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Menstrual Cycle Related Disorders

Volume 7: Frontiers in Gynecological Endocrinology

INTERNATIONAL SCHOOL OF GYNECOLOGICAL AND REPRODUCTIVE ENDOCRINOLOGY
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1 The Hypothalamic-Pituitary-Ovarian Axis and Regulation of the Menstrual Cycle

Frederick Naftolin, Ashraf Khafaga, and Margaret Nachtigall

1.1 Introduction

This chapter presents a focused, comprehensive, and rational framework for cataloging, considering, and assessing normal menstrual function, developmental changes, abnormalities, and downstream effects of normal and abnormal regulation. In this way it furnishes a framework upon which new diagnostic methods and treatments may be applied. The material is presented as a well-annotated lecture. The text is driven by figures, as would be the case in a formal presentation. References are used that support the message as well as furnishing a repository of fact.

1.1.1 The Menstrual Cycle

While the menstrual cycle has many "moving parts," it is the rational outcome of straightforward, hierarchical inducer-product feedback loops. Knowing the loops allows expectation of the function of the female reproductive system and forecasts the effects of breaks. The feedback loops are themselves products of predictable development, maturation, and senescence of the main organs contributing to the feedback loops, the hypothalamus, adenohypophysis, and ovary. As the function of each is not set in stone, their activity may regress or be overdriven by the individual women's current status.

The uterus, breasts, bones, metabolic tissues, central nervous system, and immune system are the most obvious targets of the ovarian steroids and therefore

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are the most obviously affected by breaks in the menstrual cycle. However, none of the tissues and systems in the body are indifferent to the sex steroids. Some of the most obvious effects will be cited as examples of normal/abnormal function.

Finally, although comprehensive, this introductory chapter has constraints of detail and scope; these will be addressed by the chapters that follow.

Normal reproductive function: A complete menstrual cycle includes the ovarian cycle (repetitive cycles of follicle development, ovulation, and the formation and demise of the corpus luteum) and the endometrial cycle (proliferation of the endometrium and differentiation of the gland epithelium to receive the implanting embryo). Failure of conception leads to shedding of the secretory functional layer of the endometrium which results in menstruation. Ensemble, these cycles average 28 days, depending on the length of the preovulatory phase of the ovarian cycle. The luteal phase is rather steady at 12 days (Fig. 1.1).

The menstrual cycle can be interrupted: Interruptions/failures in the cycle usually signal the lack of the succeeding events in that cycle. The interruptions can be permanent, as occurs after destructive procedures (surgery), infection, or organ failure such as ovarian failure due to exhaustion of follicles, or temporary. Examples of the latter include pregnancy, in which case the gonadotropin from the placenta drives the corpus luteum to continue to secrete estrogen and progesterone and maintain the secretory endometrium past the time that implantation has occurred, and the embryo is safe from menstrual shedding, and superfetation is blocked by the lack of

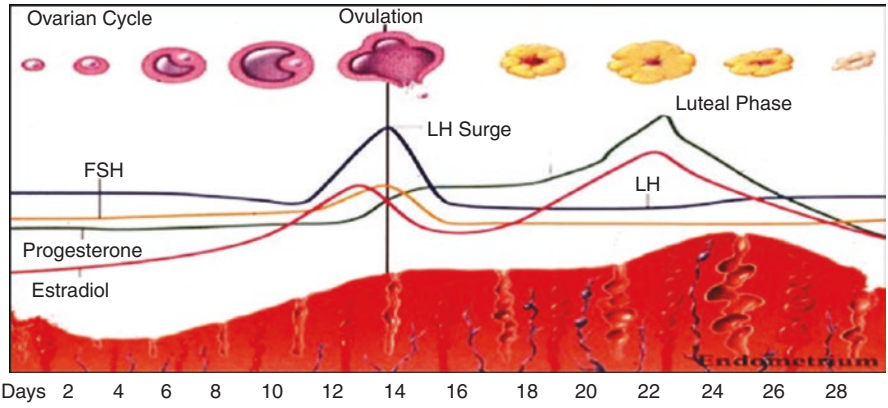


Fig. 1.1 The normal menstrual cycle. This is the reference for normal reproductive function. Since it includes ovulation, this drawing represents the mature (postpubertal) reproductive system and its interaction with the central axis (hypothalamus and adenohypophysis). The sex steroids from the ovarian follicle(s) and the gonadotropins from the central axis, are shown in temporal accordance with their respective origins and targets. Any failure of the cycle derails the menstrual cycle and the cycles that follow. There is no Day 0 because the menstrual cycles are continuations of the preceding cycle

ovarian follicle development and ovulation. Another common example is the regression to the prepubertal state, secondary hypothalamic amenorrhea (see below).

1.2 The Neuroendocrine Feedback Regulating the Menstrual Cycle

Control of the menstrual cycle requires three interwoven layers of hormonal regulation (see Fig. 1.2). The gonadotropin-releasing hormone (GnRH), a decapeptide which is secreted by hypothalamic neurons, must reach the GnRH receptors on the pituitary gland's gonadotrophs. GnRH is almost immediately metabolized in the blood; therefore, it is critical that GnRH is directly secreted by the GnRH neurons into the porous short pituitary portal vessels and goes directly to gonadotrophs [1]. The pituitary gonadotrophs express cell-surface GnRH receptors that regulate the production and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). LH and FSH are secreted into the systemic circulation and bind to receptors on ovarian stroma-theca cells and granulosa cells, respectively, to induce the enzymes that metabolize cholesterol to the ovarian steroid hormones, estrogens, androgens, and progesterone.

There are "interior" or secondary interactions that miter the overall regulation of the gonads. Inhibin B (follistatin) and activin are members of TGFβ family of glycoproteins that are secreted by the granulosa cells of developing follicles and act directly on the gonadotrophs to inhibit (inhibin) or activate (activin) the secretion of FSH. They appear not to affect hypothalamic function.

The ovary recovers after the completed menstrual cycle: During the days of this "follicular phase," the concentrations of the gonadotropins begin to wane, due to the rise of the circulating estradiol. Both FSH and LH, especially FSH, induce accelerated growth of 6–12 primary follicles. This appears to require the intermediary angiotensin, which is induced by the rising LH binding to receptors on the stromal cells, converting them to androgen-secreting thecal cells [2]. FSH induces differentiation of the inner layer of thecal cells to express estrogen receptors and to convert androgens to estrogen. Following this early proliferative phase of growth, which normally lasts for a few days, the mass of granulosa cells of the follicles develops a cavity, or antrum, with follicular fluid which is rich in estradiol.

Aromatase and follicle rescue: What determines the number of dominant follicles? The number of primary oocytes is always greater than the final number of surviving or "dominant" follicles that will go on to be ovulated. The determinant in the survival of ovarian follicles is the rate at which the granulosa cell-toxic androgens can be detoxified. The remaining follicles undergo atresia. This detoxification of androgens is accomplished by a demethylation enzyme, estrogen synthetase (aromatase) [3]. FSH induces aromatase, thereby determining the number of rescued follicles and offspring per cycle.

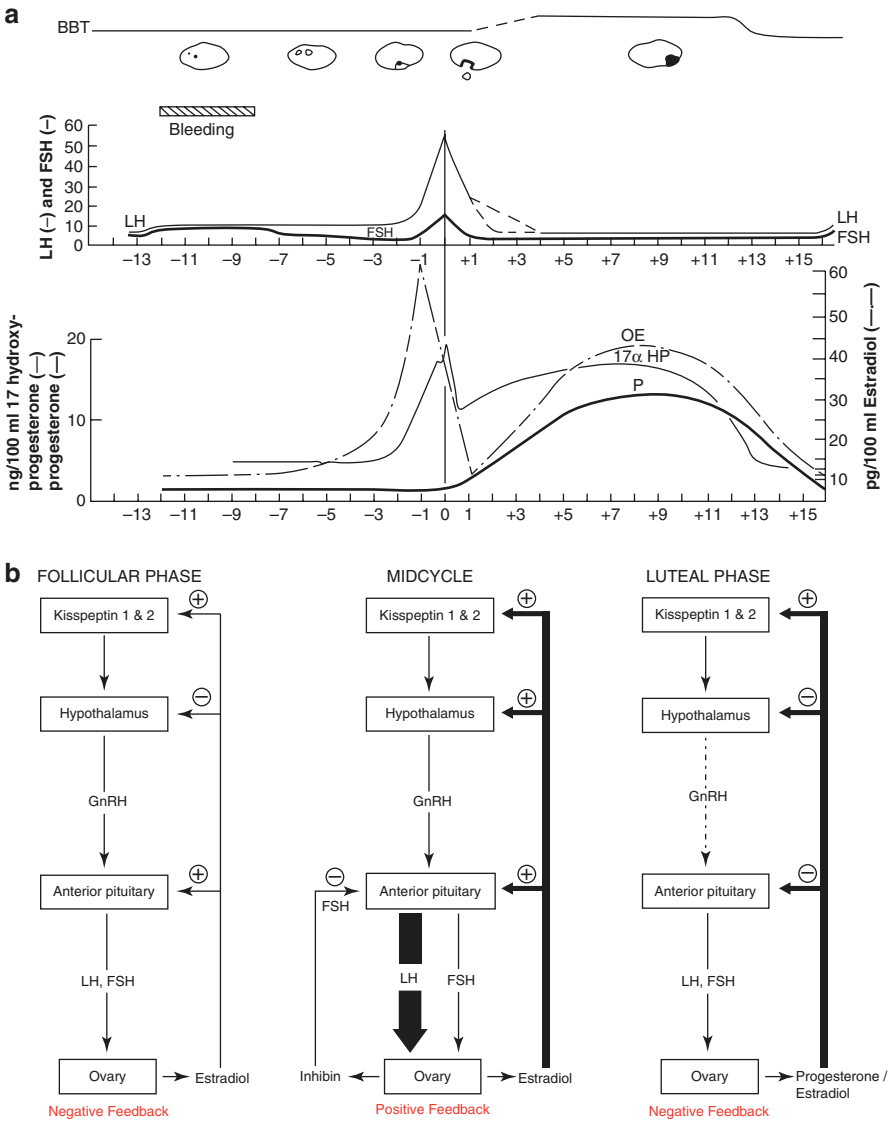


Fig. 1.2 The main elements of neuroendocrine control of the gonadotropins. The gonadotropins are secreted by the gonadotrophs in the anterior pituitary gland. The size and frequency of the pulses are regulated by the secretion of the hypothalamic peptide GnRH. The main regulator of this "negative feedback" is estradiol that is secreted by the developing ovarian follicles. In the postovulatory period, the combination of estradiol and progesterone secreted by the corpus luteum suppresses the GnRH sufficiently to achieve the lowest levels of gonadotropins in the cycle. During the 2–4 days preceding the LH surge, the estradiol levels rise dramatically, resulting in a precipitous fall of GnRH and LH, while the expression of GnRH receptors increases. At the point of maximal estrogen secretion, on day 12 or 13, GnRH secretion increases, releasing massive pulses of LH (positive feedback)

1.3 Feedback Regulation and Gonadotropin Secretion (Figs. 1.2 and 1.3)

The relationship between the central axis (hypothalamus and adenohypophysis) and the gonad (ovary) has been characterized using engineering terms. Although not perfectly accurate, the use of the term "gonadostat" is so widely understood that these terms are easily recognized descriptors of the feedback control of the gonadotropins by the ovarian secretions.

Among the ovarian sex steroids, estradiol has the strongest effect on the secretion of LH and FSH. In the main, this effect is through its effect on GnRH secretion (see below). This inhibitory effect is augmented by progesterone, even though progesterone by itself has a very limited effect on gonadotropin secretion. In this chapter, unless noted, effects of estradiol are used in describing feedback relationships between the central axis and the ovary.

The hypothalamic-pituitary-gonadal (central) axis exerts fundamental and multilevel effects on the female reproductive system.

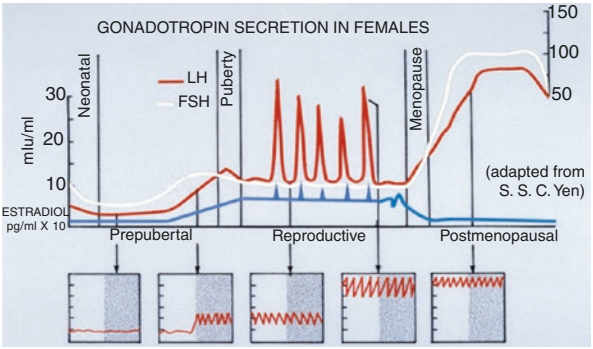


Fig. 1.3 A descriptive overview of the neuroendocrine interactions during a woman's life. The lower panel is a magnified picture of the gonadotropin pulses and resultant circulating levels during the 24-h day. Except for the dramatic peaks during the reproductive period, the relationship between estradiol and the gonadotropins is negative or reciprocal feedback, rising levels of estradiol are met with lowering of the gonadotropins. The higher sensitivity of the central axis/gonadostat to estradiol during childhood/prepuberty may be compared to the much less sensitive feedback post-menopause when the same low levels of estradiol drive the gonadotropins to their highest levels. The dampening effect of inhibin on FSH secretion is seen during the reproductive period. Midcycle positive feedback is represented by the peaks of both estradiol and LH/FSH during the reproductive period. Note that the high levels of LH are the product of augmented peaks of LH rather than increased frequency of the peaks. Syndromes such as hypothalamic secondary amenorrhea, in which there is high sensitivity to estradiol, pass through diurnal variation as they regress to the prepubertal picture and pass back through a puberty-like dynamic as they improve

The hypothalamus: Originating in the hypothalamic neurons that extend axons into the median eminence, the master hormone GnRH is secreted directly into porous vessels of the pituitary portal venous system [1]. The GnRH is secreted in pulses lasting 5–25 min and which occur every 1–2 h (Fig. 1.3) [4]. The neurons connecting to the GnRH neurons are sensitive to the negative feedback induced by estradiol [5–7].

The adenohypophysis: GnRH controls the synthesis and the release of FSH and LH from gonadotrophs in the glandular anterior hypophysis (adenohypophysis). The secretion of LH is under the control of the pulsatile GnRH [8]. FSH and LH are dimers consisting of a common α-subunit plus a hormone-specific β-subunit. They are secreted directly into the systemic circulation, and cleared by the kidney, the product of the secreted hormone less the cleared hormone constitutes the momentary circulating level [9].

The ovary: FSH and LH bind to receptors in the ovarian target cell membranes, which leads to stimulation of ovarian follicle development and proliferation, ovulation, and corpus luteum development and function. All these actions lead to both cellular differentiation and multiplication plus secretion of sex steroids (progestins, androgens, and estrogens) and, in the case of the granulosa cells, activin and inhibin B [3, 10].

The corpus luteum: The corpus luteum is an autonomous structure that secretes estrogen and progesterone. The secretion is dependent on the number of luteinized granulosa cells in the corpus luteum. The normal life span of the corpus luteum is 12–14 days. As its cells undergo apoptosis, the lack of sex steroid support of the endometrium results in degeneration and sloughing of the functionalis layer of the endometrium, menstruation.

If conception occurs, the chorionic gonadotropin (hCG) secreted by the trophoblast delays luteal cell apoptosis for up to 6 weeks. This is an effect of the secretion of hCG by the embryo's trophoblast.

The continuation of corpus luteum steroids also blocks the growth of activated Graafian follicle, avoiding superfetation [11].

1.3.1 Negative or Reciprocal Feedback

The principal source of the steroids controlling the gonadostat is the ovarian follicle(s). The follicular phase of the menstrual cycle is characterized by a sustained and marked increase in estradiol secretion and a decrease in FSH level, compared to the subtler fall in LH. This more acute response of FSH may be due to the inhibin B which is secreted by the developing granulosa cells. The rising levels of estradiol induce endometrial proliferation.

The circulating estrogen induces GnRH receptors on the surface of the gonadotrophs, sensitizing them to GnRH. But, the secretion of GnRH is simultaneously decreased by the circulating estradiol, a situation in which ovarian estrogen is both tensioning the bowstring of gonadotropin release (GnRH receptors) and staying the release of the arrow, GnRH.

1.3.2 Positive Feedback

At the peak of follicle development, the oocyte has passed through it first meiotic division and is being restrained from completing the second meiotic division that will allow it to be fertilized by the sperm. This maturation division is blocked by the high levels of estradiol in the follicle fluid. The high estrogen levels in the follicle fluid also are driving the preparation of the unraveling of the follicle wall and being secreted at levels that sensitize the pituitary gonadotrophs while suppressing GnRH secretion. When all of this development is correctly aligned, a cascade of estradiol escapes the follicle to trigger the release of the gonadotropins [12]. This is known as positive feedback because the rise, not the fall, of estradiol results in an increase of LH, rather than suppressing LH release [13].

Ovulation is a process rather than an event. The LH peak requires 12 h to complete the second maturation division of the ovum and express the oocyte-cumulus complex through the weakened follicle wall. This furnishes a mature oocyte that is prepared for insemination by incoming sperm. Since the shelf life of the oocyte for insemination is short, all aspects of positive feedback and ovulation must be synchronized. Estradiol performs that synchronization.

During the first few hours after expulsion of the ovum from the follicle, the remaining granulosa and theca cells change rapidly into lutein cells. This process is known as luteinization, and the total mass of cells together is called the corpus luteum.

1.3.3 Controversy Over The Mechanism Of Positive Feedback

The mechanism of positive feedback is still controversial. Our group has revealed that the marked preovulatory rise in the estradiol level induces a fall in the ratio of inhibitory to excitatory (I/E) synapses targeting the GnRH cells. This results in stimulation of the GnRH neurons, leading to a massive augmentation of gonadotropin secretion ensues [14]. But LH surge dominates because the secretion of inhibin B from the developing ovarian follicles partially inhibits the FSH response to GnRH. Others suggested that the underlying mechanism of the positive feedback is not influenced by the changes in the GnRH levels, estradiol by itself is capable of inducing the LH surge in experimental monkeys whose hypothalamus is cauterized, and the GnRH is replaced by constant pulses of GnRH [4, 5, 15]. However, though widely accepted, this is not a tenable explanation; it has been proven that GnRH expression and secretion fall during the preovulatory surge of estradiol and surge at the time of the rise of LH [12].

The picture of positive feedback has been further illuminated by the discovery that estradiol induces the secretion of the hypothalamic peptide kisspeptin that induces GnRH secretion [16]. This implies that the increased excitatory to inhibitory ratio of synapses on the hypothalamic neurons at the time of the estrogen-induced synaptic plasticity and the LH surge may be made up of kisspeptin synapses. The possibility is presently under study.

By the formation of the corpus luteum and the corpus luteum granulosa, cells start to produce estradiol and progesterone, the luteal phase. This includes the differentiation of the endometrial epithelial cells to secrete mucus-containing proteins that support the embryo until it begins to transcribe its own DNA. There also is an inflammatory reaction (Arias-Stella) that supports implantation [17].

The high levels of estradiol and progesterone result in fewer pulses of the gonadotropins, contributing to the lowest average levels during the cycle [9]. In the absence of conception, the corpus luteum degenerates, with a decrease in the level of estradiol and progesterone, resulting in the shedding of the endometrium, and this is the beginning of the next menstrual cycle. If pregnancy intervenes, the continued secretion by the corpus luteum will halt follicle development. This avoids superfetation.

1.4 Puberty

Puberty refers to the changes from a period of inactivity of the reproductive system to adult activity. It does not occur until the pre-pubertal child is able to develop fertile eggs, has the apparatus to support implantation, carry a pregnancy, deliver the fetus and raise the child until it can be on its own, see below. It is a time when hypothalamic maturation and synaptogenesis occur, which support the menstrual cycle [18]. This ushers in the synchronization of the elements of the menstrual cycle to achieve adult, reproductively competent individuals. Since reversion to a prepubertal feedback control of the gonadotropins is a common cause of amenorrhea and infertility, it is clinically important to understand the mechanics of the transition from childhood to adulthood (see Fig. 1.3).

Fetal life and childhood are periods during which there is oocyte activation without continuing folliculogenesis. This is due to the low levels of FSH during these periods. The cause is low expression of GnRH [19]. Also, in the absence of circulating estradiol, the gonadotrophs are not sensitized, as shown by the weak response to administered GnRH [20]. During puberty these conditions are reversed, and the gonadotropins rise in response to the low levels of estradiol; this results in complete folliculogenesis and the development of secondary sex characteristics, including menstruation, if ovulation occurs. If not, there is breakthrough bleeding due to the overgrowth of the endometrium.

At age 9–12 years, the adenohypophysis begins to secrete progressively more FSH and LH [21]. The pulsatile pattern of circulating gonadotropins concentration indicates that gonadotropins secretion is episodic, with pulses generally occurring at 30–120-min intervals [22]. With the onset of puberty, the gonadotropins initially rise at night, resulting in diurnal rhythm. As puberty proceeds, the levels of the circulating gonadotropins during the day rise to meet the nocturnal levels [23].

Expression of kisspeptin by hypothalamic neurons connecting to the GnRH neurons plays a major role in the transition from a noncyclic to a cyclic

reproductive endocrine system. Kisspeptin acts as the gatekeeper of puberty as it modulates the gonadostat set point to require more circulating estradiol to suppress the GnRH and gonadotropins [16, 24]. Kisspeptin is a potent stimulator of GnRH release and is encoded by Kiss 1 gene. In this manner, kisspeptin neurons transmit signals regarding the likelihood of successful reproduction to the GnRH cells.

Signals that regulate the onset of puberty have been known for some time. Expression has been known for some time to include body composition. Frisch studied pubertal girls and reported that weight and body fat mass reach a critical point at the onset of puberty [25]. While our studies on puberty in monkeys also showed metabolic indicators of the onset of puberty, the reaching of a critical level of muscle mass was a more accurate predictor of puberty than body fat [26]. Fat and muscle are now known to express proteins that are thought to be messengers of the metabolic state of the individual [27]. Recently, several "new" hormones have been proposed to regulate kisspeptin expression and could be the intermediaries between metabolism and puberty [28].

1.5 The Climacteric

Around 35 years of age, follicular development starts to deteriorate, and fertility begins to wane [29]. This is the beginning of the last portion of reproduction. At about 45–55, the cycles become erratic, and the amounts of estradiol secreted begin to show great variation (see Fig. 1.3). This results in wide swings in FSH and LH [30]. There are often pronounced menopausal symptoms, such as hot flushes, sleep disturbances, mood changes, vaginal atrophy, and loss of libido, during this instability. Finally, between 50 and 54 years of age, the last responsive activated ovarian follicles are exhausted, and there is cessation of menstruation or breakthrough bleeding. After 1 year, the milestone of menopause is reached. This is a normal form of secondary amenorrhea and characterized by the high levels of gonadotropins and low levels of estradiol (Fig. 1.3).

1.6 Menstrual Cycle Abnormalities

As an introduction to the following chapters of this book, we have developed the following three figures. Figure 1.4 summarizes the interplay of the involved organs and their function-linked regulation that lead to normal menstruation (defined as uterine bleeding after an ovulatory cycle). Figure 1.5 summarizes common breaks in the system and shows the downstream effects that follow these breaks. The concept of downstream disease opens the way to examining both the causes and effects of dysfunctional or disrupted interactions between the factors that make up adult reproduction. Figure 1.6 lays out the basic elements of diagnosis, upon which the following chapters are built.

In order to assess central axis disorders that may cause an abnormal ovulation-menstruation cycle, LH and FSH assay is mandatory. Suspected cases of abnormal prolactin can be assessed by prolactin level assay. Ovarian tumors can be excluded by ultrasound, as well as the pelvic exam. Specific products of functional ovarian

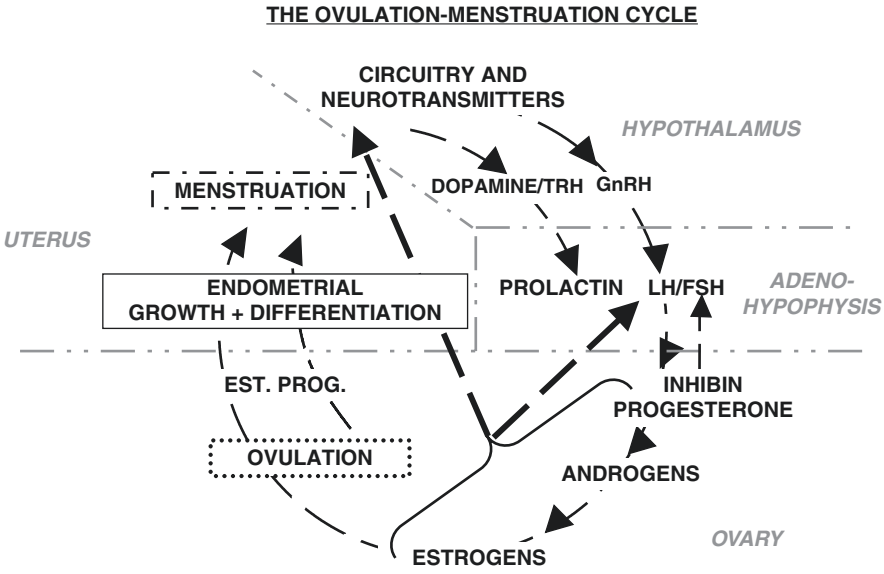


Fig. 1.4 The ovulation-menstruation cycle requires proper function of the organs in gray, the hypothalamus, the adenohypophysis, the ovaries, and the uterus. The hypothalamic neurons are the primary drivers for the menstrual cycle. They produce the master hormone GnRH which induces the secretion of FSH and LH from the anterior pituitary gland adenohypophysis. Additionally, dopamine and thyrotropin-releasing hormone (TRH) regulate the release of prolactin from the adenohypophysis. Both FSH and LH regulate ovarian follicle development, ovulation and postovulation preparation for conception and implantation, or menstruation to clear the way for the next menstrual cycle. The primary ovarian hormones progestins, androgens, and estrogens regulate the regeneration of the functionalis in the uterus and the postovulatory conversion to the secretory endometrium. Since the purpose of the menstrual cycle is successful reproduction, in the event of a non-fertile cycle, menstruation is the means of cleaning house and moving on to the next cycle

Fig. 1.6 The basic work-up of menstrual disorders is founded on the history of disease, reproductive history, and physical and laboratory exams. Some of these are listed in Fig. 1.6. It is important to keep in mind that there will be downstream consequences of the break on the chain of organ regulation. A good example is the secondary amenorrhea of the climacteric (menopause). The failure of ovarian folliculogenesis results in genital atrophy, as in the prepubertal child, and may have severe effects on sexual function. As well, the lack of estrogen deprives the liver of the stimulus to express thyroid hormone binding globulin, making for rapid clearance of thyroxine and functional hypothyroidism

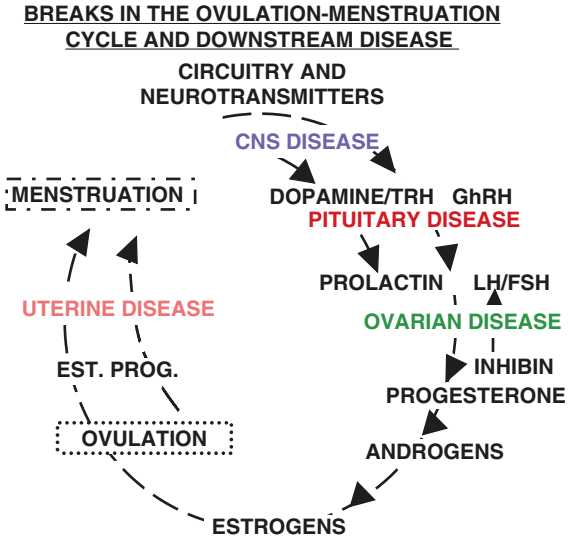
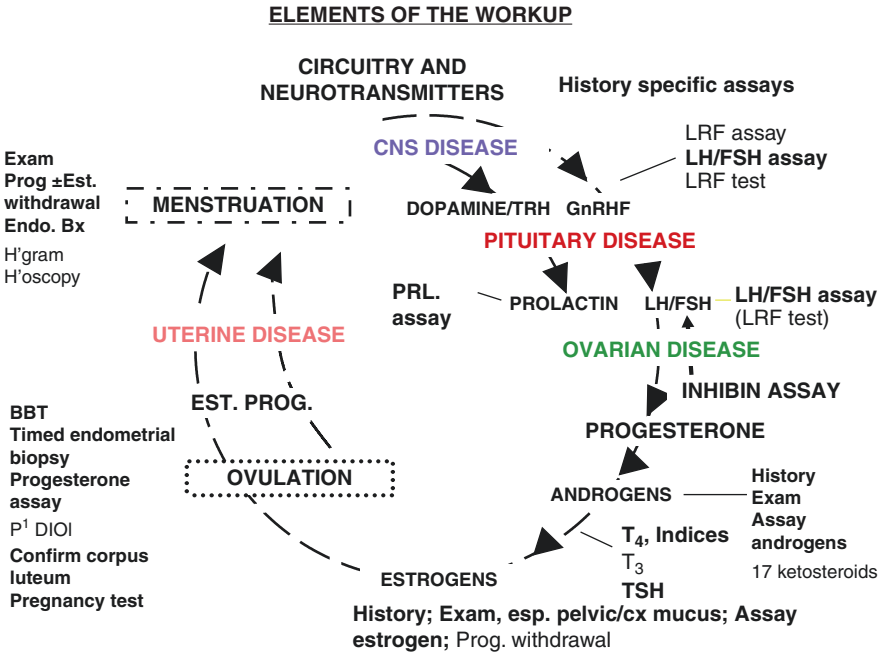


Fig. 1.5 Breaks in the ovulation-menstruation cycle lead to downstream disorders of the dependent components. The pre-pubertal child is an instructive form of primary hypothalamic amenorrhea. The hypersensitive set point of the gonadostat maintains very low gonadotropins in the face of almost unmeasurable levels of circulating estradiol. The results are an infantile genitalia, ovaries that lack follicles and corpora lutea, and an endometrium that is too atrophic to menstruate. The disease analog is secondary hypothalamic amenorrhea. Just as in puberty, correction of the underlying disease opens the way to re-establishing the normal organ relationships and menstruation



tumors may be tested, e.g., estrogen in the case of granulosa cell tumors and androgens in the case of comas. Abnormal androgen levels can be diagnosed by medical history and physical examination and confirmed by androgen assay. Thyroid disorders are assessed by monitoring the TSH and free T4 levels. Disorders affecting estrogen levels can be suspected from the history, physical examination, and ultrasound evaluation of the endometrium. Surprisingly little additional is needed if a very careful history and physical examination are performed, with most additional testing used to confirm or deny the preliminary diagnosis.

1.7 Conclusions

Reproduction is critical to the survival and fitness of our species; the reproductive system is evolved for maximally efficient reproduction. However, the contemporary times have allowed women to control reproduction in both directions, pausing it or stimulating the system to meet their needs. This ability has brought increased definition and urgency to the task of understanding the normal menstrual cycle and the diagnosis and treatment of abnormal menstrual function. While this burden is sometimes challenging, proper knowledge of the workings of the system will improve the success of diagnosing and treating menstrual disorders.

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Hypothalamic Amenorrhea

2

Sarah L. Berga

2.1 Introduction

Functional hypothalamic amenorrhea (FHA) is a form of hypothalamic hypogonadism that presents clinically as amenorrhea due to chronic anovulation. FHA is a diagnosis of exclusion and must be distinguished from all other causes of amenorrhea and anovulation. The proximate cause of chronic anovulation is insufficient hypothalamic GnRH drive to support folliculogenesis. Specifically, GnRH pulse frequency is too slow to drive sufficient release of pituitary LH and FSH secretion required to initiate and then sustain folliculogenesis. Chronically low levels of estradiol and progesterone lead to lack of endometrial development and thus absence of menses. The term functional implies that the condition is reversible and that the cause of the reduced GnRH drive is related to potentially modifiable factors that are viewed as stressors because they activate the hypothalamic-pituitary-adrenal axis and lead in increased cortisol secretion. FHA represents an adaptation to chronic stress that results not only in reduced GnRH drive but also a constellation of concomitant neuroendocrine adjustments including hypothalamic hypercortisolism and hypothalamic hypothyroidism [1]. The constellation of endocrine adaptations associated with FHA represents an allostatic state that diverts and directs metabolic and psychological energy to acute and chronic challenges. Given the energetic expense of reproduction, it is no surprise that metabolic factors play a fundamental role in gating reproductive function.

Evidence that stress is the cause of FHA includes the demonstration that cortisol levels are higher in women with FHA than in those who are eumenorrheic and ovulatory [1] and also higher than in women with other forms of anovulation [2].

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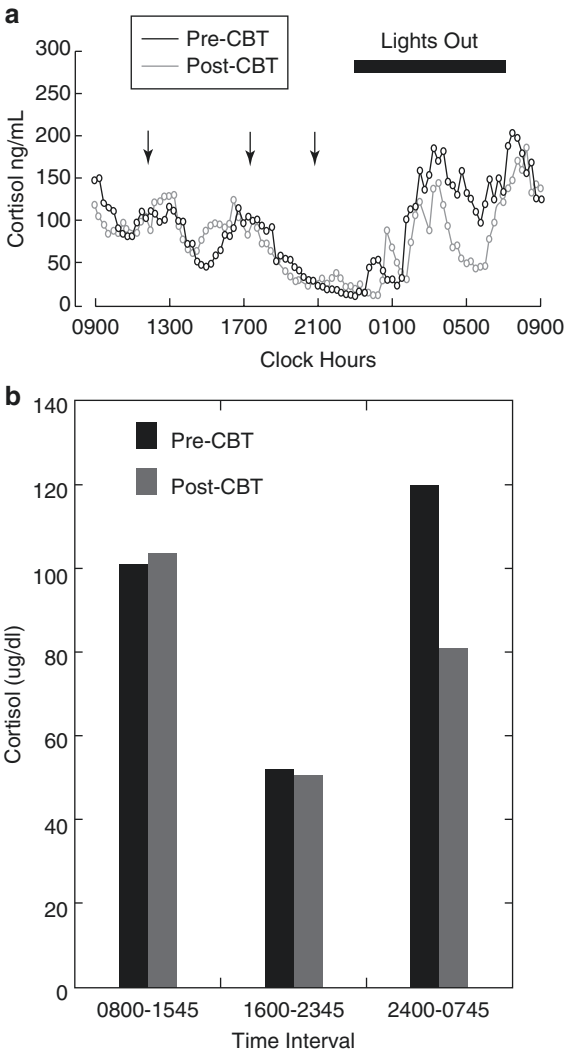

Fig. 2.1 (**a**) Circulatory cortisol concentrations (ng/ml) in 15 min intervals over 24 h in a woman with FHA before (pre) and after (post) CBT (cognitive behavior therapy). Meals are indicated by arrows. (**b**) Circulatory concentrations of cortisol displayed as 8-h mean in the same woman before (pre) and after (post) CBT [3]

Additionally, as shown in Fig. 2.1, recovery from FHA is associated with a reduction in circulating cortisol levels [2, 3]. In practice, detecting hypercortisolemia is difficult clinically because cortisol is primarily increased at night [1, 3] and the cortisol level from a single daytime blood sample is unlikely to be outside the normal range (Fig. 2.1). FHA may also follow chronic or severe illness such as cancer or pneumonia. Indeed, the term functional means that the hypothalamic GnRH-pituitary-gonadotropin apparatus is anatomically and physiologically intact and therefore capable of generating LH pulses and causing pituitary secretion of FSH. In clinical practice, it is often difficult to exclude insufficient GnRH drive due to mutations in genes critical to GnRH ontogeny and function, and it has been hypothesized that women with genetic mutations may be more sensitive to commonplace stressors [4].

2.2 Differential Diagnosis

FHA is a diagnosis of exclusion, so the following must be excluded before the diagnosis of FHA is rendered. The differential diagnosis includes (1) Mullerian anomalies, congenital and acquired, that block the outflow tract, including vaginal agenesis, imperforate hymen, and Asherman syndrome; (2) ovarian causes of anovulation such as gonadal agenesis, Turner's syndrome (45, XO), polycystic ovary syndrome, and premature ovarian insufficiency (premature menopause); (3) other endocrine conditions such as primary hyper- and hypothyroidism and Cushing's syndrome and disease; (4) pituitary causes such as pituitary adenomas and hyperprolactinemia; and (5) organic central causes such as meningioma and Kallmann syndrome and variants. Table 2.1 presents the endocrine concomitants of common causes of amenorrhea due to anovulation [5].

Defining reproductive tract anatomy is the first step in excluding anatomic causes of amenorrhea, particularly if the patient presents with primary amenorrhea. Outflow tract anomalies often present as primary amenorrhea and require a physical exam and imaging such as ultrasound or MRI to exclude and/or define anatomic anomalies. Asherman syndrome may present as secondary amenorrhea and is due to injury of the endometrial basal layer that is necessary for the generation of the endometrial functional layer. Findings may include intrauterine scarring, synechiae, and/or adhesions. A history of dilation and curettage (D&C) for miscarriage, retained products of conception, or postpartum bleeding and/or pelvic infection should raise the index of suspicion for endometrial injury, and hysteroscopy is typically needed to establish the diagnosis of Asherman syndrome. Polymenorrhea may be due to intrauterine polyps or intramural fibroids rather than functional hypothalamic hypogonadism. A karyotype is needed to evaluate the possibility of gonadal dysgenesis and Turner's syndrome, although often physical stigmata are present. Amenorrhea in the setting of a 46, XY karyotype may reflect Mullerian agenesis and either (1) 5-alpha reductase deficiency causing insufficient production of dihydrotestosterone (DHT) from testosterone or (2) androgen insensitivity syndromes (AIS) due to variable androgen receptor sensitivity. In both of these conditions, the uterus regressed during development and the gonad is a testis, but its location may be inguinal or pelvic. If the karyotype is 46, XX, a testosterone level will be in the male range in 5-alpha reductase deficiency and AIS and very low in gonadal dysgenesis (Swyer syndrome).

History is important because physical examination often shows normal reproductive anatomy and external genitalia. Delineating the timing of pubertal milestones such as adrenarche and thelarche provides clues as does a history of weight gain and loss, dietary habits, and athletic endeavors. History must be supplemented with testing hormone levels as outlined in Table 2.1 to establish the diagnosis. A panel that includes LH, FSH, estradiol (E2), progesterone, TSH, thyroxine, prolactin, and androstenedione detects most important causes if properly interpreted. The pattern of hormone levels is more critical than absolute values. In FHA, FSH will be in the normal range, and typically it will be slightly higher than LH, which will also be in the low-normal range, with E2 less than 50 pg/ml and progesterone

Table 2.1 Differential diagnosis of anovulation (Adapted from [5])

	LH IU/L	FSH IU/L	LH: FSH	E2 pg/ml	P4 ng/ml	AMH ng/ml	Prolactin	TSH	T4	A'dione	DHEAS	17OHP	Testosterone
Functional hypothalamic anovulation	<10	<10	~1	<50	<1	>1	Low nl	Low nl	Low nl	Low nl	Nl	Nl	Low nl
Ovarian insufficiency; menopause	>15	>15	FSH > LH	<50	<1	<0.5	Nl	Nl or ↑	Nl or ↓	Low nl	Nl	Nl	Low nl
Polycystic ovary syndrome	<15	<10	LH > FSH	<50	<1	Nl or ↑	High nl	Nl	Nl	High nl or ↑	High nl	Nl	High nl or slight↑
Attenuated CAH	<15	<10	LH > FSH	<50	≤1	Nl	Nl	Nl	Nl	Low nl or nl	High nl	↑	↑
Hyperprolactinemia	<10	<10	LH < FSH	<50	<1	Nl	↑	Nl or ↑	Nl	Nl	Nl	Nl	Nl
Hypothyroidism	<10	<10	LH < FSH	<50	<1	Nl or ↓	High nl or ↑	↑	↓	Low nl or nl	Low nl or nl	Nl	Nl

less than 1 ng/ml. Very low LH and FSH levels suggest organic HA due to genetic mutations affecting GnRH ontogeny and function or central causes such as brain or pituitary tumors. Anosmia indicates Kallmann syndrome, which is failure of GnRH neurons to migrate from the olfactory placode to the hypothalamus. Androstenedione (or testosterone) is typically in the lower range of normal except when FHA is superimposed on polycystic ovary syndrome, both TSH and thyroxine will be in the lower range of normal indicating hypothalamic hypothyroidism or sick euthyroid syndrome, and prolactin will be in the low normal range. It is critical to exclude chronic health conditions that may be a cause of undernutrition such as food allergies including gluten enteropathy (celiac disease).

In contrast, elevated LH and FSH with low E2 and progesterone indicate low or absent ovarian reserve or premature menopause. High LH and FSH with E2 > 150 pg/ml and progesterone less than 2 ng/ml indicates a midcycle gonadotropin surge. If TSH is low and thyroxine is high, then one must consider the possibility of autoimmune hyperthyroidism (Grave's disease). Similarly, if TSH is in the upper limit of normal with thyroxine in the lower range of normal, then autoimmune thyroiditis and hypothyroidism must be considered, and the next step would be to measure antithyroid antibodies such as thyroid peroxidase and thyroid-stimulating immunoglobulin. If frank hyperprolactinemia is found, additional evaluation is needed. Prolactin levels are elevated by food, sleep, exercise, coitus, nipple stimulation, physical examination, lactation, and many medications. Acromegaly may present with oligo- or amenorrhea and an elevated somatomedin-C (IGF-1). Diabetes may present as oligo- or amenorrhea due to reduced GnRH drive. Amenorrhea and anovulation associated with Cushing's syndrome and disease is also due to reduced GnRH drive.

While the cause of FHA is stress, the increase in cortisol secretion in FHA is less than that seen with Cushing's syndrome and disease, and the circadian pattern is preserved, so the cortisol increase is highest at night during sleep and in the early morning hours before intended wake-up time [1, 3]. If Cushing's is suspected, a 24-h urinary free cortisol (UFC) is a reasonable screening test. Secondary adrenal insufficiency may present as fatigue and anovulation. Serum dehydroandrosterone sulfate (DHEAS) will be in the lower range for age. The differential diagnosis of low normal DHEAS includes Sheehan's syndrome with partial or complete pituitary apoplexy. Provocative stimulation testing helps to establish pituitary hypofunction due to injury, tumor, autoimmune hypophysitis, or other CNS conditions. History is essential for elaborating the differential diagnosis and guiding the evaluation.

MRI of the pituitary and the brain should be considered to exclude or confirm serious CNS conditions [6]. A history of trauma should raise the index of suspicion to include pituitary stalk damage. The differential diagnosis of central lesions and conditions is extensive. A high index of suspicion and a low threshold for obtaining an MRI or other relevant neuroimaging are recommended.

Certain medications and drugs of abuse suppress GnRH drive or cause other endocrine perturbations. Chronic drug use is often a marker of stress and undernutrition. An evaluation for syndromal psychiatric conditions such as eating disorders,

depression, and personality disorders is critical. Formal psychiatric evaluation may be indicated. Psychiatric conditions are associated with activation of the hypothalamic-pituitary-adrenal axis, and appropriate treatment may reverse the functional suppression of GnRH drive. Unfortunately, stress often drives individuals to take drugs and medications that alone may suppress GnRH drive. Opioid use is both mechanistically and clinically linked to suppression of GnRH and the development of hypothalamic hypogonadism in both men and women [7]. Psychotropic use may induce hyperprolactinemia which can secondarily suppress GnRH drive [8]. Marijuana and alcohol have been linked to inhibition of GnRH drive [9]. Exercise and dietary restrictions and excesses also gate GnRH drive. In short, medications, drugs, and lifestyle adjustments initiated in response to psychological stress may interact synergistically to suppress ovarian function. If the root cause for substance use and abuse is stress, then clearly the management must involve stress as well as addiction management. The suppression of GnRH by a variety of stresses can be blocked by CRH antagonists, suggesting a pivotal role for endogenous CRH [10, 11].

2.3 Pathophysiology

Functional hypothalamic amenorrhea is more than an isolated disruption of GnRH drive. Indeed, in FHA, activation of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis disrupts GnRH pulsatility and simultaneously suppresses the hypothalamic-pituitary-thyroidal (HPT) axis to thereby reduce thyroxine (T4) and thyroxine (T3). Activation of the HPA increases GABAergic input to the hypothalamus that is reversed by a CRH receptor antagonist [11]. The constellation of neuroendocrine alterations characteristic of FHA reflects altered feedback sensitivity to estradiol, cortisol, and thyroxine. Other feedback sensitivities may be altered, including leptin and ghrelin signaling. Increased circulating and CSF cortisol levels are specific to FHA [2, 12]. Many of the clinical consequences of FHA such as osteoporosis reflect the clinical impact of the full constellation of neuroendocrine aberrations that accompany FHA causing a catabolic rather anabolic state [1, 12]. At the tissue level, cortisol directly interferes with estrogen action [13]. Further, as shown in Fig. 2.2, chronic hypercortisolemia may deplete hepatic glycogen stores to the point that the hepatic glycogenolysis needed to release glucose to meet energetic demands to acute challenges such as exercise cannot be met. Indeed, intermittent brain glucopenia in response to the challenges may be the critical signal that elicits the constellation of neuroendocrine adaptations that characterize FHA.

Reduced GnRH drive decreases circulating LH and FSH to levels too low to fully support folliculogenesis to the point of ovulatory adequacy. Amenorrhea is the most clinically recognizable manifestation of insufficient GnRH and occurs when there is sustained suppression of GnRH pulsatility to less than 50% of expected [1]. Because the suppression of GnRH exists on a spectrum, so too does the clinical presentation. More clinically occult forms result from intermittent suppression of GnRH that causes partial folliculogenesis that may manifest clinically as anovulatory cycles or luteal insufficiency. In luteal insufficiency, embryo-endometrial asynchrony may impair or prevent implantation. Unexplained infertility may well be infertility in the

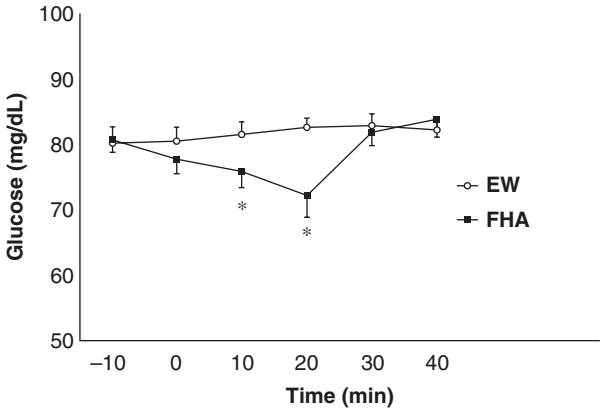

Fig. 2.2 Fig. 2.1: Mean ± SEM circulating glucose levels before ($t = -10$, 0 min), during ($t = +10$, +20 min), and after ($t = +30$, +40 min) a submaximal exercise challenge in eumenorrheic women (EW, ○) and women with functional hypothalamic amenorrhea (FHA, ■). *$p < 0.05$ for significant difference between groups (t-test). (Sanders KM, Kawwass JF, Loucks T, Berga SL. Heightened Cortisol Response to Exercise Challenge in Women with Functional Hypothalamic Amenorrhea. Am J Obstet Gynecol. 2017 Nov 20. pii: S0002-9378(17)32346-3. https://doi.org/10.1016/j.ajog.2017.11.579. PubMed PMID: 29170001)

present normal menstrual cycle intervals but insufficient progesterone to adequately prepare the endometrium for implantation. To complicate recognition, FHA and PCOS may coexist. Men can also develop functional hypothalamic hypogonadism due to insufficient GnRH drive that results in oligoasthenospermia with or without reduced testosterone levels [14].

