51
  - definition, 49
  - differential diagnosis
    - exogenous androgen exposure, 52
    - non-classical congenital adrenal hyperplasia, 52
    - precocious puberty, 51, 52
    - virilizing adrenal tumors, 52
  - examination of girls with, 51
  - management, 53–54
  - non-classical congenital adrenal hyperplasia, 52
  - precocious puberty, 51, 52
  - virilizing adrenal tumors, 52
- Premature sexual development. *See also* Precocious puberty (PP)
  - etiologies and mechanisms, 26
  - patient evaluation, 28–33
- Premature sexual maturation, 25, 26. *See also* Precocious puberty (PP)
- Premature thelarche, 26, 32, 41–42
- Preoptic area (POA), 6, 7
- Primary amenorrhea, 111, 112, 127
- Primary ovarian insufficiency (POI). *See also* Functional hypogonadal hypogonadism; Hypothalamic hypogonadotropic hypogonadism
  - aetiology
    - autosomal rearrangements, 70
    - genetic disorders, 68
    - X-autosome translocations, 69
    - X chromosome numerical defects, 68–69
    - X chromosome structural abnormalities, 69
  - amenorrhea, 67
  - androgen deficiency, 97
  - cognitive function, 96
  - description, 67
  - DHEA therapy, 98–99
  - hormonal replacement therapy
    - bone mineral density, 90–91
    - choice of, 79
    - considerations, 89
    - estrogens used, 80
    - mammary gland development, 90
    - oral micronized progesterone, 87–88
    - progestins, 88–89
    - puberty induction (*see* Puberty, induction)
    - uterus development, 90
  - pathophysiology, 68
  - with post-menarche onset
    - bone mineral density, 94–96
    - hormonal therapy, 92–94
    - psychological consequences, 96
    - testosterone replacement therapy, 97–98
- Procarbazine, 166
- Progesterone receptor modulator (PRM). *See* Ulipristal acetate (UPA)
- Progestogen only pill (POP), 194, 195

- Prostaglandins, 195
- Proximal symphalangism and multiple synostose syndrome, 71
- Puberty. *See also* Delayed puberty; Normal puberty
- adrenarche role, in maturational process, 50
  - critical fat mass, 60
  - description, 133
  - hypothalamic-pituitary-gonadal axis, 57, 58
  - induction
    - with 17 $\beta$ -estradiol (E2), 84, 85
    - with conjugated equine estrogens, 85, 87
    - with depot 17 $\beta$ -estradiol cypionate, 83
    - with ethinyl estradiol (EE2), 84–86
    - gonadal dysgenesis, estrogen replacement therapy, 80
    - with oral estrogens, 83–84
    - with percutaneous 17 $\beta$ -estradiol, in hydroalcoholic gel, 83
    - protocols, 81, 82
    - with transdermal 17 $\beta$ -estradiol, 80–82
  - leptin role, 60–61
  - metabolic control, 60–61
  - neuronal control
    - GnRH neurons, 58–59
    - kisspeptin neurons, 59
    - sex steroid stimulation, 58–59
  - normal variants of, 32–33
  - onset, 57
    - KNDy neurons, 60
    - neurokinin B agonist senktide, 59, 60
    - sex steroid levels, 60
  - repressive control, 61, 62
  - sequence of events, in girls, 134
  - upstream mechanisms, 61
- R**
- Radioimmunoassay (RIA) methods, 30
- Radiotherapy effects, on ovary and uterus, 167–168
- Rothmund-Thomson syndrome, 72
- S**
- Secondary amenorrhea, 111, 112, 127
- Secondary ovarian insufficiency, 151–154
- Secondary sexual characteristics, 39, 40
- Sex chromosomes, 2
- Sex steroid determinations, PP, 30
- Sexual differentiation
  - in central nervous system, 2
  - 17- $\beta$ -estradiol, 3
  - dehydroepiandrosterone, 4
  - estrogens, 2–4
  - testosterone levels, 3
  - for mammals, 1
- Sexual dimorphism, of human brain, 3
- Sexually dimorphic nucleus of the preoptic area (SDN-POA), 6
- SF1 gene mutation, 15
- Slowly progressive forms, of precocious puberty, 32–33
- Sox9 gene abnormalities, 15
- Spontaneous primary ovarian failure
  - causes, 181
  - family history, 184
  - pregnancy rate, 186
  - steroidogenic cell autoimmunity, 182
- SRY gene abnormalities, 15
- Steroid hormones, 2
- Steroidogenic factor-1 (SF-1), 1
- T**
- Tanner stages, 40
- Taxanes, 166
- Testicular feminization. *See* Androgen insensitivity syndrome (AIS)
- Testosterone production, defects in
  - 17-alpha-hydroxylase, 16
  - 3-beta-hydroxysteroid dehydrogenase, 16
  - 17-beta-hydroxysteroid reductase, 16
  - hypoplasia, 17
  - Leydig cell agenesis, 17
  - P450-oxydoreductase deficit, 16–17
- Therapeutic abstinence, precocious puberty, 32
- Transdermal 17 $\beta$ -estradiol, 80–82
- Transdermal contraceptive patch, 205
- Transdermal patch, 219–220, 222
- True hermaphroditism. *See* Ovotesticular DSD
- Turner syndrome (TS)
  - absent/delayed puberty, 158
  - bone fragility, 91
  - bone mineral density, 91, 92
  - clinical presentation, 158
  - description, 158
  - impact on fertility, 158–159
  - incidence, 157
  - karyotype, 158
  - oral estrogen treatment and uterus development, 90
  - pregnancy, 159
  - related mechanisms, 92
  - skeletal growth, 91