Risk factors for the development and persistence of FHA include any factors that chronically activate the HPA axis. The impact of psychological stressors is amplified when those stressors lead to energy expenditure that exceeds availability. Commonplace examples include excessive exercise and nutritional restriction of protein and fats due to notions about what constitutes a healthy diet. Attitudes that activate the LHPA include unrealistic expectations of self and others, perfectionism, high need for social approval, and conditional love [15–17]. We found that the endocrine impact of stressors is synergistic rather than additive [18–20]. We also demonstrated that addressing problematic attitudes and behaviors using cognitive behavior therapy (CBT) not only restored ovarian function [21] but also reduced cortisol levels [2, 3, 22]. Recovery from FHA included amelioration of hypothalamic hypothyroidism and an increase in leptin levels independent of weight gain [3]. These data underscore the tight link between metabolic and reproductive function.

2.4 Acute and Chronic Health Consequences

Many of the health consequences linked to FHA are likely due to the combined alterations in metabolism, neuroendocrine function, and anovulation [3]. Health conditions that accrue from chronic FHA putatively include osteoporosis, syndromal psychiatric

conditions, and infertility. Longer-term health consequences may include an increased risk of cardiovascular diseases [23] and neurodegenerative diseases due to persistent stress and hypercortisolism [12]. Treatment of osteoporosis with bisphosphonates in women intending to become pregnant is discouraged because the bisphosphonates incorporate into maternal bone and can then be mobilized during pregnancy and incorporated into fetal bone. Hormone replacement regimens also have limitations and may not permit full bone accretion because glucocorticoids abrogate the impact of estrogens by competing for transcriptional co-activators and interfering with estrogen action [5, 13]. There is no reason to assume that chronic stress is benign or that hormone replacement regimens can fully counteract the clinical impact of chronic stress or fully reverse the constellation of neuroendocrine aberrations that accompany FHA.

2.5 Management

As the term functional implies, functional hypothalamic amenorrhea improves or even remits altogether when the allostatic load is reduced. Thus, when all other causes of FHA have been excluded, stress management is indicated. The goal of treatment is to manage rather than eliminate stressors and thereby reduce their neuroendocrine impact. Appropriate management of stress has been shown to ameliorate hypothalamic hypercortisolism and reverse concomitant neuroendocrine adaptations [3, 18, 21, 22]. Since stress is an inevitable consequence of living, psychoeducation about how to manage typical and specific stressors is more practical than trying to avoid or eliminate stressors. Our research indicates that most stressors associated with the development of FHA are seemingly minor. However, to the individual, the challenge may threaten critical elements of identity and worth. For practical purposes, stressors are often categorized as metabolic and psychogenic or behavioral, but it is important to recognize that the neuroendocrine cascade that follows is similar regardless of the behavioral antecedents [18]. Psychogenic stressors reflect cognitive expectations and often present as unrealistic attitudes that compromise coping with daily demands that are to some extent externally imposed. Typically, individuals with FHA report a combination of both metabolic and psychogenic variables [15–17] that we have shown interact synergistically [18, 20] to induce chronic hypothalamic hypogonadism. A simple way to think about stressors is that they threaten the individual locus of control and thus elicit behaviors that attempt to restore locus of control to self.

Women with FHA often display greater neurobiological reactivity to commonplace stressors than individuals who are ovulatory and presumably are more stress-resilient. Metabolic stressors such as exercise and nutritional restriction are often undertaken to reduce psychological stress and yet have the potential to serve independently as metabolic or even psychogenic stressors. As shown in Fig. 2.2, exercise elicited a greater rise in cortisol in women with FHA than ovulatory women, and the rise in cortisol was accompanied by a drop in circulating glucose during exercise in women with FHA that was not observed in eumenorrheic, ovulatory women who instead showed a rise in circulating glucose during exercise [19]. Clinicians would do well to remember that what is stressful to one individual may be less or more so to

another due to personal valences and underlying neurobiological reactivity. Identifying stressful attitudes and behaviors and teaching better coping styles have been shown to reduce the neuroendocrine impact of stressors [21, 22] and reverse the neuroendocrine concomitants including reduced leptin independent of weight gain [3].

Stress management can be undertaken before or concurrently with other interventions, including infertility therapy. In our pilot study, 75% of women with FHA randomized to cognitive behavior therapy (CBT) designed to address problematic attitudes and behaviors regained ovulatory ovarian function, whereas only 25% of those randomized to observation did [3, 21]. Clomiphene citrate is less effective in eliciting an increase in FSH release if the cause of anovulation is hypothalamic hypogonadism because of reduced feedback sensitivity to hypoestrogenism. The efficacy of aromatase inhibitors has not been studied in women with FHA. While antidepressants reduce hypercortisolemia in those with depression and PTSD, their use for treatment of FHA has not been studied. It is important to screen for syndromal psychiatric disorders, particularly eating disorders, and refer individuals so identified to appropriate psychiatric care.

The Endocrine Society recently published a clinical practice guideline on FHA that comprehensively addressed therapeutic options [5]. The authors concluded that oral contraceptive pills should not be used for the sole purpose of improving bone mineral density and patients who do use oral contraceptive pills should be cautioned that their use may not prevent ongoing bone loss. A meta-analysis included in the clinical practice guideline reported that there were no reliable data examining the relationship between hormone use and bone fractures and very limited data on their impact on bone density in women with FHA. The authors concluded it is unlikely that hormone use will improve bone density or foster bone accretion in women with FHA because ongoing hypercortisolism, hypothyroidism, and undernutrition place women with FHA in a catabolic rather than anabolic state. As noted earlier, bisphosphonates should not be used in women intending to become pregnant as they may become incorporated into the fetal skeleton. Further, there are good data demonstrating that cortisol directly interferes with estrogen action [13, 24]. Since there are no data to suggest that instituting hormone therapy for other indications is harmful and women with FHA may spontaneously recover, those wishing to avoid pregnancy should use some form of birth control. However, hormonal contraception may not confer the same benefits such as cardioprotection as touted for eumetabolic women, and hormone use should not be expected to reverse the metabolic impact of stress. The situation is similar for vitamin and nutrient supplementation. Regardless of menstrual status, women need sufficient dietary calcium intake and vitamin D levels. However, neither exogenous vitamin D nor increased calcium intake will overcome the hypoestrogenism and catabolism induced by chronic stress.

Women with FHA often seek treatment for infertility. However, clomiphene and letrozole may prove ineffective as the hypothalamic GnRH drive is suppressed by mechanisms other than estradiol feedback inhibition and antagonizing estradiol action does not lead to increased GnRH drive. In our recent Endocrine Society clinical practice guideline on FHA [5], we recommended exogenous pulsatile GnRH be offered for ovulation induction because the approach is more physiological and more

likely to result in monofollicular development and less likely to result in multiple gestation than is the use of injectable gonadotropins [25]. However, many patients with FHA undergo gonadotropin therapy and assisted reproductive therapies (ART) to treat the infertility associated with FHA. The pros and cons of these approaches are open to debate. The risks are many, including multiple gestation and high expense. At the very least, women with low body mass index should not be treated for infertility. In our recent Endocrine Society clinical practice guideline on FHA [5], we recommended a minimum BMI of 18.5 kg/m^2; however, we also noted that women with a BMI < 20 kg/m^2 have a fourfold higher risk of preterm labor [26] and the infants have lower birth weights [27]. Therefore, we recommended that clinicians limit ovulation induction to women of satisfactory body weight. Independent of weight, potential maternal and fetal consequences of ongoing maternal stress and undernutrition include preterm labor [28], intrauterine growth restriction [27], and fetal neurodevelopmental disorders such as learning disabilities and autism spectrum disorders. ART is likely to increase, rather than decrease, metabolic and psychogenic stress. Indeed, controlled ovarian hyperstimulation with gonadotropins has been demonstrated to increase TSH levels and presumably metabolic demand due to multiple folliculogenesis associated with ovarian hyperstimulation [29]. Women conceiving by ART had a greater increase in TSH earlier in gestation, indicating a greater need for thyroxine early in gestation [30]. Thus, ART may amplify the neuroendocrine consequences of pre-existing stress, exposing oocytes, the mother, and the fetus to persistent neuroendocrine aberrations including lower thyroxine and higher cortisol levels. Since the mother is the sole source of thyroxine in the first trimester and since thyroxine is critical for fetal neuronal migration and differentiation [31, 32], fetal neurodevelopment may be compromised [33]. Further, even intermittent hypercortisolemia may accelerate placental aging as indicated by telomere shortening [34] and induce epigenetic changes in fetal DNA. Ultimately, the consequences of these exposures may be to potentiate the fetal origins of adult disease.

2.6 Conclusions

Functional hypothalamic amenorrhea (FHA) is a reversible form of anovulation that is not due to organic causes. While the proximate cause is insufficient GnRH drive, FHA is more than an isolated reduction in GnRH. Behavioral and psychological factors such as undernutrition, excessive exercise, and unrealistic cognitions serve as stressors that elicit hypothalamic hypercortisolism and a cascade of neuroendocrine adaptations including hypothalamic hypothyroidism. The neuroendocrine constellation conserves and diverts energy to perceived challenges and thereby promotes short-term survival at the expense of longer-term health. Behavioral interventions such as cognitive behavior therapy that address problematic attitudes and behaviors reduce hypothalamic hypercortisolism and restore ovulatory eumenorrhea. Hormonal therapies offer less promise, but contraception should be offered for those wishing not to conceive as spontaneous recovery of ovulation precedes menses. Infertility treatment should be undertaken with

caution. The role of psychotropic medications has not been appropriately studied. Because of the many health consequences of chronic stress and hypoestrogenism, women with FHA should be closely followed by an appropriate team of physicians and other health-care providers. FHA is a prototypic example of psychoneuroendocrinology and illustrates how cognitions and emotions drive behaviors that eventuate in neuroendocrine allostasis.

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Kisspeptin Role in Functional Hypothalamic Amenorrhea

3

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3.1 Functional Hypothalamic Amenorrhea (FHA)

3.1.1 Definition

FHA is the term used to describe amenorrhea that results from low energy availability (from decreased caloric intake and/or excessive energy expenditure) and stress. Such states are common causes of hypogonadotropic hypogonadism in women. FHA is diagnosed after excluding other etiologies of amenorrhea [1, 2].

FHA is a disorder that, by definition, excludes organic disease. It is based on functional disruption of pulsatile, hypothalamic gonadotropin-releasing hormone (GnRH) secretion. The abnormal GnRH secretion characteristic of FHA leads to decreased pulses of gonadotropins, absent mid-cycle surges in luteinizing hormone (LH) secretion, absence of normal follicular development, anovulation and low serum oestradiol (E2) concentrations, i.e. hypothalamic-pituitary-gonadal axis (HPG) failure. Variable neuroendocrine patterns of LH secretion can be seen. Serum concentrations of follicle-stimulating hormone (FSH) are low or normal and often exceed those of LH, similar to the pattern in prepubertal girls [2].

3.1.2 Epidemiology

FHA is responsible for approximately 25–35% and 3% of secondary and primary amenorrhea cases, respectively. Primary amenorrhea is often classified as absence of menarche by age 15 years, while secondary as absence of menses for more than 3 months in females who previously had regular menstrual cycles or 6 months in girls or women who had irregular menses. Amenorrhea lasting 3 months or more

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and oligomenorrhea (fewer than 9 menstrual cycles per year or cycle length greater than 35 days) require investigation [3].

Multiple factors may contribute to the pathogenesis of FHA including:

- Both weight loss below a certain target level (approximately 10% below ideal body weight), with nutritional deficiencies or eating disorders, such as anorexia nervosa
- Excessive exercise, often associated with weight loss (especially running, ballet dancing, figure skating or gymnastics)
- Emotional stress and stress induced by illness (e.g. myocardial infarction, severe burns)

In female athletes, the combination of low energy availability, subsequent HPG axis inhibition resulting in menstrual dysfunction and low bone density is called the "female athlete triad" [4].

There is marked interpatient variability in the degree of weight loss or exercise required to induce amenorrhea. This may in part be due to an underlying genetic predisposition in susceptible individuals. Heterozygous mutations in some of the same genes (*KAL1*, *FGFR1*, *PROKR2*, *GNRHR*) have been identified in women with functional hypothalamic amenorrhea [5].

3.1.3 Signs and Symptoms

Because women with FHA are oestrogen deficient, the symptoms are the consequences of oestrogen deficiency, including:

- Primary or secondary amenorrhea, anovulatory infertility. FHA is a reversible cause of infertility, which resolves over a period of time after energy availability normalizes or any underlying stress contributing to FHA resolves. The most subtle menstrual cycle abnormality associated with FHA is an abnormal luteal phase, which may prevent implantation or nourishment of the developing embryo [6].
- Low bone density, impaired bone accrual during adolescence, low bone density during adulthood and increased risk of bone fractures [7].
- Breast and vaginal atrophy (which is associated with dyspareunia) and sexual dysfunction [8].
- Psychiatric comorbidities, including anxiety, stress and mood disorders, correlated with LH pulse patterns [9].
- Cardiovascular consequences as effects on adverse lipids, endothelial function, vascular resistance, nitric oxide production and angiographic evidence of coronary artery disease (CAD) in women with prolonged, exercise-induced amenorrhea [10].
- Excess mortality—low body weight is associated with excess mortality [11, 12].

The precise mechanisms underlying the pathophysiology of FHA are very complex and unclear. Numerous neuropeptides, neurotransmitters and neurosteroids

play an important role in the physiological regulation of GnRH pulsatile secretion, and there is evidence that these substances may be involved in the pathophysiology of FHA. Recent discoveries show an important role of abnormal kisspeptin secretion in this pathology [13].

3.2 Role of Kisspeptin in Hypothalamic Control of Menstrual Cycle

3.2.1 Kisspeptin and GPR54 Receptor

KISS1 gene was discovered in 1996 in nonmetastatic melanoma cells as human malignant melanoma metastasis-suppressor gene [14]. It encodes kisspeptin (KISS1), protein responsible for suppression of metastatic potential. *KISS 1* gene is located on chromosome 1q32 and consists of four exons from which first two are not translated during protein synthesis. The precursor peptide consists of 145 amino acids and is subsequently shortened by variable proteolytic modifications to 54 amino acid chain [15]. This 54 amino acid peptide is called metastin due to ability to inhibit chemotaxis and invasion of metastases [16]. Metastin is in turn cleaved into 14, 13 and 10 amino acid fragments. These short peptides and 54 amino acid precursor peptide have 10 amino acid-long (kisspeptin amino acid 112 to 121) common RF-amide C terminus Arg-Phe-NH_2 sequence and together form the family of kisspeptines [17]. This 10 amino acid end is necessary for proper function and binding the specific kisspeptin receptor. All kisspeptines display the affinity to the orphan G protein-coupled receptor 54 (GPR54) [16, 18]. Human GPR54 exhibits high (81%) sequence homology to the rat galanin receptor-like orphan and belongs to G protein-coupled receptors (GPCRs) family.

In humans *GPR54* gene is placed on chromosome 16p13.3 and consists of five exons and four interrupting introns. *GPR54* gene encodes peptide composed of 398 amino acids and 7 transmembrane domains [19]. Kisspeptin activates GPR54 receptor leading activation of phospholipase C and subsequent stimulation of inositol 1,3,4-trisphosphate and protein kinase C. These processes lead to release of calcium from cell stores. Increased calcium concentration inhibits calcium-sensitive potassium channel and activates calcium-sensitive non-selective cationic (NSC) channels [20]. Kisspeptin stimulation leads to biphasic increase in intracellular calcium. Acute rapid increase of first phase is followed by more sustained second phase. Internalized complex of kisspeptin-GPR54 is degraded internally within 1 h, while complex on the cell surface is decomposed much slower [21]. Inactivated GnRH cells with GPR54 on its surface exhibit spontaneous baseline oscillations in intracellular calcium concentration. Activation by kisspeptin leads to increase in frequency of calcium spiking and summation of individual spikes [22]. All these processes of internalization and degeneration are necessary to maintain susceptibility of GPR54 and protect against desensitization of cells with GPR54. Human GPR54 is highly expressed in the brain and pituitary gland, placenta, pancreas and spinal cord, suggesting a role in the regulation of endocrine function [17]. Kisspeptin neurons are located in humans mainly in the infundibular nucleus. Axons of

kisspeptin neurons form dense plexuses and contact with bodies, dendrites and axons of GnRH neurons in infundibular stalk [23].

In rodents there are two main population of kisspeptin neurons, in arcuate (ARC) nucleus and anteroventral periventricular nucleus (AVPV) of the preoptic area (POA). In murine model kisspeptin neurons in AVPV present sexual dimorphism, and the amount of kisspeptin-synthesizing neurons is higher in females than in males [24]. In humans great majority of kisspeptin neurons is located in infundibular nucleus (homologous to rodent ARC).

Rare kisspeptin neurons are present also in POA, although population homologous to AVPV is still not identified [25]. In humans sexual dimorphism in number and location of kisspeptin neurons is also observed. Magnocellular part of the paraventricular hypothalamic nucleus is suspected of being an equivalent of murine rostral periventricular area of the third ventricle. In this nucleus there is prominent group of kisspeptin neurons in human females, but not in males, which indicates presence of sexual differences in anatomy of kisspeptin system [24].

The role of KISS1 in regulation of reproduction was strongly suggested after discovery of mutation in *GPR54* gene, leading to pubertal failure and normosomic hypogonadotropic hypogonadism [26, 27]. In murine model mutations in *GPR54* gene led to abnormalities of both male and female genitalia and histopathological changes in tissues which normally contain sexually dimorphic features [27]. Further analysis revealed that mutations inactivating *KISS 1* gene or *GPR54* gene lead to hypogonadotropic hypogonadism, failure of estrous cycle, infertility, reduced gonadal size and delayed puberty, while activating mutations cause precocious puberty and premature thelarche [28–31]. Nevertheless mice with iatrogenic lack of kisspeptin cells have smaller ovaries, while puberty onset and fertility in females are unaffected [32].

3.2.2 Regulation of GnRH Secretion by Kisspeptin

In humans hypothalamic POA and infundibular nucleus are the main areas in the central nervous system containing GnRH neurons. Axons of GnRH neurons lead from those two nuclei to the median eminence, where GnRH is released in a pulsatile manner into the portal circulation, therefore stimulate secretion of FSH and LH by gonadotropes in anterior lobe of pituitary gland [33]. Numerous neuropeptides such as neuropeptide Y (NPY), corticotrophin-releasing hormone(CRH), leptin, ghrelin, orexin A and β-endorphins are involved in regulation of GnRH secretion [34]. Nevertheless latest studies suggest that kisspeptin/neurokinin B/dynorphin neurons (KNDy) play a dominant role in the regulation of GnRH neurons. KNDy neurons in the infundibular nucleus express dynorphin, NKB, kappa opioid peptide receptor (the receptor for dynorphin) and neurokinin B (NKB) receptor. 77% of kisspeptin neurons bodies and 56% of kisspeptin axon fibres located in infundibular nucleus coexpress NKB [24]. However, regulation of KNDy neurons is likely more complex; it is suspected that NKB and dynorphin autosynaptically regulate pulsatile secretion of kisspeptin. Kisspeptin in turn coordinates GnRH, LH and sex steroid secretion. Some of infundibular KNDy project axons to the median eminence and

infundibular stalk, where they form dense pericapillary plexus [24]. Kisspeptin neurons of the infundibular nucleus project to the external zone of the median eminence [35]. Synaptic contact of KNDy neurons and GnRH cell bodies and axons in the in the mediobasal hypothalamus leads to depolarization of GnRH neurons and pulsatile GnRH secretion (see Fig. 3.1) [36]. In autoregulatory process KNDy

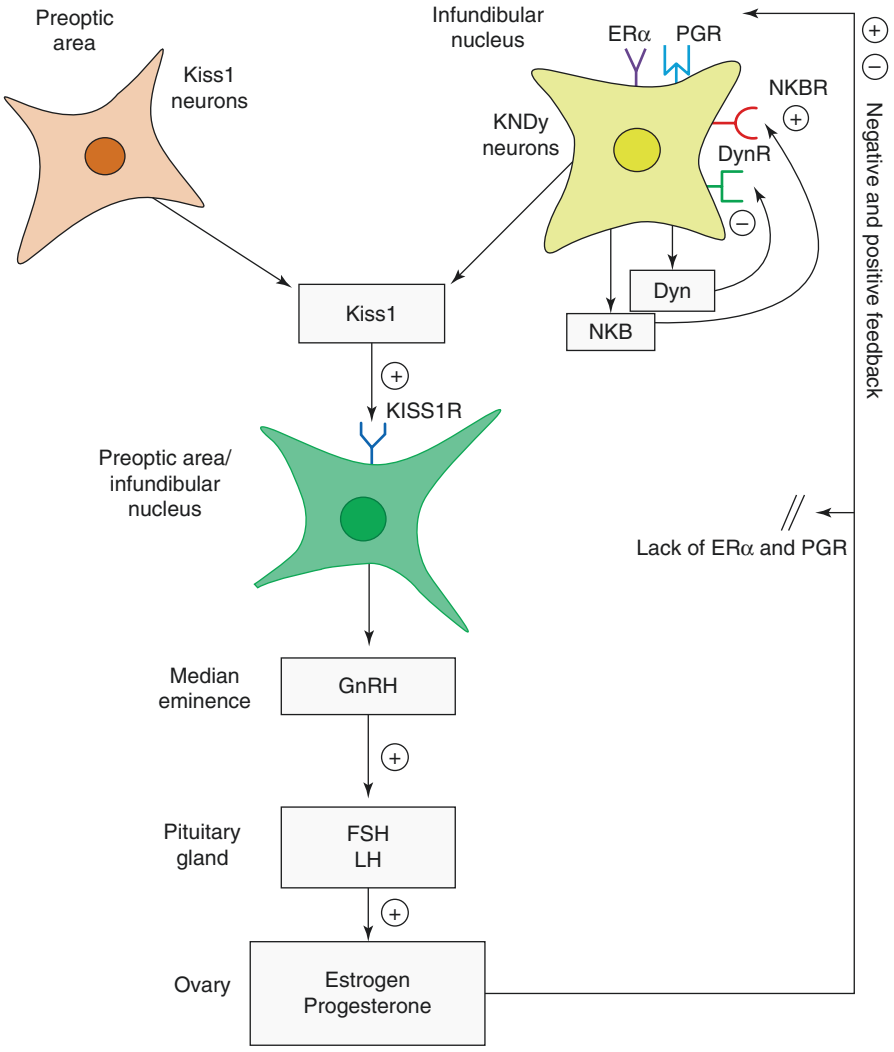

Fig. 3.1 KNDy of infundibular nucleus secrete NKB, dynorphin and KISS1. Connection between KNDy neurons leads to autosynaptic coordination of their neuroactivity. NKB exert a stimulatory effect on KISS1 secretion, while dynorphin plays an inhibitory role. Secretion of NKB increases dynorphin secretion, which in turn inhibits KISS1 production. Both KNDy and kisspeptin neurons of preoptic area synthesize KISS1 and stimulate GnRH neurons of median eminence to GnRH and simultaneously LH and sex steroid secretion

autosynaptically coordinate neuroactivity of other KNDy, leading to GnRH pulse secretion. A majority of studies suggest that NKB plays a stimulatory role on KISS1 secretion, while dynorphin exerts an exhibitory influence [23, 37]. Dynorphin secretion is triggered by NKB synthesized by KNDy. Dynorphin in turn inhibits KNDy and KISS1 secretion [38]. It is suggested that KISS1 stimulates GnRH neurons, not KNDy, because there is no expression of kisspeptin receptors on KNDy, and they are located only on GnRH neurons of infundibular nucleus and POA [39, 40]. However there is a variety of regulatory paths between KNDy and GnRH secretion, while not all GnRH neurons receive kisspeptin contact [41]. Moreover complex regulation is likely to be related to the fact that administration of senktide, highly potent and selective NK3R agonist, to kisspeptin-knockout mice resulted in secretion of GnRH from median eminence. This strongly suggests that GnRH may be secreted in kisspeptin-independent manner [42].

3.2.3 Regulation of KNDy by Ovarian Steroids

KNDy express on their surface not only receptors for NKB and dynorphin but also oestradiol receptor α (ERα) and progesterone receptor (PR). Recent studies evaluated that GnRH neurons express neither ERα nor PR receptors; therefore there are premises that KNDy are central node of feedback loops in the reproductive system [23]. Evidence has accumulated in parallel that GnRH neurons synthesize GPR54 mRNA, therefore express GPR54 on its surface and collect stimuli from KNDy neurons, not from the ovary [39, 43]. Expression of KISS1 mRNA in the hypothalamus seems to be regulated by ovarian steroids. In turn KISS1 stimulates secretion and creation of GnRH pulses. In early follicular phase, steroids produced in the ovary inhibit GnRH secretion. Opposite effect is noticed during late follicular phase, when oestradiol increases GnRH secretion and subsequently creates LH pulse [44]. In rats, oestradiol inhibits kiss neurons and mediates negative feedback on KISS1 mRNA synthesis in ARC nucleus (homologous to infundibular nucleus in humans). Opposite processes occur in AVPV, where oestradiol activates kiss neurons and mediates positive feedback on KISS1 mRNA synthesis leading to formation of LH surge [45, 46]. ARC nucleus, where negative feedback takes place is likely to be quite conservative between species. Inversely, hypothalamic nodule, where positive feedback of steroid hormones occurs is more diverse between species [23].

Recent discoveries provided new findings on progesterone role in the regulation of KNDy. It is noticed that oestradiol synthesized in the ovary increases synthesis of progesterone by hypothalamic astrocytes (neuroP) [47]. In mice neuroP acting on progesterone receptors in rostroperiventricular continuum of the third ventricle activates kisspeptin neurons, leading to formation of LH surge. Additionally, knockdown of progesterone receptor in mice attenuates LH surge [48]. All these findings reinforce the hypothesis that KNDy play fundamental role in mediation of negative and positive feedback of both oestradiol and progesterone on GnRH and subsequently in creation of LH pulses.

3.3 Kisspeptin as a HPG Regulator in Stress and Undernutrition from Basic Science to Clinics

The function of HPG axis is strictly dependent on the exogenous conditions, and it is known that it may be temporarily suppressed by stressing factors. Stress may include psychological conditions, undernutrition, strenuous physical activity or generalized inflammation. These mechanisms are present in animals and in human and seem to protect individual from reproduction, which requires high energetic supplies, in suboptimal environmental conditions.

Central regulation of reproductive functions is known to be driven by hypothalamic-pituitary axis, mainly by synchronized, pulsatile release of GnRH in hypothalamic nuclei and subsequently gonadotropins (see above). Many studies have shown that stress can interfere with GnRH secretion [49]. Until nowadays, the exact mechanism of stress-driven inhibition of HPG axis remains uncovered. However, discoveries regarding kisspeptin as the gatekeeper of this axis shed some light of possible links between stimuli from environment and reproductive functions.

There is a large body of evidence that stress interferes with HPG axis through activation of hypothalamic-pituitary-adrenal axis [50]. Particularly, CRH of hypothalamic origin is able to inhibit GnRH pulsatile secretion.

Animal studies have shown that intracerebral administration of CRH downregulates the expression of kisspeptin and its receptor in hypothalamus. CRH inhibits expression of kisspeptin signalling molecules in both ARC and POA nuclei. Other stress factors, such as restraint, insulin-induced hypoglycaemia or lipopolysaccharide stress, also resulted in decreased expression of kisspeptin and its receptor in these areas [50]. Moreover, further studies revealed corresponding distribution of CRH receptors and kisspeptin in ARC [51]. Studies in human are lacking so far, but it seems plausible that kisspeptin release is inhibited by CRH in similar fashion. On the other hand, overactivation of hypothalamic-pituitary-adrenal axis in FHA patients [12].

It is well known that body composition and nutritional status are essential for reproductive function. Many neuroendocrine factors regulating metabolism have been studied in recent years, and there is growing body of evidence that kisspeptin is transitional signal between nutrition and reproduction.

Leptin is a peptide secreted from tissue and is recognized as endocrine indicator of body's nutritional status. Interestingly, kisspeptin neurons express the leptin receptor. Mice lacking leptin gene show reduced kisspeptin expression in ARC, and this can be reversed by exogenous leptin administration [52]. The leptin deficiency conditions, such as undernutrition, are related to suppression of kisspeptin expression in animal models. Reversal of this status by leptin administration increases kisspeptin mRNA levels (reviewed in [53]). Morelli et al. [54] showed in human cell line in vitro experiments the ability of leptin to increase Kiss1 gene expression. Taken together, experimental data shows that leptin is an important regulator for kiss neurons, and this group of neurons may be a key element of interaction between nutrition and HPG axis. Full understanding of this interplay requires further studies,

since other authors provided ambiguous results regarding the regulatory role of leptin for kisspeptin neurons. Cravo et al. [55] showed that only approximately 15% of ARC kisspeptin neurons are responsive to leptin, whereas neurons in POA are entirely unresponsive to leptin. Luis et al. [56] identified populations of neurons expressing leptin receptors in close relation to the kiss1 neurons, which may be involved in indirect action of leptin on kisspeptin.

Another important group of hypothalamic neurons in control of body nutrition status is groups of ARC neurons expressing POMC [57]. This group of neurons seems to be key sensor for energy reserves of organisms. Lack of POMC neurons leads to obesity, but this effect might be reversed by leptin administration [58]. One of the neurotransmitters secreted by POMC neurons is α-MSH, which has been shown to positively influence LH secretion [59]. Recent studies showed that α-MSH on GnRH/LH release is, at least partially, dependent on kisspeptin neurons [59]. Further studies are required to elucidate this relationship.

The ARC nucleus contains also significant number of neurons secreting orexigenic signals, i.e. NPY and agouti-related peptide (NPY/AgRP neurons). This group of neurons has synaptic connection to the GnRH neurons, and NPY and AgRP are able to decrease GnRH and LH release [60]. Contrary, depending on the endocrine state, developmental stage and pattern of administration, the same neurotransmitters may enhance GnRH secretion [61]. Taken together it seems that NPY/AgRP neurons act as restrain for the HPG axis in adverse nutritional conditions [62]. Relationships between this group of neurons and kiss1 neurons are relatively poorly described. It is known that in mammals there are connections between these two groups of neurons [63]. It is suggested that increased AgRP simulation is necessary for the suppression of Kiss1 and, hence, GnRH neurosecretion in conditions of unfavourable nutritional status [64].

Clinical data regarding kisspeptin secretion in stress conditions in humans is scant. However, few studies provide some evidence of possible role of abnormal kisspeptin secretion in different stress situations on HPG axis failure.

Our group assessed mean serum kisspeptin concentrations in 41 women diagnosed with FHA (aged 17–28 years) in comparison to age-matched 40 healthy controls. The mean serum concentrations of kisspeptin in patients with FHA were 0.17 ± 0.11 ng/mL, while in control women were 0.3 ± 0.36 ng/mL. There was a statistically significant difference between the mean concentrations of kisspeptin in serum in FHA patients and the control group (p = 0.0271) [unpublished data]. However, we failed to find any significant correlations between body mass and body mass index in the study group.

These results are consistent with previously performed investigation on kisspeptin concentration in amenorrheic patients. Bacaopoulou et al. [65] showed negative correlation between peripheral kisspeptin levels and BMI in anorectic patients. In the same study, authors revealed lower serum kisspeptin concentration in amenorrheic adolescents, although difference was no statistically significant. In anorexia nervosa patients, kisspeptin has been shown to correlate positively with body weight, body mass index and fat mass [66].

Novel studies provide interesting information regarding opposite relationship, i.e. showing kisspeptin as an anorexigenic factor. Neurons secreting kisspeptin have

axonal junction to regulatory neurons in ARC. There, kisspeptin is responsible for direct stimulation of POMC/CART, and indirect inhibition of NPY/AgRP cells modifies eating behaviour [67].

Other than undernutrition, stress factors also interfere with kisspeptin signalling and HPG axis. The first studies showing this relationship have been done on female rat models. When lipopolysaccharide has been injected (stress factor), the kisspeptin mRNA expression has been suppressed, which resulted in low LH levels. The LH secretion could be restored by co-administration of kisspeptin [68]. Later, other stress factors like food deprivation, continuous overnight illumination, 24 h wet bedding [69] and psychosocial stress [70] were shown to decrease kisspeptin expression.

Similarly as described above bidirectional relationship between nutrition and kisspeptin, reverse links can be shown for psychological stress and physical activity.

Kisspeptin may exert the anxiogenic effect, which has been shown on rats. Intracerebroventricular kisspeptin injection causes anxiety and stimulates the hypothalamic-pituitary-adrenal axis [71].

Recently, Hofman et al. [66] reported negative correlation between kisspeptin serum concentrations and physical activity, i.e. number of steps and total energy expenditure. Authors speculated that this negative relationship may be a compensatory mechanism to prevent physical activity and body mass loss in anorectic patients.

Tolson et al. [72] analysed potential metabolic function of kisspeptin in mice model, using KISS1R knockout females. Interestingly, animals lacking receptor gene presented decreased locomotor activity and energy expenditure in comparison to wild-type mice. Other authors observed stimulation of locomotor activity after injection of kisspeptin into the cerebrospinal fluid of male rats [71].

3.4 Kisspeptin Administration in Hypothalamic Amenorrhea Patients

Kisspeptin plays and essential role as a component of GnRH pulse generator and potent GnRH stimulator. These findings lead scientific approach to possible use of this peptide as a therapeutic tool. At first such studies were performed on the animal models. They revealed that administration of kisspeptin stimulates LH and FSH release in all mammalian species tested to date [73, 74].

Kisspeptin-54 was used numerous times in women with different endocrine disturbances in the last decade. However it should be stressed that kisspeptin administration was first time used in hypothalamic amenorrhea. It was in 2009, and the aim was to evaluate the potential therapeutic role of kisspeptin in FHA [75]. This study assessed the effect of acute and chronic kisspeptin administration on serum gonadotropin, oestradiol levels, LH pulsatility and ultrasound measurements of ovarian activity. Acute kisspeptin administration caused tenfold increase in LH and 2,5 increase in FSH secretion. Augmentation of gonadotropin secretion did not correlate within increase in serum estradiol level which was very low what reflected quiescence in folliculogenesis. The stimulatory effect of chronic kisspeptin

(2 weeks) administration was abolished, related to tachyphylaxis. However, this study showed for the first time that kisspeptin can be potentially used as a new tool in the therapy of different endocrinological disorders, particularly hypothalamic amenorrhea.

The second study on the improved use of kisspeptin-54 in patients with hypothalamic amenorrhea was published by the same group in 2010 [76]. In this study, after earlier experience, women with FHA were treated with twice-weekly kisspeptin-54 administration or saline administration. Women in whom kisspeptin was administered, presented significantly higher serum levels of gonadotropin after 8 weeks of treatment than women in whom placebo was administered. Important information from this study is also referred to lack of side effects during kisspeptin treatment.

The open question was related to possible use of kisspeptin administration in healthy women and biological effects of this administration. Therefore Jaysena et al. [77] evaluated the impact of chronic kisspeptin administration on normal menstrual cycle in healthy women. Injection of kisspeptin or saline was performed twice daily during follicular phase of menstrual cycle in healthy women. Leading occlusion from this study was that 7 days of kisspeptin-54 treatment does not abolish normal menstrual cycle in healthy subjects.

Further approach to treat with kisspeptin the patients FHA was based on its intravenous administration, because previous studies used subcutaneous administration [78]. Main results of this study were as follows: LH pulsatility was augmented in all FHA patients; the mean peak number of pulses during kisspeptin-54 administration was threefold higher in comparison with vehicle. Additionally mean serum levels of FHS and estradiol were also significantly elevated during an infusion of highest doses of this peptide when compared with vehicle. Authors of this paper for the first time reported that constant intravenous kisspeptin-54 administration may temporarily increase both basal and pulsatile LH release in patient with HA. They also developed the dose range within which kisspeptin-54 therapy is able to restore LH secretion (both basal and pulsatile).

Drug administration such as kisspeptin should be not only effective but above more safe. Studies using in vitro models revealed that kisspeptin can cause vasoconstriction and can inhibit angiogenesis and can stimulate glucose-induced insulin secretion [79]. However studies which were conducted in healthy human volunteers did not present side effects related to cardiovascular system and glucose-induced insulin secretion [80].

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4 The Role of Androgens for Body Composition and Physical Performance in Women

Angelica Linden-Hirschberg

4.1 Androgen Biosynthesis and Secretion in Women

Androgens are secreted by both the ovaries and adrenal glands in women. The major androgens produced in the ovary are androstenedione and testosterone. Androstenedione acts as a precursor for both estrogen and androgen synthesis and can be converted to testosterone and the more potent dihydrotestosterone (DHT) in the ovary and in peripheral tissues [1, 2]. The adrenal gland produces dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, testosterone, and small amounts of DHT. Depending on availability of steroidogenic enzymes in the tissue, the precursors DHEA and DHEAS can be peripherally converted to estradiol, testosterone, and DHT [1, 2].

In premenopausal women, about 50% of circulating testosterone arises by direct secretion from the ovary and the adrenal gland of equal amount (Fig. 4.1). The remaining 50% is produced from peripheral conversion by adrenal and ovarian androgen precursors. Testosterone secretion is stimulated by luteinizing hormone (LH) and displays menstrual cycle variation in fertile women, with a peak during the mid-cycle LH-surge [1, 2]. Testosterone also displays circadian variation with peak levels in the early morning hour [2]. Serum testosterone declines gradually with age, but do not change specifically at menopause [3]. Liquid (or gas) chromatography-mass spectrometry is considered the golden standard method for analysis of serum testosterone, while immunological methods are limited by cross-reactivity with other steroids and insufficient sensitivity.

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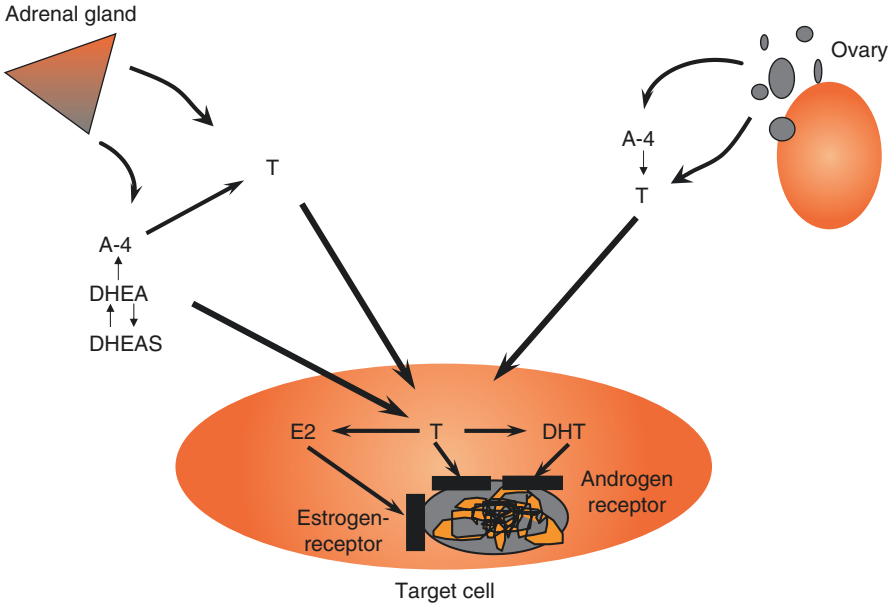

Fig. 4.1 Androgen secretion in women. For explanation see the text. *A-4* androstendione, *DHEA* dehydroepiandrosterone, *DHEAS* dehydroepiandrosterone sulfate, *DHT* dihydrotestosterone, *E2* estradiol, *T* testosterone

Sex hormone-binding globulin (SHBG), produced in the liver, is a major determinant of the bioavailability of testosterone. Around 65–70% of circulating testosterone is bound and inactivated by SHBG, 30–35% is loosely bound by albumin, and only 0.5–3% represents freely circulating testosterone [4]. Since the binding of testosterone to albumin is rather weak, the free and albumin-bound fractions are defined as bioavailable testosterone. The ratio between total testosterone and SHBG, i.e., free androgen index, is used as a measure of circulating free testosterone. Circulating DHT is even more strongly bound and inactivated by SHBG than testosterone.

Testosterone and DHT are considered the two bioactive androgens which can bind to the androgen receptor. A larger part of these active androgens is synthesized in peripheral tissue (e.g., in liver, kidney, muscle, fat, skin) from DHEA within the cell. DHEA is therefore considered a major tissue-specific source of testosterone and DHT in women [5].

Recently, it was demonstrated that the androgen derivates 11-ketotestosterone and 11-ketodihydrotestosterone from the adrenal gland also are potent agonists of the human androgen receptor [6]. Future studies are needed to investigate the relation between serum levels of these hormones and specific symptoms.

4.2 Regulation of Body Composition by Androgens

4.2.1 Muscle

Testosterone and DHT exert anabolic effects in nonreproductive tissues by binding to the androgen receptor. In muscle tissue this leads to increases in muscle fiber numbers and size, muscle satellite cell numbers, numbers of myonuclei, and size of motor neurons [7]. There is also experimental evidence that testosterone increases skeletal muscle myostatin expression, mitochondrial biogenesis, myoglobin expression, and insulin-like growth factor content [8], which may enhance skeletal muscular activity.

Studies in men have demonstrated positive correlations between endogenous testosterone levels, muscle mass, and strength [9, 10]. Furthermore, interventional studies in young and older men, whose endogenous testosterone is fully suppressed by gonadotropin-releasing hormone, have shown that increasing doses of testosterone cause a dose-dependent increase in lean mass, size, and strength in relation to increasing levels of serum testosterone [11, 12].

In postmenopausal women, serum levels of endogenous testosterone and lean body mass are related [13]. Little is known if the corresponding relationship exists in young women. However, in nonathletic women with mildly elevated testosterone levels due to polycystic ovary syndrome (PCOS), studies have shown that muscle mass is increased in proportion to circulating testosterone [14]. Limited studies have administered increasing doses of testosterone in women due to ethical concerns about risks of adverse effects. However, in postmenopausal women a few randomized studies have demonstrated significant increases in muscle mass and strength by high doses of testosterone [15].

4.2.2 Bone

Androgens are major determinants of bone metabolism in both women and men. Bone formation is stimulated by androgens directly and partly by local aromatization to estrogen [16]. Experimental studies have shown that androgens stimulate proliferation and differentiation of osteoblasts and inhibit apoptosis by specific androgen receptor mechanisms [17]. Furthermore, androgen-stimulated increase in muscle mass and strength induces mechanical factors that favor bone formation [17]. The net result is increased bone mass and bone strength.

Epidemiological studies have demonstrated a positive correlation between endogenous androgens and bone mineral density (BMD) in both adolescent and adult women of fertile age [18, 19]. Moreover, studies in premenopausal women have shown an independent and possibly additive effect of androgens and estrogens on bone mass [17]. Women with mild hyperandrogenism, e.g., those with hirsutism and PCOS, have increased BMD compared with control women [20]. Testosterone treatment increases BMD in both surgically and naturally postmenopausal women [17].

4.2.3 Fat Mass and Distribution

There is evidence that androgens also regulate fat mass and distribution. In women, endogenous androgen excess, as in PCOS, is associated with accumulation of abdominal fat [20]. In turn, abdominal fat predisposes to insulin resistance, dyslipidemia, and hypertension, i.e., the metabolic syndrome leading to increased long-term risk of diabetes mellitus and cardiovascular disease [21]. Noteworthy, the situation appears to be exactly the opposite in men. Thus, larger depots of abdominal fat in men are associated with lower testosterone levels because gonadotropin secretion is reduced, while upon weight loss testosterone levels and insulin sensitivity return to normal [21]. Furthermore, long-term treatment of obese men with testosterone causes them to lose fat and gain lean body mass [22]. However, the effect of exogenous androgens on fat mass in postmenopausal women is inconclusive, and both an increase and decrease and no change have been reported [22].

4.3 Possible Athletic Performance-Enhancing Effects of Androgens

Several effects of androgens could be beneficial for physical performance in both women and men (Table 4.1). Thus, it is obvious that muscle growth and the increase in strength and power it brings have a performance-enhancing effect. For instance, testosterone administration in postmenopausal women reaching mean serum testosterone 7.3 nmol/L resulted in muscle mass increase by 4.4% and muscle strength increase by 12–26% [15]. Likewise, stimulation of bone size and strength could be beneficial in certain sports such as jumping, throwing, and other explosive activities.

Furthermore, testosterone stimulates the formation of new blood cells and increases circulating hemoglobin [23]. The mechanism seems to involve increased secretion of erythropoietin, as well as suppression of hepcidin [24]. Increased amount of hemoglobin in the blood has the effect of increasing oxygen transport and consequently enhance aerobic capacity. Experiments from the seventies showed a strong linear relationship between changes in hemoglobin and maximal exercise-induced oxygen consumption [25]. In postmenopausal women treated with testosterone, hemoglobin levels increased in a dose-dependent fashion [15].

Androgens may also have behavioral and psychological effects of importance for athletic performance. Exogenous testosterone in men has been reported to increase

Table 4.1 Possible beneficial effects of androgens for athletic performance

Increase in lean body mass and muscle strength
Stimulation of bone size and strength
Increase in hemoglobin and oxygen uptake
Behavioral and psychological effects including increased mental drive and competitiveness
Increased visuospatial ability?

behavior of competition and dominance and to reduce fear [26]. Endogenous testosterone in men has also been positively associated with high-risk behavior [26]. Women tend to be more risk aversive, less competitive, and more prosocial than men. It has been demonstrated that women are more risk aversive during the ovulatory phase, when estradiol is high, in comparison with other cycle phases [27]. Furthermore, androgens may affect cognition and mood. In general, men tend to excel women in spatial ability tests, while women outperform men in episodic memory and verbal fluency [28]. Certainly, high spatial ability plays a role in many sports, such as pole vault and hammer throw. However, correlation studies in women have not been conclusive regarding relationship between testosterone levels and visuospatial skills.

4.4 Exogenous Androgens and Sport Performance in Women

Exogenous androgens have since the 1950s been used to enhance athletic performance with reports of increased power, strength, and training capacity [26]. These substances are therefore banned from sports and labeled as doping agents. Studies regarding exogenous anabolic androgens in women athletes are few, the exception being experiments performed in the former German Democratic Republic (GDR). The now disclosed documents revealed that GDR athletes were exposed to exogenous androgens from an early age [29]. Although these experiments were clearly unethical, they prove the positive effects of administered androgenic hormones on performance and that the performance-enhancing effects were especially prominent in female athletes. As the authors of the paper state: "special emphasis was placed on administering androgens to women and adolescent girls because this practice proved to be particularly effective for sports performance." They also note: "The effects of the treatment with androgenic were so spectacular, particularly in female athletes in dependent events, that few competitors not using the drugs had a chance of winning" [29]. After 1989, the top performances of the world's best in several sports fell markedly.

Still, anabolic-androgenic steroids (AAS) are the most prevalent doping substances among both female and male athletes despite continuous improvement of analytical methods and strategies for detecting doping [26, 30]. It is well-known that high doses of exogenous androgens may induce substantial and sometimes irreversible masculinizing effects of the female body including acne, hirsutism, breast atrophy, clitoral enlargement, uterine atrophy, infertility, and deepening of the voice [31]. There is also support for adverse mental and behavioral effects including increased irritability, aggressiveness, depression, maniac behavior, paranoid psychoses, and suicide thoughts by AAS [31]. Life-threatening adverse effects such as cardiac arrhythmias and sudden death have also been reported. Therefore, detection of AAS abuse in female athletes is important to avoid serious health risks and to ensure fairness in competition.

4.5 The Role of Normal Endogenous Androgen Levels in Training and Sport

What is the role of normal endogenous androgen levels for athletic performance? In men, several studies lend support for an association between endogenous testosterone levels and physical performance in both nonathletes and athletes [10, 32]. However, until recently surprisingly little is known about the influence of endogenous androgens on physical performance in women. One reason for this could be the need to take into account the hormonal variations of the menstrual cycle and use of hormonal contraception.

One study showed that serum levels of testosterone at rest correlated with explosive performance in female athletes [33]. In a large study, serum androgen levels and their relation to performance were studied in more than 2000 elite athletes participating in the 2011 and 2013 International Association of Athletics Federations (IAAF) World Championships [33]. The athletes were classified in tertiles according to their free testosterone (fT) concentration, and the best competition results achieved in the highest and lowest fT tertiles were then compared. Female athletes with the highest fT tertile performed significantly better in 400 m, 400 m hurdles, 800 m, hammer throw, and pole vault with margins of 1.8–4.5% than those with low fT values. In another study, more than hundred Swedish Olympic female athletes were compared with age- and BMI-matched sedentary controls for their muscle and bone mass, muscular strength, and androgen steroid profile in serum and urine [34]. The athletes displayed higher levels of some precursor androgens (DHEA) and higher muscle and bone mass than the sedentary control women, with strength tests correlating strongly with muscle mass and androgen precursors and androgens. The results suggest that endogenous androgens are associated with a more anabolic body composition and enhanced performance in women athletes.

4.6 Hyperandrogenism in Female Athletes

4.6.1 Polycystic Ovary Syndrome

It has been demonstrated that polycystic ovary syndrome (PCOS is a common disorder in female elite athletes [35–39]. PCOS is the most frequent hormonal aberration in women of fertile age and is characterized by increased testosterone production, disturbed ovulation, and polycystic ovaries. What causes PCOS is largely unknown, but there is strong evidence for a genetic predisposition, although environmental factors also play a part. Endocrine studies have shown enhanced diurnal secretion of LH and testosterone in athletes with PCOS [36] (Fig. 4.2). In contrast, athletes with functional hypothalamic amenorrhea due to energy deficiency, display completely abolished LH pulsatility and low levels of testosterone [36]. The levels of testosterone in PCOS typically remain within the normal upper range for women and are seldom pathologically increased. Athletes with PCOS also demonstrate a more anabolic body composition with more muscle mass and higher

bone mineral density than other athletes [35]. Hyperandrogenism appears to provide good protection from bone loss despite oligomenorrhea/amenorrhea and relative estrogen deficiency in PCOS.

There are data to support that PCOS is advantageous for physical performance. For instance, endurance athletes with PCOS have shown higher maximal oxygen uptake and performance levels than athletes without PCOS (Fig. 4.3) [35]. It has also been reported that PCOS is overrepresented and is the most frequent cause of menstrual disorders among Olympic sportswomen [37]. These studies suggest that mild forms of hyperandrogenism like PCOS may be beneficial for physical performance and could play a role in the recruitment of women to competitive sport activities. There is no support for the opposite, i.e., that sport could induce PCOS.

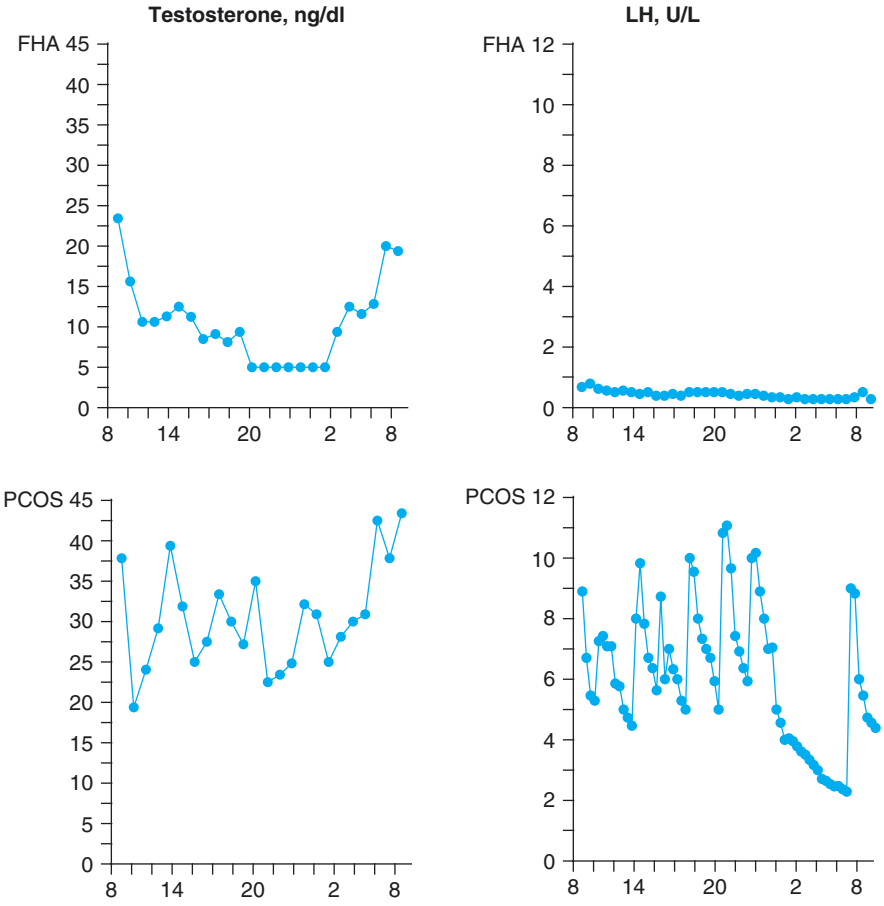

Fig. 4.2 Typical diurnal hormonal profiles in individual female athletes and a sedentary control woman. *FHA* athlete with functional hypothalamic amenorrhea, *PCOS* athlete with polycystic ovary syndrome, *RM* athlete with regular menstruation, *CTR* sedentary control

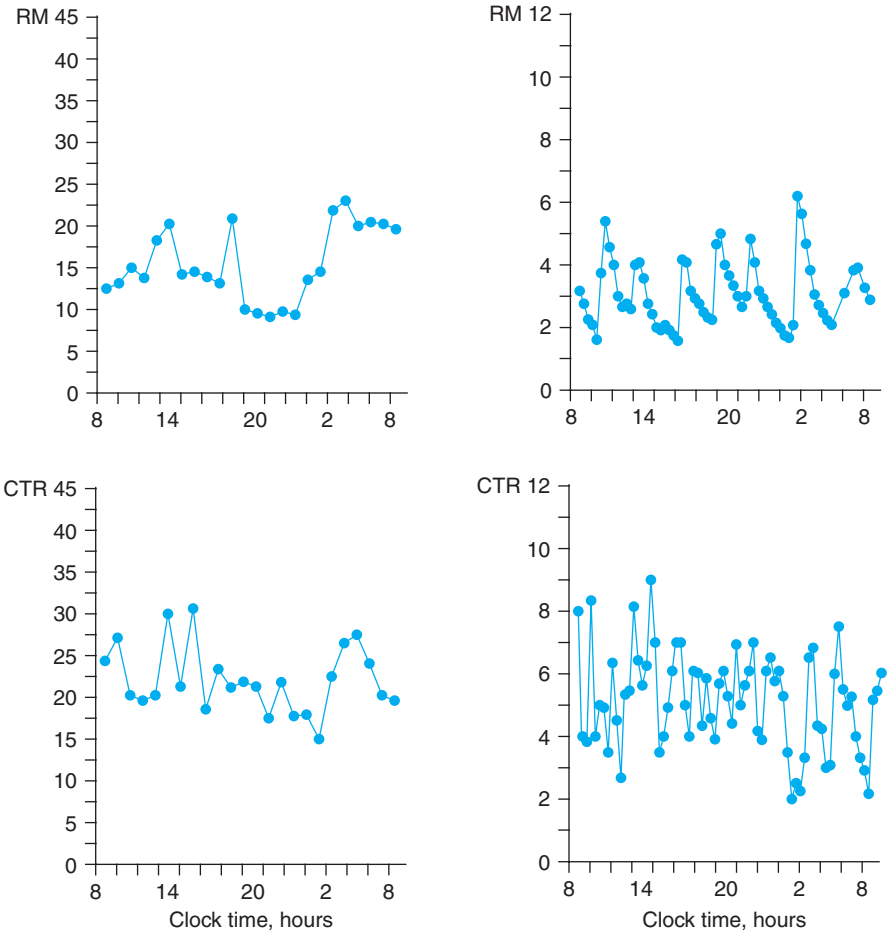

Fig. 4.2 (continued)

4.6.2 Disorders of Sex Development

Men have 10–20 times higher concentrations of testosterone in the blood as compared to women [40], which is likely one reason to sex differences in physical performance. However, a few women are born with rare conditions, named disorders of sex development (DSD) in which the development of chromosomal, gonadal, and anatomic sex is atypical. Congenital disorders in this category are, e.g., 5α reductase deficiency, complete and partial androgen insensitivity, and congenital adrenal hyperplasia (Table 4.2). These conditions may cause a greatly increased production of testosterone in the male range. If the individual has normal sensitivity to androgenic hormones, her muscle mass will develop as in males, along with increasing signs of virilization such as increased body hair, deepening of the voice, breast atrophy, and clitoromegaly.

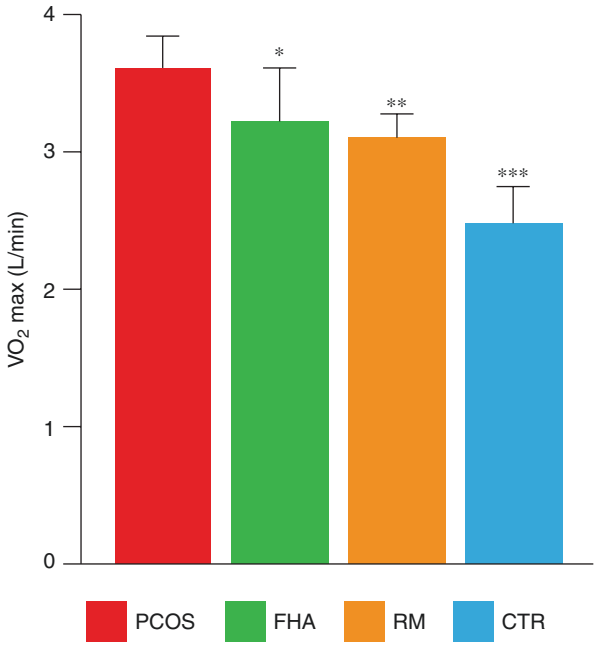

Fig. 4.3 Maximal oxygen uptake in groups of athletes with polycystic ovary syndrome (PCOS), functional hypothalamic amenorrhea (FHA), and regular menstruation (RM) and in a sedentary control group (CTR)

Table 4.2 Some conditions in women that may cause hyperandrogenism

Condition	Incidence/ prevalence	Karyotype/comment	Advantage in sports?
Polycystic ovary syndrome (PCOS)	10%	XX Testosterone levels are often within the normal female range	Yes
Congenital adrenal hyperplasia (CAH)	Classic form 1/12,000 Nonclassic variable. 1/100 in some areas	XX Classic form: Virilized at birth Nonclassic: Not virilized, may be missed	Yes, but only if undertreated with glucocorticoids
5α-reductase deficiency type 2	Rare	XY Individuals may be assigned female sex at birth. Virilization proceeds at puberty	Yes
Complete androgen insensitivity (CAIS)	1/50,000?	XY Completely female external genitalia. No effect of high testosterone levels	No
Partial androgen insensitivity (PAIS)	1/30,000?	XY Ambiguous genitalia at birth. From puberty high testosterone, but with very poor effect	Yes
Ovotesticular DSD	Very rare	XX or XY Have both ovaries and testes. Born with ambiguous genitalia. Virilization continues at puberty	Yes
Aromatase deficiency	Extremely rare	XX Individuals are born virilized, which continues at puberty	Yes

The prevalence of such rare conditions is estimated to be about 140 times increased among elite female athletes [41]. Most of these women have already been diagnosed and treated in early childhood, but in some areas with limited medical resources, they might enter puberty undiagnosed. Since sports are divided into male and female classifications, many female athletes consider it unfair if they must compete against a woman who has the advantage of a male physiology. This lead the IAAF and the International Olympic Committee (IOC) to establish regulations for management of hyperandrogenism in female athletes. It was stated that for female athletes to be eligible to compete in female events, the athlete must be legally recognized as a woman and maintain serum testosterone less than 10 nmol/L (the lower normal male range) provided she has normal androgen sensitivity. In 2015 the regulations were challenged by a female athlete before the Court of Arbitration of Sport (CAS), which suspended the regulations pending strengthened scientific basis. Since then, further evidence of the role of endogenous androgens for athletic performance in female athletes has been published [33, 34]. The regulations are currently under revision.

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Polycystic Ovary Syndrome

5

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5.1 Introduction

Polycystic ovary syndrome (PCOS) is a very common endocrine disorder in women of reproductive age, characterized by chronic anovulation and hyperandrogenism. Women with PCOS present menstrual cycle disturbances and signs of hyperandrogenism, such as hirsutism and acne [1]. From a metabolic point of view, insulin resistance is a cardinal feature of PCOS. Many women with the syndrome are obese and develop metabolic disturbances, such as dyslipidemia, hypertension, and glycemic dysregulation [2]. PCOS is usually clinically manifested early in female life but seems to persist even after menopause. Although several criteria exist for women of reproductive age, PCOS in adolescent and postmenopausal women is not well defined. Reproductive disorders are the main issue of concern during reproductive life. On the contrary, the crucial question for postmenopausal women is if they are at increased risk for metabolic and cardiovascular disease [3].

The purpose of this chapter is to discuss the criteria and definition of PCOS, to present current epidemiological data, to analyze the hypotheses for the pathophysiology of the syndrome, to discuss the appropriate diagnostic algorithms with employment of clinical examination accompanied with laboratory and/or imaging techniques, and to propose causative treatment of various parameters of the syndrome, including metabolic disease, menstrual disturbances, hirsutism, and infertility.

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5.2 Definition and Prevalence

The definition of the syndrome is currently based in three distinct criteria, namely, chronic anovulation, hyperandrogenism, and polycystic morphology of the ovaries [4], although the involved scientific societies apply them in different ways (Table 5.1). More specifically, the National Institute of Health (NIH) recommended in 1990 that the diagnosis of PCOS should be reached when a woman presents concomitant chronic anovulation and clinical or biochemical hyperandrogenism [5]. In 2003, a joint European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) consensus meeting held in Rotterdam proposed that the presence of two out of three criteria (chronic anovulation, hyperandrogenism, and polycystic ovarian morphology) is enough for the diagnosis of the syndrome [6]. In 2006, the Androgen Excess Society (AES) suggested that hyperandrogenism is the hallmark of PCOS, while the second essential criterion for the diagnosis could be either chronic anovulation or polycystic ovaries [7]. This variety of definitions can result in a broad spectrum of patients with different phenotypes having the same diagnosis. These discrepancies are highlighted in recent expert panels and position statements [4, 8, 9], where the maintenance of the broader Rotterdam diagnostic criteria is recommended [6], focusing, however, on the identification of the specific PCOS phenotypes [9]. Four different phenotypes have been identified: (A) women with hyperandrogenism and chronic oligo- or anovulation and polycystic morphology of the ovaries (HA + OA + PCO), (B) women with hyperandrogenism and chronic oligo- or anovulation (HA + OA), (C) women with hyperandrogenism and polycystic morphology of the ovaries but without oligo- or anovulation (HA + PCO), and (D) women with chronic anovulation and polycystic morphology of the ovaries but without hyperandrogenism (OA + PCO) [4, 9] (Table 5.1). Because of the different criteria applied, the PCOS prevalence varies in different cohorts from 6% to 20% (lower prevalence with the NIH criteria, higher prevalence with the Rotterdam criteria, and intermediate with

Table 5.1 Definition of PCOS according to various criteria

Society	Oligo-ovulation	Androgen excess	US morphology	Exclusion criterion
NIH 1990	+	+		+
ESHRE/ASRM 2003[a]	(+)	(+)	(+)	+
A (frank)	+	+	+	+
B (classic)	+	+		+
C (ovulatory)		+	+	+
D (mild)	+		+	+
AES 2006	(+)	+	(+)	+

AES Androgen Excess Society, *ASRM* American Society for Reproductive Medicine, *ESHRE* European Society of Human Reproduction and Embryology, *NIH* National Institute of Health
[a]Two out of three criteria must be present

the AES criteria). Irrespective of the criteria used, PCOS represents a very common endocrine disorder of women of reproductive age [4, 8].

5.3 Pathophysiology

The underlying mechanism of PCOS remains to be elucidated, and various hypotheses regarding the genetic and environmental components exist. There is some evidence that PCOS may depend on genetic defects, but these are more likely to be oligo- or polygenic [10, 11]. Various genome-wide association studies (GWAS) have been conducted so far, involving genes which participate in androgen biosynthesis and action, in insulin action and effectiveness, as well as in the synthesis and function of inflammatory cytokines [12]. Intrauterine growth restriction (IUGR) or exposure to androgens have been associated with the development of PCOS in adult life [13]. Through effects on programming in the hypothalamus-pituitary-gonadal and adrenal axes, as well as through impact on insulin secretion, sensitivity, and effectiveness, early events that take place even during intrauterine life may result in later luteinizing hormone (LH)/follicle-stimulating hormone (FSH) derangement, androgen excess production by the ovaries or the adrenal glands, or hyperinsulinemia, which leads secondarily to increased androgen production [14, 15].

In women with the syndrome, early follicular growth is excessive, with increased population of antral follicles present; nevertheless, the selection and maturation of a dominant follicle from this increased pool are disturbed (follicular arrest) [16]. Many of these accumulated follicles remain capable of steroidogenesis, producing estrogens and progesterone. In fact, women with PCOS produce both androgens and estrogens in excess. Numerous studies have provided evidence that LH concentrations, but also frequency and amplitude of its secretion, are increased in PCOS [4]. These changes, along with increased responsiveness of smaller diameter than normal follicles to LH, may lead to inappropriate differentiation of granulosa cells and disorganized follicular development [17]. Furthermore, steroidogenic enzymes are higher expressed under the stimulation of LH, while granulosa cells seem to display resistance to FSH. Intraovarian modulators, such as IGF-binding proteins, may act locally and decrease the effects of FSH [16–18].

Another pathophysiological hypothesis is that androgen excess represents a fundamental disorder in these women, resulting from increased activity of enzymes that participate in steroidogenesis. The larger proportion of ovarian androgens is produced in the theca cells, and CYP17α represents the key complex for this biosynthetic pathway. Normally, most of the produced androgens diffuse into granulosa cells and are rapidly converted to estrogens; however, this may not be the case in women with PCOS [19]. Adrenal glands can also participate in the pathogenesis of the syndrome, as dehydroepiandrosterone sulfate (DHEAS) concentrations are found elevated in at least 30% of patients with the syndrome, although adrenocorticotropic hormone (ACTH) concentrations remain within reference range. The adrenal androgens can be transferred to ovaries and converted there into more potent forms [20].

Insulin resistance and hyperinsulinemia are frequently identified in women with PCOS. The primary defect lies in the insulin post-receptor signaling pathway and can be due to various factors secreted by adipose tissue in obese women; nevertheless, it can represent an intrinsic event, as it is also evident in lean women with the syndrome. Insulin can act as a co-gonadotropin in the ovary, stimulating the CYP17α activity and leading to androgen overproduction [2]. Furthermore, insulin, being a modulator of adrenal secretory activity, can increase the secretion of 17(OH)progesterone and DHEAS [20]. In addition, insulin directly inhibits sex hormone-binding globulin (SHBG) production; hyperinsulinemia is associated with low SHBG concentrations and, hence, increased concentrations of circulating free bioavailable androgens [2, 21].

5.4 Diagnosis

Although PCOS is a very common disorder, the definite diagnosis of the syndrome necessitates often the employment of a combination of clinical skills, accompanied by appropriate laboratory techniques, and should always be set after the exclusion of other causes of androgen excess, such as Cushing's syndrome, congenital adrenal hyperplasia (CAH), ovarian or adrenal androgen-secreting tumors, and acromegaly [4, 8].

5.4.1 Clinical Picture

Women with PCOS usually present with menstrual disturbances. Oligomenorrhea (menstrual cycle length more than 35 days) and secondary amenorrhea (absence of menses for more than 6 months) are the most typical manifestations of oligo-anovulation. These manifestations often start early in puberty [19]. The diagnosis of the syndrome during puberty can be difficult and should be based on both hyperandrogenism and persistent oligomenorrhea. Anovulatory symptoms and polycystic morphology are evident even in normal stages of reproductive maturation and are not sufficient for diagnosis [8]. Primary amenorrhea is an uncommon manifestation of PCOS; still, up to 20% of girls evaluated for primary amenorrhea are diagnosed with the syndrome [22]. Primary amenorrhea that accompanies PCOS is reversible and is not associated with pubertal delay.Irregular and/or heavy bleeding (dysfunctional uterine bleeding, DUB) can also be observed in some women with PCOS; however, endometrial hyperplasia or cancer should be excluded, especially in older women [19]. Although there are no definitive criteria for the diagnosis of PCOS in perimenopausal or postmenopausal women, it can be based on a well-documented long-term history of hyperandrogenism and oligomenorrhea during reproductive life [8]. About 20% of patients with the diagnosis of PCOS may report regular menstrual cycles, but approximately 20% of these cycles are anovulatory [23]. Infertility can be another major problem in women with PCOS, as they may have 2 to 6 ovulatory cycles per year on average. Of course, this pattern of ovulation allows some of these patients to conceive spontaneously, especially when other causes of male or female infertility are not present [19].

Hyperandrogenism is often clinically manifested by hirsutism or acne, while acanthosis nigricans and alopecia can also be present. The prevalence of hirsutism in the general population ranges from 5% to 15% with differences in various ethnicities [7], but from the patients with hirsutism more than 70% are usually diagnosed with PCOS [24]. The most common method for the assessment of hirsutism is the modified Ferriman-Gallwey score [25, 26]. According to this scale, nine body areas are assessed and then rated from 0 (no terminal hair) to 4 (extensive terminal hair), and the sum of the numbers of each area are added. A score ≥6 defines hirsutism [26, 27]. Acne is also common in women with PCOS, especially during adolescence, with reported prevalence varying from 14% to 25%, while androgenic alopecia is rather rare [28].

It is well documented that hyperandrogenism is positively associated with the metabolic syndrome, insulin resistance, and other metabolic biomarkers that increase cardiovascular disease risk. There is a positive correlation between androgen concentrations and degree of insulin resistance [29]. However, recent studies indicate that the presence of adrenal hyperandrogenism, with DHEAS as the main representative, prevents further deterioration of the metabolic profile, including glucose and lipid homeostasis, in young, middle-aged, and postmenopausal women with PCOS [30, 31]. Of women with PCOS, 30–70% are obese, while dyslipidemia, hypertension, and glycemic disorders are often present. The pattern of dyslipidemia most usually met in women with PCOS is that of elevated triglycerides and decreased high-density cholesterol (HDL-c) concentrations. Glycemic dysregulation presents mainly as impaired glucose tolerance (IGT) in 30–35% or type 2 diabetes mellitus (T2DM) in 3–10% of the cases [21]. Indeed, women with PCOS have 5 to 10 times higher risk for T2DM. It is interesting that lean women with PCOS present still high for their age and body mass index (BMI) prevalence of IGT (10–15%) and T2DM. The accumulation of various risk factors can increase the cardiovascular disease risk, and these patients should always be evaluated accordingly [32, 33].

5.4.2 Laboratory Evaluation

Measurement of total testosterone concentrations should be the main laboratory test for the evaluation of hyperandrogenemia in women, as testosterone represents the main circulating active androgen. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is considered the gold standard technique. Free testosterone (fT) measurement should not be performed, as the available routine techniques are not accurate enough [4, 8, 34, 35]. After measurement of SHBG, free androgen index (FAI), which is the ratio of total testosterone to SHBG, can be estimated and provide an estimation of fT [4, 35]. Adrenal androgens can also be measured (androstenedione and DHEAS), even if this is not usually necessary in everyday routine. Very high serum testosterone (>150–200 ng/dl) and/or DHEAS (>6000 ng/ml) concentrations are more in favor of an androgen-secreting tumor of ovarian or adrenal origin, respectively [34]. The presence of symptoms along with absolute biochemical hyperandrogenism requires utilization of relevant imaging modalities to exclude such a tumor, e.g., ovarian magnetic resonance imaging (MRI) or adrenal computed

tomography (CT) [8]. Gonadotropin measurement, especially LH, can be helpful, especially for the differential diagnosis of hypothalamic amenorrhea. As gonadotropins should be measured in the early follicular phase of the menstrual cycle, we tend to perform all measurements at the same time, even if testosterone concentrations present very low variability. In the case of amenorrhea, measurements can be performed at any time [4]. When there is a need to document ovulation, progesterone should be measured 7 days before the probable day of the following menstruation, and this should be performed in two consecutive cycles. Progesterone concentrations ≥3 ng/ml are usually indicative of an ovulatory cycle [4, 8]. The measurement of thyroid-stimulating hormone (TSH) and prolactin can help in the differential diagnosis of the syndrome, while more specific clinical phenotypes may be indicative of another underlying endocrinopathy, such as Cushing's syndrome or acromegaly, and necessitate the use of specific tests. When congenital adrenal hyperplasia (CAH) is suspected, a stimulating dynamic test with ACTH (Synacthen) with measurements of 17(OH)progesterone should be performed [4, 8]. Women with PCOS should also be evaluated for fasting lipids and glucose levels, while some guidelines indicate the need of performance of an oral glucose tolerance test (OGTT) with 75 g of glucose, especially in obese women or in older women with a history of gestational diabetes mellitus or family history of T2DM [8].

5.4.3 Ovarian Morphology

With the use of high-resolution ultrasonography and intravaginal probes, the ovarian pictures in everyday clinical practice are of high quality. The traditional definition for polycystic ovarian morphology requires 12 or more follicles measuring 2–9 mm in diameter and/or increased ovarian volume (>10 cm^3); "only one ovary meeting these criteria is sufficient to define PCO" [6]. The marked improvements in ultrasound resolution have resulted in suggestions of raising the diagnostic thresholds substantially to 19 or even to 26 follicles per ovary [19].

5.5 Treatment

The therapeutic targets for a woman with PCOS can be varied and include metabolic disorders, menstrual disturbances, hirsutism, or infertility. The targets change from woman to woman and from time to time for the same woman. Various options are available for each target in the therapeutic armamentarium (Fig. 5.1).

5.5.1 Treatment of Metabolic Disorders

Insulin resistance is the metabolic hallmark in women with PCOS, even in lean ones; therefore, means that can improve insulin sensitivity are of great importance [2, 36]. Lifestyle intervention with diet and physical exercise to lead to appropriate weight

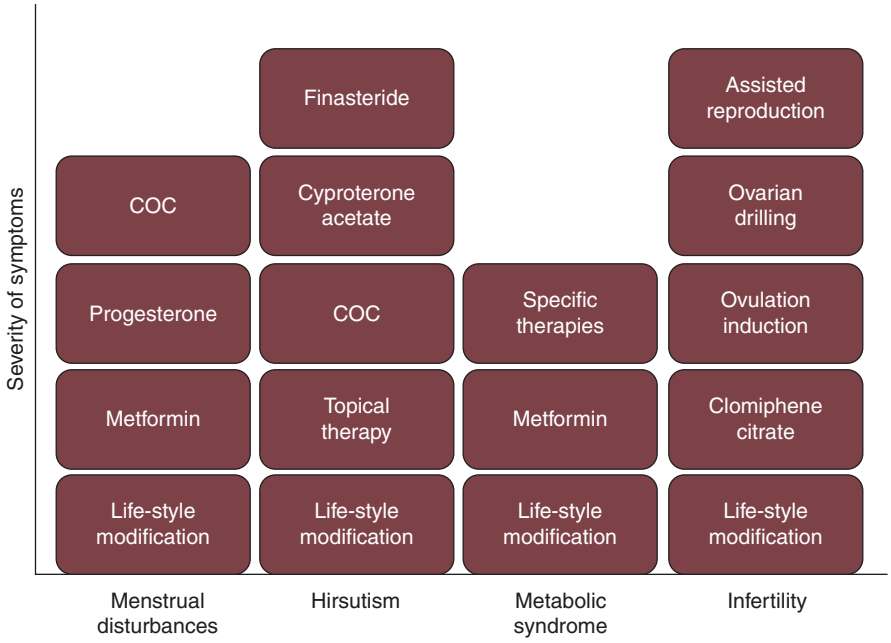

Fig. 5.1 Treatment targets and treatment options for women with PCOS

loss, especially in overweight and obese patients, is the cornerstone of the therapeutic strategy. No particular diet pattern substantially benefits weight loss in women with the syndrome [37, 38]. Lifestyle programs that achieve an energy deficit of 500–750 kcal per day or provide 1200–1500 kcal per day for these women should be recommended. Exercise can act additively with diet and present beneficial effects on weight loss and parameters of glycemic metabolism [39]. Bariatric surgery is increasingly used for the management of morbidly obese patients. Current guidelines recommend bariatric surgery in patients with BMI > 40 or even >30 kg/m^2, in case they present a relevant medical comorbidity [40]. Women with PCOS experience great improvement in symptoms after bariatric surgery; therefore, such a surgery could be an additional therapeutic tool for the management of the syndrome, even if there is only limited evidence from trials to assess the benefit-to-risk ratio [41].

Metformin primarily decreases hepatic glucose production by inhibiting key enzymes for gluconeogenesis and secondarily enhances peripheral insulin sensitivity. These two actions result in a reduction of insulin concentrations and consequently in an improvement of the metabolic parameters. As insulin can act as a co-gonadotropin in the ovaries, the reduction in insulin levels can result in a decrease in endogenous androgen production [2, 42]. Indeed, various studies and a meta-analysis have provided evidence that treatment with metformin results in a significant reduction in total testosterone and fT and in a significant increase in SHBG concentrations [43, 44]. Metformin has a favorable safety profile and most caregivers are very familiar with its use. It carries only a small risk of lactic acidosis, but

this danger would exist only among women with impaired renal function. Gastrointestinal symptoms, including anorexia, nausea, and diarrhea, are the most common and can be ameliorated by a gradual increase of the dosage [21]. Thiazolidinediones present a favorable effect on insulin sensitivity, and their use has resulted in improved glucose tolerance and reduction of circulating androgens. However, as they may present serious adverse effects, including weight gain, hepatotoxicity, and unfavorable pregnancy outcome, their use is limited in women with PCOS [21, 45].

5.5.2 Treatment of Menstrual Disturbances

Lifestyle intervention and insulin sensitizers can improve menstrual disturbances, as they result in the reduction of insulin and androgen concentrations. Hence, women with PCOS that present abnormal menstrual cycles should be advised accordingly [19, 21]. Oral contraceptive pills (OCPs) have been the mainstay of management in women with PCOS, although, there are only a few well-designed studies in such populations. OCPs can reduce effectively endogenous androgen production, through various physiological pathways, such as suppression of gonadotropins and increase of SHBG concentrations [46]. In PCOS women with severe hirsutism, the ideal OCP should also present antiandrogenic effects, and, hence, the combination of ethinyl estradiol with cyproterone acetate is very effective. In the mild case of if cyproterone acetate is not available, the use of a progestin with relative low androgen effects, such as drospirenone, would be the treatment of choice [19, 47]. Physicians should always consider the restrictions on the use of OCPs, according to age and the presence of various cardiovascular risk factors [48]. Instead of using of OCPs, oral medroxyprogesterone acetate has been proved to suppress gonadotropins and result in the reductions of circulating androgens in women with PCOS. Even simple treatment with progesterone only for 7 to 10 days could be very successful when menstrual disturbances are the only concern. Furthermore, progestin-only oral contraceptives can protect the endometrium, but they are associated with high incidence of breakthrough bleeding [19].

5.5.3 Treatment of Hirsutism

Lifestyle intervention and agents that suppress androgen production, such as OCPs, when used alone, usually have a modest effect on hirsutism [46]. Their effect is enhanced, if they are combined with appropriate mechanical and cosmetic local treatments or with peripheral androgen blockers [26, 47]. Shaving, bleaching, and chemical depilation are useful to ameliorate hirsutism. Plucking or waxing can cause discomfort and lead to folliculitis in these women. Eflornithine (Vaniqa) is a local enzyme inhibitor in the form of cream that reduces the rate of hair growth, making it less visible and coarse. Laser epilation and repeated sessions of electrolysis can be used to achieve a more permanent loss of unwanted hair [47]. The

pharmacological antiandrogen agents include androgen receptor blockers, namely, spironolactone, cyproterone acetate, and flutamide, as well as finasteride, which is a 5α-reductase inhibitor [26, 36]. All these agents present similar efficacy, which is around 30% and needs 9–12 months to develop a successful result. Possible adverse effects include hyperkalemia (spironolactone) or liver toxicity (cyproterone acetate, flutamide, finasteride). Furthermore, these agents present teratogenic potential and, therefore, should always be combined with a form of contraception to avoid an unwanted pregnancy. This combination also reduces the risk of irregular menstrual bleeding [26, 47].

5.5.4 Treatment of Infertility

In patients with PCOS, proper ovulation and fertility can be induced by various means [49]. Lifestyle modifications should be the first step therapeutic approach, especially in women with increased BMI. A 5–10% decrease of initial body weight results in spontaneous ovulation and seems to optimize the results of other therapeutic means [50–52]. Insulin sensitizers, such as metformin, can also increase the rate of spontaneous ovulation and regular menstruation in women with insulin resistance. Following lifestyle changes and in combination with them, the pharmaceutical agent of choice for women with PCOS is clomiphene citrate (CC) [19, 49]. Recent randomized trials that examined ovulation induction in PCOS women have documented that CC alone is superior to metformin alone [51, 53, 54, 55]. Clomiphene is an antiestrogen that acts on the hypothalamus-pituitary axis and stimulates the secretion of FSH. The starting dose varies from 50 up to 150 mg/day and can be initiated from day 2 to day 5 of the menstrual cycle for 5 days. The ovulation rate is about 75–80%, but the pregnancy rate is significantly lower [49]. Clomiphene resistance is defined when there is no ovulation on maximal doses, while clomiphene failure is defined when there is no conception after six ovulatory cycles. Weight loss or insulin sensitizers can improve resistance, and addition of metformin should be considered when treatment with CC alone is unsuccessful [19, 49, 54].

Another option to induce ovulation in PCOS women is aromatase inhibitors [49]. An initial meta-analysis [56] showed that there was no difference between CC and letrozole in ovulation and pregnancy rates. However, a recent RCT showed that the cumulative birth rate was significantly higher in PCOS women treated with letrozole as compared to CC, primarily in those with BMI > 39.4 kg/m^2 [57]. However, it should be noted that the use of aromatase inhibitors for ovulation induction is off-label.

The use of exogenous gonadotropins for ovulation induction is well-established using the low-dose protocols [49]. The rationale of these protocols is to mimic the natural cycle by raising the serum FSH concentrations gradually, aiming to develop 1 to 3 follicles that will become dominant. Practically, FSH or human menopausal gonadotropin (hMG) is injected each day from day 2 for 2 weeks. The initial dose (50–75 IU/day) can be upgraded at 7-day intervals if no dominant follicle is detected. When a mature preovulatory follicle is obtained, human chorionic gonadotropin (hCG) is injected intramuscularly [19]. For women with PCOS that do not respond

to standard means of ovulation induction or even for those who hyper-respond or have additional infertility factors, assisted reproduction by in vitro fertilization (IVF) can be an effective way for achieving pregnancy [19, 49].

When CC and gonadotropins were not available, ovarian wedge resection was used to induce ovulatory cycles. Nowadays, laparoscopic ovarian drilling (LOD) is performed by ovarian multi-perforation with diathermy or laser [49]. Although the specific mechanisms of action are still unknown, a meta-analysis has shown that LOD is associated with similar live birth and miscarriage rates as compared to gonadotrophin stimulation but with significantly less multiple pregnancies [58].

5.6 Conclusions

PCOS is a very common endocrine disorder in women of reproductive age and usually presents with menstrual cycle disturbances, signs of hyperandrogenism, such as hirsutism and acne, as well as infertility. Many of these women manifest insulin resistance and develop metabolic disturbances. The diagnosis of the syndrome can be set only when various criteria are fulfilled, after exclusion of relevant diseases, a process that necessitates the employment of a combination of clinical skills and appropriate laboratory and imaging techniques. The therapeutic targets are diverse and include metabolic disorders, menstrual disturbances, hirsutism, or infertility. The targets change from woman to woman and from time to time for the same woman. Lifestyle intervention with diet and physical exercise should be the cornerstone of management of all targets, while metformin, antiandrogens, local skin therapies, and ovulation induction or even assisted reproduction techniques are available and can address successfully the reproductive and health problems associated with PCOS.

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Metabolism, Obesity, Thinness, and Reproduction

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6.1 Introduction

The physiology of reproduction, which is very complex, is regulated by an elaborate interplay of various molecular signaling pathways. In the past few decades, various neuropeptides have been demonstrated to be involved in the stimulation or inhibition of reproduction. These signaling pathways act on three components: the hypothalamus, the pituitary, and the gonads of the hypothalamic–pituitary–gonadal axis. Gametogenesis and steroidogenesis are regulated mainly by the pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of pituitary gonadotropins is regulated by hypothalamic gonadotropin-releasing hormone (GnRH) [1], and this hormone is regulated by many central and peripheral excitatory and inhibitory signals mediated by neurotransmitters, neuromodulators, and neuropeptides, such as ghrelin, leptin, neuropeptide Y (NPY), agouti-related protein (AgRP), cocaine- and amphetamine-regulated transcript (CART), alpha-melanocyte-stimulating hormone (α-MSH), corticotropin-releasing hormone (CRH) [2], kisspeptin [3], and γ-aminobutyric acid (GABA) [4]. In 2000, gonadotropin-inhibitory hormone (GnIH), a new hypothalamic neuropeptide, was identified and demonstrated to inhibit gonadotropin secretion [5].

Many endocrine diseases such as hyperprolactinemia, adrenal and thyroid diseases, and hyperandrogenism interfere with the release of GnRH from hypothalamic neurons, as do metabolic conditions that compromise GnRH secretion such as obesity, weight loss, or undernutrition, and obviously also many systemic diseases.

To better understand how the reproductive system works, we need to focus on our metabolism, starting from the evolution of our biology. In its very early beginnings, mankind was able to survive in very difficult times when little or no food was

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available. Primitive prehistoric peoples often had very little food available and could not eat every day, perhaps only once a week. So, our metabolism has evolved from this early origin: indeed, our biology evolved to store energy when little food was available, to save the body's energy for times of fasting, which was a frequent condition.

Thus, it is absolutely relevant that our genotype prioritizes saving and storing energy: if bodily energy is stored, it can be utilized later in times of food deprivation or excessive fasting. Clearly, such a system is mainly the result of the adaptive evolution that allowed humans to evolve from primates to *Homo neanderthalensis* and later to *Homo sapiens*.

Our species is probably not the most efficient in either reproduction or survival. This point is illustrated by the fact that our population began increasing in number consistently only when living conditions became significantly improved and continued to improve, starting from the end of the twelfth century but increasing after the seventeenth century. Such improvements included our feeding, living, and working conditions, medical discoveries, and the availability of drugs.

Lack of food and diseases were the main causes of a short life: indeed, it was difficult to find something to eat two times a day! Mankind lived in small and relatively organized groups. Most of these groups migrated, following the animals (i.e., for food). Much human energy was expended for this need. The main problems were to find food and to survive to the moment when food was "in hand." If no food was available, the search for food obliged humans to undertake long travels. Much energy was lost during such migrations, and for the "human body" these were very stressful times. This primitive search for food led to the modern conditions of severe energy consumption, severe "dieting," and psychological stress. Moreover, reduction of rest and sleep intervals increased psychological stress.

All these factors created conditions that obliged the homeostatic system to preserve and use energy to sustain muscle activity (running or walking); to sustain the heart, kidney, lung, brain, and vital organs; and to block the function of the reproductive system as needed to avoid a physiological condition that might reduce the chance to survive. This last function was essential: preserve the species by preserving each single person. It was logically perceived that a pregnant female was certainly weaker than the nonpregnant females of the community, and if food was not found, the embryo/fetus could kill the mother by consuming all her energy. Reproductive failure was then the only contraceptive system that nature might use to save a greater number of females during the toughest periods of the year, or during the frequent natural calamities that might oblige the human community to fast and/or be on the move for days or weeks at a time.

But what is stress nowadays? We can ascribe our actual stress to conditions such as the maniacal attention to weight control and dieting and to fitness and training, excessive work activity, family problems, and psychological weakness. All these factors can interfere with many central nervous system (CNS) functions and with many of the homeostatic controls of the hypothalamus, resulting in the induction of an adaptive solution. So, stress occurs as a specific adaptive response to adverse external stimuli: such a response usually activates endogenous biochemical

synthesis and the secretions of neurotransmitters, neuromodulators, and neuropeptides, and, obviously, the activation or modulation of the many pathways these agents control or modulate [6].

We can distinguish between acute stress and chronic stress. Stress that is quite rare or infrequent, that is, acute, causes minimal changes in CNS and hypothalamus activities, but a persistent, or chronic, stress condition induces important changes in CNS and hypothalamus activities when the duration of the stressful condition(s) is longer than expected for an acute stress event—this is subjective [7, 8]. Physical, metabolic, or psychological stress is elaborated by the cerebral cortex, the area of primate cognitive elaboration, with synthesis and release of inhibitory neurotransmitters, neuropeptides, and neurohormones that affect the hypothalamus, with negative effects on reproductive function. Women with functional hypothalamic amenorrhea are sensitive to stressors at central and neuroendocrine levels. This sensitivity is in great part mediated by cognitive characteristics, so stress has a different effect on each person. In this case, amenorrhea is the result of altered control or modulation as a defensive system created by impaired gonadotropin secretion as a consequence of stress: it is a functional blockade of the reproductive function [7, 8]. Obviously, we have to exclude systemic causal factors, CNS disease/lesion (tumors, trauma), and endocrine diseases. The modulation of opioid, dopamine, serotoninergic, and GABA systems on GnRH secretion leads to abnormal LH secretion, causing amenorrhea.

Intake of food is frequently reduced under chronic stress as a psychological reaction, causing the onset of several dysfunctions. The association of stressant conditions, adaptive responses, lack of adequate feeding, or excess in energy consumption (induced by stressant conditions) determines a severe adaptive condition [9]. Sometimes the lack of energy (food) or willful fasting determines a severe deficit of glucose and proteins for homeostatic activity. Stress also affects many different endocrine systems, indeed increasing GH, prolactin, and adrenal gland hormones while decreasing thyroid hormones and LH. LH pulsatility returns to a hypo-LH condition with LH pulses of lower amplitude and of minimal biological effect. Opioids have an important inhibitory role in GnRH secretion, such as cortisol from the adrenal gland: elevation of cortisol levels overlaps with the occurrence of “true” hypothalamic amenorrhea [10]. In hypogonadotropic amenorrhea, we find also higher levels of insulin-like growth factor (IGF)-binding protein, which binds insulin and IGF-1, leading to a reduction of their plasma levels and a consequent low consumption of sugar. IGF-1 stimulates GnRH release, whereas cortisol has a negative effect on GnRH discharge [also via corticotropin-releasing factor–beta-endorphin (CRF-βEP) action]. Thus, there is a strong interaction between the hypothalamic and pituitary axes (Fig. 6.1).

Patients with hypothalamic amenorrhea are characterized by higher IGFBP-1, cortisol, and GH plasma levels: this is especially true of athletes, whose intensive chronic physical exercise related to training reduces bioactive IGF-1, increases hypothalamic–pituitary axis (HPA) activity and CRF release, and increases GH. Training before the pubertal period can interfere with a normal menarche and later reproductive ability [11].

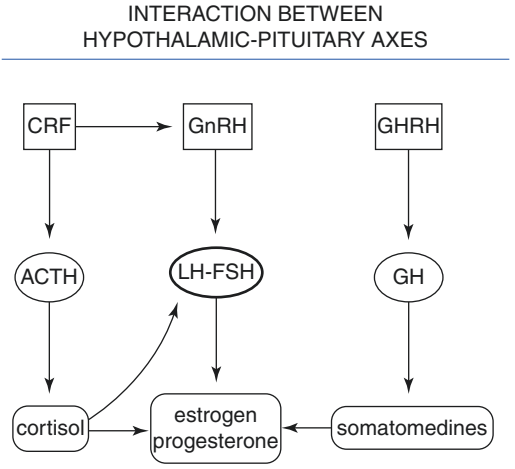

Fig. 6.1 Interaction between hypothalamic and pituitary axes

In normal conditions, free fatty acids (FFA) inhibit GH release, and IGF-1 and insulin stimulate GnRH release. An extreme weight disorder such as obesity or malnutrition creates an altered balance in such substances, thus interfering with the reproductive axis: in both cases we find high FFA levels, low IGF-1, and low insulin levels in undernutrition but high levels in obesity. A body weight reduction of 10% to 15% is equal to a 30% fat reduction, and this brings on anovulation [11].

6.2 Metabolic Factors That Affect GnRH Secretion

Numerous studies on animal models and on humans have shown that the proper function of the hypothalamic–pituitary–gonadal axis is affected by metabolic and nutritional factors [12, 13], and a minimum body weight is necessary for pubertal development and reproduction [12]. The identification of peripheral hormones (such as leptin, insulin, and ghrelin) that signal the metabolic status to the reproductive axis has expanded our knowledge in regard to the neuroendocrine mechanisms linking metabolism and reproduction. Nonetheless, our understanding of how such dynamic connections take place remains incomplete.

6.2.1 Satiation Signals

Ghrelin, a peptide predominantly secreted by the stomach, has been postulated to be a peripheral signal for energy insufficiency, acting as a potent orexigen [14]. Ghrelin has direct actions on the brain and the pituitary, where it has an inhibitory effect on gonadotropin pulsatility and decreases LH responsiveness to GnRH, and on the other hand it has a stimulatory effect on prolactin secretion, probably involving direct action on somatomammotrophic cells [15]. Ghrelin from the stomach both acts on the vagus nerve and stimulates neurons in the arcuate nucleus of the

hypothalamus (ARC) directly. Cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and distension of the stomach and intestine trigger nerve impulses through sensory nerves up to the hindbrain. These satiation signals reach neurons in the nucleus of the solitary tract (NTS), where they modulate the amount of food intake.