**U**

- Ulipristal acetate (UPA). *See also* Emergency contraception (EC)
  - availability, 191
  - contraception after, 194–195
  - effectiveness, 190
  - mechanism of action, 191
  - safety and efficacy, 191

**V**

- Vaginal administration, of micronized progesterone, 88
- Vaginal contraceptive ring, 217–219
- Venous thromboembolism (VTE), 204–205
- Ventromedial nucleus of hypothalamus (VMN), 6–7
- Vinca alkaloids, 166
- Virilizing adrenal tumors, 52

**W**

- Werner syndrome, 72
- Wilm's tumor-related gene-1 (WT-1), 1
- Women's Ischemia Syndrome Evaluation (WISE) study, 128
- WT1 gene abnormalities, 15

**X**

- X-autosome translocations, 69
- X chromosome numerical defects, 68–69
- X chromosome structural abnormalities, 69

**Xenoestrogens, 46**

- 46,XX DSD, 12–14
  - exogenous hyperandrogenism, 14
  - fetal hyperandrogenism, 12–14
  - gender orientation, choice of, 20–21
  - gonadal determination, abnormalities in, 14
- 46,XY DSD, 14
  - androgen-resistance disorders
    - androgen insensitivity, 17–18
    - 5 $\alpha$ R deficiency, 18
    - environmental endocrine disrupting chemical exposure, 19
    - MALD1 gene mutations, 19
  - gender orientation, choice of, 20–21
  - gonadal dysgenesis
    - chromobox homolog 2 gene mutation, 16
    - DAX1 gene, 15–16
    - NR0B1 gene, 16
    - renal dysfunction, 15
    - SF1 gene mutation, 15
    - Sox9 gene abnormalities, 15
    - SRY gene abnormalities, 15
    - WT1 gene abnormalities, 15
  - testosterone production, defects in
    - 17-alpha-hydroxylase, 16
    - 3-beta-hydroxysteroid dehydrogenase, 16
    - 17-beta-hydroxysteroid reductase, 16
    - hypoplasia, 17
    - Leydig cell agenesis, 17
    - P450-oxydoreductase deficit, 16–17