6.2.2 Adiposity Signals

Signals related to body fat content such as leptin and insulin act on neurons that synthesize proopiomelanocortin (POMC) or neuropeptide Y (NPY) and agouti-related protein (AgRP). ARC neurons in turn project to other hypothalamic areas, including the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA). The net output of the PVN is catabolic and enhances the potency of satiation signals in the hindbrain. The net output of the LHA, on the other hand, is anabolic, suppressing the activity of the satiation signals [16].

Leptin, an adipocyte-derived hormone, is a satiety factor secreted in proportion to the amount of body energy stores and was one of the first factors observed to link metabolism with the reproductive axis [17]. Serum leptin concentrations and leptin mRNA concentrations in adipose tissue are associated positively and very closely with fat mass [18]. The attainment of appropriate leptin concentration is essential for the maturation of the hypothalamic–pituitary–gonadal axis, normal pubertal progression, and the maintenance of fertility.

Another systemic hormone with a major role in the regulation of reproduction is the pancreatic hormone insulin [19]. Various studies suggested that insulin acts on the hypothalamic–pituitary–gonadal axis at the level of the hypothalamus to directly or indirectly modulate GnRH secretion (probably via kisspeptin), as well as at the level of the pituitary gonadotropic cells [20]. Insulin is a major regulator of leptin production; therefore, some of the positive effects of insulin on the reproductive system might derive from its ability to stimulate leptin secretion [21]. However, the precise site of action of insulin at the hypothalamic level in vivo remains uncertain [22]. IGF-1 is structurally homologous to insulin, and findings suggest that it is also involved in GnRH regulation [23], as studies show an increase in IGF-1 expression during pubertal maturation [24].

6.2.3 Caloric Restriction

Caloric restriction elevates brain-derived neurotrophic factor (BDNF) plasma levels, suggesting that low calorie intake might mediate the effects on synaptic plasticity. Reducing calorie intake to approximately 40% of control nominal values in mice from weaning to 35 months of age decreases the deficits in motor and cognitive function that are associated with aging. Alternate-day feeding ameliorates age-related deficits in cognitive function in a mouse model of Alzheimer's disease when the feeding program is maintained between 3 and 17 months of age [25]. Regular diet and exercise have a positive effect because these can affect

mitochondrial energy production, which is important for maintaining neuronal excitability and synaptic function. The combination of certain diets and exercise can have additive effects on synaptic plasticity and cognitive function. Adenosine triphosphate (ATP) produced by the mitochondria might activate BDNF and IGF-1, which support synaptic plasticity and cognitive function. Excess energy production caused by high caloric intake or strenuous exercise results in the formation of reactive oxygen species (ROS). When ROS levels exceed the buffering capacity of the cell, synaptic plasticity and cognitive function are compromised, probably as a consequence of a reduction in the actions of signal-transduction modulators such as BDNF [25].

In undernutrition that is a condition of glucose deficit, we observe ketoacidosis, lipid catabolism (total cholesterol, triglyceride, β-hydroxybutyric acid, non-esterified fatty acids (NEFA; higher levels), reduction of IGF-1 and insulin, and liver sensitivity to GH reduction. The final point is protein catabolism and metabolic acidosis: indeed, short-term changes in glucose deficit conditions include degradation of glycogen, synthesis of glucose from proteins (liver and muscles), lipid degradation from fat tissue with low insulin levels, and elevated levels of glucagon, adrenaline, and cortisol. Clinically, most of these conditions occur whenever a forkhead-associated domain (FHA) situation begins.

It is relevant to remember that these patients often show the so-called low T3 syndrome, a sort of defensive behavior that our biology adopts when chronic starvation/abnormal feeding/excess of training occurs. In this case, thyroid-stimulating hormone (TSH) is normal, but the fT3 levels are low or very low (less than 2.2 pg/ml). Because the "low T3 syndrome" is a defensive adaptation resulting from lack of energy, it is not appropriate to treat these patients with L-thyroxine because the real treatment for these subjects is a more adequate feeding program and a consistent reduction of physical activity (jogging, walking, working out at the gym, etc.) [26].

Moreover, in weight loss-related hypothalamic amenorrhea, we observe low LH levels and tiny, frequent LH pulses. The lack of any effect on the ovary is related to the low pulse amplitude of each LH secretory burst [27] (Fig. 6.2).

As evident, our species started millions of years ago as primates that had to face starvation. Evolution made us learn how to produce food, but nowadays we even produce too much! Indeed, if in the past the problem was fasting, nowadays obesity has become a global epidemic, affecting more than 600 million adults worldwide [28]. Certain risks associated with obesity target this cohort, including menstrual irregularity, endometrial pathology, and infertility. Obese women also have higher rates of many complications in pregnancy, including hypertensive disorders, gestational diabetes, preterm birth, and higher rates of cesarean delivery [29].

Obesity has a negative effect on reproductive potential, primarily thought to be caused by functional alteration of the hypothalamic–pituitary–ovarian (HPO) axis. Obese women often have higher circulating levels of insulin, which is a known stimulus for increased ovarian androgen production [30]. These androgens are aromatized to estrogen at high rates in the periphery because of the excess adipose tissue, leading to negative feedback on the HPO axis and affecting gonadotropin production

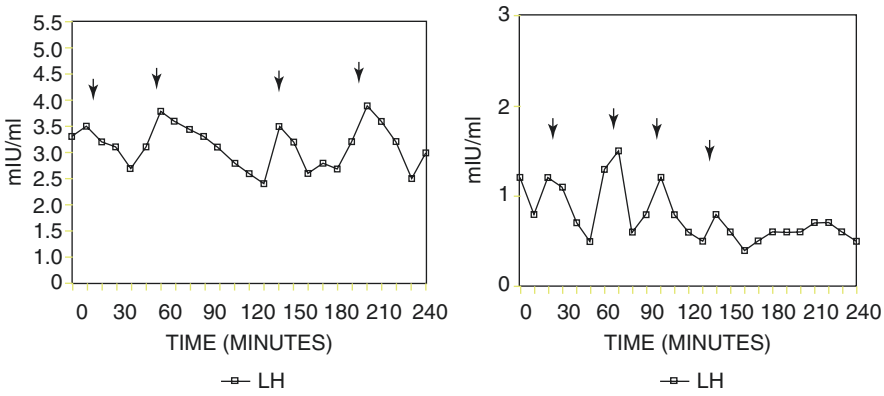

Fig. 6.2 Many peripheral signals arrive at the central level and interact with the neuroendocrine control of the cortex as well as that of the hypothalamic/limbic areas. (Modified from Ref. [24])

[31]. Thus, menstrual abnormalities and ovulatory dysfunction are triggered. Hyperinsulinemia is highly implicated in the pathogenesis of the polycystic ovarian syndrome (PCOS), characterized by oligomenorrhea and hyperandrogenism. Obesity contributes to insulin resistance and appears to exacerbate the symptoms of PCOS, with obese women often demonstrating a more severe phenotype [32, 33].

During pubertal development, fat tissue increases in terms of both mass and cell number. After pubertal maturation, only the fat stored in each cell can increase, with no further increase of cell number. Increase of body weight before 9 years of age mainly increases the number of cells of fat tissue (hyperplastic obesity; no increase in the fat content of each cell) and exposes the child to the risk of being obese during adult life (hyperplastic-hypertrophic obesity). Constitutional factors might increase such risks of being overweight or obese (i.e., insulin resistance in lean subjects that start to be overfed and then become obese).

Being overweight or obese during adolescence may depend on a combination of various factors, such as neonatal or postnatal development conditions, abnormal attitudes and behavior toward food, a familial predisposition to metabolic diseases (i.e., diabetes), and no or little physical activity. When overweight/obesity is a long-lasting feature, this may lead to a number of adaptative abnormalities that might predispose to severe diseases such as the Metabolic Syndrome (Fig. 6.3).

We must remember that our biology did not evolve in a situation of excess food, and this induced a specific biological ability to store energy for potential fasting in the future. This ability has remained as a built-in mechanism to today, exactly when our skills let us store and consume food of any kind at any moment. We achieved this "evolution" in only a few centuries although over millions of years our biology has enabled us to survive fasting. Too fast, indeed! our physiology allows us to store energy from food, notably from all excess food, thus triggering the overweight condition and then obesity. When overweightedness or obesity begins, specific metabolic signals change, such as sensitivity to insulin, which decreases with a concomitant

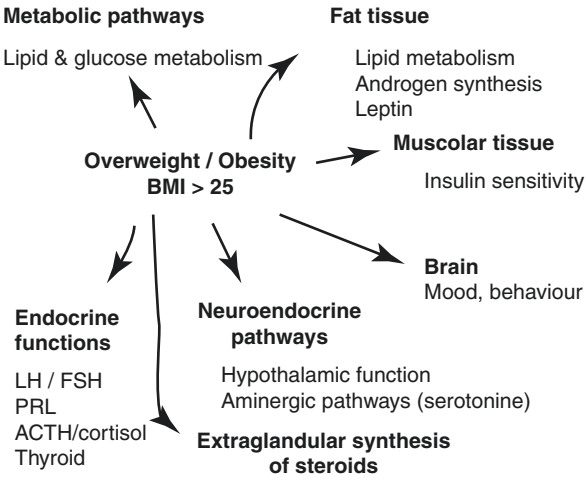

Fig. 6.3 Effects of overweightedness or obesity on many biological functions

compensatory hyperinsulinemia. An excess of insulin amplifies the binding of LH on its receptor, leading to higher ovarian androgen production; moreover, high levels of LH and a hyperandrogenic environment contribute to increased cyclic adenosine monophosphate (cAMP) levels within granulosa cells and increased cytochrome P-450 side chain cleavage enzyme (cP450scc) activity, consequently inducing the arrest of follicle growth and increased peripheral synthesis of estradiol.

Predisposition to obesity can start very early in human life. Limited glucose input in the prenatal stage, caused by malnutrition, and the consequent low insulin production leads to low levels of IGF-1 and to intrauterine growth retardation (IUGR). Later, during postnatal life, adequate nutrition produces a catch-up growth phase because of the excessive activation of IGF system(s) and increased insulin production, with consequent hyperinsulinemia, insulin resistance, and a correlated progressive weight increase. Ibanez in 1998 observed a direct correlation between a very low birth weight, precocious pubarche, hyperandrogenism, and hyperinsulinism [34]. This obesity rebound is characterized by reduced insulin sensitivity, greater storage of visceral fat, and increased androgen levels. Nowadays, about 5% of adolescents shows metabolic syndrome. The metabolic phenotype of IUGR babies is determined by slow growth before birth with accelerated growth in early postnatal life, and a consequent development of glucose intolerance and insulin resistance, thus predisposing the child to obesity and type 2 diabetes. Moreover, the early insulin environment and intracellular availability of free fatty acids and glucose in the visceral adipocytes, skeletal muscle cells, and hepatocytes are triggers of the progression toward obesity [35]. The criteria used to define the risk of metabolic syndrome during adolescence are given in Fig. 6.4.

Practically speaking, there is a high risk for the occurrence of metabolic syndrome for those who are obese or overweight during prepubertal age or adolescence, especially if familial risks (i.e., obesity or diabetes) or a history of IUGR is present. It is relevant to say that most of these triggers derive from reduced insulin

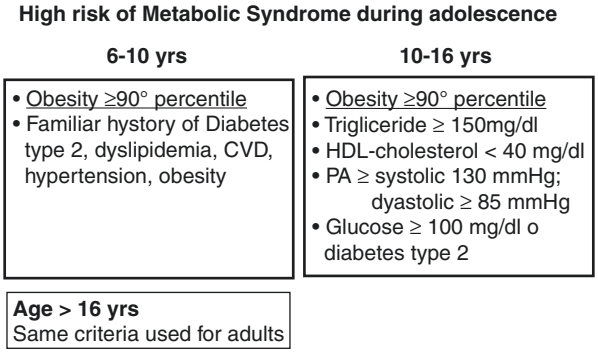

Fig. 6.4 Factors inducing the risk of metabolic syndrome during adolescence

sensitivity or increased insulin resistance. In addition, insulin production becomes higher and insulin sensitivity becomes lower during the transition from prepuberty to puberty because this development requires much energy, similar to the premenopausal transition [36, 37]. It is evident that being obese during the pubertal transition predisposes women to obesity and to type 2 diabetes later in adult life.

Polycystic ovary syndrome (PCOS) probably results from the overlapping of many factors: the maternal environment and numerous endocrine disturbances with insulin in the central role, mediated by genetic predisposition and activation prenatally, that leads to altered central regulation [38]. For these reasons, PCOS is considered a combination of diseases (from developmental origin) and adaptation (changes and impairments from an abnormal milieu): the interactions of insulin resistance and hyperandrogenism affect the central process that subserves reproduction and metabolism [39].

Insulin resistance is typical of PCOS, is not found in hyperandrogenic women, and is partially dependent on body weight. Indeed, we observe that 60% to 75% of overweight/obese PCOS patients have insulin resistance and also that 18% to 30% of normal-weight PCOS patients show insulin resistance. It is well known that insulin resistance is caused by the reduction of peripheral sensitivity to insulin, which determines a compensatory hyperinsulinemia.

In hyperandrogenism, body weight is relevant in the modulation of hypothalamus-pituitary functions, because the fat tissue is a "factory" of steroids without any control and includes 25% of circulating androgens; thus, only weight reduction can reduce such production. PCOS indeed is a multigenic condition in which the phenotype is modulated by external lifestyle factors. Hyperandrogenism and hyperinsulinemia affect the follicular microenvironment, leading to reduced ovarian development of immature oocytes, and the result is chronic anovulation.

Obese patients are characterized by chronic anovulation, and we can find signs and symptoms such as oligomenorrhea/amenorrhea, hyperandrogenism, altered body mass index (BMI), hyperinsulinemia, infertility, and stress: all these are very common in PCOS.

In obesity, we observe an inappropriate feedback system: androgen excess increases peripheral conversion to estrone, and this hyperestrogenemia alters gonadotropin secretion, mainly the release of FSH.

Plasma levels of estrone, a weak estrogen with biological activity 100-fold less than that of estradiol, are increased by the peripheral conversion of androstenedione by aromatase activity. Excess of estrone leads to a hyperestrogenic state, which might predispose the patient to endometrial proliferation and to a higher risk for endometrial cancer [40].

Compared to normal women, obese women have characteristics similar to PCOS and amplified pulsatile LH release: hyperandrogenism and hyperestrogenemia can augment pituitary sensitivity to GnRH, mainly increasing LH pulse amplitude, and this causes abnormal ovarian stimulation. Most such neuroendocrine impairment results from abnormal endogenous opioid peptides (EOPs) and dopamine (DA) modulation at the hypothalamic level, thus impairing GnRH.

The other central element in obesity is hyperinsulinemia, which might be central in the pathogenesis of the syndrome because it can induce higher ovarian androgen production and anovulation [41], sustained also by the abnormal LH secretion, with a higher frequency of menstrual abnormalities than in normoinsulinemic PCOS patients [42]. Being a PCOS patient is an additional risk factor for insulin resistance: as seen in Fig. 6.5, the risk for insulin resistance increases as more risk

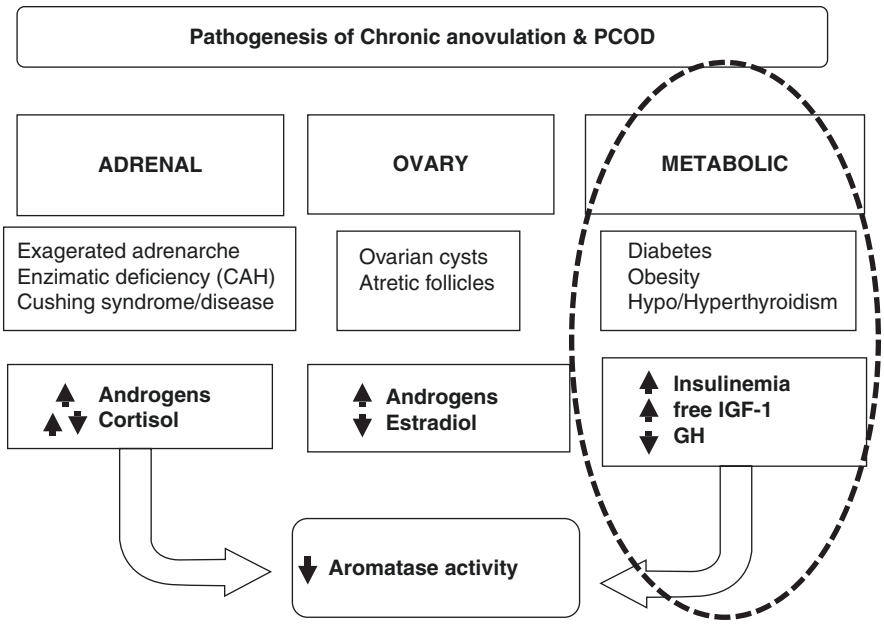

Fig. 6.5 At least three endocrine situations can induce abnormal (reduced) function/expression of aromatase, but the role of metabolism is now considered central for polycystic ovarian syndrome (PCOS)

factors are present, so surely the obesity associated with PCOS enhances the typical conditions for insulin resistance [43].

Screening for glucose intolerance (oral glucose tolerance test, OGTT) in patients with PCOS is recommended by the American Society of Reproductive Medicine, the European Society of Human Reproduction, and the Endocrinology PCOS Consensus Workshop Group, especially those patients with a family history of type 2 diabetes or those who are overweight or obese [44].

Nevertheless, we need to remember that insulin resistance could be present not only in obese PCOS but also in lean patients, induced by genetic factors. In particular, PCOS patients might have altered insulinemia because of altered sensitivity to insulin, altered insulin signal transduction, altered receptor activity, altered receptor number, or beta-cell dysfunction (pancreas). A diabetic or glucose-intolerant parent or relative is present in 30% to 50% of hyperinsulinemic PCOS patients.

At this point it is relevant to observe that greater availability of food, coupled with an abnormal everyday lifestyle (excess sedentarism, low physical activity, junk food) and any clinical/genetic predisposition to insulin resistance represent a higher risk of compensatory hyperinsulinemia. Such a condition is at the basis of the well-known metabolic syndrome that can induce elevation of lipid profiles, hypertension, and a greater risk of type 2 diabetes. Again, it appears clear that our physiology is not yet "adjusted" to overcome such epigenetic and environmentally induced diseases. Patients, and all clinicians, should be aware that everyone should keep their diet (i.e., quality of food) and the amount of food ingested under control. Integrative treatments [45, 46] can help greatly, but we must always keep in mind that dieting and body weight control are not only a matter of putting food into the mouth but also require optimal brain control.

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Management of Adolescent Hyperandrogenism: Still a Challenge?

7

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

7.1 Introduction

Androgen excess during puberty produces a variety of clinical signs and symptoms that must be appropriately recognized, evaluated, and treated.

Since acne, hirsutism, and obesity are outward signs of androgen excess, they are more than cosmetic concerns: body image, matters of sexual identity, and peers interaction are critical for the evolving personality.

Hyperandrogenism must be considered in an adolescent with:

- Excessive development of acne
- Hirsutism (≠ hypertrichosis) [1]

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- Menstrual irregularities: oligomenorrhea, dysfunctional uterine bleeding, and amenorrhea [2, 3]
- Abdominal obesity [4]
- Masculinization of the body habitus and/or virilization (= clitoromegaly, severe hirsutism) [5]

Early and late consequences of hyperandrogenism are well-described [6] and listed below:

- Increase of visceral fat, adipocyte proliferation, and inflammation (white adipose tissue)
- Decreased energy expenditure (brown adipose tissue)
- Increased systemic oxidative stress (macrophages)
- Decreased insulin sensitivity (muscle)
- Increased insulin secretion (beta pancreatic cells)
- Decreased central leptin sensitivity (brain)

A rapid diagnosis and subsequent treatment of hyperandrogenism at adolescence are thus mandatory [3].

The management of an adolescent girl with hyperandrogenism is still a challenge. It must achieve three main goals [7]:

- Distinguish the so-called «physiological» hyperandrogenism of puberty (Fig. 7.1), which will resume within 1–2 years after menarche, from an endocrine cause
- Eliminate severe cause of hyperandrogenism, such as ovarian and adrenal tumors or nonclassical congenital adrenal hyperplasia
- Recognize PCOS early in order to initiate the prevention of obesity and insulin-resistance and to start adequate therapy

7.2 Causes of Adolescent Hyperandrogenism

Main etiologies of hyperandrogenism are reported on Fig. 7.2 [8].

During the last 20 years, we managed 194 adolescent girls referred to our clinic for hyperandrogenism. The different causes are listed below:

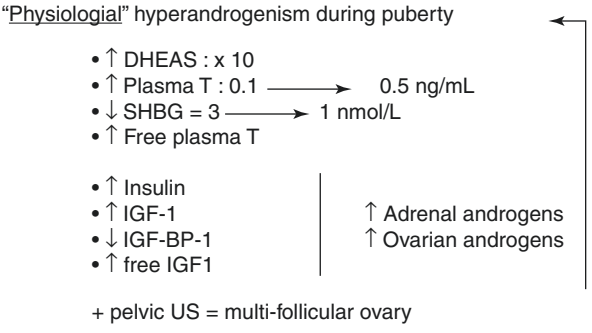

Fig. 7.1 Factors involved in the so-called physiological hyperandrogenism that occurs during pubertal development in girls

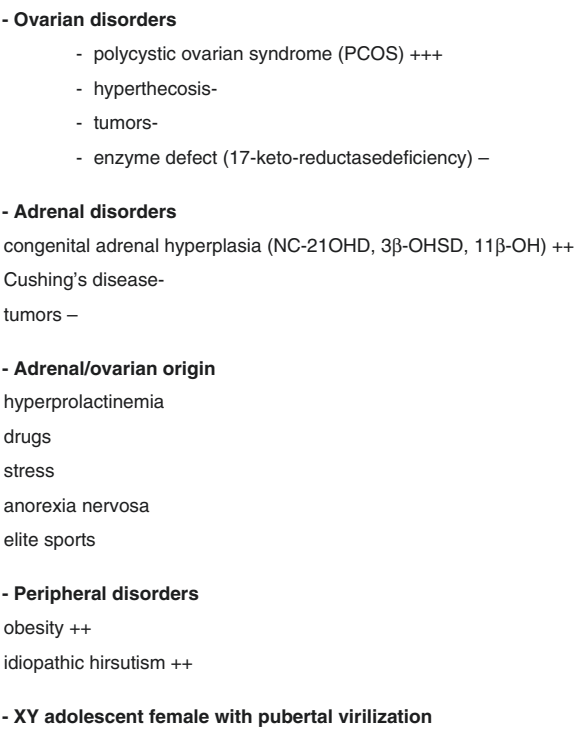

Fig. 7.2 Main etiologies of hyperandrogenism in adolescent girls

- Idiopathic hirsutism ($n = 26$)
- Abdominal obesity ($n = 17$)
- Transitory (reversible) hyperandrogenism ($n = 29$)
- Anorexia nervosa ($n = 10$)
- Late-onset 21OH deficiency ($n = 30$)
- Hyperprolactinemia ($n = 3$)
- Ovarian/adrenal tumors ($n = 3$)
- Elite sports ($n = 5$)
- Pubertal virilization in XY-DSD female ($n = 5$)
- PCOS ($n = 73$)

7.3 Clinical Expression of Hyperandrogenism in Adolescent Girls

7.3.1 PCOS During Puberty

The well-known long-term sequelae of PCOS now present a challenge for endocrinologists and gynecologists to make an early diagnosis (in the pubertal period) and to treat these teenagers both symptomatically and prophylactically [9].

The striking trend toward adolescent obesity should reinforce our responsibilities.

Menstrual irregularities in adolescents in the early post-menarchal years can be an early sign of PCOS.

In obese adolescents who subsequently develop glucose intolerance, there is an overall clinical impression that PCOS has become a prevalent cause of hyperandrogenism/menstrual disorders.

According to the International Consensus Meeting [10], the criteria for the diagnosis of PCOS in adolescents are:

- Hirsutism (progressive)
- Irregular menses/oligomenorrhea (*m* > 2 years)
- Testosterone concentration > 45–55 ng/dL (foll. phase)
- Other signs (obesity, insulin resistance, risk factors) are optional
- PCO morphology (US) is helpful

We actually recommend diagnostic criteria (≥4) [11]:

- Oligo-/amenorrhea in the early >2 post-menarchal years (obese)
- Clinical signs of hyperandrogenism
- Biochemical evidence of hyperandrogenism (T > 50 ng/mL) along with dysovulation (LH/FSH > 2)
- Insulin-resistance
- Enlarged ovaries with a hypervascularized stroma and small multiple peripheral cysts

Besides the typical post-menarchal PCOS, PCOS can occur in premenarchal time, in familial cases, after central precocious puberty, precocious pubarche in girls with IUGR, and after pubertal intensive sport. It can also be revealed by early metabolic expression [12].

The aims of treatment of adolescents with PCOS are to regulate menses and to improve androgenic concerns and lifestyle issues.

- Lifestyle intervention and weight loss are beneficial in many areas.
- The association of antiandrogen (cyproterone acetate, 50 mg/day for 20 days) plus natural estrogens (Provames 2 mg/day for 20 days) has been proved useful in many European countries.
- Insulin sensitizers are sometimes discussed, but there is no large-scale, double-blind, placebo-controlled studies in the adolescent group [13].

7.3.2 Nonclassical CAH

Nonclassical CAH [14] occurs on 1 out of 1000 individuals (prevalence 0.1–0.26%), but this frequency is higher among Ashkenazi Jews (prevalence 1–2%).

Moreover, higher prevalence is observed in Mediterranean, Middle-Eastern, and Indian populations.

Many patients with nonclassical CAH are asymptomatic.

According to pubertal development, nonclassical CAH is reported among 5–30% of girls with premature pubarche.

During adolescence, nonclassical CAH is revealed by hirsutism (60% of cases), oligomenorrhea (53%), or acne (30%).

Regarding adult reproductive function, many women are relatively fertile, but the success rate of women seeking pregnancy ranges between 60% and 70%: a greater risk of subfertility is related to ovulatory dysfunction secondary to elevated progesterone that impacts the quality of cervical mucus during follicular phase and induces inadequate endometrial maturation during preovulatory phase.

The diagnosis of nonclassical CAH is sometimes difficult: random 17-OHP may be within the normal range.

The largest multicentric trial reported that baseline 17-OHP level above 10 ng/mL is a sensitive criterion for nonclassical CAH.

To enhance screening efficiency, an ACTH test using 250 μg cosyntropin has been developed: with a >10 ng/mL cutoff, nearly 100% of patients with genetically proved nonclassical CAH could be identified. This accurate cutoff is routinely used for nonclassical CAH in hyperandrogenic states.

Individuals with nonclassical CAH are most commonly compound heterozygotes with different mutations on each allele:

- Seventy percent of nonclassical CAH are associated with Val 281 Leu (50–82% loss of 21OHase).
- Other missense mutations (P30L, P4535, R339H) are also reported.

For adolescents and adult women, goals of therapy include:

- Regularization of menstrual cycle
- Prevention of progressive hirsutism
- Preservation of fertility

OCP alone is discussed in oligomenorrheic, hyperandrogenic adolescents and women not seeking fertility.

Antiandrogens are sometimes useful when hyperandrogenism is severe.

Glucocorticoids are limited for symptomatic individuals.

7.3.3 Idiopathic Hirsutism

In an adolescent who shows clinical evidence of hyperandrogenism, contrasting with a normal plasma T level, normal ovarian morphology, and regular ovulation, a diagnosis of idiopathic hirsutism can be discussed [15].

Idiopathic hirsutism is related to an higher sensitivity of the pilo-sebaceus unit to androgens: increased 5-α reductase activity, that converts T into DHT, the active androgen, of higher sensitivity (increased androgen receptor transcriptional activity) to normal plasma androgen level.

Its prevalence ranges between 10% and 17%, but it is usually reported in adolescents from Mediterranean origins.

7.3.4 Abdominal Obesity

Recent data have disclosed a high prevalence of hyperandrogenism among peripubertal girls with obesity [4], suggesting that such girls are indeed at risk of PCOS. Abdominal obesity and subsequent insulin resistance and compensatory hyperinsulinism are associated with an augmentation of adrenal/ovarian production of androgens, a reduction of SHBG, and an increase of androgen bioavailability [3]. Altered LH secretion is usually reported. Besides, it is well-known that expanded fat mass enhances androgen production. BMI is significantly and positively correlated with free T. Compared with normal weight controls, mean free T in girls with abdominal obesity was reported to be elevated two- to ninefold, depending of pubertal stage.

Weight reduction appears to be the gold standard treatment for reducing hyperandrogenism in adolescent girls with abdominal obesity: according to some investigators, the reduction of free T is up to 50% after a weight reduction of 10%!

7.3.5 Androgen-Producing Tumors

Recent onset and rapidly progressive symptoms of hyperandrogenism usually suggest the presence of an androgen-producing tumor: severe hirsutism and virilization (deepening of the voice, clitoral hypertrophy), over the last 6 months, should be managed adequately.

In the majority of cases, they are androgen-producing ovarian tumors, a benign ovarian tumor. In this case, plasma total T is found to be above 100–200 ng/dL. Pelvic US is useful, but in some cases, MRI is mandatory.

In most cases, these ovarian tumors are benign, and surgical removal is associated with a resumption of hyperandrogenism.

7.3.6 Hyperprolactinemia

Hyperprolactinemia is known to disturb Gn-RH production and pulsatility and impair LH and then FSH secretion. This leads to relative hyperandrogenism. In an adolescent who recently become oligomenorrheic or amenorrheic, the diagnosis of hyperprolactinemia should always be discussed. Evaluation of prolactin serum level should systematically be done.

7.3.7 Cushing's Syndrome

It is caused by the excess of glucocorticoids with per se can not be the cause of hyperandrogenism. Cushing's syndrome is a very rare cause of hyperandrogenism, but it

must be discussed in an adolescent girl with recent onset of hyperandrogenism, associated with signs of hypercorticism: facial fullness, weight gain, and truncal obesity.

Measurement of a 24 h urinary free cortisol is usually the easiest way to confirm Cushing's syndrome. Performing the dexamethasone overnight suppression test is mostly informative.

7.3.8 Female XY-DSD

In adolescents with severe hirsutism that increases during pubertal development, along with some evidences of virilization and behavioral disorders, a karyotype must be systematically performed, in addition to an endocrine investigation (plasma T, D4-androstenedione, DHA-sulfate).

On some rare cases, adolescent hyperandrogenism may actually occur in YX-DSD females. In this condition, it is associated with a gonadal dysgenesis, a 17-β hydroxysteroid dehydrogenase defect, or an androgen resistance syndrome (partial androgen insensitivity syndrome or 5α-reductase deficiency) [16].

7.4 Patient Evaluation

7.4.1 Clinical Assessment

The first visit must include a thorough record of history and a careful physical examination [7, 10].

Is acne composed of obstructive, inflammatory, or cystic lesions? Clinical scoring of acne must be calculated.

Hirsutism must be quantified through the Ferriman-Gallwey score. Hyperandrogenism is defined by a Ferriman-Gallwey score above 9.

Some clinical symptoms may draw the clinician's attention toward a severe disease, such as rapidly growing hirsutism, symptoms of hypercorticism, galactorrhea, and virilization.

The initial history of hyperandrogenism must be precised: what is the relation of hyperandrogenism with the onset of puberty, with menstrual cycles? What is the rapidity of the symptoms? Is there any family history of hyperandrogenism and what is the ethnic background?

7.4.2 Hormonal Investigations

Most consensus statements recommend measuring total T as a first-line investigation of hyperandrogenic state. Is plasma total T the relevant hormone for identifying hyperandrogenic states [17].

Direct immunoassay is the most widely used method easy to use with a short assay time, suitable for automated platforms, and with a good intra- and inter-assay reproducibility. However, it presents low accuracy, lack of specificity, and some interference with binding proteins.

Conversely, LC-MS methodology presents accuracy, high specificity, and sensitivity but needs some expensive equipment and shows some variability between labs and technical pitfalls.

Measuring the protein-unbounded fraction of T provides a better marker of biological activity of T. Unfortunately, available immunoassay for free T shows inadequate accuracy and precision [18].

Routinely, evaluation of androgen overproduction is based on plasma T (total or free) and plasma 17OH progesterone.

According to most investigators, plasma T value above 50 ng/dL defines biological hyperandrogenism in adolescent girls.

Evaluation of ovulation disorders includes measurement of plasma LH and FSH and performing a pelvic US (ovaries structure and morphology).

In some patients, provocative tests, such as corticotropin stimulation test, LHRH test, and insulin resistance test, are discussed.

7.4.3 Decision Algorithm

The decision algorithm for managing adolescent hyperandrogenism is reported in Fig. 7.3.

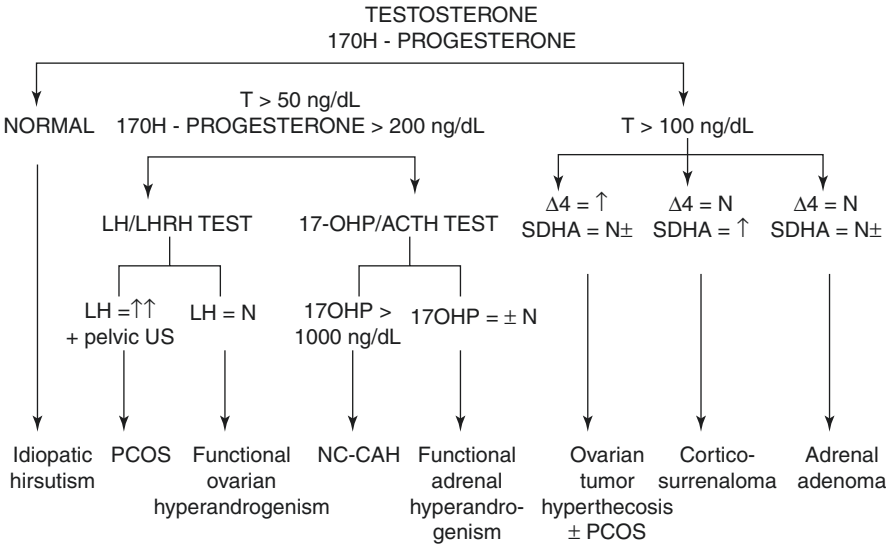

Fig. 7.3 The decision algorithm for managing adolescent hyperandrogenism

7.5 Therapeutic Options

- Management of excess ovarian production is based on antiandrogen treatment (inhibition/LH secretion), combined oral contraceptive pill, and/or insulin-sensitizing agents (metformin).
- Corticosteroid therapy is usually prescribed to reduce excess of adrenal production.
- Antiandrogens are usually discussed to reduce the intracellular androgen biodisponibility.
- Whatever the cause of hyperandrogenic state, optimization of weight and managing hirsutism (bleaching, electrolysis, laser) are frequently suggested to improve body image and self-esteem in adolescents with psychological concerns.

7.6 Conclusion

Hyperandrogenic states encompass several clinical situations [19], associated with excess androgen production from androgen-producing tumors, genetic disorders, polycystic ovaries, and obesity, with prevalence ranging between 5% and 18% in adolescent girls. It raises the question of how laboratory investigations should be conducted for its diagnosis. Adolescents with hyperandrogenism are actually at increased risk for metabolic complications, cardiovascular diseases, and infertility. Hyperandrogenism concerns all health professionals who treat adolescents [20]. Intervention may prevent/reduce major health sequelae and can contribute to psychological well-being. It is however still a challenge!

In conclusion, can the relationship between androgens and puberty be considered as a necessary evil?

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Hormonally Related Headaches

8

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8.1 Introduction

Sex hormones modulate a number of brain functions [1], which are relevant to the clinical expression of primary headaches, especially migraine, across the reproductive life of women [2]. Headache is among the most common neurological conditions with a current worldwide prevalence in the general population of 47% [3]. The Global Burden of Disease Study 2015 ranks headache disorders (migraine, tension-type headache, and medication-overuse headache) as the third leading cause of life lost to disability worldwide in the 15–49 age group [4]. Both age and gender contribute significantly to the chronification of headache [5]. In a population-based study, the frequency of migraine without aura and tension-type headache peaked within a

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few days of onset of menses. Interestingly, migraine with aura did not appear to have a strong relationship to the hormonal fluctuations associated with onset of menses [6], but with the high estrogenic state [7]. That being so, reproductive milestones (menarche, pregnancy, and menopause), as well as the use of hormonal treatments (hormonal contraception, hormonal replacement therapy, other hormonal manipulations), may inflect frequency, intensity, duration of head pain and clinical response to pain medications and pharmacological preventive strategies [8, 9].

In here, we will summarize briefly the role of hormonal fluctuations in the pathogenesis of migraine related to menstruation, and we will focus on hormonally related headaches associated with the use of exogenous hormones in clinical practice.

8.2 Menstrual-Related Migraine

The most recent version of the International Headache Society (IHS) Classification of Headache Disorders [10] includes migraine related to menstruation in the appendix to underline the need for more research to better characterize the relationship between neuroendocrine events driving the activity of the hypothalamus-pituitary-ovarian axis (HPO) and mechanisms involved in the temporal pattern of migraine across the menstrual cycle. Headache experts state that many women overreport an association between attacks and menstruation. Thus, a diary-documented, prospectively recorded evidence over a minimum of three cycles is necessary to confirm the diagnosis [11]. Menstrual migraines can be divided into two categories: pure menstrual migraine (PMM) and menstrual-related migraine (MRM). PMM occurs exclusively on day 1 ± 2 of menstruation in at least two out of three menstrual cycles and at no other time of the cycle. MRM occurs on day 1 ± 2 of menstruation in at least two out of three menstrual cycles and additionally at other time of the cycle. PMM is far less frequent, concerning 7–12% of women [12, 13], whereas MRM occurs in about 60% of women who report other nonhormonal triggers for head pain throughout the month [13]. The recognition of a 5-day menstrual migraine window [14] is based on clinical data [6, 12] and on the early observations that a period of several days of exposure to high estrogen levels is necessary before estrogen withdrawal can result in migraine at the time of menstruation [15]. Migraine is an inherited disorder that involves central pain modulating dysfunction via a complex interplay between neurotransmitters, inflammatory peptides, and vasculature modulated by the trigeminovascular system [16]. Estrogen variations are highly implicated in modulating the threshold to challenges by altering neuronal excitability, cerebral vasoactivity, pain sensitivity, neuroendocrine axes, etc., across the menstrual cycle and not only at the time of menstruation [17]. Women with both PMM and MRM have faster late luteal phase-conjugated urinary estrogen decline (greater absolute rate of decline and greater percent of change) compared with controls [18]. Then, it is likely that women with migraine are more vulnerable to hormonal fluctuations at multiple levels of the pain control system, especially following estrogen withdrawal at menstruation. However, many clinicians are under the impression that migraine attacks related to menses are more severe, long-lasting, and refractory to both acute and prophylactic treatments [6,

19, 20]. Migraine attacks may cover the entire perimenstrual period, from day −2 to day +7, because head pain lasts longer and is less responsive to acute treatment [20]. Moreover, migraine at menstruation is different in terms of severity from non-menstrual attacks, even within individuals, and the highest severity is evident on day 1–3 when menstrual bleeding starts [14]. Interestingly, as compared with women with typical duration of PMM attacks (4–72 h) and controls, ovulatory women with menstrual status migrainosus, an extremely severe migraine attack lasting longer than 72 h, display a blunted neuroendocrine response to a challenge with meta-chlorophenylpiperazine (m-CPP) [21]. By using this 5-HT agent with a high affinity for several subtypes of 5-HT receptors, especially 1 and 2, the same findings were found in women with a clear window of vulnerability to migraine due to the abrupt withdrawal of synthetic estrogens, as it occurs during the hormone-free interval of combined oral contraception [22]. The ability of transdermal estradiol supplementation to restore the neuroendocrine response to m-CPP confirmed a crucial role for estrogen withdrawal in the modulation of pain threshold within the menstrual migraine window [22]. A very recent data in women with headache attacks of the migraine type recurring during the hormone-free interval confirmed that estrogen withdrawal is associated with an increased sensitivity to somatosensory stimuli [23].

8.3 Secondary Headaches Related to Exogenous Hormones

Hormonally related headaches may occur as a result of natural hormonal fluctuations or in response to the exposure to exogenous hormones or following their discontinuation [24]. The most recent version of the IHS Classification of Headache Disorders [10] includes them among secondary headaches, either migraine or tension-type headache, attributed to a substance or its withdrawal. Headaches may occur for the first time, or pre-existing types may become chronic or more relevant (usually meaning a twofold or greater increase in frequency and/or severity) in close temporal relation to exposure to or withdrawal from a substance such as hormonal contraception containing estrogens and hormonal replacement therapy (HRT).

The previous diagnosis 8.1.12 Headache attributed to exogenous hormone is now coded 8.1.10 Headache attributed to long-term use of non-headache medication [10]. This definition describes headache developing as an adverse event during long-term use of a medication for purposes other than the treatment of headache, i.e., with exogenous hormones. It is not necessarily reversible. Evidence of causation of headache (present on ≥15 days/month) should be demonstrated by at least two of the following:

1. Headache has developed in temporal relation to the commencement of medication intake.
2. One or more of the following:
   (a) Headache has significantly worsened after an increase in dosage of the medication.

(b) Headache has significantly improved or resolved after a reduction in dosage of the medication.
(c) Headache has resolved after cessation of the medication.
3. The medication is recognized to cause headache in at least some people, during long-term use.

Headache or migraine developing within 5 days after daily consumption of exogenous estrogen for 3 weeks or longer, which has been interrupted (usually during the pill-free interval of combined oral contraception or following a course of replacement or supplementary estrogen), is coded as 8.3.3 Estrogen-withdrawal headache, which replaces code 8.4.3 Estrogen-withdrawal headache. The present version of the IHS Classification [10] states that evidence of causation should be demonstrated by both of the following:

1. Headache or migraine has developed within 5 days after the last use of estrogen.
2. Headache or migraine has resolved within 3 days of its onset.

Prescribers of combined hormonal contraceptives (CHCs) and HRT should be aware that hormonal compounds may trigger the occurrence of migraine and/or tension-type headache or exacerbate pre-existing attacks, especially around the time of withdrawal bleeding [24].

8.4 Decision-Making Process in Hormonally Related Headaches

Reproductive life is characterized by the need of reliable and convenient methods of contraception, whereas menopausal years may be associated with climacteric symptoms requiring personalized HRT. The prescription of CHCs may have different effects on migraine with not univocal results because of many methodological limitations (diverse hormonal combinations, variable research settings, retrospective and/or cross-sectional designs, lack of a clear phenotyping of the headache according to IHS criteria, inadequate duration of observation) [25–27]. Historically, combined oral contraceptives (COCs) is a category best studied in migraineurs with an aggravation of migraine reported in 18–50% of cases, an improvement in 3–35%, and no change in 39–65% [28]. A more recent cross-sectional study on a large population found that migraine is significantly associated with COCs assumption, but no causal relationship between exposure and disease could be demonstrated [29]. Analysis on the different effects of the COCs on the two forms of migraine revealed that MA worsen more (56.4%) than MO (25.3%) [30]. Furthermore, women can present MA for the first time during the initiation of COCs [31]. Several evidences indicate that CHCs may be used in the majority of women with headaches, but migraine deserves accurate diagnosis and recognition

of the impact of different methods on such condition [32]. Prescription should be guided by the recognition of cardiovascular and thromboembolic risk factors (smoking, hypertension, obesity, diabetes, dyslipidemia, thrombophilia, family history) in women with migraine without aura, especially older than 35 years [33]. Migraine with aura represents a contraindication to the use of CHC, and progestogen-only contraception should be preferred [33–35]. In general, the absolute risk associated with CHC use is very low in healthy young women with no additional risk factors and mostly related to the estrogen dose [36]. The recent consensus statement of the European Headache Federation and European Society of Contraception and Reproductive Health did not make any distinction among types of estrogen or characteristics of progestogens in CHCs and their associated risk of stroke [33]. However, it recognized that the decision-making process should take into account additional benefits of specific CHCs on some reproductive disorders, namely, polycystic ovarian syndrome and endometriosis [33]. Over the year, several hormonal strategies have been proposed to improved hormonally related headaches in women taking CHCs. Recognizing that headaches are triggered by estrogen withdrawal, transdermal estradiol supplementation has been proposed for the management of MRM and other hormonally associated headaches [22, 37, 38]. For CHC users reporting estrogen-withdrawal headache during the 7-day free hormone interval, a different approach has been to shorten the interval from 7 to 4 days or to administer low-dose combined oral contraceptives (COCs) in extended/flexible regimens to prevent withdrawal bleeding [39, 40]. Even the use of 26/2 regimen with natural estradiol has been proposed considering also its less metabolic and vascular impact [41, 42]. That being so, a very recent consensus statement summarized recommendations for all the exogenous hormonal compounds with available evidence in terms of possible effects on migraine course and on possible treatment of headache associated with the use or with the withdrawal of hormones. Overall, quality of current evidence is low, and further research is needed [43]. In order to decrease duration, frequency, intensity, and disability of hormonally related headaches, short-term prophylaxis or mini-prophylaxis with standard options, including NSAIDs (naproxen) or triptans (frovatriptan, naratriptan, zolmitriptan) daily starting 2–3 days prior to onset of expected bleeding up to 3–7 days post-onset of bleeding, should be considered [44].

In perimenopausal and postmenopausal women requiring HRT, the picture is even more complex because of the older age and of the presence of potential vascular risk factors in women with migraine [45]. Quality of available data is poor, but maintaining a stable estrogen environment with estrogen replacement can benefit estrogen-withdrawal migraine particularly in women who would also benefit from relief of vasomotor symptoms [46]. In women with migraine with or without aura, using only the lowest doses of transdermal estrogen necessary to control vasomotor symptoms minimizes the risk of unwanted side effects. Cyclical progestogens can have an adverse effect on migraine by inducing withdrawal bleeding [47, 48]. Therefore, continuous progestogens, as provided by the levonorgestrel intrauterine system or in continuous combined transdermal preparation, are preferred [46].

8.5 Conclusion

Hormonally related headaches are frequent in routine practice and require accurate counseling. Headache classification is evolving, and more hormonal options are available for women. However, there is a need for better management of headaches related to the use of hormones or their withdrawal.

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Diagnosing Abnormal Uterine Bleeding: The Standard of Care Has Changed

9

Ashraf Khafaga and Steven R. Goldstein

9.1 Introduction

Abnormal uterine bleeding (AUB) is a common gynecologic complaint which accounts for one-third of the outpatient visits to gynecologists and represents more than 70% of all gynecological consults in the perimenopausal and postmenopausal years [1]. A US population-based survey of women ages 18–50 years reported an annual prevalence rate of AUB as 53 per 1000 women [2]. The estimated annual direct cost of AUB in 2007 was approximately $1 billion, with indirect economic costs of $12 billion [3]. The overwhelming problem of AUB is due to its tremendous impact on women's quality of life, productivity, and utilization of healthcare services, and thus diagnosis and treatment of this condition needs to be undertaken judiciously. Therefore, it should be clear that evaluation of patients with AUB aims (1) to exclude serious underlying pathology such as carcinoma or complex atypical endometrial hyperplasia and (2) to diagnose the cause of bleeding so an appropriate management can be implemented.

9.2 Etiology

The definition of AUB is "flow outside of normal volume, duration, regularity, or frequency" [1]. AUB can be caused by uterine structural abnormalities or nonstructural causes. In 2011 the International Federation of Gynecology and Obstetrics (FIGO) introduced a new classification system for abnormal uterine bleeding that was endorsed by the American Congress of Obstetrics and Gynecology in 2012, as

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an effort to standardize the terminology used to describe AUB, and eventually this system has now become widely accepted [4]. This system, known by the acronym PALM-COEIN, distinguishes abnormal uterine bleeding based upon the suspected etiology: *p*olyp, *a*denomyosis, *l*eiomyoma, *m*alignancy and *h*yperplasia, *c*oagulopathy, *o*vulatory dysfunction, *e*ndometrial, *i*atrogenic, and *n*ot yet classified [4]. The PALM portion of the PALM-COEIN covers the structural causes of abnormal uterine bleeding. In contrast, the COEIN acronym represents the nonstructural, hormonal, or systemic causes of abnormal uterine bleeding.

Descriptive terms are paired with AUB to indicate the bleeding patterns. Heavy menstrual bleeding (AUB/HMB) is now used instead of the term menorrhagia, and intermenstrual bleeding (AUB/IMB) has replaced the term metrorrhagia [1]. AUB is further denoted by the qualified letter or letters to indicate the underlying etiology such as AUB-P for AUB-polyp, AUB-L for AUB-leiomyoma, etc. Leiomyomas may be subclassified as either submucosal (AUB-L_{SM}) or those that do not affect the uterine cavity (AUB-L_O). Abnormal bleeding associated with the use of exogenous steroids (i.e., hormonal treatments), intrauterine systems (IUSs) or devices, or other systemic or local agents is classified as iatrogenic, whereas the remainder of rare or ill-defined causes is categorized as not yet classified. Ovulatory dysfunction (AUB-O) is usually related to exposure to unopposed estrogen by different mechanisms such as PCOS or oligo-ovulation which is common in the perimenopausal years.

The term dysfunctional uterine bleeding is usually used to indicate AUB which is caused by nonstructural abnormalities, and it is not a part of the PALM-COEIN, so the American Congress of Obstetrics and Gynecology recommended to discontinue using this term [1].

9.3 Premenopausal and Perimenopausal Women

AUB most frequently occurs in women aged 19–39 as a result of pregnancy, structural abnormalities such as leiomyoma and polyps, anovulatory cycles (e.g., PCOS (polycystic ovarian syndrome)), hormonal contraceptive, and endometrial hyperplasia [1]. Endometrial carcinoma is less common at this age group, but it may occur [5]. In women aged 40 years to menopause, AUB is most likely due to anovulatory bleeding, as a result of the exhaustion of the functioning ovarian follicles. It also may be due to endometrial hyperplasia or carcinoma, endometrial atrophy, and leiomyomas [1].

9.4 Postmenopausal Bleeding

Postmenopausal bleeding (PMB) is defined by any uterine bleeding in a menopausal woman who is not taking cycling postmenopausal hormone therapy. It represents 5% of office gynecology visits [6]. Even though, the most common cause of PMB is atrophy of the vaginal mucosa or endometrium [7], and in clinical practice, only 3–7% of women presenting with PMB will ultimately be found to have cancer; all women with PMB should be evaluated for endometrial cancer. Endometrial cancer is

the most common type of gynecological cancer in the United States. In 2017, the incidence of uterine cancer was estimated as 61,380 cases, with a mortality of 10,920 cases [8]. Most cases of uterine cancer occur in the endometrium and have been reported to represent 92% of cases [8]. Additionally, vaginal bleeding is the presenting sign in more than 90% of postmenopausal women with endometrial cancer [9].

9.5 Diagnosis in Women Presenting with Abnormal Uterine Bleeding

The evaluation of women with AUB includes a through medical history and physical exam and appropriate laboratory and imaging tests as indicated. A medical history should be guided by the PALM-COEIN system and include inquiries about the menstrual bleeding pattern, the amount, the presence of pain, any family history of AUB or underlying bleeding disorders, medication or herbal preparations that might affect bleeding in general such as ginseng, ginkgo, motherwort, contraceptives, nonsteroidal anti-inflammatory drugs (NSAIDs), and warfarin or heparin derivatives [10, 11]. One of the most important aspects of the medical history will be careful assessment of the bleeding pattern, although, admittedly, many women will not be aware of exactly how often or how long they bleed. However, when possible, for instance, very cyclic heavy menstrual bleeding without any intermenstrual bleeding would be unlikely to be carcinoma or even hyperplasia. Most often, an irregular bleeding pattern is not associated with any structural abnormality as mentioned above but clearly one must be excluded.

The physical examination may also reveal findings that contribute to AUB. Physical signs suggestive of an underlying cause include excessive weight, hyperprolactinemia (galactorrhea), signs of polycystic ovaries syndrome PCOS (e.g., acne, hirsutism), signs of thyroid disease (e.g., thyroid nodule or goiter), and signs of bleeding disorders (e.g., ecchymosis and petechiae). Additionally, a pelvic exam using a speculum should be performed to exclude lower genital tract causes as cervical or vaginal etiologies of bleeding, and bimanual assessment of size and contour of the uterus should be performed as well.

Laboratory testing should be ordered depending on the patient's history and physical examination. In general, the initial laboratory assessment of AUB should include complete blood count (CBC) to ascertain whether anemia is present, in attempt to assess the severity of bleeding, pregnancy testing, as well as assessment of underlying bleeding disorders if concerning or suspected. Thyroid-stimulating hormone (TSH) level assessment and cervical cancer screening may also be appropriate. In some cases, testing for *Chlamydia trachomatis* may also be necessary to rule out AUB associated with infection.

Uterine evaluation for AUB may also include endometrial biopsy and imaging studies when indicated. The best initial imaging test of the uterus to assess AUB is transvaginal ultrasound (TVU). If transvaginal ultrasound images are not adequate or further evaluation is required, then sonohysterography which is also called saline infusion sonography SIS (the installation of fluid or gel into the endometrial cavity

to further delineate endometrial anatomy) or hysteroscopy is recommended. Hysteroscopy is more expensive, requires more anesthesia, and, if performed, is preferably done in an office setting [1]. Newer disposable hysteroscopy, recently developed, makes this recommendation easier to follow.

One study of 443 women [9] used transvaginal ultrasound and saline infusion sonohysterography as the first step in triage reported 79% of women between 35 years old and menopause with AUB had no anatomic pathology, presumably secondary to anovulatory bleeding. Some, whose AUB is heavy menstrual bleeding, may have an enlarged cavity with increased surface area due to increasing parity, uterine hypertrophy secondary to leiomyoma with no submucous component, or adenomyosis without endometrial abnormality. In that study, endometrial abnormalities included hyperplasia, polyps, and submucous myomas.

9.6 Endometrial Sampling

As stated in ACOG 2012, the current recommendation is that endometrial sampling should be the first-line test for tissue sampling in patients presenting with AUB who are older than 45 years. Additionally, it should be also done in patients who are younger than 45 years, in the case there is a history of unopposed estrogen exposure commonly seen in obesity and PCOS patients, or failed medical management, or persistent AUB or those who have any irregularity in the appearance of endometrium on TVU, and in women at high risk of endometrial cancer (e.g., tamoxifen therapy, Lynch or Cowden syndrome) [1].

Choosing 45 years of age as the cutoff point for increased concern regarding endometrial neoplasia is supported by evidence that the risk of endometrial hyperplasia and carcinoma is quite low prior to age 45 years and increases with advancing age, as the incidence rate of 16.3% was reported in women aged 45–54 years compared with an incidence rate of 5.4% in those aged 35–44 years [12, 13].

In postmenopausal women, the endometrial evaluation is essential in triaging patients to no anatomic pathology or anatomic pathology and then, furthermore, whether such pathology is focal in nature and needs to be distinguished from more global processes. Historically, this is used to utilize dilatation and curettage as the primary diagnostic test. In fact, it was the most common surgical procedure in women during much of the twentieth century. More recently, endometrial biopsy in an outpatient setting has gained great regularity. The aim of such endometrial sampling was expected to diagnose the presence of carcinoma or premalignant lesion.

After a single study by Stovall and colleagues [14], blind endometrial sampling with disposable suction piston devices became the standard approach to patients with AUB. Stovall performed such an outpatient biopsy on 40 patients with known carcinoma in the week prior to their hysterectomy and obtained endometrial carcinoma in 39 of the 40 samples, thus reporting a 97.5% accuracy. This was widely publicized, marketed, and promoted and was rapidly accepted as "standard of care." In a similar study, Guido and colleagues performed such blind endometrial sampling in 65 patients with known carcinoma in the operating room just prior to their

hysterectomy [15]. They missed 11/65 cancers (sensitivity only 83%) but, upon opening all those uteri, they reported that, when the cancers occupied 50% or more of the endometrial surface, the biopsy was 100% accurate. Others did similar studies to those of Stovall and Guido.

In women with known carcinomas, the sensitivity of blind sampling was only 84% [16] and 68% [17] in those studies, yielding a false-negative rate of 16% and 32%, respectively! And again, these were blind biopsies done on women with known carcinoma. In trying to understand why such biopsies failed in non-global pathology, one needs to look no further than the pre-hysterectomy study by Rodriguez and colleagues [18] in which the Pipelle brand sampled an average of 4% of the endometrial surface area (range 0–12%). All the previous data were as a red flag of using blind sampling as the standard care.

Finally, in 2012, the American College of Obstetricians and Gynecologists (ACOG), in their Practice Bulletin [1], acknowledged "the primary role of endometrial sampling in patients with AUB is to determine if carcinoma or premalignant lesions are present." The Bulletin goes on to state that endometrial biopsy has "high overall accuracy in diagnosing endometrial cancer when an adequate specimen is obtained and when the endometrial process is global. If the cancer occupies less than 50% of the surface area of the endometrial cavity, the cancer can be missed by blind endometrial biopsy. Therefore, these tests are only an endpoint when they reveal cancer or atypical complex hyperplasia." This has tremendous ramifications for clinical practice. Certainly, healthcare providers, especially in low-resource areas, can begin the evaluation with a blind biopsy, but if the results do not indicate cancer or atypical hyperplasia, the evaluation is not adequate and cannot be accepted as an endpoint, especially if bleeding persists, so further testing becomes necessary [8]. Thus, the concept of distinguishing "global" from "focal" pathologies by using SIS is becoming increasingly utilized.

To conclude, the biopsy shortcomings and ACOG acknowledgment of this non-trivial problem represent a major shift in how blind endometrial sampling should be reviewed and has led to a fundamental change in the standard care of AUB patients.

9.7 Imaging Techniques

The decision to proceed with imaging technique should be based on the medical history, physical exam, patient's age, and the clinician's assessment.

9.7.1 Transvaginal Ultrasonography (TVU)

TVU is the best initial imaging study in women presenting with AUB, as it is a safe and cost-effective method of diagnosing structural causes of abnormal uterine bleeding by exploring the uterine cavity, so it is a substantial diagnostic tool to exclude the PALM portion of the PALM-COEIN system. The vaginal probe provides a degree of image magnification as if we were doing ultrasound through a

low-power microscope and can be considered a form of "sonomicroscopy" [19]. The use of TVU in the assessment of endometrial thickness is not an optimal tool to assess abnormalities in premenopausal woman as compared to its use in exclusion of malignancy in postmenopausal [20–22]. Endometrial thickness varies during the menstrual cycle as a result of the dynamic hormonal changes, leading to a limited application of endometrial thickness as a diagnostic tool in *premenopausal* women.

There are insufficient data collected on *perimenopause* women with AUB. Perimenopause is defined as "the period around the onset of menopause that is often marked by various physical signs such as hot flushes and menstrual irregularities" [23]. One potential pitfall in perimenopause women is that their cycling of the endometrium is dependent on erratic estrogen production of perimenopausal ovaries. Thus, the use of TVU in such patients must be timed to the end of bleeding episode when the endometrial echo will be as thin as one would expect throughout the whole month. Additionally, this prevents misinterpretation of endometrial "moguls," which can occur because of the heterogeneity of the topography of the endometrium's functionalis as it proliferates.

In a study of 433 perimenopausal patients [24] aged 37–54, 10.2% required sonohysterography because the unenhanced transvaginal ultrasound done at the end of a bleeding cycle was inadequate effectively to characterize and measure the endometrium.

In postmenopausal women, the earliest reports comparing transvaginal ultrasound (TVU) with endometrial sampling in women with PMB consistently showed an endometrial thickness 4–5 mm or less reliably excluded endometrial cancer [25]. Since that time, a number of confirmatory multicenter studies have been performed. Accordingly, ACOG in 2009 stated that when TVU reveals a thin, distinct endometrial echo 4 mm or less, the risk of malignancy is 1 in 917, and therefore, endometrial sampling is not required [26]. When the endometrial thickness is less than 4 mm there was a greater than 99% negative predictive value for endometrial cancer [8]. Thus, the initial evaluation of women with PMB may begin with a TVU, and if sufficiently distinct and thin, no further workup is necessary. In fact, if one does attempt endometrial sampling in such women, often no tissue is present, and if present, it is often insufficient for histologic evaluation [26]. Since rarely cases of endometrial carcinoma, particularly type II cancers, can present with an endometrial thickness of less than 4 mm, in cases of persistent or recurrent uterine bleeding, furthermore extensive evaluation irrespective of the endometrial thickness is indicated [8].

Additionally, an endometrial thickness greater than 4 mm that is incidentally diagnosed in postmenopausal women without bleeding should not prompt automatic further evaluation, unless the clinician's assessment is concerning for other cancer risk factors [8].

9.7.1.1 Limitation of TVU

Unfortunately, the main drawbacks of using TVU are the low sensitivity and specificity for assessing the intracavity lesions as they were reported as only 56% and 73%, respectively [27]. In addition, transvaginal ultrasound does not adequately

image the endometrial cavity in all women with PMB. An axial uterus, obesity, coexisting myomas, adenomyosis, or previous uterine surgery can preclude satisfactory endometrial evaluation. Failure to adequately identify a thin, distinct endometrial echo in a postmenopausal woman with bleeding should trigger an alternative method of evaluation.

9.7.2 Sonohysterography (Saline Infusion Sonography (SIS))

Saline infusion sonography (SIS) is a very useful adjunct to the traditional TVU. As stated in ACOG 2012, when TVU is insufficient in cases of endometrial echo not sufficiently thin to exclude pathology or the endometrial thickness is inadequately visualized (as previously mentioned in cases of axial uterus, marked obesity, coexisting myomas, previous surgery, or adenomyosis), then SIS or hysteroscopy, preferably in an office setting, can be employed [1]. Many studies [27, 28] have concluded that SIS is a more valuable tool than TVU to assess the intracavity lesions such as polyps and submucosal leiomyoma.

Saline infusion sonohysterography involves instillation of a small amount of saline through a special catheter under ultrasound guidance. By distending the endometrial cavity, SIS highlights the endometrial contents, revealing causes of AUB/PMB, including endometrial polyps, intracavitary (submucosal) fibroids, and focal endometrial abnormalities more concerning for hyperplasias or carcinoma. A sonohysterogram demonstrating uniformly smooth endometrial surfaces without intracavitary masses provides reassurance that organic pathology is not present.

Only SIS can differentiate between focal and global thickening of the endometrium. A localized thickening of the endometrium is considered an obstacle to obtain an adequate endometrial sampling with blind biopsy. Therefore, using SIS can be a turning point in the decision of performing an endometrial biopsy under direct vision of hysteroscopy in cases of focal endometrial thickening or obtaining a blind endometrial biopsy which is ultimately appropriate for the cases of global endometrial thickening.

One study [27] compared the accuracy of several diagnostic modalities in the evaluation of AUB case showed that the effectiveness of using SIS is not inferior than performing hysteroscopy in detecting structural abnormalities. “Some data suggested that three-dimensional SIS is more accurate than two-dimensional SIS in determining the size and depth of myometrium invasion of submucosal leiomyoma, which may help predict the success of hysteroscopic resection” [1].

SIS should not be done during active bleeding which may produce false-positive results, as shedding endometrial lining and small clots clinging to the wall may appear similar to other intrauterine pathology such as endometrial polyps. If the patient is bleeding so heavily or so often that it is difficult to achieve the correct timing, it may be beneficial to perform a “medical curettage” with progestin inducing a withdrawal bleed and then timing the ultrasound evaluation to that bleeding episode. An alternative to SIS involves using new disposable office hysteroscopes that facilitate direct endometrial visualization in the office setting.

9.7.3 Hysteroscopy

Hysteroscopy is a technique that allows direct visualization of the uterine cavity and taking directed biopsies by placing a thin endoscopic instrument through the cervix into the uterus [29]. It shows high accuracy in detecting endometrial cancer, but it has a limited use in diagnosing endometrial hyperplasia [30].

9.7.4 Magnetic Resonance Imaging (MRI)

There is no indication for routine use of MRI in evaluation of AUB cases. It can be used as a tool to guide the treatment of cases of multiple myomas, especially when the uterus is diffusely enlarged. However, the benefit-cost ratio has to be weighed when considering its use.

9.8 Summary

Abnormal uterine bleeding (AUB) is a common gynecologic problem. A prompt diagnosis and evaluation of patients presenting with AUB aims to exclude serious underlying pathology such as carcinoma and to diagnose the cause of bleeding, so an appropriate management can be implemented. The standard of care of patients with AUB has changed due to the results of many studies that showed that blind uterine biopsy can often have a high false-negative rate which can be explained by the presence of focal versus global endometrial findings. Endometrial cancer can be misdiagnosed by the blind uterine sampling if it does not occupy more than 50% of the endometrial thickness. In 2012, ACOG acknowledged this nontrivial problem and recommended that blind endometrial biopsy cannot be an endpoint unless it shows cancer or endometrial complex atypical hyperplasia, especially if bleeding persists, so a further testing such as saline infusion sonohysterography or hysteroscopy, preferably in an office setting, is an appropriate choice. Blind endometrial sampling still remains the first-line test for endometrial in patients presenting with AUB who are older than 45 years of age or those patients who are younger with concerning risk factors for endometrial hyperplasia or cancer, keeping in mind the limitations of such blind sampling when negative, especially in cases of persistent bleeding.

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Hormone Replacement Therapy in Premature Ovarian Insufficiency

10

Vincenzina Bruni and Francesca Pampaloni

10.1 POI Definition

Various terms have been used to define deviations from normal, healthy ovarian function. The term "premature ovarian insufficiency" (POI) suggests that ovarian follicular activity may resume, even years after diagnosis, leading to pregnancy in some women. Other terms used for such cases are transient ovarian failure, premature ovarian dysfunction, transient ovarian insufficiency, occult ovarian failure, and incipient ovarian failure [1, 2]. Instead, the term "premature ovarian failure" (POF) is used when the loss of ovarian follicular activity is definitive.

This dysfunction is estimated to affect 1–2% of all women. In 76%, it manifests as secondary amenorrhea with onset after a period of regular menstrual cycles, sometimes after suspension of contraceptive hormone treatment, immediately after a pregnancy, or after a period of irregular menstrual cycles. It occurs as primary amenorrhea in 10% of cases [3]. There may be some residual, difficult to predict ovarian function in about 30% of cases, with occurrence of spontaneous pregnancy in 4–6% of cases, but in many cases, onset of POF is sudden, unexpected, and definite [4].

10.2 POI Etiology

- Premature onset of ovarian insufficiency may be familial and is related to genetic disorders in 10–13% of cases [3, 5–11].
- Autoimmune conditions account for approximately 4% of cases [12], but a concurrent autoimmune disease is present in 10–30% of cases [13].

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- Infections (mumps and oophoritis, in particular) may be the cause; there are various anecdotal reports of POI due to other viral and microbial infections [14].
- Latrogenic causes, such as surgery and chemo- and radiotherapy for either malignant or benign diseases, may be involved.
- Environmental factors may play a role in some cases [15–18].
- Lifestyle is important; for example, smoking increases the risk of POI development.
- There might be an association between prematurity and POI risk. The factors that contribute to premature birth and some of the postnatal events and conditions that are direct results of premature birth could lead to early follicle depletion [19].
- Finally, however, we note that the majority of cases of POI and POF are idiopathic.

The pathogenic mechanisms that lead to POI development involve disorders of primordial follicle endowment: X chromosome dysfunction, autosomal mutations, enzyme deficiency, depletion or destruction of ovarian follicles (spontaneous follicle loss, treatment-associated follicle loss), and dysfunction of ovarian follicles (signaling defect) [13].

10.3 POI Common Denominators

- FSH—The increase must be >25 U/L, with measurements done at least 1 month apart in the setting of 4–6 months of amenorrhea. FSH is not an ideal diagnostic tool: it rises only in the later stages of follicle depletion, and it is a poor predictor of reproductive status.
- AMH (ng/ml)—This hormone is a direct marker of ovarian reserve.
- Inhibin α and inhibin β—Decreases are an expression of ovarian follicle reserve depletion.
- Ovarian follicle reserve levels—Measured by ultrasound.

10.4 POI Short-, Medium-, and Long-Term Repercussions

Vasomotor symptoms (hot flushes and night sweats) are the most common menopausal symptoms reported by women with POI, who may also experience insomnia, joint pain, mood changes, low energy and low libido, as well as impaired memory and concentration. The most important medium- and long-term repercussions involve:

- The urogenital tract (genitourinary syndrome) with repercussions on sexuality
- The cardiovascular system and metabolic status in general (impaired endothelial function and increased triglyceride and LDL cholesterol levels)
- Bone mass (osteopenia, osteoporosis, increased fracture risk, arthrosis)

- Cognitive function (cognitive dysfunction, especially memory and concentration problems and increased risk of dementia)
- Mood
- Reproductive function (temporarily or definitively compromised)

POI is known to be associated with an increased risk of premature death, especially in oophorized patients [20–25].

10.4.1 Genitourinary Syndrome

The data regarding this condition in normal postmenopausal women is abundant, but information specifically related to POI is scarce. Menopausal symptoms in patients with POI are a consequence of the premature decline in the number of ovarian follicles and dysfunction of the residual follicles resulting in hypoestrogenemia and, to a lesser extent, decrease in ovarian testosterone. A reduction in estrogenic trophic effect in the vulvar and vaginal tissue is correlated with a reduction in elastic fibers and collagen, tissue hydration, and vaginal wall thickness. Vaginal PH increases (5–7.5); glycogen storage is reduced with increases in hydrogen peroxide, bacterial growth, and risk of vaginitis and cystitis [26]. Subjects who experience spontaneous POI generally present fewer and less frequent menopausal symptoms than those who undergo medically induced premature menopause. The main exception is sexual function, which is worse in more than half of the patients in both groups [27].

10.4.2 Sexual Function

Deteriorated sexual function in POI women is accentuated by genitourinary syndrome and involves in particular dissatisfaction, inadequate lubrication, lack of orgasm, lack of arousal, and pain [28–31]. Arousal dysfunction is often due to having a partner with sexual problems, dyspareunia, or dissatisfaction with the partner as a lover [31]. Female sexual dysfunction is not uncommon in physiological menopause, but the prevalence is higher, 32%, in so-called spontaneous POI. In cases of surgically induced menopause, that is, after bilateral salpingo-oophorectomy, the abrupt loss of ovarian hormones, not only estrogens but also progesterone and androgens, leads to more important problems [32] that are not resolved even with accurate, appropriate treatment [33].

10.4.3 Cardiovascular Risk

Numerous investigations show that the risk of developing cardiovascular conditions is twice as high for women who experience onset of POI before age 40 than their normal healthy peers [34]. The Multi-Ethnic Study Atherosclerosis (MESA) demonstrated

increased risk for coronary disease (HR 2.08, 95% CL, 1.17–3.70) in 28% (693) of 2509 women without baseline coronary condition; a similarly significant risk was evidenced in women who developed POI following surgery [35]. A Chinese review of relevant studies in electronic databases (up to February 28, 2015) reported that POI is associated with noticeably higher risk of ischemic heart disease (IHD) (RR 1.48, 95% CI 1.02–2.16) and all-cause mortality ((RR) 1.39, 95% (CI) 1.10–1.77); early natural menopause (ENM) presented only slightly higher risk of IHD mortality (pooled RR 1.09, 95% CI 1.00–1.18) [25]. The study on the 22,256 postmenopausal women in the population-based Swedish Mammography Cohort (1997–2011), with information on age at natural menopause and a mean follow-up of 13 years, showed increased risk of heart failure (HR 1.40; 95% CI, 1.19–1.64) in women who experienced early natural menopause (age 40–45 years) compared to those with onset of menopause at age 50–54 years; the authors also report a significant relationship between age at natural menopause and smoking ($p = 0.019$) [36]. A systematic search in PubMed (1966–2012) and EMBASE (1980–2012) shows that the POI subjects (age < 40 years) presented higher IHD risk (HR 1.69, 95% CI 1.29–2.21, $p = 0.0001$) and total CVD risk (HR 1.61, 95% CI 1.22–2.12, $p = 0.0007$), with no relation to stroke (HR 1.03, 0.88–1.19, $p = 0.74$) [24].

Subjects with POI have been shown to present early alterations of endothelial vascular function with reduced flow-mediated dilatation (FMD) [37, 38]. Women with POF presented increased thickness of the carotid intima media compared to controls (0.67 ± 0.17 vs 0.43 ± 0.10, $p < 0.05$) [38]. We know that the decline in endothelial function starts in the early phases of menopause; this decline continues with the progressive loss of ovarian function and prolonged estrogen deficiency [39].

The increased risk of cardiovascular events is related to the metabolic conditions in POI. These women present a higher prevalence of metabolic syndrome [40, 41] with modified lipid profile due to the increase in triglycerides and HDL cholesterol [40]. The high levels of triglycerides are significantly correlated with FAI, but not with the time lapse from onset of POI or with E2 levels [42]. Less recent findings showed a correlation between lipid profile and estrogen deprivation [43]. Another study, on a smaller number of subjects, showed a significant negative correlation between estradiol levels and triglyceride and LDL cholesterol levels ($r = -0.291$, $p = 0.047$) [44].

Early onset of ovarian insufficiency, both natural and surgical, was associated with increased risk of diabetes, reaching a hazard ratio of 1.83 in women under age 40 [45]. The risk for type 2 diabetes is unrelated to other intermediate risk factors for this condition, including BMI, glucose, and insulin levels as well as levels of endogenous sex hormones and SHBG [46]. These are findings of the population-based Rotterdam Study involving 3639 postmenopausal women divided into groups based on age at onset of menopause: premature menopause, <40 years; early, 40–44 years; normal, 45–55 years; and late, >55 years.

10.4.4 Cardiovascular Risk in Ovariectomized Women

Premenopausal unilateral oophorectomy (UO) significantly reduces the age of menopause, by 1.8 years. The younger the patient is at the time of UO, the earlier

the onset of menopause. Multiple chronic conditions and accelerated aging are manifest in subjects with bilateral oophorectomy [23, 47, 48]. The metabolic alterations as well as all other conditions related to hypoestrogenism vary depending on the etiology of POF and the time lapse between the removal of the gonads and patient evaluation. Impaired carbohydrate metabolism was detected in 30 ovariectomized premenopausal patients (mean age 47.13 + 3.2 years, range 40–54 years) with a significant increase of insulin responses to the glucose tolerance test (p\0.005) [49].

The risk of CVS following prophylactic removal of the ovaries in cases of ovarian cancer remains controversial. One recent study on 50 women with surgically induced menopause and 50 women undergoing natural menopause aged 40–50 years did not show any statistically significant differences in growth differentiation factor 15 (GDF-15), β-type natriuretic peptide (BNP), ischemia-modified albumin (IMA), lipid profile (total cholesterol, LDL-C, HDL-C, triglycerides), fibrinogen, and RCP [45]. Another study on 825 premenopausal women (baseline mean age 32 years) in the Coronary Artery Risk Development in Young Adults Study, 1990–1991, 317 of whom underwent surgical menopause (SM) (34% had bilateral oophorectomy), shows that the adverse left ventricular structure and function observed in the women who experienced surgical menopause could be explained by their unfavorable presurgical cardiovascular disease risk factor (CVDRF) profiles [50]. High individual premenopausal cardiovascular risk factors predispose women with SM to elevated future cardiovascular disease risk. We note that too few women subjected to ovariectomy during childhood, adolescence, or young adulthood have been included in dedicated studies to allow one to draw any definitive conclusions regarding this group of subjects.

10.4.5 Bone Mass Repercussions

Reduced bone mass has been widely documented in both POI women under 40 years old with normal karyotype [51–54] and women who experienced early menopause at 40–45 years of age [55]. The time lapse between onset and diagnosis conditions BMD: the sooner the diagnosis, the sooner the treatment begins, leading to better BMD. A study on 4725 postmenopausal women showed that women with POI are at higher risk of fractures after age 50 [56].

10.4.6 Bone Mass in Ovariectomized Women

Women who develop POI following surgery are at greater risk for bone mineral density loss and fractures than women who experience natural menopause, most probably due to the acute, abrupt onset of estrogen and androgen deficiency [57]. Women with surgical POI present rates of bone density loss that are almost double those of women in natural menopause [58].

Low bone mineralization is common in Turner syndrome (TS) subjects. The causes are related to hypoestrogenism as well as altered mechanical strength of the

skeleton probably related to haploinsufficiency of genes located on the X chromosome (SHOX gene), with specific characteristics of the bones that are smaller than normal and have altered trabecular geometry. Girls and young women with Turner syndrome present high osteoclastogenic potential associated with elevated FSH serum levels [59]. The risk of fractures in TS subjects is higher than average, especially during childhood and after age 45 [60, 61]. These subjects are also at higher risk of falling because they have spatial orientation difficulties due to hearing problems and frequent sedentary lifestyle. Also, low vitamin D levels, in some cases related to vitamin D receptor gene polymorphism (genotype Bsml or FokI) [62], and the relatively frequent association of thyroid disorders and immunological diseases exercise negative influences on BMD.

10.4.7 Arthrosis and POI

It is well known that estrogens exercise a protective effect on synovial membrane which has estrogen receptors [63]. Certain polymorphisms of these estrogen receptors are related to arthrosis risk [64]. Premature menopause is associated with increased risk of joint pain [65]. Experiments have shown that estrogens and estrogen-correlated drugs (certain SERMs) facilitate the synthesis of glycosaminoglycans, reducing turnover of articular cartilage, production of inflammatory cytokines in the synovia, and proliferation of arthritis-like synovial cells, in addition to exercising beneficial effects on bone and muscle tissues [66]. In physiological menopause, the protective effect of estrogens is controversial, in particular because different studies evidence involvement of different joints; the effect of SERMs is better documented [66, 67]. Low dosages of estrogens probably exercise a more favorable effect, and high dosages have a more marked pro-inflammatory effect [68].

Subjects with idiopathic juvenile arthritis that persists over time present a higher risk of developing POI independent of medications assumed [69]. Onset of menopause in women younger than 45 years old has been shown to be associated with rheumatoid factor positivity [70] and disease risk, but in a milder form [71]. Some observational studies have demonstrated that HRT can have a protective effect against RA in physiological menopause [67].

10.4.8 Neurological Function

Numerous findings suggest an association between neurological function and increased risk of dementia [72], with particular evidence of this association in surgery-induced POI [73–77]. This increased risk appears to be most apparent in the domains of global cognitive and verbal memory tests. Cognitive deficiencies in POI due to genetic disorders may be more likely to have a genetic basis than to be caused by effects of sex steroids on the brain [78]. Findings related to the loss of cognitive function after chemotherapy or GnRH analogue treatments are mixed.

10.4.9 Mood

Anxiety and depression are the most common disorders observed in POI patients. Patients require psychological support given the difficulty or impossibility of conceiving, worsening symptoms sometimes due to the very cause of the condition, and the knowledge that cure may be impossible or that treatment will be very long term. Women with idiopathic POI often search spasmodically for the cause and a cure. In POI following ovariectomy, whether for benign or malignant causes, the rapid onset of clinical symptoms, sometimes severe, can lead to post-traumatic stress disorder (PTSD) [79]. HRT has a positive effect on serotonin levels [80] and improves mood in the majority of patients. Antidepressants, in particular serotonin reuptake inhibitors (SSRIs), are efficacious, and the response is enhanced by estrogen treatments.

10.5 Targeting Hormonal Therapy

In cases of postpubertal onset of POI, recovery of normal estrogen levels and achievement of peak bone mass are the main objectives of treatment. These objectives are of particular importance in the prevention of both systemic and local (genital tract) long-term negative effects of estrogen deficiency. The specific etiology of POI or POF (e.g., genetic causes, autoimmune disease, surgery for benign or malignant conditions, chemotherapy, radiotherapy) together with age of onset orients the choice of estrogen therapy. Attention should also be directed to correction of certain baseline factors that may have had an influence on the development of the condition (lifestyle, including diet, smoking, and other risk factors).

In cases of prepubertal onset, the aim is to achieve good pubertal endocrine status to induce adequate secondary sexual characteristics, proper maturation of the lower genital tract (uterine development), achievement of peak bone mass, and menstrual cycles [3].

10.5.1 Estrogens, Progesterone, and Progestins

Natural estrogens (E2, E2V, and the synthetic estrogen ethinylestradiol) can be administered as hormone replacement therapy (HRT).

10.5.2 Natural Estrogens for HRT

- 17β-Estradiol (transdermal, transcutaneous cream or gel, spray) [81, 82].
- 17β-Estradiol (oral); in Italy, this substance is available only in certain EP combinations with dydrogesterone, cyproterone acetate, norethisterone acetate, and nomegestrol acetate. A product containing 1.5 mg estradiol associated with 2.5 mg nomegestrol acetate was recently marketed for contraceptive use in 24 + 4 regimen.

- Estradiol valerate (oral; available alone and in 2 mg or associated with medroxyprogesterone acetate or cyproterone acetate). This product is marketed also in a four-phase version, with E2V and dienogest, for contraceptive use in 26 + 2 regimen.
- Conjugated equine estrogens (ECE) [83] (not available in Italy for HRT).
- An association of estetrol (E4, an estrogen of fetal origin) and drospirenone is currently under investigation for contraceptive use [84]; this combination has minimal metabolic impact [85, 86].

10.5.3 Ethinylestradiol, the Synthetic Estrogen

The only synthetic estrogen available, ethinylestradiol is characterized by its slow inactivation. The C=CH group prevents oxidation of the 17β OH group, hindering transformation to estrone; furthermore, oxidation of the C=CH group by cytochrome P 450 3 A 4 induces formation of an intermediate metabolite capable of inhibiting that very cytochrome P 450 3 A 4. The molecule is then hydroxylated in position 2 forming a 2 OH derivate that is methylated/glucuronidated and then excreted in the feces and urine [87].

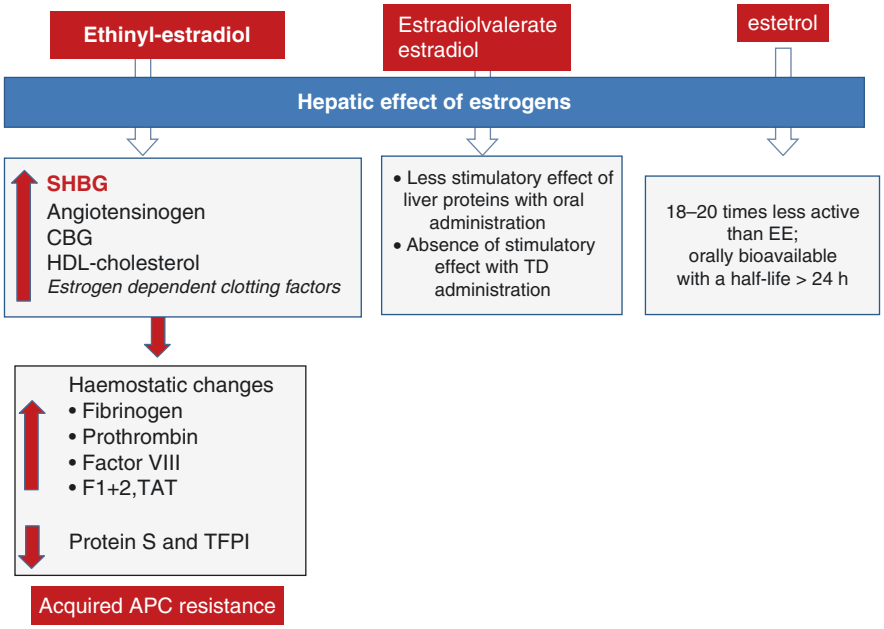
This "accumulation" effect explains the higher potency of EE compared to E2 on all estrogen-correlated activities, including the synthesis of certain liver proteins. Oral estradiol has less effect on liver cells than ethinylestradiol; the transdermal patch is the method that exercises the least influence on coagulation factors, with no increased risk of VTE, even in subjects with thrombophilic diathesis [88]. Thus, currently, transdermal estradiol is the treatment of choice for ovarian insufficiency with postmenarchal onset and conscientious patient compliance (see Table 10.1).

10.5.4 Progesterone and Progestins

In women who have not undergone hysterectomy, estrogen treatments should be accompanied by either progesterone or continuous or sequential (minimum 10 days per cycle) progestin administration to prevent the onset of endometrial hyperplasia which occurs with estrogen alone [89]. The pharmacokinetics of progesterone in vaginal suppository (dosage 100 or 200 mg) evidence good intestinal absorption with under-curve progesterone levels of 5.7–20.9 ng/ml, with no significant differences in relation to dosage [90]. The capsules dissolve in the vagina in 7–15 min, and absorption is rapid; peak levels are reached in plasma in 2 h, and therapeutic levels are stable for 12 h [91]. The lowest systemic absorption is found with progesterone in polycarbophilic vaginal gel, with irrelevant or even no effect on the CNS.

Micronized formulations of the hormone are efficacious with oral administration [92, 93]: a 10 mm particle suspended in oil and packed in a gelatin capsule [94]. Oral administration results in varied concentrations in plasma from subject to subject, with possible formation of metabolites active on the GABAergic receptors, with *sedative* and *hypnotic* effects, probably due to individual variations in enzyme levels related to gastric and intestinal microbe balance. A recent review of the

Table 10.1 Estrogens used in HRT



literature [95] has confirmed that oral micronized progesterone provides protection for the endometrium when taken in sequential regimen for 12–14 days, 200 mg/day for more than 5 years. Vaginal administration provides protection in sequential regimen for at least 10 days per month, 4% (=45 mg/day) or a daily dose of 100 mg for 3–5 years (off-label use).

The synthetic progestins differ depending on their affinity to steroid receptors. 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 [96, 97]. The non-androgenic progestins are preferred because they have less effect on metabolic factors and the mammary glands [98–101].

In particular, the pregnane derivative dydrogesterone is a retroprogesterone formed from progesterone by UV light exposure. It is currently on the market and does not have clinically relevant androgenic, estrogenic, glucocorticoid, or mineralocorticoid effects, and it resembles progesterone mainly in its progestogenic effects [102]. The metabolite half-life is 17 h, with a relative binding affinity for progesterone receptor of 75%; metabolite configurations remain stable also after oral administration [103]. Thus, dydrogesterone can be considered an ideal oral supplement progestogen in hormone replacement therapy. In addition, it has minimal side effects, as demonstrated in a recent review in the literature [104], its beneficial effects [105] and safety in hormone replacement therapy have been widely

Table 10.2 Progestins: pharmacological characteristics

Progestogen	Partial effect pattern	Half-life (fundamental for endometrial stability)	Equivalent doses (for secretory endometrial transformation)
Cyproterone acetate (structurally related to 17-hydroxyprogesterone)	Antiandrogenic +++ Glucocorticoid +	48	1 mg
Chlormadinone acetate (structurally related to 17-hydroxyprogesterone)	Antiandrogenic + Glucocorticoid +	34–40	3 mg
Nomegestrol acetate (structurally related to 19-nor-preganes)	Antiandrogenic ± Proestrogenic − Glucocorticoid −	30–50	(1) 2.5–5 mg
Drospirenone (spironolactone derivative)	Antiandrogenic Antimineralocorticoid	30	3 mg
NETA (estranes, structurally related to testosterone)	Proandrogenic + Proestrogenic + Antiestrogenic +	8–26	2.5–5–10 mg
Dienogest (estranes, structurally related to nortestosterone, ethynyl group replaced by cyanomethyl group in 17-α)	Antiandrogenic ++ Glucocorticoid −	11	2–3 mg
Dydrogesterone	Antiestrogenic − Antiandrogenic ± Antimineralocorticoid ±	17 (considering metabolites)	10–20 mg
Micronized progesterone		18.3 ± 3.5 ore	200–300 mg

confirmed [106], it has been used as support therapy in place of micronized progesterone in the second phase of the menstrual cycle [107], and when administered with transdermal E2, it does not interfere with bone turnover [108].

Table 10.2 presents a pharmacological classification of the progestins administered in HRT. We note that the half-life of the progestin influences the stability of the therapy [109, 110].

10.6 Protocols Proposed for HRT

There are essentially two lines of hormone replacement treatment for POI [111]. One is the so-called "physiological sex steroid replacement" (pSSR) therapy which entails transdermal administration of estradiol (100–200 mcg) associated with micronized natural progesterone for non-hysterectomized women. The other is the "standard hormone replacement therapy" (sHRT) involving ECE + progestin or E2 + progestin, both associated with a hormonal contraceptive. Another alternative is a progestogen intrauterine device (IUD) associated with continuous 17β-estradiol (transdermal or oral); this can be an optimal choice for some patients with POI, including adolescents who want to take a contraceptive [112].

10.7 HRT in POI: Combined Hormonal Contraception

Residual fertility in nonsurgical POI may create problems. We know that POI patients on so-called physiological hormonal therapy, pSSR, which does not have contraceptive effects, have a 5–10% possibility of conceiving. Ovulation after diagnosis of POI has been reported in 20% of cases, and 1–10% of these women are intermittently fertile [113, 114]. A young woman with POI who still has her ovaries and does not want to risk pregnancy may choose to take a combined hormonal contraceptive. In such cases, taking an oral contraceptive has a sort of psychological value; it removes or masks the perceived stigma of early menopause. Adolescents who make this choice appear to be like their peers, and this strengthens compliance in a choice that has other advantages, such as protection from cancer of the ovaries, endometrium, and colon as well as lymphatic and hematopoietic cancers (see UK RCGP Oral Contraception Study on General Population: 46,022 women were recruited in 1968 and 1969 and followed up for 44 years) [115].

The relationship between thromboembolism (VTE) and estroprogestin in contraceptives has been known for many years. There are ample epidemiological data regarding combinations with ethinylestradiol [116–120], and data is now available for dienogest/E2V [121]. The increased risk for thrombosis seems to be related to an acquired APC resistance; increased levels of prothrombin, factor VIII, and fibrinogen in plasma; and decreased levels of protein S and TFPI in plasma, all induced by the estrogen-progestin combination. The associations containing progestins with residual androgenic activity [118, 119] that cause lower increase in SHBG [122] appear to be less risky. In fact, a correlation between SHBG and resistance to C-reactive protein has been documented repeatedly [123]. The progestin with residual androgenic activity is probably able to limit the pro-coagulative action of EE via APC resistance; reduction of protein S, TFPI, and plasminogen; and increase in factor VII; there have been no reports of modifications of fibrinogen, AT III, prothrombin, F 1 + 2, D-dimer, and F 1 + 2/D-dimer. A study that compared the E2V and dienogest association with E2V plus LNG reported a hazard ratio for VTE, adjusted for age, BMI, family history of VTE, and duration of use, of 0.4 (0,2-1,1) (see INAS/SCORE Study, ICPE, Montreal 2017).

Women who take combined hormone contraceptives (COCs) are also at increased risk for other cardiovascular conditions, such as ischemic stroke and myocardial infarct. The risk of ischemic stroke has been estimated to be 1.8–2.7 times higher in these women [124], especially in relation to first-generation progestins (less for second- and third-generation products) according to the database of the French national health insurance agency report on 4,945,088 women aged 15–49 years [125]. The EE dose appears to be the most important factor in the risk for ischemic stroke and myocardial infarct; it is considered prudent to limit the dosage to 20 mcg when possible and in any case not to exceed 30 mcg. A recent Cochrane review attributed the increased risk (1.6%) for myocardial infarct with COC to the estrogen dosage in the formulation and not the type of progestin; according to this review, the risk increases with EE ≥50 mcg [126]. Another study regarding myocardial infarct found a RR of 0.88 (0.22–5.35) for the association of EE 20 mcg + drospirenone, RR 1.53 (1.26–1.87) for EE 20 mcg + desogestrel, and RR 1.70 (1.37–2.12) for EE 20 mcg + gestodene [127].

The LASS (Long-term Active Surveillance Study for Oral Contraceptives) study reports lower incidence of arterial thromboembolic events and antihypertensive drugs with use of EE + DRSP compared to other combinations. This investigation involved 59,510 women enrolled in 1113 studies with 10-year follow-up; 28% of the women used EE + DRSP, 26% EE + LNG, and 45% other COC combinations [128].

Available epidemiological data regarding E2V + dienogest show an adjusted RR of 0.1 (0.0–0.6) regarding risk of arterial disease compared to combinations with LNG and RR of 0.1 (0.0–1.2) compared to other combinations. The metabolic neutrality of COCs with natural estrogens reduces the differences between HRT and traditional EE contraceptive products.

10.8 Intrauterine Contraception with LNG in Combination with Estradiol

An intrauterine device (IUD) with progestogen associated with continuous 17β-estradiol (transdermal or oral) may be a good alternative choice [112]. A progestogen IUD containing 52.5 mg that releases 20 μg/24 h is registered for HRT use: the T-frame dimension is 32 × 32 mm, and maximum duration of use is 5 years; the LNG content found in the endometrium is 470–1500 ng/g and in plasma 0.1–0.4 ng/ml (100–400 pg) [129]. The strong endometrial action of the LNG IUS suppresses the endometrium, preventing endometrial hyperplasia even when associated with continuous administration of estradiol.

Another IUD, which contains a total 19.5 mg LNG and releases 12 μg/24 h, is now available. The T-frame dimension is 28 × 30 mm, and the maximum duration of use is 5 years. Pooled data from the phase II and pivotal phase III studies showed a mean LNG concentration of 214 pg/ml 11 days after placement of the device; the concentration declines to 125 pg/ml at 1 year, 95 pg/ml at 3 years, and 64 pg/ml at 5 years [130]. The strong concentration gradient leads to high drug exposure in the endometrium and low systemic exposure. The endometrium/myometrium gradient is >100-fold; the endometrium to serum gradient is >1000-fold. These smaller systems are needed for young nulliparous women with small uterine cavities, average ~27 × 27 mm [131]; insertion has been successful even in young adolescents [132]. In a cohort of 965 participants, 213 (22.1%) received local anesthesia, including paracervical blockade; another 213 (22.1%) were administered with paracervical blockade before the procedure, two (0.2%) when the procedure proved difficult, and one (0.1%) when the procedure proved both difficult and painful. Analgesics were administered to 354 participants (36.7%) before the procedure, two participants (0.2%) when placement proved difficult, and 42 participants (4.4%) when they experienced pain. This device is currently used only for contraception; its hypothetical use as a progestin delivery system in HRT has not been supported by research to date.

10.9 Topical Estrogens

The choices are 17-β estradiol in a vaginal ring or vaginal pills, estriol in gel or cream, and promestriene in cream or capsules. The *vaginal ring* (55 mm in external diameter, transversal 9 mm, nucleus 2 mm) contains 2 mg of estradiol hemihydrate (equal to 1.94 mg estradiol) and releases an average of 7.5 mcg estradiol in each 24-h period for 90 days. Remember that normal menopausal estradiol levels are ≤20 pg/ml. The 7.5 mcg silastic ring achieves plasma levels of 55 pg within 3 h of placement, ≤20 within 4 h of placement, and ≤10 within 2–3 days of placement. The mean thickness of the endometrium is 2.7 mm within 3 days of placement [133]. Endometrial biopsy after 12 weeks of treatment evidenced atrophy in 60% of patients and moderate proliferation and simple hyperplasia in 2% [134].

The *vaginal pills* contain 10 mg estradiol, and they achieve hematic levels of 6.56 pg/ml after 14 days and 2.4–12 pg/ml after 1 year. Treatment comports a significant increase in superficial cells, decrease in basal and parabasal cells, and decrease in pH in women receiving 10 μg of estradiol daily for 2 weeks and then twice per week thereafter [135].

Estriol and *promestriene* have been used in the local treatment of lower genital tract atrophy.

Estriol is produced naturally via peripheral metabolism of ovarian estrogens in the nonpregnant female. This short-acting estrogen has a proliferative effect on vaginal, urethral, and bladder epithelium as well as the surface epithelium of the cervical os. Estriol has low affinity for estrogen receptor alpha (ERα) (dominant in the endometrium and glandular breast tissue), but it exercises a proliferative effect on both vaginal and urethral epithelium via ERβ. Ultra-low dose estriol vaginal gel formulations (20 mg/g (T1) and 50 mg/g (T2)), can reverse vaginal atrophy and present a highly favorable safety profile, producing very low systemic absorption of estriol (AUC0-t: 171.65 ± 80.18 (T1) and 406.75 ± 199.53 (T2) pg/mlh). After multiple administrations, estriol exposure (AUCss: 36.33 ± 30.52 (T1) and 73.71 ± 46.86 (T2) pg/mlh) is significantly lower than after single-dose administration. One could hypothesize that absorption of estriol decreases due to the estrogenic effect of estriol on the vaginal mucosa and consequent maturation of the epithelium [136].

Promestriene is a 3-propyl and 17b-methyl ether of estradiol that exercises efficient action on vaginal atrophy with minimum absorption [137]. Its effectiveness in relieving atrophy has been confirmed in almost 40 years of use in 34 countries, with millions of prescriptions; reports of negative side effects have been rare. Penetration in the basal membrane and concentrations in plasma are low [137, 138]. Thus, this could be prescribed as first choice for patients who need minimal or ideally no vaginal absorption, especially patients with symptomatic cancer [139].

10.10 Androgen Replacement Therapy (ART)

Also testosterone levels are reduced in POI, especially in women who have had bilateral ovariectomy. It is important to measure the levels to differentiate these women from those who have experienced natural menopause and program appropriate treatment. Androgen treatment is recommended only in particular cases, such as when extremely reduced levels are associated with modifications in sexual function or other androgen-dependent conditions [140].

The efficacy and safety of transdermal testosterone treatment (300 mg/day/6 months) were demonstrated to be efficacious and safe in 132 women in menopause due to surgery enrolled in two randomized, controlled trials ($n = 1094$) [141]. There are no reports of trials involving girls with postmenarchal POI. However, investigators have demonstrated improvement in body composition, neurocognition, and quality of life with ART in a randomized double-blind study on 14 Turner syndrome patients (ages 17–27 years, 21.3 ± 3.1 years) treated for 1 year with placebo or oral (1.5 mg/day) methyl testosterone followed by the alternative for another year, without a washout period given the long term of each arm [142]. There is some debate about the effects of ART-HRT on bone mineral density [143, 144].

Two other treatments used in POI are dehydroepiandrosterone (DHEAS) and its sulfate ester DHEAS, the most abundant steroid hormones in humans. DHEA is an endogenous steroid that originates from the zona reticularis of the adrenal cortex and the ovarian theca cells in women; it is an essential prohormone in ovarian follicular steroidogenesis. Women with POI present an increased risk of low levels of testosterone, DHEAS, and delta-4 androstenedione. A recent meta-analysis showed reduced DHEAS levels in POI women, but the levels were higher than in postmenopausal women [145]. DHEA has been used to improve ovarian response in women with ovarian insufficiency. The conclusions of a Cochrane database review were that pretreatment with DHEA or testosterone may be associated with improved live birth rates for women identified as poor responders who undergo assisted reproductive procedures [146], but the evidence is of moderate quality. The findings of a recent prospective observational study on 31 women with POI to evaluate the effect of 12-month DHEA supplements (25 mg/3/day) on menstrual pattern and ovarian reserve markers (ovarian volume, antral follicle count, AMH, FSH, E2, T, liver function, and hemoglobin level) did not show any significant improvement in ovarian function at the end of 12 months [147].

10.11 Goals of HRT in POI

10.11.1 Cardiovascular Protection

No longitudinal data are available regarding the protective effects of HRT in POI. Observational and randomized studies have shown that early administration in healthy women with menopausal symptoms reduces the risk of cardiac disease and death as well as death for all causes [148, 149]. Lifestyle, meaning no smoking,

adequate amounts of physical activity, and normal BMI, together with good management of lipid levels and hypertension all participate in the reduction of CVS.

Regarding hormone replacement therapy, transdermal administration of estradiol (E2) is currently considered the most metabolically neutral choice for women with POI. Unfortunately, the ample data reported in the literature regard prevalently treatment of women who have experienced normal menopause, and it is not appropriate to apply the findings across the board to POI.

10.11.2 Lipid Profiles

Post-menopause long-term HRT has a generally positive influence on lipid profiles, with some differences depending on the method of administration. A study on 64 women randomized to receive transdermal HRT (age 52.4 year; 17β-estradiol 50 mcg + NETA 025 mg/day/2 weeks) or oral (age 54.3 year; 17β-estradiol 2 mg for 12 days, then E2, 2 mg + NETA, 1 mg for 10 days, followed by estradiol 1 mg/day for 7 days) showed that the transdermal administration had a favorable or neutral effect on lipid levels, with an increase in HDL2 concentrations; the oral administration produced significant decrease in LDL cholesterol, no changes in triglyceride levels, and a significant increase in hsRCP [150]. Another study involving 223 women in menopause (age 49.8 ± 3 year at first observation) demonstrated a trend to increase in HDL cholesterol and decrease in triglycerides after 10 years of HRT with transdermal E2 (50 mcg) + oral micronized progesterone (200 mg) [151]. On the other hand, oral E2 caused an increase in C-reactive protein, triglycerides, and SHBG, albeit less than ethinylestradiol, and decrease in IGF1 and IGFBP 3 [152–155]. Another study on 185 women (average age 50.71 years) under treatment with ECE (0.625 mg) and MAP (2.5–5 mg) evidenced a significant reduction in total cholesterol and no changes in other parameters at 2–3 years of follow-up [156].

The findings regarding adult women with Turner syndrome are controversial [157]. These women do not appear to benefit from any improvement in lipid profile with HRT. After an average 18 (7–32) years of treatment, a group of 30 subjects aged 32 ± 4 years showed higher than normal frequency of visceral obesity, IR, hypercholesterolemia, and hypertension. There were no signs of benefits on lipid metabolism in another study on 165 TS women (24.9 ± 7.7 year) recruited between 1995 and 2011, but the use of growth hormone in childhood had favorable effects on their lipid profiles in adulthood [158]. A review published in 2016 reports that the route of administration, type, and dose of 17β-estradiol (E2) used to feminize girls with TS are not well established and that these subjects present increased HDL cholesterol independent of dosages of estradiol and without changes in total cholesterol, LDL, and triglycerides [159].

10.11.3 Glucose Metabolism

Worsening of glucose metabolism is frequent in women in menopause. The risk of diabetes, prediabetes, and metabolic syndrome is increased in POI and early menopause, both natural and following ovariectomy.

Important observational studies in POI and various controlled randomized trials reviewed in two meta-analyses agree that there is a significant reduction in the incidence of diabetes in HRT subjects. The majority of the findings regard oral treatments, with standard doses equivalent to 0.625 mg ECE [160].

A study on the use of transdermal E2 in women with type 2 diabetes has evidenced improvement of insulin resistance with no negative modifications in cardiovascular risk factors [161].

10.11.4 Hypertension

Hypertension increases in 7% of women in the perimenopause period and 12% of women post-menopause according to a large study on 1684 women (age 45–65 year) analyzed by age groups depending on age at onset of menopause (early, late, post-menopause) [162]. The findings evidenced that hypertension and metabolic changes are the result of estrogen deficit, and they are clearly distinguishable from the onset of those factors due to aging. Another important study demonstrates that transdermal administration of HRT is associated with lower systolic and diastolic blood pressure, improved renal function, and less active renin/angiotensin system. This was a controlled randomized trial comprising 42 POI women from 19 to 39 years old to compare transdermal E2 therapy and oral E2 associated with EE + NETA [163]. The results allow one to hypothesize that there is reduced CVS risk with transdermal E2. It is important to follow up on the patient over time to counter noncompliance.

10.11.5 Hemostatic System

Oral E2, oral ECE, and transdermal E2 all appear to have various repercussions on the hemostatic system. However, the data available regard only postmenopausal women.

Oral administration of estrogens (E2 or ECE) comports diminishing of tissue factor pathway inhibitor (TFPI) that is the principal inhibitor of the "tissue factor-factor VII" complex which activates the coagulation cascade. Low levels of TFPI increase the risk of thrombotic events and their recurrence. Women with a history of thrombotic events present a 30–50% reduction in TFPI. The fibrinolytic system is also activated, and there is a reduction in plasminogen activator inhibitor-1 (PAI), reported by various authors [164–167], without significant modifications in di-dimero.

With transdermal administration, there are no or only minor modifications in TFPI and no modifications in PAI-1, and di-dimero levels remain stable. One study involving 45 healthy women aged 49 ± 6 years, recruited 12 weeks after hysterectomy and ovariectomy, showed significant reduction in TFPI and PAI-1 levels (less significant for PAI-1) in the oral treatment arm. There were no significant changes in the markers investigated in the transdermal E2 arm, except for AT III which,

however, remained in the norm [168]. Other observational studies confirm that the risk of developing a venous thrombotic event or having a stroke or myocardial infarct is lower in women during treatment with transdermal estradiol [169, 170].

10.11.6 Bone Health

The protocols for inducing puberty in girls with hypergonadotropic amenorrhea due to genetic causes (there are specific data for subjects with Turner syndrome) are well defined to achieve adequate peak bone mass [171–173]. A longitudinal study of 54 Turner syndrome women (22–65 years, average 37 years) treated with oral E2 (2 mg) associated with a progestin (NETA 1 mg, MAP 10 mg, LNG 0.25 mg for 10 days/cycle) for 5–9 years ("conventional HRT") evidenced only a reduction in bone resorption markers, with no changes in markers of new bone formation. All the subjects were encouraged to follow good lifestyle and took vitamin D supplements [174]. We note that good results were obtained with transdermal E2 (25 mcg for 6 months followed by 37.5 mcg for the subsequent 6 months) in 12 subjects (14.0 ± 1.7 year) recruited for a randomized study comparing that regimen with ECE (0.3/day for the first 6 months and 0.625 for the subsequent 6 months) [175].

Table 10.3 lists the major studies on ECE (currently not available in Italy) which evidence the importance of beginning therapy as early as possible to achieve good BMD response.

In patients with so-called spontaneous POI, the best BMD results were obtained with pSSR (physiological sex steroid replacement) therapy. A randomized study of 18 women (mean age 27 years, range 19–39) comparing transdermal 17β-estradiol (100–150 mcg) plus vaginal progesterone (200 mg/14 days) with COC (30 mcg EE plus 1.5 mg NETA mg) showed an increase in lumbar BMD and markers of new bone formation with reduced markers of bone resorption in the transdermal E2 plus natural progesterone arm. The only finding of note in the COC arm was a reduction in bone resorption markers [181].

A study involving 30 women from 18 to 44 years old compared sHRT with oral estradiol plus 75 mcg LNG and COC; the result was increased lumbar BMD in the sHRT patients but no improvement in the COC arm [182]. Another interesting finding came from a randomized study of 72 POI patients treated with transdermal E2 (100 mcg) plus oral MAP (10 mg) compared with 73 treated with the same substances plus a testosterone patch (release 150 mcg/day). The control group comprised 70 women with normal BMD. The transdermal E2 plus oral MAP group showed good results, with BMD levels equal to those of the control group. The testosterone supplement did not produce any advantages [143]. More recently, a study of 132 women with spontaneous POI (mean age at last menstruation 31.0 ± 7.3 years, mean age at BMD evaluation 37.4 ± 7.3) who were not undergoing HRT at the time of enrollment evidenced that standard replacement therapy with 17β-estradiol (1 mg) or conjugated equine estrogens (0.625 mg) plus NETA or MAP does not improve BMD [183]. We note that these patients started HRT approximately 3 years after their last menstrual period.

Table 10.3 Major studies on ECE

Author; year of publication	N° subjects; age (years)	Treatment	Duration	Results
Lanes et al. (1999) [176]	8 subjects 18.25 ± 1.06 years	ECE (0.625 mg/21 days) + MPA (5 mg/day/10 days)	4.1 ± 1 years	Normal peak bone mass not achieved with TOS begun in adolescence; no changes in BMD after age 6 years
Benetti-Pinto et al. (2002) [177]	38 subjects 16–35 years (average 24.6)	ECE (0.3–0.625 mg/21 days) + MPA (10 mg/day/10 days)	4.5 ± 3.6 years	Positive correlation between BMD and treatment duration + BMI
Chan et al. (2006) [178]	20 subjects with hypoestrogenism 36 ± 3.4 years	ECE (0.625 mg/21 days) + MPA (10 mg/day/10 days)	12.2 ± 3.4 years	Bone turnover similar to that of normally menstruating women
Kodama et al. (2012) [179]	67 subjects Pre untreated group 21.6 ± 5.5 years Pre low-dose group 16.8 ± 0.8 years pre adult-dose group 22.4 ± 4.9 years	ECE (0.625 mg/21 days) + DYG (10 mg/day/11 days)	July 1, 1992 to December 31, 2005	Treatment improves BMD and can be efficacious if initiated at age < 18 years
Nakamura et al. (2015) [180]	100 subjects 31.8 ± 9.1 years; no preceding therapy 32.0 ± 7.4 years; preceding therapy	ECE (0.625 mg/21 days) + MPA (5 mg/day/10 days)	No preceding therapy: 5.1 ± 3.3 years Preceding therapy: 11.2 ± 7.1 years	BMD improved only when therapy initiated early (<18 years)

Ovariectomized POI (actually POF) patients achieve recovery of bone density to preoperative levels if estrogen therapy is begun within 3 years after surgery. Estrogen therapy begun up to 6 years after ovariectomy can stop bone loss and stabilize it, but bone density is not restored to preoperative levels in these cases [184].

In brief, women with spontaneous POI benefit most from transdermal E2 therapy compared to standard treatment. Delay in the diagnosis of POI and thus in treatment contributes to loss of bone mass. Table 10.4 shows the dosages of transdermal E2 necessary to ensure estrogenization that corresponds to the late follicular phase in a normal menstrual cycle, 100 mcg.

The data available regarding treatment of women in normal menopause show that even standard doses of E2 (see Table 10.5) are able to control bone turnover and reduce the risk of fractures. Thus, for women who develop POI after a long period of normal ovarian function and present normal BMD or only slight osteopenia at the time of diagnosis, a 50 mcg patch should be adequate. We emphasize the importance of immediate evaluation of BMD at the time of POI diagnosis in all patients, young and old, especially those who present osteoporosis risk factors (eating disorders, low BMI) and when diagnosis is made prior to peak bone mass accrual. Rapid treatment is crucial. In fact, at 18 months from diagnosis without treatment, there is a 47% reduction in BMD at the neck of the femur, seen in 89 32-year-old (range 20–39 years) POI women, compared to 213 controls (regularly menstruating women of the same age) [51]. Young POI subjects who have not achieved peak bone mass at the time of ovarian damage should be treated with the 100 mcg patch.

In addition, monitoring of POI patients must include information and advice regarding lifestyle, vitamin D3 cholecalciferol (1000–2000 IU daily) intake either through dietary sources or supplements, and constant attention to compliance regarding prescribed therapy. One French study in particular has raised an alert about the high rates of patients who abandon treatment (42.6% within a year); the

Table 10.4 E2 dosages and plasma levels in different menstrual cycle phases

E2 plasma levels with matrix patch		E2 plasma levels during ovulatory menstrual cycle	
25 mcg	30–45 pg/ml	Follicular phase	10–98
50 mcg	40–80 pg/ml	Ovulatory phase	170–770
100 mcg	90–140 pg/ml	Luteal phase	190–340
		Post-menopause subjects	10–38

Table 10.5 Comparison of estrogen dosages

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

women in question presented BMD alterations at follow-up controls [185]. To date, there is a lack of consensus regarding frequency of surveillance, especially for adolescents [186]. Certain European guidelines recommend BMD evaluation within 5 years of the original measurement for POI subjects with a diagnosis of osteoporosis who are taking estrogen replacement treatment or other therapies [187, 188].

10.11.7 Sexual Function

Improvement of sexual function starts with HRT and simultaneous attention to correction of genitourinary syndrome conditions. Women must be informed about testosterone supplements, although we know that the medium- and long-term effects are not well defined. There have been reports regarding beneficial effects of estrogen therapy on sexual function in ovariectomized POI women [189–193]

In situations presenting genitourinary syndrome, women may continue to experience symptoms of vaginal dryness and superficial dyspareunia notwithstanding appropriate HRT. In these cases, also local treatments should be prescribed: daily application of topical estrogens for 2 weeks and then twice weekly. This treatment can be continued indefinitely. When it is interrupted, the symptoms of urogenital atrophy often recur. Treatment with vaginal estrogens comports little risk because they rarely cause adverse effects; however, any unexpected bleeding should be reported. There is no need for routine monitoring of endometrial thickness [194].

10.11.8 Cognitive Function

Hormone replacement therapy reduces the risk of modifications in cognitive function and must be continued until the patient reaches the age of natural menopause. We note that a healthy lifestyle (eating habits, exercise, etc.) is also important in ensuring continued good cognitive function. The data on the cognitive benefits of HRT all refer to older women in menopause, and there are to date no studies demonstrating cognitive benefits of HRT in young women with POI. Thus, any potential benefits in this group of patients must be extrapolated from studies on older women and animals.

10.12 POI: Variables to Consider When Choosing HRT

10.12.1 POI Etiology: Cancer

Hormone treatments cannot be prescribed in cases of hormone sensitive cancers, such as breast cancer (past or in course), sarcoma of the endometrial stroma (a neoplasm that has estrogen receptors), and granulosa cell tumors, no matter the age of the patient at POI onset. There are no contraindications for women with the

BRCA1/2 gene mutation who have no personal history of breast cancer after prophylactic BSO [194]; thus, these women with POF due to surgery can use HRT.

There are no contraindications for patients who have undergone treatments for ovarian carcinoma of any stage, except for endometrioid cancer of the ovary. HRT can also be prescribed after surgical treatment for early stages of endometrial cancer [195]. MHT can be prescribed for squamous cell carcinoma of the cervix and vulva which are not hormone-dependent [196].

There are some data indicating that HRT can be administered to survivors of colorectal cancer and localized malignant melanoma and in women with premature ovarian insufficiency resulting from treatment of hematologic cancers. Attentive counseling and accurate information should be provided to subjects who undergo thoracic irradiation for Hodgkin's disease because of the increased risk of developing breast cancer [197].

Systemic HRT is not recommended for vaginal dryness caused by hormone deficiency related to certain cancers, but low-dose, local vaginal estrogen application is a feasible treatment option [196]. The latter treatment is safe, and data from postmenopausal women using vaginal estrogens are reassuring in regard to the low risk of cardiovascular disease and breast or endometrial cancer [198].

10.12.2 POI Patients with Specific Cardiovascular Conditions

It is well known that POI subjects affected with Turner syndrome are at risk for CVS [199–202]; they require regular monitoring of blood pressure, BMI, WH ratio, lipid and glucose profiles, glycated hemoglobin, and lifestyle (smoking is tabu) during HRT. Adequate physical activity is recommended. The POI women in this particular group who present hypertension should use transdermal HRT preparations.

10.12.3 Other Specific Conditions

Continuous combined HRT can prevent relapse in women who have undergone ovariectomy due to endometriosis [203]. There are no contraindications for HRT use by women with uterine fibroma, and there are no documented reports of any significant increase in the development of fibroids in postmenopausal women on HRT. Accurate gynecological anamnesis must always be done, specifically regarding the characteristics of the patient's menstrual cycle before POI onset and evaluation of the site of the fibroid(s). A regimen of combined/continuous treatment with regular sonographic monitoring is advisable. In cases of submucosal fibroma, particular caution is required in the event of reappearance of pre-POI symptoms during HRT. There have been reports of development of symptomatic fibroids in two patients on hormone replacement therapy with primary ovarian failure secondary to prepubertal gonadotoxic cancer treatment [204].

Transdermal treatment is to be preferred in patients with gallstones, hypertension [205], dyslipidemia, and diabetes; for women who still have their uterus, the

treatment should be associated with micronized natural progesterone (preferably vaginal or oral dydrogesterone).

10.12.4 Duration of HRT Treatment

It is imperative that POI women be encouraged to use HRT at least until the presumed age of physiological menopause [7, 186, 187, 206, 207]. Annual checkups should be scheduled to evaluate patient health and compliance to therapy. No routine tests are necessary unless the patient presents conditions that require investigation.

10.13 Conclusions

Women with POI need a broad-spectrum treatment that takes into consideration the causes of the condition. The choice of HRT depends on bone age and chronological age, short- and long-term symptomatology, and social and psychological factors. Treatments with important side effects should be avoided, and any treatment must be acceptable to the patient, including the method of administration.

Psychological support is always necessary, and it is particularly important in case of infertility. Patients must be informed that lifestyle is always important. It should be made clear that women who smoke have lower estrogen levels and a greater risk of osteoporosis; transdermal regimens are the best for these patients [208, 209]. The question of testosterone and DHEA supplements in relation to improving mood and sexual function as well as preservation of eventual residual ovarian function remains open to debate.

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Premature Ovarian Insufficiency: Practical Management Approaches

11

Sophia Tsiligiannis, Nick Panay, and John C. Stevenson

11.1 Introduction

There is a lack of international consensus on diagnostic criteria for POI, with different age limits and elevated follicle-stimulating hormone (FSH) cut-off levels used to define hypogonadism. According to the National Institute for Health and Care Excellence (NICE) guidance, diagnosis is based on menopausal symptoms and no or infrequent periods, taking into account whether the woman has a uterus and raised FSH (>40 IU/L) in two blood samples taken 4–6 weeks apart [1]. Raised gonadotropins are typically accompanied by low oestradiol.

The lack of consensus may result in diagnostic confusion and a subsequent delay in diagnosis. Over half of patients see three or more clinicians before the diagnosis is made, and in a quarter, the diagnosis takes more than 5 years [2]. This could negatively impact all long-term implications of POI particularly cardiovascular risk and bone density. Equally this could exacerbate the psychological distress of the diagnosis.

The prevalence of spontaneous POI has been traditionally quoted at 1%. Some data suggest that the figure may be higher and population characteristics such as ethnicity may affect the prevalence [3]. Establishing causation may have implications on the management strategy and long-term consequences of POI and is

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therefore essential as is attention to modifiable risk factors which may include smoking, gynaecological surgical practice and treatment regimens for malignant and chronic disease [3].

POI does not necessarily develop by the same mechanism as the normal menopause, which is due to follicle depletion. POI can be caused by chromosomal and genetic defects, including fragile X syndrome and autosomal gene defects. For example, some women develop POI due to mutations in the follicle-stimulating hormone (FSH) receptor; these women have follicles in the ovary that are unable to function [4]. POI can also be associated with autoimmune disorders or infections. Adrenal autoimmunity is the most frequent type observed in 60–80% of patients with autoimmune POI [5]. Another example is steroidogenic cell autoimmunity lymphocytic oophoritis as a cause of POI—this is a specific autoimmune attack against growing ovarian follicles [4]. Iatrogenic causes include surgery, chemotherapy or radiotherapy. Lastly, environmental factors are also implicated as determinants of the age of menopause and therefore could be a causative factor in POI.

In up to 90% of women diagnosed with spontaneous POI, the causative factor remains elusive, and the term idiopathic POI is used [6]. There is however a strong heritability of age at menopause [7], and approximately 10–15% of women with POI will have a first-degree relative who is also affected [8].

A recent review of the aetiology of POI cases managed at the West London Menopause and PMS Centre (London, UK) demonstrated the percentage of genetic/chromosomal cases, benign cases (autoimmune/infectious), malignant cases (as a result of cancer treatment) and idiopathic cases [9] (Fig. 11.1).

Life expectancy in women with POI is 2 years less on average than those who have menopause over 55 years, and this is thought to be due to an excess of deaths due to CVD, osteoporosis and neurocognitive decline [10].

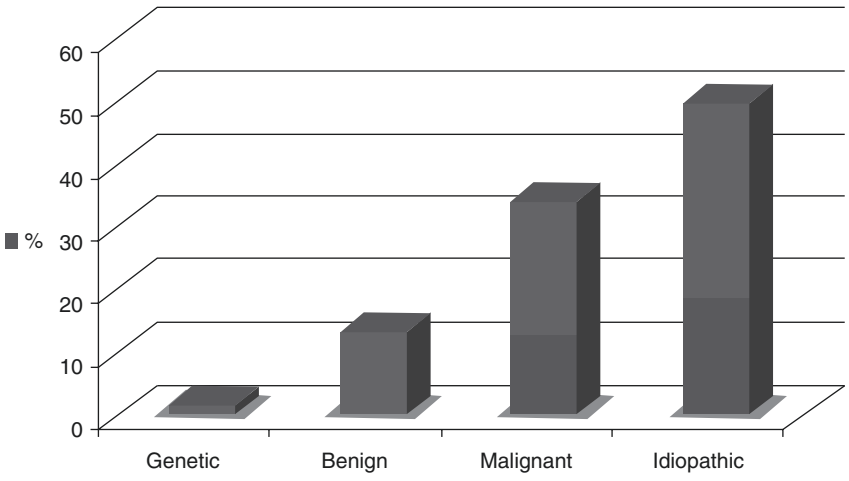

Fig. 11.1 Aetiology of premature ovarian insufficiency cases managed at the West London Menopause and PMS Centre, London, UK

11.2 Hormone Replacement Therapy

NICE recommends women with POI should receive either menopausal hormone therapy (MHT) or combined estrogen/progestogen contraceptive pills (COCP), and this should be continued until at least the average age of menopause (52 years). At present there is only limited data in support of particular hormone therapy regimens, and therefore much variation in practice exists [11]. The aim of treatment is to control menopausal symptoms, to maintain sexual function and to minimize the risk of cardiovascular disease and osteoporosis and possibly reduce the risk of cognitive impairment associated with POI [12]. Hormone therapy also minimizes the long-term effects and in adolescents with POI will be required to help induce secondary sexual characteristics.

As is the case for management of normal menopause, estrogen-only MHT has a significant risk of endometrial hyperplasia which can result in malignancy, and it therefore must be combined with a progestogen in women with an intact uterus [13]. Most preparations of MHT have been designed for women experiencing menopause around the expected age, whereas POI patients typically require higher doses of estrogen than older postmenopausal women with the aim to achieve serum oestradiol levels equivalent to premenopausal mid-follicular levels (approximately 300–500 pmol/L) [4]. As a result of recent studies in the POI population, there is a shift towards physiological estrogen replacement via the transdermal route. The transdermal route has the advantage of avoiding first-pass hepatic metabolism and the subsequent effect on clotting factors, which reduces the risk of thromboembolism [14].

Progesterone is most commonly micronized progesterone 200 mg daily for 12 days/month sequential combined or 100 mg daily continuous combined. The levonorgestrel-releasing intrauterine system (LNG-IUS) can be used for endometrial protection, particularly where contraception is required. The effect is more likely to be adverse for cardiovascular health if androgenic progestogens are used; the most neutral effect metabolically is reported with progesterone or dydrogesterone (available in some European countries) [15]. Further studies are needed to determine the optimum type of progesterone/progestogen [16]. Sequential combined regimes are usually administered in the first instance as intermittent return of ovarian function may result in unscheduled breakthrough bleeding on continuous combined regimens.

The risks attributable to MHT used by women with POI are likely smaller and the benefits potentially greater than those in older women who commence MHT beyond the typical age of menopause [17]. Importantly women with POI should be informed that MHT has not been shown to increase the risk of breast cancer before the age of natural menopause [18].

The COCP may be used continuously until the expected time of the menopause, but data are lacking regarding impact on bone and CVD. There are also limited studies comparing MHT with the COCP. Although the COCP causes more suppression of FSH [19], MHT is associated with beneficial effect on blood pressure [20], less hyperinsulinaemia [19], increased bone formation markers [21] and improved

lumbar spine bone mineral density [22]. Thus, the small randomized trials suggest that metabolic and bone health are more effectively maintained with MHT, but this requires confirmation. NICE guidance states that combined oral contraceptives are often prescribed when this might not be the best treatment in terms of quality of life and preservation of bone density and cardiovascular health. This forms the basis for the recommendation by NICE for large studies into short- and long-term outcomes of MHT versus combined hormonal contraceptives in women with premature ovarian insufficiency [1].

Non-hormonal therapies such as selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors or gabapentin may have a role in the management of vasomotor symptoms in women who decline MHT or in whom MHT is contraindicated, but they will have no benefit on future risk of osteoporosis and CVD.

There is no evidence for the use of complementary or herbal preparations in POI, and therefore hormone replacement forms the mainstay of pharmacological management.

In women with POI, ovarian function can return intermittently, and around 5% of women conceive naturally after the diagnosis. MHT is not contraceptive unless estrogen is combined with a LNG-IUS; it may therefore be more practical for the COCP to be used for the first few years following diagnosis of POI in those wishing to avoid pregnancy [23].

11.3 Cardiovascular Consequences

Cardiovascular disease is the leading cause of death in women overall, and proportionally this is increasing in many parts of the world. Multiple large epidemiological studies have reported a negative correlation between age at menopause and CVD morbidity and mortality in the general population [10, 24]. Multiple meta-analyses have highlighted the link between POI and increased risk of ischaemic heart disease [25]. Longitudinal studies have also demonstrated an 80% increased risk of mortality from ischaemic heart disease in women with POI compared to those with menopause at 49–55 years [26]. A recent meta-analysis of 32 observational studies found premature or early-onset menopause in women younger than 45 years were associated with an increased risk of coronary heart disease and all-cause mortality [27].

Furthermore the onset of CVD has been shown, in many cohort studies, to be earlier in women with natural POI before the age of 40 with the effect being more pronounced in artificial menopause [28]. Women undergoing bilateral oophorectomy before the age of 40 consistently showed an increased risk for CVD in a number of studies [29].

This increased risk of CVD in POI is not thoroughly understood although it is thought to be at least in part due to decreased sex hormone levels. This is considered to be the case as longer exposure to endogenous estrogens is thought to protect against cardiovascular diseases [30] and the overall risk is more pronounced in those who have never used estrogens. In addition to hormonal changes, loss of

ovarian function at the time of menopause is associated with activation of the renin-angiotensin-aldosterone system. This leads to downstream endothelial dysfunction, inflammation and immune compromise which may cause or contribute to the vascular damage [31].

The increased risk and earlier onset of CVD may be due to direct effects on the endothelium [32] or through alterations to traditional cardiovascular risk factors such as adverse effects on lipid profile [33], reduced insulin sensitivity [34] and metabolic syndrome [35].

One recent cross-sectional case study compared the cardiovascular risk profile between women with POI and premenopausal controls of comparable age. This found that women with POI demonstrated an unfavourable cardiovascular risk profile including a trend towards increased hypertension, impaired kidney function, higher abdominal fat and elevated chronic inflammatory factors compared to controls. However, no signs of increased subclinical atherosclerosis in women with POI were observed [36].

A recent review of the available evidence has suggested a significant increased risk of stroke associated with POI, with a protective role for estrogen used until the average age of menopause [37]. Additionally, data from the Women's Health Initiative Observational Study showed that surgical POI was associated with 13% increased risk of stroke, rising to 44% increased risk in those who did not use hormone therapy [38]. These figures did not reach statistical significance, although, as the authors acknowledge, the study was underpowered to detect differences within the POI group.

Aside from estrogen deficiency, POI is accompanied by decreased circulating androgen concentrations [39]. These lower levels of endogenous androgens may further impair CVD risk in women with POI, due to the association with increased dyslipidaemia and atherosclerosis shown in postmenopausal women. It has been demonstrated that in postmenopausal women endogenous steroid precursors and androgens are inversely related to carotid intima-media thickness, an established marker of atherosclerosis. Therefore normal androgen levels may benefit the carotid artery wall [40].

Progestogens have differing effects on lipids and lipoproteins depending on their androgenicity and potentially their dosing. The addition of progestogens to estrogen therapy has no adverse effects in terms of lowering of LDL, since although they may increase LDL production, they also increase its clearance [41].

Both menopause and MHT have a profound effect on metabolic risk factors for CHD as loss of ovarian function leads to adverse changes in lipids, glucose and insulin metabolism and body fat distribution. Estrogen replacement decreases total and low-density lipoprotein (LDL) cholesterol and increases high-density lipoprotein (HDL) cholesterol [42]. Triglycerides are increased with oral estrogen but decreased with transdermal administration, and the effects may be modified with the addition of progestogens. Oral estrogens increase coagulation activation and are therefore associated with a transient increase in venous thromboembolism, although this may be avoided with the use of transdermal estrogen [43]. Atherogenic lipid profile changes have been demonstrated in women with POI, which could contribute to this

increased CVD risk, with one study showing higher fasting triglycerides and marginally lower HDL [33].

11.4 Bone Mineral Density

It is well established that women with POI have significantly lower BMD and increased fracture risk [44]. There is now evidence to suggest that hormone therapy use (transdermal oestradiol 100 μg/day) not only preserves BMD but can actually restore BMD to levels comparable to control groups [45] and continuing use for at least 3 years may reduce fracture risk [46].

A recent randomized, double-blind controlled trial by Popat et al. demonstrated that the use of 100 μg of transdermal oestradiol plus oral medroxyprogesterone acetate (MPA) 10 mg/day for 12 days/month restored BMD to that of normal controls over 3 years [45].

In an open-label randomized controlled crossover trial by Crofton et al., women with POI were randomized to 4-week cycles of either physiological replacement of oestradiol (transdermal oestradiol 100 μg daily for week 1, 150 μg for weeks 2–4 with vaginal progesterone 200 mg twice daily for weeks 3–4) or standard hormone replacement treatment (oral ethinyloestradiol 30 μg and 1.5 mg norethisterone daily for weeks 1–3 and a 'pill-free' week 4) for a total of 12 months. Lumbar spine BMD *z*-score increased by +0·17 (CI +0·07 to +0·27) in response to the physiological dose of oestradiol ($P = 0·003$), compared with +0·07 (CI −0·03 to +0·18) during standard treatment ($P = 0·2$). Bone formation markers (bone-specific alkaline phosphatase and procollagen type 1 amino-terminal propeptide) increased in the physiological treatment arm but decreased in the standard hormone replacement group. Both treatments suppressed a marker of bone resorption (CrossLaps) [21].

A further 2-year open randomized trial by Cartwright et al. compared the effects of MHT (oestradiol 2 mg daily, with the addition of levonorgestrel 75 mcg for 12 days a month) and COCP (Microgynon 30; ethinyloestradiol 30 mcg and levonorgestrel 150 mcg taken daily for 21 days followed by a 7-day break) on BMD and turnover in women with spontaneous POI. In comparison with COCP, treatment with MHT increased BMD at the lumbar spine at 2 years (+0.050 g/cm^2; 95% confidence interval 0.007–0.092; $P = 0.025$) [22].

Bisphosphonates are inhibitors of bone resorption with proven efficacy in the prevention of vertebral and hip fractures. However some safety concerns are relevant to women with POI as there is an association suggested between atypical femur shaft fractures and over-suppression of bone turnover in patients exposed to bisphosphonates for longer than 3–5 years [23]. Bisphosphonates are also not recommended in women who wish to achieve pregnancy as they have a long skeletal retention time and the fetal effects are unknown.

In addition to treatment with MHT, general lifestyle and dietary measures should be advised to reduce the risk of osteoporosis. This includes adequate dietary intake or supplementation of calcium (1000 mg) and vitamin D (800 IU), regular weight-bearing exercise and reduction in smoking, alcohol and caffeine [12].

11.5 Psychological Impact

The psychological impact of a diagnosis of POI has been shown to increase anxiety, depression and somatization and reduce both self-esteem and overall life satisfaction. Studies have also shown that overall women with POI were less satisfied with their sexual life. One study demonstrated that sexual contact was associated with less sexual arousal, reduced lubrication and increased pain. However, despite women with POI having lower levels of oestradiol, total testosterone and androstenedione, multiple regression analysis revealed that androgen levels had only a weak influence on sexual functioning [47].

Most women are emotionally unprepared for the diagnosis of POI, and feelings of anger, sadness, guilt and shame can be present as women realize the implications of the diagnosis. It is therefore important to refer patients to additional accurate sources of information and to assess the strength of their social support network. Referral to a psychologist and advice regarding patient support groups such as The Daisy Network (www.daisynetwork.org) can also be helpful.

11.6 Fertility

One of the principal differences between managing women with natural menopause and with POI is the subject of fertility. POI can pose fertility difficulties which need to be addressed from a physical and psychological point of view.

At present IVF with donor oocytes presents the highest chance of pregnancy with success rates of around 40–50% per cycle. Embryo donation, surrogacy and adoption are further options which may be considered. Up to 50% of patients may have intermittent return of ovarian function for many years, and approximately 5–10% conceive spontaneously and unexpectedly [48]. It has been hypothesized that exogenous estrogen administration may help reduce endogenous gonadotropin levels, resulting in upregulation of FSH receptors in remnant follicles and potentially ovulation. However no particular characteristic appears to be of good predictive value for the resumption of ovarian activity in patients with POI [49].

11.7 Neurological Function

Early data demonstrating an increased risk of cognitive impairment [50] following premenopausal oophorectomy have now also been observed in women with POI [51]. In a French population-based cohort study of 4868 women, both spontaneous and iatrogenic POI were associated with negative effects on cognitive function in later life including increased risk of poor verbal fluency and impaired visual memory. There was no clear evidence that use of MHT reduced the risk of cognitive decline, but MHT use was self-reported at the age of at least 65 years, and therefore recall bias may have affected the results [52].

11.8 Conclusion

POI is a complex condition involving multiple physical factors and psychological needs. Health providers involved in the care of women with POI will likely include gynaecologists and endocrinologists, fertility specialists, oncologists, haematologists, psychologists, pharmacists and dieticians. Patient support groups such as The Daisy Network can give further support to women with POI. Ideally patients should be seen initially in specialist units which can facilitate an integrated and holistic approach to their care.

MHT will form the mainstay of treatment until at least the natural age of menopause along with monitoring of BMD and modification of cardiovascular risk factors. Additional studies are required to identify specific determinants of long-term CVD risk in women with POI.

Much of our knowledge on the treatment of this condition is derived from studies involving women experiencing menopause at the natural age. For many aspects of POI research, a prospective international database recruiting many thousands of cases is the only realistic way in which meaningful data can be gathered to answer many of the questions for which there is only speculation at present. Regarding treatment, questions which urgently need to be answered include whether the type of MHT matters, body-identical versus other types of MHT, oral versus transdermal estrogen, dosage of oestradiol, progesterone versus androgenic progestogens and the impact of androgens on both short-term quality of life and long-term outcomes. The database would also give the opportunity for the role of unproven fertility interventions in POI to be studied, such as dehydroepiandrosterone and the use of low-dose HRT and the contraceptive pill to suppress levels of follicle-stimulating hormone in order to facilitate ovulation of any remaining oocytes [53].

As is the case with a number of other centres, we have been collecting data from our cohort of women with POI for a number of years. More than 850 patients have already been recruited to the registry, and 70+ registrations have been received to enter data globally thus far. We hope this will ultimately lead to better understanding of the condition and the establishment of refined guidelines for the targeted care of young women with POI to optimize both short- and long-term outcomes.

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Management of Transsexuality in an Outpatient Gynecologic Area

12

Iuliia Naumova and Camil Castelo-Branco

12.1 Introduction

At present, people with sexual identity disorders are increasingly appearing in the world. A clear increase in the prevalence of transgender individuals is consistently observed in all studies that have been conducted over the past 50 years [1–3]. This fact indicates that the society has increased tolerance to people with nontraditional sexual orientation and gender identity. In this regard, transsexuals feel more openly and often seek professional help [4].

Despite the improvement of the overall situation, in many countries where transsexualism is considered a mental disorder, society, by and large, is simply not ready to treat people tolerant and understanding [5]. While acknowledging the improvement in the situation with transsexual medical care in countries with advanced human rights protection, it must be recognized that there are multifactorial problems around the world that are associated with providing medical care to this group of patients.

Transgenders remain one of the most underserved subgroups in many countries around the world. Unemployment rate, clinical depression, anxiety disorders, interpersonal violence, family abandonment, physical and mental violence, suicide risk, substance abuse, and serious diseases such as human immunodeficiency virus (HIV) are the highest among these population groups [6–9].

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Phobic attitudes against trans people are present in many health facilities [10–12]; noted the inaccessibility of medical assistance, the lack of qualified medical professionals, and high cost of services [13, 14]; inconsistency of existing mechanisms to assist transgender people with the principles of human rights, imperfection of the legal system among others [12, 14]. The shortage of skilled health workers and medical information on trans-health care is quoted as one of the main reasons for understanding the limitations suffered by trans-people seeking medical care [15, 16]. And, as a result of this inaccessibility to qualified medical care combined with the frequent suicidal mood and long social disadaptation, some individuals carry out hazardous practices for their health such as attempt to self-castration and uncontrolled hormone therapy [17, 18].

Transsexual subjects need to receive an effective and safe treatment. The goal of such therapies is to rehabilitate them as a member of the society in the gender area with which they are identified. According to the "Standards of Care for the Health of Transsexual, Transgender, and Gender-Nonconforming People," the options for medical treatment include:

- Changes in gender expression and role (which may include episodic or permanent life in another gender role that coincides with gender identity person).
- Hormone therapy for feminization or masculinization of the body.
- Surgical correction for the purpose of changing primary and/or secondary sexual characteristics.
- Psychotherapy in order to explore gender identity, role, and self-expression, work with the negative impact of sexual dysphoria and stigma on mental health, facilitate internal phobia, and increase social support and mutual assistance.
- Improvement of body image or development of stress resistance [19].

12.2 Clinical Management of Transgender People

Medical services for transgenders should be provided by a multidisciplinary team consisting of a psychologist, social worker, psychiatrist, endocrinologist, and surgeon (gynecologist, plastic surgeon, urologist) [20]. The psychologist and psychiatrist should diagnose transsexualism and recommend hormonal treatment; the endocrinologist, in turn, initiates and controls cross-sex hormonal treatment and participates in determination of indications for surgery. Finally, the surgeon must be responsible for the gender-affirming operation that is required to complete the transsexual transition [19, 20].

With the accumulation of experience, medical specialists recognized that although some people require both hormone therapy and surgical procedures to alleviate gender dysphoria, others need only one of these treatments, and others do not require any of them. In some cases, with psychotherapeutic support, subjects cease to feel the need to undergo feminizing or masculinizing surgeries. Some patients may need hormonal treatment, the possibility of changing the gender role, but not surgical correction; others may need to change their gender role along with

surgical correction, but not hormonal treatment. Thus, the treatment of this condition became more individualized [19].

Feminizing or masculinizing drug therapy is based on the administration of exogenous hormones that cause changes in physical appearance [18]. Since hormone therapy is inexpensive compared to surgery and very effective in the development of secondary sexual characteristics (e.g., facial and body hair in transgender men (female-to-male, FTMs) or breast growing in transsexual women (male-to-female, MTFs)), hormone therapy is often the first and sometimes the only intervention available to transgender people who seek to develop male or female characteristics according to their gender identity. In some cases, hormone therapy may be required before sex-affirming operation [12, 16]. The change in the physical characteristics of a person with hormone therapy is considered a necessary medical intervention for many transgender people and can alleviate the psychological suffering associated with gender dysphoria, reduce mental disorders, and improve the quality of life of patients [20, 21]. The effectiveness of hormone therapy in the elimination of mental disorders associated with gender dysphoria has been largely confirmed by clinical practice and evidence of low level [22–24]. According to the Standards of Care for the Health of Transsexual, Transgender, and Gender-Nonconforming People, the following criteria must be met for conducting gender-affirming hormone therapy in adults: persistent, well-documented gender dysphoria/gender discrepancy, the ability to take a fully informed decision and consent to treatment, and age of majority in a particular country; mental health problems, if any, should be sufficiently well controlled [19].

12.3 Management and Therapeutic Options for Transgender Adults

12.3.1 Male-to-Female Transsexual Subjects

Patients with MTF transformation need complex hormone therapy aimed at suppressing secondary sexual characteristics inherent in the inborn sex and the induction of sexual features compatible with gender identification. The main hormones in gender-affirming therapy for transgender women are estrogens. For these purposes, pharmacological preparations of estrogens, including oral, injectable, transdermal, and intravaginal forms as monotherapy or in combination with progestins, are used in a variety of dosages and administration routes [17]. According to the latest recommendations, preferable are oral conjugated equine estrogens or 17β-estradiol and transdermal 17β-estradiol [20].

Before the initiation of therapy, it is recommended a routine blood test with blood cell count and basic metabolic panel (glucose, liver enzymes, electrolytes, and lipids). The measurement of testosterone, prolactin, and hemostasis is also recommended [25].

It is important to rule out family history of breast cancer and to encourage the patient in the need for breast self-control. The personal history of breast cancer or

other estrogen-dependent neoplasms are absolute contraindications for the use of estrogen therapy [26]. It is necessary to explain to patients the possibilities of hormone therapy, the risks of complications, and the effects that hormone therapy cannot achieve. It is also necessary to discuss with the patient the possibility of maintaining fertility [27, 28].

The effects of feminizing estrogen therapy in the first 3–12 months in transgender women are breast growth, some redistribution of adipose tissue according to the female type, a weakening of the musculature of the upper body, softening of the skin, a reducing of skin oiliness, a decrease in hair growth on the body, and less erections [26, 29–31]. With time, atrophy develops in the testis and prostate.

The full effect of hormone therapy on physical appearance in transgender people may not be attained in the first 2 years of therapy. However, the complete disappearance of the changes induced by the hormones of their biological sex is almost impossible to achieve.

Estrogen monotherapy does not lead to a decrease in testosterone levels in MTF transgenders up to standard female values [25, 29], and most studies highlighted the need to prescribe additional antiandrogenic drugs [29, 32, 33]. It is noteworthy that some progestins, such as cyproterone acetate, may have antiandrogenic properties [17, 26, 34]. Data exist on the effect of cyproterone acetate reducing or eliminating the effects of androgens on target organs such as the growth of hair on the face and body and the production of skin sebaceous glands. Moreover, this drug may induce a weakening of sexual desire [34] and stimulate the growth of mammary glands [35].

Spironolactone and flutamide are drugs that block the effects of testosterone at the androgen receptor level [36]. Different studies report good results using these drugs with a testosterone-suppressive goal in the schemes of MTF hormone therapy [35, 37]. Gonadotropin-releasing hormone (GnRH) agonists in combination with estrogen have shown in some studies a high efficacy and safety achieving an antiandrogenic effect in MTF patients [17, 38, 39].

Additionally, data exist on the combined use of 5-alpha-reductase inhibitors (finasteride and dutasteride). These drugs block conversion of testosterone to potent androgenic dihydrotestosterone and therefore may provide a more pronounced feminizing effect [40]. Inhibitors of 5-alpha-reductase may be a good choice for intolerance or the presence of contraindications to the use of spironolactone. 5-Alpha-reductase inhibitors may also be an option for use as monotherapy in patients requiring partial feminization or for those who have signs of virilization against antiandrogen therapy or gonadectomy [41].

Cross-sex therapy should be selected individually, paying special attention to the characteristics and wishes of each patient, and be effective and as safe as possible. Gender-affirming hormone therapy in MTF transsexuals is carried out in two stages. The first stage—before—sex reassignment surgery is aimed at the reverse development of secondary sexual characteristics of the inborn sex and the formation of those that are specific for selected sex, and the second stage—after orchiectomy—is necessary for further feminization of the patient and prevention of post-castration

syndrome development. Commonly, cross-sex therapy should be started at least 6 months before the planned surgical intervention and ceased 3–4 weeks before surgery since prolonged immobilization may increase the risk of thromboembolism. After surgery, when physical activity is recovered, hormone therapy should be resumed [25].

Recently the North American Endocrine Society published the recommended therapeutic doses and routes of administration to achieve the feminizing effect [20] (Table 12.1).

Exceeding the recommended daily dosage of estrogens is allowed for a short period of time in case of insufficient decrease in testosterone levels with lack of antiandrogenic effects and insufficient growth of the mammary glands [17]. The most dangerous adverse event of estrogen therapy in MTF transgenders is thromboembolism. From 2% to 6% of MTF individuals during the first year of therapy will develop thrombotic complications; however, frequency decreases to 0.4% afterward [17]. In addition, single cases of pulmonary artery embolism [42] and cerebral thrombosis [43] have been published. The higher risk of thrombosis is associated with oral administration of ethinyl estradiol [17, 26, 33], smoking, and the presence of cardiovascular and thrombophilic diseases [20, 44]. By oral route, estrogens undergo active metabolism in the liver that stimulates the production of coagulation factors and triglycerides [25]. Parenteral forms of estrogen administration bypass this first step, reducing the risk of thrombotic complications, and are the forms of choice for patients in the age group over 40 years [45]. In spite of this concern, administration of oral ethinyl estradiol to MTF transgenders at a dose of 0.03–0.1 mg/day in the composition of COC appears to be safe with a good feminizing effect [17, 25].

A slightly increase of prolactin level in the blood has been detected in some MTF transgenders. However, in cases of significant excess of prolactin and galactorrhea, prolactinomas should be ruled out [29].

Table 12.1 Recommended therapeutic doses and routes of administration to achieve the feminizing effect in MTF transgender individuals (Endocrine Society, US)

First stage before the operative removal of gonads			
Drug	Dose	Route	Freq.
17β-Estradiol	2.0–6.0 mg/day	Oral	Per day
Estradiol valerate	2.0–6.0 mg/day	Oral	Per day
17β-Estradiol patches/gel	0.025–0.2/day	Transdermal	Per day
Estradiol valerate/cypionate	5–30 mg	Intramuscularly	Every 14 days
Estradiol valerate/cypionate	2–10 mg	Intramuscularly	Every week
Antiandrogens			
Cyproterone acetate	25–50 mg/day	Oral	Per day
Cyproterone acetate	3.75 mg/month	Subcutaneously	Every month
Spironolactone	100–300 mg/day	Oral	Per day
Gonadotropin-releasing hormone agonists	11.25 mg	Subcutaneously	Monthly for 3 months

Interestingly, a large percentage of depression (10%) in MTF transgenders under hormone therapy has been reported [17].

At the second stage, after surgical removal of the gonads, estrogen monotherapy is usually continued. However, some patients even after the surgery have excessive growth of hair on the face and body. Such patients can be recommended to continue taking antiandrogen [26].

Individuals under MTF hormone therapy should be checked to evaluate the efficacy and safety of such a therapy. It is recommended a clinical assessment every 3 months during the first year of therapy and then every 6–12 months [17, 46]. The physical examination includes weight, blood pressure, breast augmentation, hair body involution, redistribution of fat deposits, and testicular atrophy (if not removed). Blood samples should be performed every 6–12 months to determine the level of LH, FSH, testosterone, estradiol, prolactin, hepatic serum enzymes, blood coagulation factors and lipid profile, and blood count. The levels of serum estradiol and testosterone should ideally correspond to those of premenopausal women (100–200 pg/mL and <50 ng/dL, respectively) [47]. Finally, bone absorptiometry and breast ultrasound should be performed regularly (Fig. 12.1).

12.3.2 Female-to-Male Transsexual Subjects

The goal of hormone therapy in FTM transgenders is the development of secondary sexual characteristics, inherent to men. Masculinizing effect can be achieved by using various testosterone pharmacological preparations [29, 33, 35]. The aim of

First control	6-12 months monitoring (every 3 month during 1 st year of therapy)
Weight	Weight control
Blood pressure	Blood pressure
Hemogram	Physical examination (breast augmentation, hair body involution, redistribution of fat deposits and testicular atrophy)
Metabolism (glucose, liver enzymes, electrolytes and lipid profile)	Hemogram
	Metabolism (hepatic serum enzymes, lipid profile)
	Blood coagulation factors
Testosterone, prolactin	LH, FSH, testosterone, estradiol, prolactin
Hemostasis	**3-5 years monitoring**
EKG, Abdominal ultrasound	Bone absorptiometry
	Breast ultrasound

Fig. 12.1 Management of MTF transgender subjects

cross-sex hormone replacement therapy in this case is to achieve normal male testosterone blood levels, usually within the range of 320–1000 ng/dL [48].

Commonly, in the management of FTM transgenders, therapeutic doses and routes of administering testosterone vary upon subjects. Injectable preparations of short-acting testosterone esters, injections of long-acting forms of testosterone undecanoate, transdermal patches and testosterone gels, subcutaneous implants, and oral testosterone undecanoate are prescribed.

Table 12.2 records the recommendations of the Endocrine Society for cross-sex therapy in FTM transgenders [20].

Injections of short-acting testosterone do not mimic the physiological circadian rhythms of testosterone production, and it is not uncommon that in the first days of use, supraphysiological levels were observed leading to the development of adverse effects, such as aggressiveness, increased libido, and sweating [49]. These supraphysiological peaks of testosterone and most of its adverse effects are not present with the long-acting injectable forms which are significantly better tolerated by patients [50]. However, the use of long-acting forms of testosterone is often limited by their high cost compared to the short-acting forms. On the other hand, transdermal testosterone systems simulate the physiological daily rhythms and have a reasonable cost [17].

Expected effects of testosterone therapy include increased muscle mass; fat tissue redistribution; voice change; body hair growth on the face, chest, and abdomen; clitoral size increase; and increased libido [29, 48]. The cease of menstrual function often occurs in 2–3 months from the beginning of testosterone therapy. In case of persisting uterine bleeding, the use of progestins or even endometrial ablation has been suggested [51]. In addition, gonadotropin-releasing hormone agonists or medroxyprogesterone may be administered to stop menstrual function before starting treatment with testosterone.

Testosterone replacement may be associated with adverse effects in FTM transgenders. Among them, acne, weight gain, aggressiveness, increased sexual desire, and hypertension are the most common [52]. Cases of venous thrombosis and thromboembolism, deterioration of lipid profile, polycythemia, insulin resistance, atherosclerosis, and breast and ovarian cancer have been also reported [29, 53]. In this sense, the results from the European Network for the Investigation of Gender Incongruence corroborate that current treatment modalities for transgenders are effective and carry a low risk for side effects and adverse events at short-time follow-up [54].

Table 12.2 Recommended therapeutic doses and routes of administration to achieve the masculinizing effect in FTM transgender individuals (Endocrine Society, US)

Drug	Dose (mg)	Route	Frequency
Testosterone enanthate/cypionate	100–200	Intramuscularly	Every 14 days
Testosterone enanthate/cypionate	100–200	Subcutaneously	50% dose weekly
Testosterone undecanoate	1000	Intramuscularly	Every 12 weeks
Testosterone gel 1.6%	50–100	Transdermal	Per day
Testosterone patches	2.5–7.5	Transdermal	Per day

First control		6-12 months monitoring (every 3 month during 1 st year of therapy)	
	Weight		Weight control
	Blood pressure		Blood pressure
	Hemogram		Physical examination (breast augmentation, hair body involution, redistribution of fat deposits and testicular atrophy)
	Metabolism (glucose, liver enzymes, electrolytes and lipid profile)		Hemogram
			Metabolism (hepatic serum enzymes, lipid profile)
			Blood coagulation factors
	Testosterone, prolactin		LH, FSH, testosterone, estradiol, prolactin
	Hemostasis	3-5 years monitoring	
			Bone absorptiometry
	EKG, Abdominal ultrasound		Breast ultrasound

Fig. 12.2 Management of FTM transgender subjects

Regular clinical and physical examinations to assess the development of signs of virilization and to detect adverse effects of hormone therapy every 3 months during the first year of therapy and thereafter every 6–12 months are recommended. Along with these clinical controls, blood pressure and weight should be recorded, and blood analysis should be performed including assessment of serum testosterone levels every 3 months until blood levels in healthy men are reached and LH, FSH, estradiol, blood cell count, and lipid profile each 3 months during the first year of therapy and 6–12 months thereafter.

In cases of lack of compliance with hormone therapy, violations of treatment schedules, or failure of hormonal replacement action, the actual risk of osteoporosis is high. For such patients, osteoporosis needs to be screened and absorptiometry recommended.

Oophorectomy and hysterectomy are recommended after hormonal transition. If mastectomy has been performed, a regular peri- and subareolar survey is mandatory; if not performed, mammogram is recommended [19, 55] (Fig. 12.2).

12.4 Conclusions

Mental health is improved through comprehensive gender-based treatment, including psychologic actions, real-life experience, hormone therapy, and surgical operations [34, 56]. Subjects who underwent sex reassignment surgery should be under supervision of an endocrinologist to monitor the adequacy of the hormone replacement therapy for the rest of their life. Only a properly selected dose of hormonal

replacement can prevent the development of adverse effects caused by the removal of gonads, i.e., post-castration syndrome [20].

For many transgender adults, surgery, which proves gender identity, can be a necessary step toward the goal of a successful life in accordance with the desired role of men or women. The type of gender-affirming operation is divided into two main categories: those that directly affect the reproduction ability and those that do not affect fertility. The first include surgery to remove the penis and testicles in men and the removal of the uterus and gonads in women. Operations that affect fertility are often regulated by laws. Other gender-affirming operations such as rhinoplasty that do not directly affect fertility are not so strictly regulated.

Sex reassignment surgical options for transwomen include non-genital surgeries such as breast augmentation, liposuction, facial feminization surgery, lipofilling, voice feminization surgery, thyroid cartilage reduction, and gluteal augmentation. Genital feminizing surgeries include bilateral orchiectomy, penectomy, and options for remodeling the genital tract via clitoroplasty, vaginoplasty, and/or vulvoplasty.

Similarly, transmen may undergo bilateral total or partial mastectomy, chest contouring, liposuction, lipofilling, liposurgery, and/or pectoral implants. Genital masculinizing surgeries include metoidioplasty (lengthening and straightening of the testosterone-enlarged clitoris to create a neophallus), phalloplasty, urethral lengthening and scrotal reconstruction with insertion of testicular prostheses with or without hysterectomy, and/or bilateral salpingo-oophorectomy [57].

For the last 10 years, sex reassignment surgical methods have significantly improved. Reconstructive surgery on the genitals, preserving neurological sensitivity, is at present time the standard, and the level of patient satisfaction after surgical correction is currently very high [21].

Finally, despite the growing awareness, the reduction of stigmatization, and the positive trend in the medical care of the transgender population, physicians and transgenders need to overcome many obstacles before reaching the long-term goal of achieving high standards of care for members of this diverse social group. Health facilities should include formal training on transgender health issues and help patients make choices from the full range of available health services, according to their clinical needs and the goals of gender expression. Further study of the etiology, pathogenesis, and manifestations of transsexualism, as well as profound understanding by physicians, especially endocrinologists, psychiatrists, and therapists, of this issue, will allow diagnosis and treatment at an earlier time, faster to withdraw patients from chronic stress, which, ultimately, will allow transgender patients to maximally improve their health, psychological well-being, and self-actualization.

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Regulation of Proliferation and Invasion in Endometriosis

13

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Endometriosis is a common disease in young women, which affects approximately 6–10% of the female German population. The disease is defined as endometrium-like glands and stroma cells outside the uterus and can cause severe and chronic pain (dysmenorrhea, dyspareunia, abdominal pain) as well as reproductive problems and infertility [1, 2]. Endometriosis triggers a decrease in the quality of life similar to other chronic diseases such as arthritis or heart conditions [3].

Endometriotic lesions are divided into three different entities that most likely have their own pathogenesis: peritoneal endometriosis, ovarian endometrioma, and deep infiltrating endometriosis. Those endometriotic lesions differ in molecular and cellular structures as well as in their invasive and proliferative characteristics. Nevertheless, there are invasive and proliferative pathways and modulators that remain equivalent, even if the impact may differ. Thus, every scientific result viable for one entity must be carefully reviewed for the remaining two [4].

The exact mechanisms of the pathogenesis of endometriosis, including the invasion and implanting in other tissues, remain unclear and subject of investigation. However, several different etiological theories are widely accepted [1, 2, 5]:

1. Retrograde menstruation resulting in implantation/transplantation of endometriotic tissue fragments.
2. Coelomic metaplasia.
3. Possible stem cell involvement.
4. Lymphovascular metastasis.

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In every proposed model, enhanced proliferative and invasive properties of the cells play a vital role in pathogenesis and progress of the disease—both clinically and concerning lesion size.

As stem cell properties and markers have been discovered in endometriotic cells, the stem cell concept describes the endometriotic lesions as deriving from ectopic displacement of cells with stem cell characteristics. Due to their characteristics, e.g., unlimited proliferation abilities and developing potential, endometrial glands, stroma cells and endothelial cells can develop [6, 7].

Invasion is enhanced through increased cell motility and decreased adhesion due to a dysregulation of cytoskeletal and cell adhesion molecules as well as proteolysis. Contributing to the invasion inflammation, angiogenesis, neurogenesis, and progesterone resistance are also enhanced [1].

Ectopic endometriotic lesions may invade the peritoneum and every pelvic organ; they have also been shown to invade perineural and intraneural structures—causing severe pain [8].

13.1 The Role of MicroRNAs in Regulation of Invasion and Proliferation

Deep infiltrating endometriotic lesions possess special characteristics regarding invasion. Invasive endometrial glands have been reported to have increased mitotic activity and fewer adhesion molecules as well as altered morphology—indicating collective cell migration as part of endometrial invasion in ectopic tissue [9, 10].

The molecular regulation of this invasive progress is still subject of investigation. MicroRNAs (miRNAs) have been classified as governing structures over parts of the transcriptional processes leading to invasive characteristics [1].

Proliferation and apoptosis in endometrial cells contribute to the progress and invasiveness of endometriotic tissues and are also partly regulated by miRNAs.

Endometriotic lesions have been found to be less vulnerable to apoptosis, contributing largely to the success of endometriotic implantation in other tissues [11].

Collected data concerning the changes of proliferation in endometriotic lesions provide inconsistent data, from an increase to a decrease in proliferation of endometriotic cells. This may be due to the different molecular structure mentioned earlier. As proliferation is determined by many factors differing between the entities, the different tissues will have different proliferation characteristics. It has been established that the different entities of endometrioses exhibit heterogeneous mRNA signatures—leading to different gene expressions and characteristics [11, 12]. Up- or downregulation of miRNAs has been connected to the changes in mRNA expression.

miRNAs are small, noncoding RNA molecules. Consisting of a singular strand, they target mRNA to inhibit protein expression through gene regulation (gene silencing) on a posttranscriptional level. The mRNA is degraded or translation is inhibited [13].

Various studies demonstrate the impact of miRNAs on every aspect of the invasion, viability, and proliferation. They affect inflammation, apoptosis, cell cycle, angiogenesis, and much more. In the following abstracts, the key miRNAs and their influence will be elaborated.

13.2 miRNA-145

A key role in endometriosis invasion and proliferation is imputed to miRNA-145. It contributes to the regulation of apoptosis, proliferation, and invasiveness in cancer [12]. In endometriosis, it has been reported to reduce stem cell activity, influence the cell cycle, repress stem cell characteristics such as pluripotency, and inhibit invasiveness and proliferation [1].

miRNA-145 is known to be commonly expressed in mesenchymal cells (e.g., fibroblasts and smooth muscle cells). As the cell composition of those varies in different tissues and forms of endometriosis, differences between the entities should be expected.

miRNA-145 levels differ in the various entities of endometriosis, also contributing to their different invasiveness. It has been reported to increase in infertile endometriosis patients and to decrease in stage III endometriotic lesions compared to eutopic endometrium. In studies, miRNA-145 dysregulation and decrease occur in more invasive and aggressive lesions [1].

It was verified that in endometriotic tissue, miRNA-145 significantly inhibits the three important mechanisms of invasion, stem cellness and proliferation. It does so by inhibiting posttranslational expression of several key molecules and might be a target for new therapeutic options [1].

13.2.1 Invasiveness

miRNA-145 reduces invasiveness through inhibition of proteolysis, cell mobility and adhesion.

It inhibits FASCIN-1 expression resulting in decreased cell mobility and proliferation [1]. Fascin-1 bundles actin as a cytoskeletal element contributing to cell motility and invasiveness. The expression of fascin-1 mRNA is downregulated by miRNA-145 resulting in an impaired cytoskeleton and decreased cell mobility. Decreased proliferation impedes lesion growth.

Additionally, miRNA-145 has been found to regulate tight junction molecules, such as JAM-A. The expression of the adhesion molecule is downregulated by miRNA-145, resulting in impaired cell junction of the tissue. In breast cancer it can reduce invasiveness due to impaired junction between the cells. The pathway suggests JAM-A as a factor for invasiveness that is controlled and inhibited by miRNA-145 [1].

Proteolysis is also regulated and suppressed by miRNA-145. Through miRNA overexpression, protease-inhibitor expression was downregulated [1].

13.2.2 Proliferation and Stem Cell Function

Markers of stem cellness indicate characteristics such as high proliferative and differentiation potential. Those properties enhance invasion and severity of the lesion.

Proliferation and stem cell abilities in endometriosis cells are reduced by miRNA-145 overexpression and might rely on similar pathways.

A common marker of stem cells is SOX-2, a transcription factor that is required for stem cell maintenance [14].

Adult stem cell determination, pluripotency, proliferation and differentiation are regulated by SOX-2, and it is expressed in mesenchymal stem cells in endometrium and endometriotic lesions, mainly in proliferative areas. It is upregulated in endometriosis, suggesting it plays a key role in proliferation and underlining the importance of stem cells in the pathophysiology of endometriosis [6, 15].

It was shown that miRNA-145 inhibits expression of SOX-2 in mesenchymal cells in endometriotic lesions. Also, other transcription factors of stem cells such as MSI2, OCT-4 and KLF4 are significantly decreased. MSI2 is upregulated in endometriotic lesions and promotes proliferation in stem cells.

It can be concluded that miRNA-145 in endometriotic lesions reduces proliferation, stem cell motility as well as markers and therefore inhibits progress of the disease [1] (Fig. 13.1).

13.3 miR-142-3p

Another important molecule regarding invasion and proliferation in endometriosis is miR-142-3p.

A constitutive factor that is repressed by miR-142-3p is the interleukin 6 (IL6) signal transducer (IL6-ST).

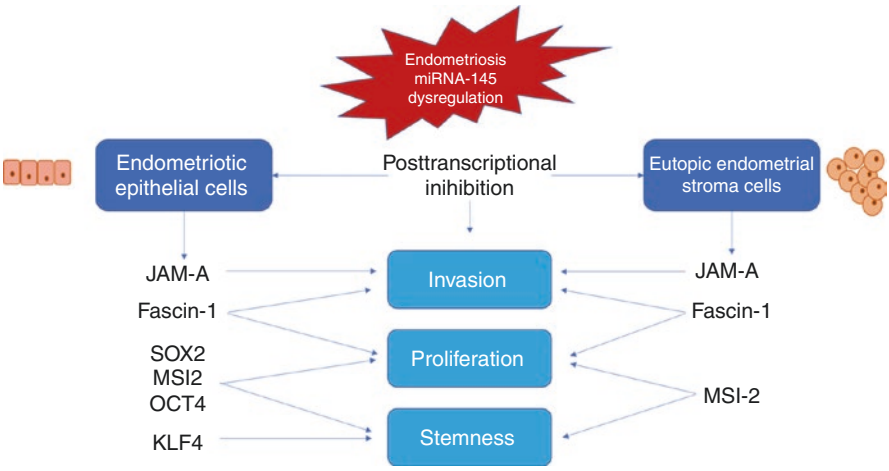

Fig. 13.1 miRNA-145 in endometriosis (Designed after Adammek et al. [1])

IL6-ST functions as a signal transducer and a component of the cytokine receptor complex. Activation is possible through binding of cytokines such as IL6 to their receptors. IL6-ST seems to play a key role in apoptosis of myocytes, therefore regulating immune responses [16]. In endometriosis it has been found to affect proliferation and migration of cells [17]. In endometriosis and various cancers, it has been shown to impede invasion and cell motility due to cytoskeletal changes.

miRNA-142-3p is reduced in endometriosis—possibly enhancing invasion and progress of the lesions through cytoskeletal changes, enhanced proliferation, immunological responses, angiogenesis and altered oestrogen levels [17]. Upregulating miRNA-142-3p levels could be a starting point for new treatments.

13.3.1 Immunology and Proliferation

The cytokine IL6 induces the activation of IL6-ST through the IL6 receptor. The IL6-ST as a complex with other molecules activates the transcription factors STAT3 and NFKB and their translocation to the nucleus. Over those pathways IL6-ST induces transcription of target genes. NFKB and STAT3 activate important target genes for invasion and proliferation [17].

STAT3 controls the expression of acute-phase proteins in immune responses and expressions of factors controlling apoptosis and cell growth [18].

NFKB indicates expression of genes that activates immune response, enhances cell proliferation and prevents apoptosis. In endometriotic lesions, a significant activation of NFKB can be detected [19].

As a summary, by repressing IL6-ST, miR-142-3p inhibits the cell proliferation and enhances apoptosis—possibly inhibiting endometriosis. Thus, impaired miR-142-3p levels promote endometriosis.

13.3.2 Cytoskeletal Function and Cell Motility

miR-142-3p reduces expression of cytoskeletal molecules linked to cell mobility [17].

A restructuring of the cytoskeleton is induced by miR-142-3p. It reduces matrix receptors and cytoskeletal elements such as integrin αV. Through regulation of WASL, integrins as well as actin and vinculin in endometriosis, the building of spikes and focal adhesion formation is reduced, resulting in diminished invasiveness. Reduced levels of miR-142-3p in endometriosis have been found and can lead to enhanced invasiveness [17].

13.3.3 Angiogenesis

The miRNA mir-142-3p induces also apoptosis of endothelial cells [20].

It is also indicated that miRNA contributes to the regulation of vasculogenesis in various cancers [21]. The impaired levels of mir-142-3p could likely enhance the building of new vascular structures in progressing endometrial lesions. Involvement of miRNAs in angiogenesis and vasculogenesis in endometriosis remains under investigation.

13.3.4 Hormonal Regulation

Endometriosis and the progression of lesion proliferation and invasion are highly influenced by oestrogen. Steroid sulfatase is an enzyme that hydrolyses progenitors of oestrogens and androgens in their biosynthesis [22]. In ectopic endometrial tissue, the steroid sulfatase expression is enhanced leading to higher oestrogen levels and proliferation. Its inhibition has been found to significantly reduce lesion size and weight [23].

miR-142-3p reduces expression of steroid sulfatase in endometrial stroma cells, indicating a regulatory role in steroid hormone and therefore oestrogen signalling. Impaired levels of miRNA-142-3p thus may cause lesion growth through altered oestrogen levels [17].

13.4 miR-200b

The third key miRNA with inhibiting effects on invasiveness, proliferation and stem cell characteristics in endometriosis is miRNA-200b. In malignant diseases and benign conditions such as endometriosis, miRNA-200b expression is reduced significantly inducing lesion growth and progress of the disease [24] (Fig. 13.2).

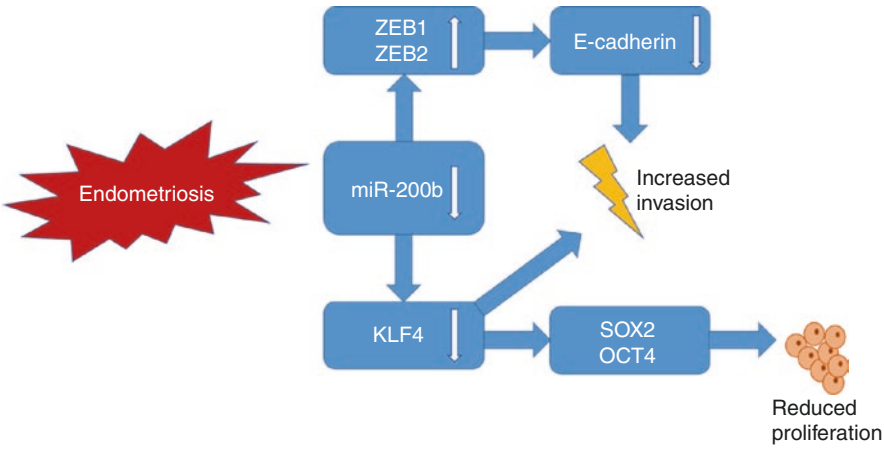

Fig. 13.2 Effect of miR-200b (Designed after Eggers et al. [24])

13.4.1 Proliferation and Stem Cell Phenotype

Krüppel-like-factor 4 (KLF-4) is a zinc finger transcription factor regulating proliferation, differentiation of cells, apoptosis and somatic cell reprogramming [25]. It is also a pluripotency factor of stem cells that, such as the other pluripotency factors SOX-2 and MSI2, is downregulated in endometriosis. Downregulation of KLF4 in endometriosis through low levels of miRNA-145 and 200b results in stemness self-renewal [1].

Under the influence of miR-200b, KLF-4 expression increased strongly and indicated significantly the influence of miR-200b on proliferation. Consecutive increased cell proliferation has been shown in endometrial cells and cells from endometriotic lesions [24].

miRNA-200b induced increased expression of KLF-4 and also increased the side population of cells detected in cell flow cytometry, a population that is associated with stemness characterisations [1].

In summary, miR-200b downregulation may reduce stem cell features and decrease proliferation through decreased levels of KLF-4 expression [24].

13.4.2 Invasiveness

Zinc finger E-box-binding homeobox 1 and 2 (ZEB 1 and ZEB 2) are transcription factors known for their repression of interleukin 2 (IL 2) expression and regulating pathways of transforming growth factor β (TGFβ) [26]. In endometriosis they are the master regulators of epithelial to mesenchymal transition (EMT) and control the expression of E-cadherin [24].

EMT describes the loss of polarised organisation of the cytoskeleton and cell-to-cell contacts in epithelial cells of endometriosis. Thus, the cells are gaining characteristics of mesenchymal cells—such as high motility. Contributing to invasion of the cells in other tissues, these changes may be very important in the formation of endometriotic lesions [27].

Cadherins are calcium-dependent transmembrane glycoproteins controlling cell-to-cell adhesions. They appear in desmosomes and adherent junctions. E-cadherin is an anti-metastatic adhesion molecule of epithelial cells [28].

miR-200b overexpression induces a significant decreased expression of the EMT-promoting transcription factors ZEB 1 and ZEB 2. As one of their key targets is inhibiting the transcription of E-cadherin, the downregulation of ZEB 1 and 2 is linked to the consecutive upregulation of E-cadherin. The treatment with miR-200b resulted also in an upregulation of E-cadherin [24]. Meanwhile E-Cadherin upregulation promotes loss of epithelial integrity and is linked to increased cell motility and invasive cell behaviour, contributing to the invasiveness of endometriosis through loss of cell-to-cell adhesions [29], while E-cadherin upregulation promotes cell adhesion and decreases cell mobility. miR-200b enhances E-cadherin expression through diminished ZEB 1 and ZEB 2 levels resulting in decreased cell mobility, decreased declamping cells from the cell layer and less invasion of endometrial cells.

miR-200b therefore inhibits invasiveness and can be a valid candidate for further treatment. Additionally, the decreased miR-200b levels in endometriosis explain the invasiveness of the tissue [24].

13.5 Summary

The pathology of endometriosis, including invasiveness and proliferation of the lesions, remains unclarified in their full extent. As research develops the regulating functions of miRNAs in epigenetic modulation of gene expression are suggested to be a central point of the mystery. A lot of contributing miRNAs have been found, but further research regarding their physiology and therapeutical consequences are needed [30].

The miRNAs in the centre of the equation at the moment seem to be miRNA-145, 200b, 142-3p and 10b. Dysregulation, in most cases downregulation of those single-stranded RNAs, leads to progress of the disease through increased invasiveness, proliferation, EMT and development of stem cell characteristics. Their epigenetic contribution is never limited to one pathway, but they all have influence on proliferation and invasion processes. The complexity of their impact and regulatory functions indicates also synergistic effects. As mRNA structures in the different entities have been found heterogeneous, it is highly likely that also the regulation of miRNAs is diverse between peritoneal endometriosis, deep infiltrating endometriosis and ovarian endometrioma.

Another conclusion should be that a drug or treatment targeted at miRNAs should always consider more than one miRNA.

Further research is needed and many mysteries are waiting to be solved.

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Hormones and Inflammation: An Update on Endometriosis

14

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14.1 Background

Endometriosis is a chronic inflammatory disorder causing pain and affecting fertility in reproductive-age women, and it is defined as the presence of functional endometrium outside the uterine cavity [1–3].

The pathogenesis of endometriosis has puzzled investigators for years and is still not fully understood. The hypothesis with the most supportive evidence is the so-called retrograde menstruation phenomenon [4]. The shed menstrual tissue is viable and then adheres, implants, and grows ectopically in women, who eventually develop endometriosis. Nevertheless, the proportion of women who experience retrograde menstruation is high (up to 90%), but endometriosis only affects a much smaller proportion of women. Other pathogenetic theories include endometrial stem cell implantation, Müllerian remnant abnormalities, coelomic metaplasia, and neonatal uterine bleeding; however, they cannot explain all the phenotypes of endometriosis, suggesting that other factors may be involved [4].

Genetics and epigenetics have been demonstrated to play a role in increasing the susceptibility of endometriosis, and, indeed, dysregulated genes are involved in hormonal function, inflammation, immune response, and cell proliferation [5, 6].

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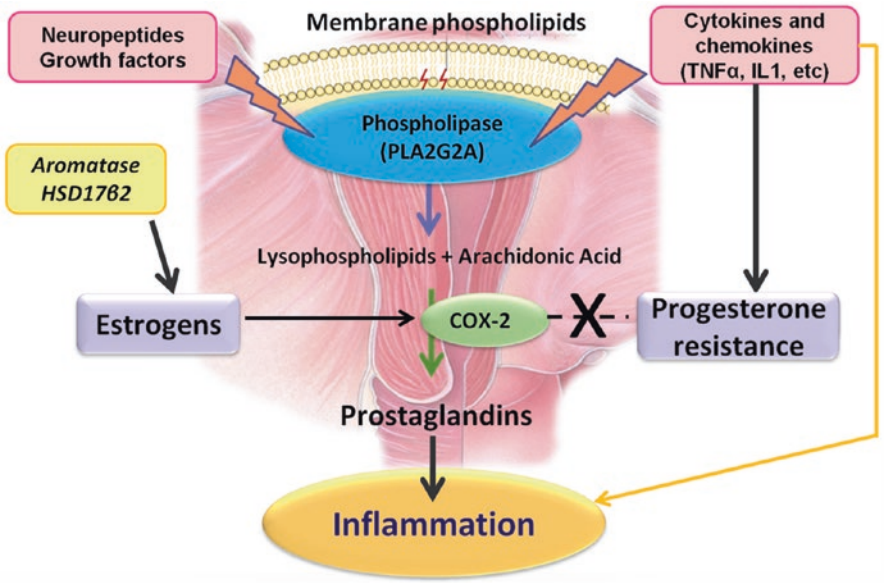

Fig. 14.1 Interaction between endocrine and inflammatory mechanisms in endometriosis

The role of sex steroid hormones has been shown to be relevant in endometriosis onset and progression [7, 8]. However, ectopic implants of endometrium due their growth and persistence to also immune dysfunction [9]. In fact, a number of immune cells, secreting inflammatory factors, such as cytokines and chemokines, are involved and have been found in endometriotic lesions [10]. Multiple cellular and molecular mechanisms, including increased endometrial cell adhesion, angiogenesis, inflammation, impaired immune response, aberrant estrogen signaling, and progesterone resistance as well as reduced apoptosis, are involved in the development, growth, and survival of endometriotic lesions [11].

This chapter will review the mainstays of pathophysiology of endometriosis, focusing on the role of sex steroid hormones and their interplay with immune and inflammatory pathways (Fig. 14.1).

14.2 Hormones and Endometriosis

Endometriosis, together with breast cancer, endometrial cancer, adenomyosis, and leiomyomas, is a hormone-dependent disease, and estrogens play a major role [12]. The relation between ovarian steroid hormones and the development and maintenance of lesions is not fully understood, although endometriosis occurs almost exclusively in menstruating women [12].

Estrogen and progesterone exert a direct effect on endometrial cells (stroma, epithelium, immune), modulating cytokine and chemokine synthesis and/or local

regulatory factor expression [13]. They may also act through the mediatory effect of a large number of locally produced growth factors and neurohormones. Local sex hormone-mediated events induce changes of the immune cell population in the endometrium, contributing to an endocrine-inflammatory link that is critical in reproductive function. In addition, patients with endometriosis have normal serum levels of gonadotropins and ovarian steroid hormones. The hormonal dysfunction is mainly local and functional: estrogen (E) and progesterone (P) imbalance has been found in eutopic and ectopic endometrium, and that may be the main trigger for the development of endometriosis [14].

14.2.1 Estrogen and Estrogen Receptors

Estrogen is associated with an increased endometrial cell proliferation and reduced apoptosis. In most human tissues and under most physiological conditions, the activation of estrogen receptor (ER) by estradiol or other ligands results in the inhibition of apoptosis [15], especially in the endometrium. In natural cycles, unopposed E stimulation in the proliferative phase is characterized by absence of apoptosis in endometrial glandular cells. Furthermore, E stimulation in vitro increases endometrial cell viability, and this effect is strongly reversed by E withdrawal. The anti-apoptotic effects of E is mediated by both nuclear and extranuclear ER signaling [14].

In endometriosis E function is impaired due to a double mechanism: the increase estrogen receptor activity and the increased local production. Firstly, endometriotic cells produce themselves E and express ER [16]. The ectopic implants also express aromatase cytochrome P450, an enzyme that catalyzes the conversion of androgens to E. The enzyme aromatase P450 is expressed aberrantly in endometriosis and is stimulated by prostaglandin E2, resulting in production of estrogen that induces prostaglandin E2 expression within endometriotic lesions. Furthermore, estrogen promotes the secretion of several inflammatory cytokines and growth factors, which contribute to the progression of endometriosis and stimulate estrogen production [17]. In addition, endometriotic tissue contains 17b-hydroxysteroid dehydrogenase (HSD) type 1, an enzyme that converts estrone (E1) to the more potent 17b-estradiol (E2), whereas they lack 17b-HSD type 2, an enzyme responsible for the inactivation of E2 to E1, resulting in raising the local estrogen activity level [18].

Moreover, ER activity is altered in ectopic cells, leading to an increased sensitivity to E [19]. The ERs are nuclear receptors existing in two isoforms (ERα and ERβ) exhibiting an E-binding domain and a DNA-binding domain. After binding to ligands, they act as transcriptional factors that upregulate or downregulate gene expression by interacting with regulatory regions of target genes. In endometriotic cells there is an increased sensitivity to the survival message mediated by estradiol. While ERα is expressed in endometriotic lesions at normal or reduced levels [20], ERβ is upregulated in endometriosis, and its levels are >100 times higher than those in endometrial tissue. Deficient methylation of the ERβ promoter results in pathological overexpression of ERβ in endometriotic stromal cells. High levels of ERβ

also suppress ERα expression. A severely high ERβ-to-ERα ratio in endometriotic stromal cells is associated with suppressed progesterone receptor and increased cyclooxygenase-2 levels contributing to progesterone resistance and inflammation [21, 22].

In endometriotic tissue there is not only an altered gene expression but also allelic variant (polymorphisms) of ER. A significantly higher frequency of allele Pvu II polymorphism as well as the multi-allele (TA)n polymorphism was observed in patients with endometriosis compared to controls [23]. Moreover, ER polymorphisms have been associated with a worst prognosis and a higher rate of recurrence especially in those with homozygosity in the allele variation but also in those with ER polymorphism in heterozygosity [16].

14.2.2 Progesterone and Progesterone Receptors

The role of P in endometriosis is complex, since the effect of this hormone in the endometrium is entangled; however, since from the beginning, it was clear that progesterone function was abnormal. Physiologically, hormone withdrawal in the late luteal phase triggers apoptosis, while progesterone supplementation may prolong endometrial epithelial cell survival and prevent the premenstrual surge of apoptosis [24]. However, in cultured endometrial cells, progesterone actually induces apoptosis [25, 26]; furthermore, progesterone physiologically downregulates endometrial Bcl-2 expression (an antiapoptotic gene) [27]. Probably, the most realistic scenario is that progesterone reduces the growth on the human endometrium by modulating apoptosis-related genes in favor of increased apoptosis [14], though in the late secretory phase, cell death is somehow precipitated by progesterone withdrawal [24].

Ectopic endometriotic tissues show an aberrant response to progesterone. The progesterone resistance observed in endometriotic lesions contribute to an increased capacity for cell proliferation and survival [28], reduced apoptosis, and impaired decidualization [1]. The abovementioned alteration in P function in endometriotic tissue is mediated by alterations in progesterone receptor isoform expression [29]. Progesterone effects on target genes are conferred primarily by nuclear progesterone receptor B (PR-B) homodimers acting as transcription factors, whereas the truncated PR-A isoforms repress the actions of PR-B. The two isoforms appear to have converse effects on inflammation, with PR-B opposing and PR-A promoting a proinflammatory environment. The levels of PR mRNA and proteins, particularly PR-B isoform, are markedly reduced in endometriotic lesions compared to matched eutopic endometrium [30]. In fact, progesterone-regulated endometrial genes are generally underexpressed, whereas proinflammatory genes are overexpressed in cases of endometriosis [31]. This observation has been further supported by evidence that the PR-B promoter is hypermethylated in endometriosis [32] and other chromatin modifications occur that may account for reduced PR-B expression.

In normal cells, PR stimulates the apoptotic pathway triggered by retinoic acid nuclear signaling, through the upregulation of the retinoic acid shuttling protein

CRABP2. Endometriotic cells have deficient retinoic acid production due to insufficient retinol uptake. In addition, they have an aberrant profile of retinoic acid shuttling proteins, leading to a paradoxical retinoic acid action mediated by fatty acid-binding protein 5 (FABP5), a prosurvival nuclear receptor [33].

Wieser et al. [34] demonstrated the constitutive activation of NF-κB, a master transcriptional regulator of inflammatory responses, in endometriotic cells. It has been suggested that NF-κB may promote the growth and survival of endometriotic lesions and its activation in endometriotic stromal cells is induced by TNF-α and estradiol and inhibited by progestogens [35], which is an additional downstream mechanism involved in steroid hormone control of apoptosis in endometriosis.

Genomic variants may also be implicated in the progesterone resistance of women with endometriosis. The PR gene polymorphism PROGINS codifies a variant PR that is less responsive to progestogens, when compared with wild-type PR, resulting in reduced biological activity. As predicted, in vitro experiments have demonstrated that P fails to induce apoptosis in endometrial cells harboring the PROGINS variant allele [36]. This finding is relevant because the PROGINS variant has been reported to be more common in women with surgically confirmed endometriosis [37, 38].

14.2.3 Stress Hormones

Stress hormones play an important role in the pathogenesis of endometriosis. Patients with endometriosis have increased level of CRH due to stress response, and in endometriotic lesions an abnormal local production of stress hormones has been found [39]. Indeed, clinical symptoms of endometriosis, such as pain and infertility, can be described as persistent stressors. Such continuous exposure to stress may severely affect the equilibrium and bidirectional communication of the endocrine and immune system, hereby further worsening the progression of endometriosis. Tariverdian et al. demonstrated in women undergoing diagnostic laparoscopy due to infertility that the TNF/IL-10 ratio, reflecting cytokine secretion by peritoneal cells, was higher in cells derived from endometriosis patients and could be further heightened by CRH stimulation. Moreover, stimulation with dydrogesterone abrogated the CRH-mediated inflammation. Finally, the expression of progesterone-induced blocking factor by peritoneal leukocytes was increased in endometriosis. Peripheral CRH, increasing upon high psychological stress, might contribute to the peritoneal inflammation present in endometriosis [40].

Endometrial corticotrophin-releasing hormone (CRH), urocortin 1 (Ucn1), and urocortin 2 (Ucn2) are neurohormones modulating stress-induced hypothalamic-pituitary-adrenal axis; they are also produced by endometrial cells (glands and stroma). The signaling of Ucn 1 and Ucn 2 is mediated by two G-protein-coupled transmembrane receptors, CRH receptors 1 and 2 (CRH-R1 and CRH-R2). An increase of CRH, Ucn, and Ucn2 mRNA expression in different types of endometriotic lesions [41] and high levels of Ucn in cystic fluid of endometrioma [42] have been described.

Ucn1 is expressed and secreted by luminal and glandular epithelial cells from endometrium with maximal abundance in the secretory phase of menstrual cycle [41]. The paracrine effects of Ucn1 promote endometrial differentiation and decidualization, which is a requisite for trophoblast invasion and blastocyst implantation [43, 44]. Patients with endometriosis may lack the cyclic variations of endometrial Ucn1 and the effects of Ucn1 on stromal cell decidualization [41].

Carrarelli et al. have demonstrated that the expression of CRH and Ucns in endometriotic tissue differs from site to site. In deep infiltrating endometriosis (DIE) lesions, the highest levels of (CRH, Ucns) and receptor (CRH-R2) were found. Moreover, they found the highest expression of cyclooxygenase 2 (COX2) and phospholipase A2, group IIA (PLA2G2A) mRNA in DIE lesions. Both PLA2G2A and COX2 are fundamental enzymes for the synthesis of prostaglandins, leading to the start and maintenance of a prolonged inflammatory response [45].

14.3 Inflammation and Endometriosis

14.3.1 Acute Inflammatory Response

Inflammation is a body's immune system's response to noxae. An inflammation is not always a helpful response of the body. In certain diseases the immune system fights against its own cells by mistake, causing harmful inflammatory responses. In other diseases, such as cancers, non-self-cells are not properly attached and killed by the immune system cells.

Endometriosis has an impaired immune system that allows ectopic endometrial tissue to invade the peritoneum and a harmful inflammatory response to the ectopic endometrial tissue proliferation. As a non-self-lesion in pelvic environment, the growth or persistence of endometriosis can also be regulated by innate immune system [46]. Endometriotic tissue can cause a deleterious inflammatory response as the endometrium is in a non-self-environment and as it is the reproductive tissue richest in inflammatory mediators. The ER/PR imbalance in women with endometriosis is associated with increased white blood cell recruitment and macrophage activation, contributing to IL-1, IL-6, and tumor necrosis factor (TNF)-α secretion. A hyper-expression of peptides or growth factors in peritoneal fluid and in endometriotic lesions is observed, and oxidative stress and endometrial cell proliferation are increased [14].

Menstruation entails a physiological but similar inflammatory response. There is an accumulation of leukocytes in the endometrium before the onset of menstruation: macrophages and natural killer cells play a role during the mid-late secretory phase; on the contrary neutrophils and eosinophils appear in the premenstrual phase only [47]. Chemoattractant cytokines, chemokines, COX-2, and prostaglandins (PGs) mediate the migration of leukocytes into the endometrium. The effect of PGs on local blood vessels causes exudation of both plasma proteins and leukocytes through the endothelium and basement membrane and permits and initiates the acute inflammation process.

14.3.2 Cytokines and Chemokines

Chemokines are a superfamily of small cytokines (70–90 amino acids) with the ability to induce directed chemotaxis in nearby responsive cells. Over the last decade, a new protein, the C-X-C motif chemokine 10, also known as interferon-γ-inducible protein-10 (CXCL10), has been investigated. CXCL10 is an anti-inflammatory chemokine involved in the Th1-type immune response. This chemokine is produced in pathological conditions in response to proinflammatory stimuli, such as IL-1, TNF-alpha, LPS, or viruses, stimulating NK cells' migration and cytolytic effect and showing antiangiogenic properties [48]. Therefore the decreased CXCL10 concentrations revealed in peritoneal fluid of advanced stages of endometriosis may contribute to the suppressed Th1 response and partially explain the decrease of NK cell's activity and support a more favorable environment for endometriotic implant neoangiogenesis [49]. Previous reports showed that RANTES, one of the major anti-inflammatory chemokines primarily identified as potent inducers of leukocyte motility, and CCR1, a CC chemokine receptor with high affinity for RANTES, are expressed in endometriotic tissues and may play important roles in the growth and development of endometriotic cells [34, 50]. RANTES and CCR1 showed a significant correlation with the severity of stage of DIE lesions and dysmenorrhea in DIE patients [51].

In ectopic endometriotic lesions, a high level of local proinflammatory mediators secreted by peritoneal macrophage has been recorded: interleukin (IL)-1β, tumor necrosis factor (TNF)-α, interferon (INF)-α, IL-6, IL-8, IL-18, and transforming growth factor (TGF)-β.

The inflammatory cytokines TNF and IL-1 reduce the expression of progesterone receptors in cultured endometrial stromal cells of women with endometriosis, contributing to progesterone resistance and a suboptimal local response to progestin therapy [52].

IL-1β, IL-8, and TNF-α activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and hypoxia-inducible factor (HIF)-1α signaling pathway, which determines an increased cyclooxygenase (COX)-2 expression in endometriosis and an antiapoptotic effect [53, 54].

The activation of NF-κB involves macrophage migration inhibitory factor (MIF) gene expression in ectopic endometrial cells in response to IL-1β. Therefore, various inflammatory factors activate NF-κB, and NF-κB further stimulates the synthesis of proinflammatory cytokines to form an autoregulatory loop: a higher expression of COX-2 causes a higher production of prostaglandin (PG)E_2, which further worsens endometriotic lesion. IL-1β seems to be higher in DIE localization especially in case of severe form of bowel localization [55]. Serum IL-33, a cytokine involved in fibrotic disorders, has been found to be abnormally elevated in women with multifocal DIE lesions with intestinal infiltration [56].

An increased level of IL-6 in the peritoneal fluid of patients with endometriosis suppresses natural killer (NK) cell cytolytic activity by downregulating cytolytic granule components, such as granzyme B and perforin, through the modulation of Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2) expression [57].

Leptin is a multifunctional hormone with immunoregulatory, proinflammatory, and angiogenic effects and plays an important role in controlling reproductive functions; it seems to be involved in the development of endometriosis. Leptin promotes CD4+ T helper I cell proliferation, macrophage phagocytosis, and the secretion of cytokines. Concentration of leptin in peritoneal fluid was found to be increased in patients with endometriosis, and this level may contribute through the activation of peritoneal macrophages to the pathological process of endometriosis [58].

Lastly, recent data has shown a possible role of inflammasome in the pathogenesis of endometriosis. Inflammasome has been described as a multiprotein oligomer complex and is considered a key regulator of the innate and adaptive host response that surveys the cytosol and other compartments into the cell. The inflammasome promotes the maturation and secretion of proinflammatory cytokines such as IL-1β and IL-18. The secretion of these cytokines results in pyroptosis, a form of programmed proinflammatory cell death distinct from apoptosis [59].

14.3.3 Cell-Mediated Mechanisms

Determination of various T- and B-lymphocyte subpopulations in blood and peritoneal fluid showed no differences between control group and patients affected by endometriosis [60]. Nonetheless, in some studies lymphocytes were found to significantly contribute to endometriotic lesion growth. The use of flow cytometry techniques with new subset markers could be a valuable tool to evaluate different T- and B-cell phenotypes in endometriosis.

T lymphocytes are classically classified into cytotoxic T cells or helper T cells. Cytotoxic T cells are capable of destroying a specific target by cytotoxic mechanism, and helper T cells transmit signals from antigen-presenting cells and enhance further immune response. Recently, a new classification for helper T-cell classification has been introduced. Based on the clusters of differentiation, these lymphocytes have been classified into Th1, Th2, Th17, and regulatory T cells [61]. A low ratio of Th1 to Th2 cells has been found to be relevant in endometriosis progression [62]. The Th1-Th2 ratio depends on stage. Once the endometriotic foci are established, in the first minimal and mild stages, Th1 cytokines become prevalent, due to the strict interaction between endometriotic and immune cells in the peritoneal fluid, whereas Th2 differentiation and Th2-related cytokines prevail in severe stages [63].

B lymphocytes are responsible for humoral adaptive immune response, principally producing antibodies against different antigens. In the pathogenesis of endometriosis, B lymphocytes have been suggested to play roles by secreting autoantibodies. Although many studies have demonstrated an aberrant production of autoantibodies in endometriosis, there is no consensus about the concentration of B lymphocytes and their role in this disease. Some studies have suggested that endometriosis has an autoimmune etiology, presenting alterations in both humoral and cellular immunity that lead to inflammatory reactions and proliferation of endometriotic cells [53, 61].

In the peritoneal fluid, macrophages are the most represented type of immune cells. Their number and activation are increased in endometriosis. Activated macrophages regulate the peritoneal environment removing red blood cells, damaged tissue fragments, and cellular debris by phagocytosis [64]. Despite the increased activation, the phagocytic activity of the macrophages is reduced in endometriosis, as they fail to eliminate the ectopic endometrial cells that reach the cavity through retrograde menstruation. Phagocytosis is, among other factors, regulated through activation of matrix metalloproteinases and expression of CD36 receptors reduced in endometriosis most likely by changes in prostaglandin E2 levels [65]. A defective scavenger function of the peritoneal macrophages that depends on their reduced attachment to extracellular matrix components could lead to the survival of ectopic endometrial cells. In addition to the reduction of phagocytosis ability of macrophages, the amount of regurgitated endometrial cells in the peritoneal cavity may be higher than the ability of the macrophages to remove them [9]. The survival and maintenance of the ectopic endometrial cells are permitted by a tumor-like cell behavior, through an increased expression of antiapoptotic factors, an abnormal proliferation, an endometrial cell invasiveness, and the production of angiogenic factors that promote neovascularization [14].

However, an upregulation of a physiologically catabolic process called autophagy, through which cytoplasmic organelles and macromolecules are degraded after lysosomal fusion, is detrimental for endometrial cell subsistence in OMA lesions [66].

The survival of ectopic implantations and its proliferation in a hostile microenvironment, based on impaired immunotolerant mechanism, are supported by an altered expression of sphingosine-1-phosphate receptor 1 (S1P). Expression of the enzymes implicated in the regulation of the S1P level balance and of its receptors is overall heavily deregulated in endometriotic lesions in favor of a decreased S1P catabolism [67].

Additionally, the eutopic endometrium from women with endometriosis was found to be more resistant to lysis by natural killer (NK) cells than the eutopic endometrium from women without disease [68].

Endometriotic cells decrease the ability of recruited immune cell populations to effectively recognize and target endometrial antigens shed during menstruation [61], and in the peritoneum NK cells seem to have a reduced concentration and killing activity, probably due to changes in cytokines and inhibitory factors.

One of the mechanisms of escape from immunosurveillance involves LFA-1-ICAM-1 pathway. In healthy women, LFA-1-positive lymphocytes adhere to ICAM-1+ endometrial cells and offer them to NK cells as a target. In women with endometriosis, soluble ICAM-1 secreted from endometrial lesions compete with the LFA-1 and subsequently block the NK cell-mediated killing [65].

A recent theory describes a possible role of innate immunity for the initial chronic pelvic inflammation in endometriosis. A disruption in the maintenance of endometrial homeostasis when there are bacterial infections can lead to a dysfunction in the endometrial barrier function in genetically susceptible hosts. A bacterial contamination in menstrual blood and peritoneal fluid may promote Toll-like

receptor 4-mediated growth of endometrial tissue originating from retrograde menstruation [69]. Toll-like receptors (TLRs) are essential components of the innate immune system that protect the host against bacterial and viral infection. It has been reported that the expression of TLR4 in the ectopic endometrium of endometriosis was higher than that in the normal endometrium and the levels of TLR2 and TLR9 mRNAs were higher in the peritoneal effusions of endometriosis than in the non-endometriosis group; the proinflammatory innate response leads to the activation of the slower adaptive immune system, leading to NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells)-dependent inflammation [70].

In addition, the peritoneal cavity of patients with endometriosis is characterized by abnormalities in the regulation of pathways of the complement system [71]. Recently, complement component C1q was found to be higher in peritoneal fluid samples of endometriosis-affected patients. C1q has been shown to induce a plethora of biological functions through the production of immunomodulatory molecules by an autocrine- or paracrine-mediated signaling [72].

14.3.4 Prostaglandins and Neuroangiogenesis

In endometriotic patients there is a higher level of prostaglandins (PGE_2 $PGF_{2\alpha}$) compared with controls that may contribute to the presence of symptoms such as dysmenorrhea, dyspareunia, and infertility.

PLA2G2A and COX2 are involved in the synthesis of prostaglandins, both initiating and maintaining a prolonged inflammatory response [73]. PLA2G2A and COX2 are highly expressed in DIE lesions, supporting a more sustained activation of inflammatory pathways in this localization [45]. COX enzyme system is the major pathway catalyzing the conversion of arachidonic acid into prostaglandins (PGs). COX-1 is constitutively expressed with the primary function of housekeeping. COX-2 is an inducible enzyme which is usually absent and rapidly expressed after stimulation in inflammatory conditions such as endometriosis [58]. PGE_2 is one of the most biologically active eicosanoids that regulates many pathophysiological processes in the development of endometriosis such as cell proliferation, angiogenesis, immune suppression, and antiapoptosis.

In peritoneal macrophages and endometriotic stromal cells, several proinflammatory cytokines induce COX-2 expression, leading to high PGE_2 levels. Usually COX-2 and PGE_2 have short half-lives: in endometriotic lesions a self-supported system based on two positive feedback loops (COX2-PGE_2-estrogen and COX2-PGE_2-cytokines such as IL-1β, TNF-α) determines that constant high level of PGE_2 is maintained. COX-2 is found to be overexpressed in DIE, SPE, and OMA lesions. The local inflammatory microenvironment sustains the growth and maintenance of endometriotic lesions through adhesion, invasion, angiogenesis, proliferation, and subsequent fibrosis. The ectopic endometriotic lesions, especially in the peritoneal microenvironment, need of a rich vascular supply in order to develop and maintain itself. Angiogenesis is modulated by estrogen and progesterone, but locally a role is exerted by chemokines.

The upregulation of TNF-α, IL-8, and MMP-3, demonstrated in patient with endometriosis, promotes proliferation and adhesion of endothelial cell (neoangiogenesis) and a local neurovascularization (neuroangiogenesis) contributing to explain the endometriosis-related pain mechanisms. Pain perception is amplified by an altered transmission of dolorific stimulus by the dorsal roots neurons due to the influence of nerve fibers in endometriotic lesions.

Activated peritoneal macrophages and endometriosis cells secrete high level of vascular endothelial growth factor (VEGF) a prominent angiogenic factor [74]. Macrophages and their products can stimulate the synthesis of nerve growth factor (NGF), which plays a crucial role for the survival, development, and function of neurons in both the central nervous system and peripheral nervous system [75].

14.4 Clinical Manifestations and Inflammatory Comorbidities

Endometriosis is a unique model of a disease where the interactions between inflammation and hormones create a various range of clinical phenotypes. Clinical manifestations of endometriosis include chronic pelvic pain, menstrual-related symptoms (dysmenorrhea and menorrhagia), symptoms related to sexual intercourse (dyspareunia and postcoital bleeding), ovarian cysts, and infertility. In the following section, we will investigate how these symptoms are related to the previously described molecular interactions.

Endometriosis is associated with several inflammatory comorbidities. Gastrointestinal symptoms (such as bloating, abdominal pain, constipation, diarrhea, nausea, and vomiting) and irritable bowel syndrome are more common in women with endometriosis than in the general population. In a large population study, women with endometriosis had an increased risk of inflammatory bowel disease (IBD) [76, 77]. Higher rates of hypothyroidism, systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), rheumatoid arthritis (RA), multiple sclerosis (MS), and fibromyalgia were found in patients with endometriosis when compared with the general population [78]. All of these autoimmune diseases have a higher prevalence in women, suggesting that female hormones have a fundamental role in the pathogenesis of these diseases, allowing a further parallelism between autoimmune diseases and endometriosis [79]. TNF-blocking agents have changed the treatment of several diseases such as RA, inflammatory bowel disease, and psoriasis. Studies performed in primates indicate that similar effects could also be expected in endometriosis. Furthermore, endometriosis shares several common characteristics with autoimmune disease, such as B-lymphocyte and T-lymphocyte abnormalities with altered immune surveillance, depressed cell-mediated immunity, altered apoptosis, tissue damage, and familial occurrence with genetic predisposition [80, 81]. Population studies found associations with specific human leukocyte antigen (HLA) variants, single nucleotide polymorphism in chemokines, and endometriosis. More specifically, CCL21 polymorphism was found associated with moderate/severe endometriosis. CCL21 is a chemokine, responsible for recruiting

CCR7-expressing lymphocytes and dendritic cells to secondary lymphoid tissues. Also HLA-DRB1, a HLA variant associated with RA, shows an epidemiological correlation with endometriosis [82].

Endometriosis patients also tend to be more susceptible to allergic manifestations and to allergy-related conditions such as asthma or atopic diseases such as eczema or allergy to medications than women without endometriosis, paralleling the aberrant immunologic response and inflammatory reaction in these women [78, 83].

Endometriosis may also be associated with a negative outcome when pregnancy occurs [84]. Miscarriages occur more frequently in patients affected by endometriosis [85]. Preeclampsia, intrauterine growth restriction, and preterm birth (PTB) have been found associated with endometriosis [86]. Local endometrial immune and endocrine system are altered in endometriosis patients, with a swift toward a proinflammatory state; this may influence the decidua/trophoblast interaction and activate the mechanisms which cause some of these common pregnancy complications [87]. A recent systematic review and meta-analysis demonstrated that endometriosis increases the risk (OR, 1.49; 95% CI, 1.30–1.70) of PTB, both in spontaneous or with assisted reproductive technology pregnancies [88].

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Endometriosis and Adenomyosis in Adolescents and Fertile and Menopausal Women

15

Leila Adamyan

Among doctors there is a version that adolescents never suffer from endometriosis. However, the possibility of developing endometriosis in adolescents is confirmed by numerous studies. According to different data, the frequency of laparoscopically confirmed endometriosis in girls aged 19–21, suffering from dysmenorrhea and chronic pelvic pain that cannot be treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and combined oral contraceptives (COCP), is 35.5 to 70–73% [2–4]. The average age of the debut of symptoms is 15.9 years, although there are known cases of disease onset even before the menarche. Lesions of the peritoneum similar to endometriotic ones (vascular proliferation, hemosiderin deposits, presence of stroma, but not endometrial glands) were detected in girls before the age of menarche with mammary gland development of I–III degree, who did not have any abnormalities of the genital tract [5]. Average age of diagnosis of endometriosis in adolescents is 17.95 ± 1.48 years, while first symptoms manifested by 15 years. According to the World Endometriosis Society, in 38% of women, symptoms of endometriosis appear before the age of 19; in 21%, up to 15 years; and in 17%, between 15 and 19 years old. It is important to note that 80.9% of those girls suffered from dysmenorrhea and 66% suffered from chronic pelvic pain. Furthermore, most often the peritoneum (47.6%), ovaries (38%), and the retrocervical region (23.8%) were already involved in the process by the time of diagnosis [6]. According to the recommendations on endometriosis of the Society of Canadian Obstetricians and Gynecologists, since the treatment of the revealed lesions contributed to the reduction of pelvic pain, the onset of thelarche can be considered as a milestone when endometriosis need to be included in differential diagnosis of pelvic pain [7].

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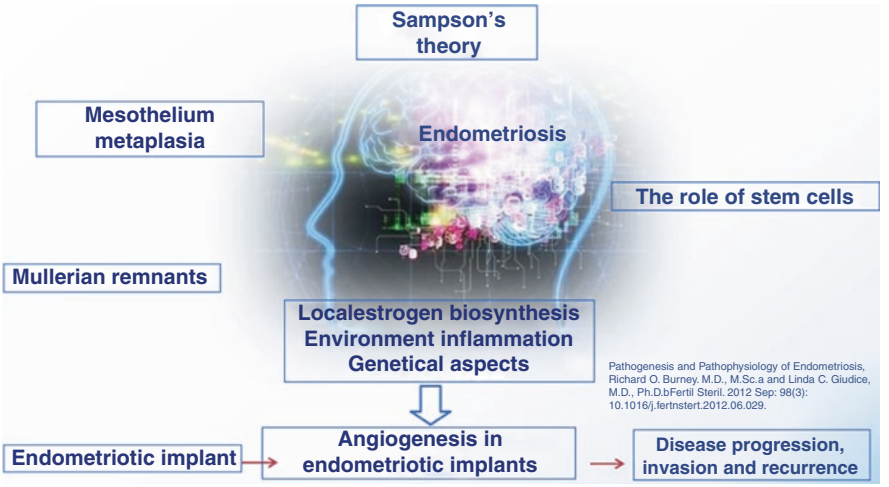
An average of 4.2 doctors are seen for patients whose symptoms starts before age 15 years [8]. The delay in the diagnosis of endometriosis from onset of symptoms is 11.8 years in the United States and 6.7 years in the United Kingdom [9]. These impressive numbers are largely due to the fact that doctors are not ready to diagnose "endometriosis" in young age.

Endometriosis in women of reproductive age is associated with pelvic pain, dysmenorrhea, and dyspareunia and leads to infertility in 30–40% of cases. Endometriosis in postmenopausal women is often accompanied by urinary incontinence, constipation, or even may present as acute abdominal syndrome. Postmenopausal women also often have different concomitant pathologies—genital and extragenital.

Peritoneal endometriosis is most typical for women of reproductive age, whereas postmenopausal women are more likely to have a bowel endometriosis and endometriotic cysts. Numerous clinical cases of endometriosis, either endometriotic cysts (more often) or extragenital endometriosis (bowel, bladder, urethra, lungs, liver, and postoperative cutaneous scars) in postmenopausal women, who didn't show any symptoms during reproductive age (chronic pelvic pain, dysmenorrhea, infertility, etc.) once again confirm the complex pathogenesis of the disease. Seventy percent of women who were newly diagnosed with endometriosis in postmenopause have bowel endometriosis with involvement of (in decreasing order of frequency) the appendix, cecum, ileum, and transverse colon.

15.1 Etiology and Pathogenesis

Despite numerous studies devoted to the various aspects of endometriosis, the etiology and pathogenesis of this disease are not fully understood. Currently, there are more than ten theories of the onset of endometriosis, including theory of retrograde menstruation and of embryonic origin, implantation theory, and coelomic metaplasia theory.



However, none of these theories can fully explain the causes of all cases of endometriosis and the variety of foci localization. A certain role in the onset of endometriosis is attributed to the following factors:

- Hereditary: researchers have found that first-degree relatives have a greater risk of developing endometriosis. In addition, more severe form of endometriosis is noted in the next generation in case of hereditary predisposition. Dalsgaard et al. analyzed data of more than 12,389 girls whose mothers had endometriosis and found that they had a 2.2-fold increase in the risk of endometriosis detection in comparison with their peers ($n = 52{,}371$), whose mothers did not suffer from this disease [10].
- Immunological changes (disturbances in T-cell immunity, autoimmune reactions): Despite many theories of endometriosis, it has been established that disruption of the hypothalamic-pituitary-ovarian system functioning plays a key role in pathogenesis of endometriosis. It was noted that level of LH in blood serum and follicular fluid is decreased in cases of small forms of endometriosis. At the same time, a sharp increase in FSH and estradiol level increased basal LH secretion in blood serum, and insufficient functional activity of corpus luteum was revealed in patients with endometriotic cysts.

True cause of endometriosis is unknown, although there are many theories of its origin that do not exclude each other. The pathophysiology of endometriosis is multifactorial and may be accompanied by the interaction of several factors [11].

Possible factors leading to the onset of endometriosis include abortions in the anamnesis, ecological factors, iron deficiency in the body, surgical interventions on the pelvic organs (including cesarean section, cauterization of erosion of the cervix), obesity, pelvic inflammatory disease, and intrauterine device usage.

Since menstrual bleeding is considered as an important pathogenetic aspect of development and progression of endometriosis, after natural or surgical menopause symptoms of the disease tend to decrease, and the general condition of women is significantly improved. However, numerous cases of recurrent endometriosis in postmenopausal women have been described, both under menopausal hormone therapy and without it, as well as cases of newly diagnosed endometriosis. According to different data, the incidence of endometriosis in postmenopausal women is around 2–5% [12]. The most important issues that physician faces in managing menopausal patients with endometriosis are the risk of malignancy (especially in infiltrative forms and in cases of endometriotic cysts) and the possibility of menopausal hormone therapy when it is indicated.

Most researchers believe that the disease can occur de novo in menopausal women due to peripheral conversion of androstenedione to estrogen or due to menopausal hormone therapy [13]. It is proved that local production of estrogen in endometriotic lesions plays a key role in the mechanisms of their implantation and subsequent survival. Surely, we cannot exclude the possibility of endometriosis, which remained undiagnosed during reproductive age and recurred in menopause. In addition, relative immunosuppression of different origin in menopausal women might be one of the pathogenetic aspects of endometriosis development. Nevertheless, if endometriosis does occur in postmenopause, it is usually present in smaller volumes and is less active [14].

15.2 Classification

The following classification was made based on the localization of endometriotic lesions:

- Genital endometriosis:
  - External (heterotopia on the ovaries, fallopian tubes, ligaments of the uterus and fallopian tubes, pelvic peritoneum, etc.).
  - Internal (lesions within uterine wall and intramural parts of the fallopian tubes): Development of this form is facilitated by pathological labor, abortions, diagnostic curettage of the uterine cavity, as well as inflammatory processes.
- Extragenital endometriosis (is not associated with the organs of reproductive system): Genital form of endometriosis is most common among adolescents.

  Severity of external genital endometriosis is assessed based on a scoring system, which takes into account the location of lesions, their depth, prevalence of the pathological process, and presence and characteristics of adhesions. According to this classification, there are four degrees of severity: minimal (first degree of spread), 1–5 points; light (second degree of spread), 6–15 points; moderate (third degree of spread), 16–40 points; and heavy (fourth degree of spread), more than 40 points.

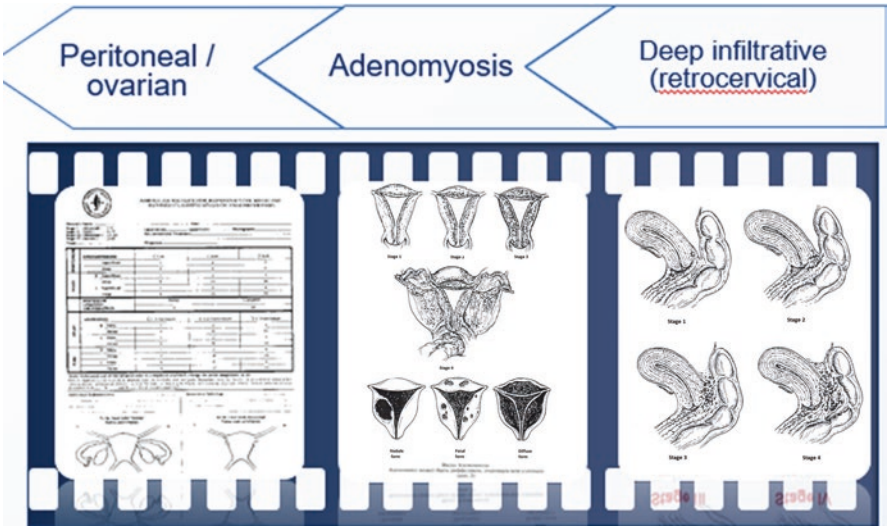


Fig. 15.1 Classification of endometriosis (ASRM), adenomyosis, and retrocervical endometriosis (Adamyan)

- Internal genital endometriosis (adenomyosis): It is characterized by the presence of endometrial glands and stroma within myometrium and junctional zone. Adenomyosis is classified by four stages: stage I, endometriotic lesions are within submucosal layer; stage II, endometriotic lesions penetrate into myometrium; stage III, involvement of entire myometrium up to serosa; and stage IV, involvement of pelvic peritoneum and adjacent organs. Adenomyosis may be diffuse or focal (nodular or cystic). Adenomyosis affects more than 60% of women in the age of 40–50. Crucial difference of adenomyosis from leiomyoma is an absence of distinct borders of the lesion [15] (Fig. 15.1).
- External genital endometriosis: Small forms of external genital endometriosis (first degree of spread) are the most common cause of chronic pelvic pain in adolescents. Adenomyosis or endometriotic cysts can be observed much less frequently in adolescents than in adults [16].
  When the cervix is affected by endometriosis, small areas of red tissue, 3–6 mm in size, are seen on its surface. Each time before menstruation, these lesions increase in size and change their color. The onset of cervical endometriosis is facilitated by cauterization of its erosions with diathermocoagulation, cervical injury after childbirth, abortion, and diagnostic curettage.
  Ovarian endometriosis is characterized by presence of lesions on the surface of ovary or within ovarian tissue. Moreover, endometriotic cysts can form, the contents of which resemble liquid chocolate, as a result of which they are often called the “chocolate” cysts.
- Deep infiltrative (retrocervical) endometriosis: This form is classified according to the involvement of the cervix and vaginal wall, uterosacral ligaments, rectosigmoid, rectal wall, and peritoneum in the rectouterine pouch [17].

As already mentioned, endometriosis can appear in various areas of the body. Risk factors of endometriosis of the scar include previous operations on organs of an abdominal cavity and the pelvis [10, 18, 19]. Endometriosis can also be represented by a skin lesion (skin endometriosis). Less frequently, endometriotic foci can be found on the diaphragm. Diaphragmatic endometriosis is rare and can cause periodic pain in the right shoulder before and during menstruation. Rarely, endometriosis can be extraperitoneal and could be detected in the lungs and central nervous system [20].

15.3 Diagnosis

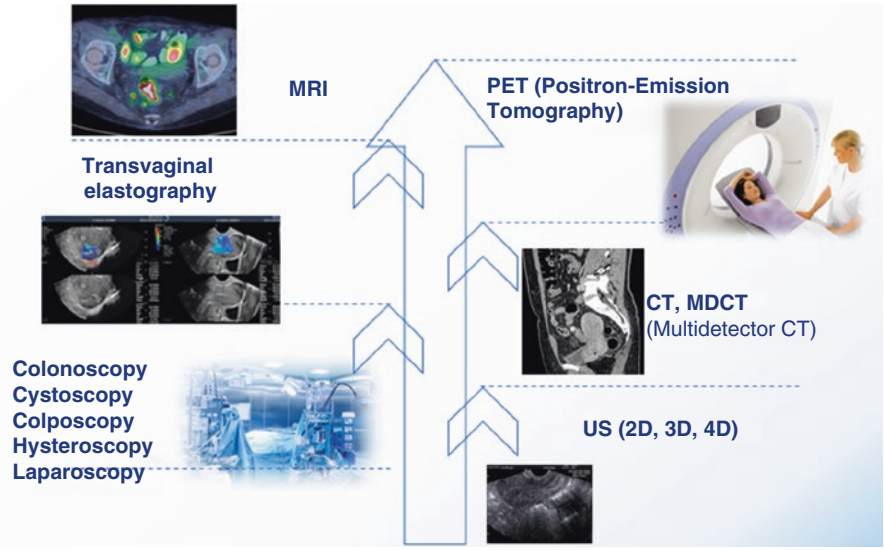
Endometriosis is characterized by a variety of manifestations—from asymptomatic to the symptoms of the "acute abdomen" [21, 22]. To a large extent, the clinical picture of this disease depends on the location of the lesions, duration of the disease, concomitant diseases, and psychoemotional characteristics of the patient. Nevertheless, all manifestations of endometriosis can be divided into typical, less typical, rarer, and very rare. Typical manifestations of endometriosis include pelvic pain, menstrual problems, pain during intercourse, and infertility (30–40% of women). Less typical features include defecation and urination problems. Other manifestations of endometriosis are rare and include frequent urination, bleeding from the rectum, hemoptysis, and fatigue.

The delay in the diagnosis of endometriosis in adolescents is often explained by the fact that initially pelvic pain is associated with primary dysmenorrhea and is considered as "normal" for the adolescents. Only when pelvic pain disrupts normal daily activity and studies, it becomes the subject of doctor's attention. However, in 10% of cases dysmenorrhea in adolescents is secondary and is associated with other disorders. Endometriosis is the most common cause of secondary dysmenorrhea in adolescents [23]. Acyclic pelvic pain seems to be more common in adolescents than in adults [24].

In adolescents, ultrasound investigation and magnetic resonance imaging (MRI) are used to detect endometriosis at early stages. The initial stage of endometriosis gives intense pulling pains; therefore, the earlier the stage of the disease is determined, the sooner it will be possible to begin a treatment. Genital endometriosis in nonsexually active patients can also be suggested based on the clinical and anamnestic data and the results of a bimanual rectoabdominal investigation [25]. If there is a suspicion of genital endometriosis, the examination should be carried out both in the first phase of the menstrual cycle and on the day before menstruation. In the days prior to menstruation, swelling and soreness of tissues in the region of internal genital organs and tension and sharp pain of the uterosacral ligaments and peritoneum in the region of broad ligament of the uterus and retrouterine pouch are noted in endometriosis. Cervical assessment could be painful, the uterus is not mobile in endometriosis, and the size of the uterus can be significantly increased in case of adenomyosis. When examination is performed during the first phase of the menstrual cycle (on the 5–7th days), this symptomatology disappears. The main method

for diagnosing small forms of external genital endometriosis is laparoscopy followed by a histological investigation. The diagnosis is verified if an endometrial epithelium, or glands, or stroma is found in the biological sample. For diagnosing an internal endometriosis, retrocervical endometriosis (extremely rare in adolescents), and endometriotic cysts, the following methods are also used:

- Ultrasound of the pelvic organs abnormal cystic cavities in myometrium, an increase in the size of the uterus, especially anteroposterior, with a general roundness of its shape; change in the size of the uterus depending on the phase of menstrual cycle (enlargement of the uterus in the second phase). Smooth-walled cystic cavities with a fine- and medium-dispersed non-shifting suspension are revealed in case of ovarian endometriosis.
- MRI (especially important for differential diagnostics of adenomyosis and uterine fibroids, the extent of spread of retrocervical endometriosis, diagnosis of disturbances of architectonics of pelvic organs due to possible adhesions, differential diagnosis of endometriotic cysts with other tumor-like formations of pelvic organs and ovarian tumors). Three-dimensional printing of infiltrating endometriosis may be a beneficial adjunct to 2D imaging and can provide further structural relationships to support surgical planning [26].



- Hysterosalpingography—when adenomyosis is suspected (rarely used in adolescents).
- Hysteroscopy is carried out in the first phase of menstrual cycle, on the 7–9th days, which sometimes allows to see endometriotic passages. Indirect hysteroscopic signs of diffuse adenomyosis include widening of the uterine cavity and change in the relief of its walls: the surface of the basal layer acquires a coarse folded character with uneven contours (the phenomenon of "wave formation").

- Laparoscopy is used not only as a diagnostic tool but also for performing an operation. This is considered as a "minimally invasive" operation [27]. Laparoscopy with biopsy is the only way to diagnose external genital endometriosis. Laparoscopy in patients under 21 years often reveals atypical endometriotic lesions such as clear vesicular, red lesions, peritoneal defects, or vesicular implants [28]. In patients of reproductive age, endometriotic lesions are usually black in color [29].

There are only few case reports about adenomyosis in adolescents. In all mentioned cases, patients presented with severe dysmenorrhea or pelvic pain [30]. In those cases, medical management with GnRH agonists appeared to be effective in the short-term follow-up. Ho et al. showed that cystic adenomyosis may be a rare cause of chronic pelvic pain in adolescents, but the physician must always be aware of this diagnosis because adenomyosis is more common among adolescents than was previously considered [31].

15.4 Differential Diagnosis

Differential diagnosis of external genital endometriosis with pain syndrome should be performed primarily with an adnexal chronic inflammatory disease and dysmenorrhea caused by hyperprostaglandinemia.

Adnexal chronic inflammatory disease is characterized by:

- The absence of a connection between the onset of pain and the phase of the menstrual cycle.
- The same results of bimanual rectoabdominal examination in nonsexually active adolescents, regardless of the phase of the menstrual cycle.
- The absence of a sharp pain in the region of typical location of endometriotic heterotopia (uterosacral and broad ligaments, peritoneum of the retrouterine space).

15.5 Treatment

The main purposes of the treatment are:

- Elimination of severe pain syndrome (due to temporary complete/partial blockage of the reproductive system) and prevention of associated severe neurological disorders.
- Prevention of the spread of endometriosis into adjacent organs.
- Preservation of reproductive function.

Treatment methods do not differ much between adolescents and women of 20–40 years of age. All available treatment options are acceptable for adolescents,

but the age of the patient and the side effects of medicines should be taken into account. Unfortunately, there are no effective methods that completely cure endometriosis.

Painkillers. Pain is the most important symptom of endometriosis in many women. To combat pain, such drugs as analgesics (e.g., acetylsalicylic acid, paracetamol), combined painkillers (a combination of acetylsalicylic acid with paracetamol or codeine), narcotic drugs (like morphine), as well as nonsteroidal anti-inflammatory drugs (ibuprofen, etc.).

Hormonal treatment. In many patients, endometriosis develops during the adolescent period and is the most frequent cause of severe dysmenorrhea, which may not be cured completely with NSAIDs and COCP treatment. Many researchers believe that estrogen plays a major role in the pathogenesis of endometriosis. The hormonal treatment is thus aimed at suppressing the estrogens production, resulting in reduced symptoms. Hormonal treatment includes contraceptive pills, progestin, aGnRH, and danazol (although now it is used less commonly). There are several concerns about estrogen component of the COCP, which can possibly increase the risk of endometriosis, mask symptoms, prolong time before diagnosis, and contribute to its progression to the deep infiltrative form. Unfortunately, hormone therapy has many side effects and, besides, provides only a temporary effect [32].

The majority of adolescent girls with chronic pelvic pain not responding to conventional medical therapy have endometriosis. In fact, there is no suitable drug for young patients, since aGnRH cannot be used for a long time due to a decrease in bone mineral density (BMD), which is especially important because adolescents need to achieve optimal bone health (peak bone mass). Results of new clinical trial on the efficacy and safety of dienogest 2 mg/day (Visanne Study to Assess Safety in Adolescents (VISADO)) are very promising. The drug was administered for 52 weeks to adolescents aged 12–17 years with surgically confirmed endometriosis and demonstrated a good efficacy profile (reduction in visual analogue scale by more than 40%) and safety (decrease in BMD, which occurred in some patients, tended to recover within 6 months after the end of the treatment course) [33]. A favorable profile of benefits and risks of dienogest 2 mg/day makes possible long-term use of this drug in adolescents.

It is very important to remember that empiric treatment of pelvic pain in adolescents using contraceptive pills may contribute to delayed diagnosis of the endometriosis and postponement of surgical assessment.

There is a limited choice of drugs for the medical treatment of endometriosis in postmenopausal women. Use of dienogest (2 mg/day) in a continuous mode for 10–11 months in patients with extragenital endometriosis (with involvement of the sigmoid colon, rectum, or bladder) showed decrease in pain intensity and size of endometriotic lesions.

There are a lot of hormonal drugs studied for endometriosis treatment besides COCP and GnRH analogs: aromatase inhibitors such as letrozole, selective estrogen receptor modulators such as bazedoxifene, and selective progesterone receptor modulators such as asoprisnil. The majority of studies evaluating the use of

aromatase inhibitors in endometriosis have determined relief of pelvic pain and effect on endometriotic implant size [34].

Fischer et al. demonstrated that treatment with a SERM caused regression of endometriotic lesions in a mouse model and has a significant endometrial-specific estrogen antagonism [35].

SPRMs induce amenorrhea through selective inhibition of endometrial proliferation and suppress production of prostaglandin in endometrium, leading to relief of endometriosis-related pain [36].

15.6 Surgery

Surgical management is beneficial in reducing pain, progression, or recurrence of endometriosis.

Indications for surgical treatment of genital endometriosis include:

- Endometriotic cysts—laparoscopy, cyst enucleation with subsequent endocoagulation of its bed, or resection of the ovaries within healthy tissues when it is impossible to separate the capsule from ovarian tissue.
- External endometriosis—laparoscopy, endocoagulation of endometriotic lesions with subsequent peritoneal lavage, and control revision of pelvic peritoneum.
- Retrocervical endometriosis—endometriosis excision.
- Contraindications to hormonal therapy.

Uterus-preserving surgery in patients with adenomyosis wishing to have pregnancy is possible in case of nodular form, but both the doctor and the patient should be aware of high risk of recurrence. Laparoscopy is used for removal of nodular or cystic forms, but hysterectomy is still the only radical treatment option. Hysteroscopic resection because of bleedings is also possible with levonorgestrel-IUS insertion thereafter. Uterine-sparing surgery for adenomyosis controls dysmenorrhea and menorrhagia in more than 81% with pregnancy rates about 50% [37]. Despite of different treatment options, recurrence rate of endometriosis is still estimated as 21.5% for 2 years and 40–50% for 5 years after laparoscopic treatment [38].

15.7 Endometriosis and Genital Malformations

In cases of genital malformations, combined endometriosis was detected in 15–77% of patients and in 20–37% of cases of symmetric anomalies (with a normal outflow of menstrual blood) [39, 40]. Presence of endometriosis in patients with a lack of functioning endometrium cannot be explained by the retrograde menstruation.

The likely theories of endometriosis development in these cases are embryonic theory or theory of coelomic metaplasia, which was developed by Meyer in 1903. A similar conclusion was reached by Kyoung et al. [41] and Troncon [42], who

describe cases of endometriosis detection in patients with Mayer-Rokitansky-Küster-Hauser syndrome in the absence of functioning endometrium.

Genital malformations, depending on its form and type, are clinically manifested by pelvic pain, which appear with the onset of menstruation, with an inability to have a sexual intercourse, and by the absence of menstruation [39, 43].

Malformations associated with a disturbances of menstrual blood outflow lead to a pronounced pain syndrome in 25.6% of cases and development of hematomas, hematocolpos, and widespread endometriosis and in some cases cause symptoms of the "acute abdomen" [44, 45].

Adenomyosis and numerous endometriotic lesions were detected in 30% of patients with a single-horned uterus, in the absence of a functioning endometrium in the rudimentary horn. This suggests the need to remove the rudimentary horn of the uterus not only when it contains any pathological changes (fibroids, pregnancy) but also in cases of a dysfunctional horn, because of the high risk of endometriosis occurring therein. This tactic is also consistent with the surgical technique of Medeiros et al. [46] and Pados et al. [47].

Research group from the Department of Operative Gynecology of the Research Center for Obstetrics, Gynecology, and Perinatology revealed a concomitant gynecological pathology in 103 patients with genital malformations. The frequency of external genital endometriosis in those patients was 53%. Authors also visualized endometriotic lesions on pelvic peritoneum in 20% of patients with uterovaginal aplasia without functional rudimental horns [48].

It is advisable to carry out complex treatment of genital malformations, including correction of the anatomical shape and accompanying gynecological pathology, excision and coagulation of endometriotic lesions, and removal of endometriotic cysts with ovarian reserve preservation.

15.8 Impact of Endometriosis and Adenomyosis on Fertility

There are a lot of changes in peritoneal fluid content in patients with endometriosis, which affects endometriotic lesions and oocytes: inflammatory changes in peritoneal fluid, proliferation of macrophages and phagocytic dysfunction, and proinflammatory and angiogenic factor release. Changes in peritoneal fluid can affect sperm-oocyte interaction [49].

Frequent errors in meiosis during in vitro maturation of oocytes in the culture medium with peritoneal fluid (1% and 10%) obtained from women with and without endometriosis were also shown. Peritoneal fluid causes disturbances in the meiosis II, directly affecting the spindle division [50].

Changes in the follicular fluid such as significant increase of IL-8, IL-12, and adrenomedullin in endometriosis correlate with a decrease in the quality of oocytes [51].

Endometriosis affects a number of oocytes as well as its quality. Large endometrioma (≥5 cm in diameter) significantly decreases the number of oocytes (more than two times). Da Luz et al. demonstrated that the PTGS2 gene expression is

significantly lower in cumulus cells in women with external genital endometriosis which leads to a decreasing the oocytes quality [52]. In endometriosis, folliculogenesis is also disturbed, which leads to the formation of oocytes with a reduced capacity for fertilization.

Endometriosis might also be associated with mitochondrial dysfunction in cumulus cells. There is a significant correlation between ATP levels in the cumulus cells and the number of mature oocytes, as well as pregnancy rates. In patients with endometriosis, significantly reduced ATP level in the cumulus cells was identified.

Adequate surgery leads to temporary negative impact on the ovarian reserve (no more than 6 months) and depends on severity of endometriosis, surgical skills, and energy used. Therefore, enucleation of capsule of endometriotic cyst, destruction (by CO_2, fiber CO_2 laser—the best one) should be performed [53].

Factors which are contributing to the infertility in the presence of adenomyosis include endometrial receptivity disturbances, increased level of prostaglandins, TNF-alpha, IL-6, IL-8, IL-1, MMP, decreased expression of integrins and E-selectins, resistance to progesterone, an oxidative stress, and hypermethylation of HOXA 10,11 (defect of decidualization) [54].

Thus adenomyosis affects fertility by gene dysregulation, decreased endometrial receptivity, impaired implantation, disturbed decidualization, abnormal concentrations of intrauterine free radicals, and an altered uterine peristaltic activity.

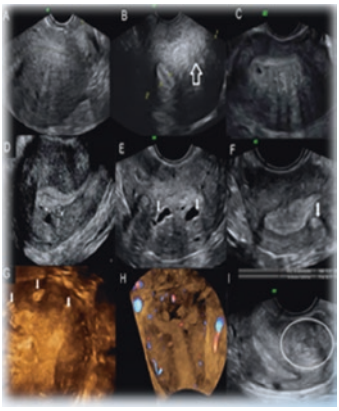
Impaired uterotubal transport determined by HSG was present only in women with adenomyosis and not in women without according to Kissler et al. [55].

Adenomyosis has detrimental effect on IVF outcome: reduces clinical pregnancy and live birth rates, as well as doubles the miscarriage rate. Long-term suppression of adenomyosis with GnRH agonists or surgery might improve IVF/ICSI outcome [56].

Adenomyosis has detrimental effect on IVF outcome:

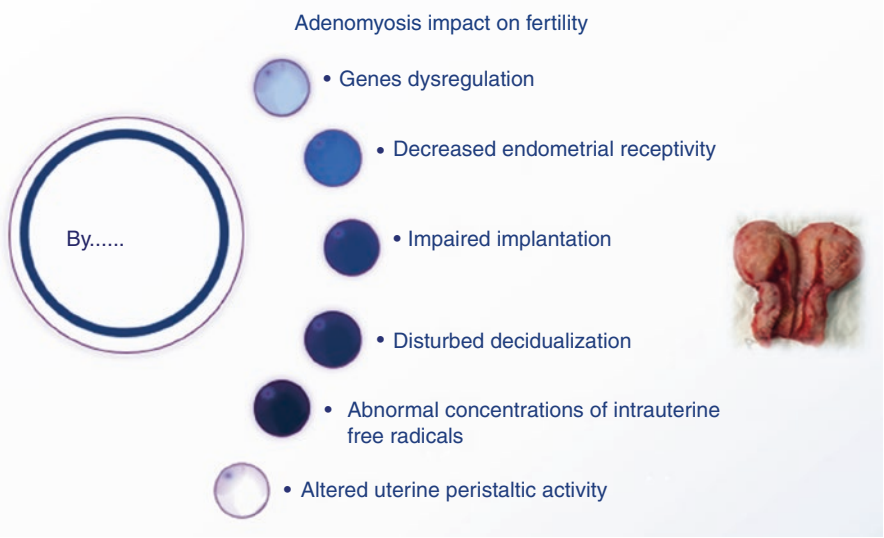
- Reduces clinical pregnancy and live birth rates
- Doubles the miscarriage rate

Long-term suppression of adenomyosis with GnRH agonists or surgery might improve IVF/ICSI outcome



Matched control studies with IVF cycles have reported higher miscarriage rate (66.7% vs 8%) and higher implantation failures (95.8% vs 37.5%) in patients with adenomyosis vs matched controls [57]. Adenomyosis is also associated with

significantly increased risk of spontaneous preterm delivery and preterm premature rupture of membranes [58].



15.9 Risk of Malignant Transformation of Endometriosis

Despite being initially considered a benign disease, the wide opinion nowadays is that endometriosis and especially endometriotic cysts are neoplastic conditions with the potential to become malignant. Especially it is important for postmenopausal women, although the frequency of malignant transformation of endometriosis is low (about 0.9%). Malignant transformation of endometriosis occurs generally in 1–3.6% of cases (Fig. 15.2).

Since infiltrative forms of endometriosis and endometriotic cysts are most typical for this category of women, physician often has to perform differential diagnostics with oncological diseases. There is a regular suspicion of colorectal cancer when bowel is involved in pathological process, and that is why it is necessary to perform a colonoscopy with biopsy of lesions in order to determine the scope of surgical intervention, to address the question of the scope of the surgical intervention. It has been established that ovarian endometriosis correlates with an increased risk of developing clear cell and endometrioid ovarian carcinoma [59]. Ovarian cancer develops in 1–5% of cases with ovarian endometriosis and in a lower percentage of cases with extra-ovarian endometriosis. At the same time, endometriosis is present in 10–15% of patients with ovarian cancer [60]. In general 19% of epithelial ovarian cancer were associated with endometriosis [61]. Presence of endometriotic cysts 9 cm in diameter is an important risk factor for ovarian cancer in women 45 years old. It should be remembered that with the persistence of endometriosis in postmenopausal women, the risk of malignancy increases. It was demonstrated that

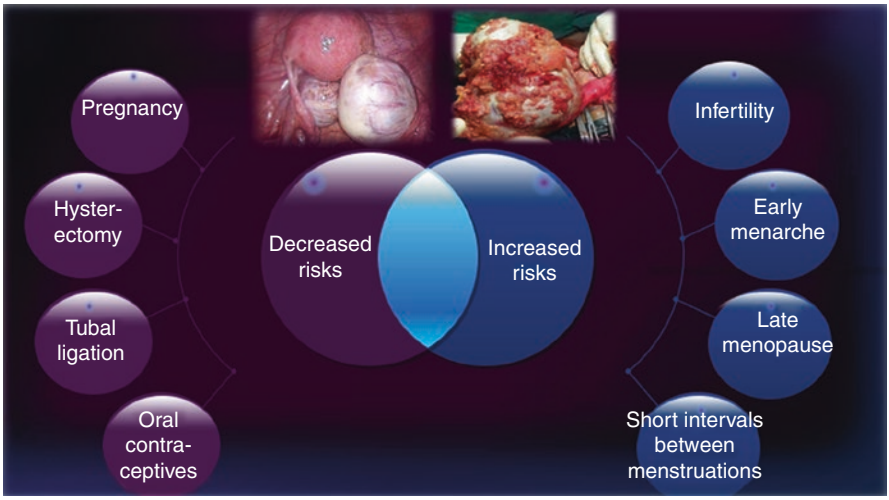


Fig. 15.2 Oncological aspects of endometriosis

miR-325 expression is significantly increased in endometriosis and ovarian cancer; therefore it can serve as a possible prognostic marker of transition from endometriosis to malignancy [62].

15.10 Prescription of Menopausal Hormone Therapy for Women with Endometriosis

MHT use in the treatment of patients with endometriosis is still very discussable, since it could possibly reactivate residual endometriosis and even stimulate the development of endometriosis de novo.

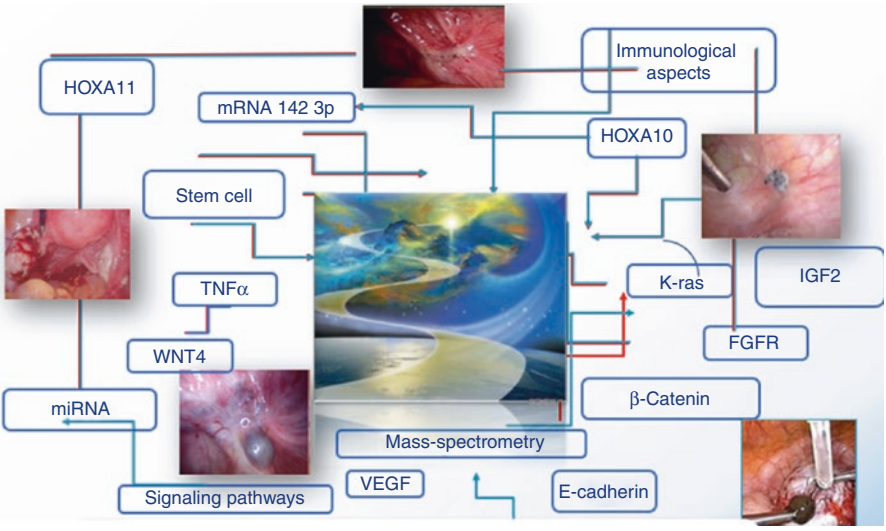
Several data in the literature indicates an increased risk of endometriosis recurrence on the background of hormonal therapy, but authors associate this possibility with the presence of residual endometriotic lesions in the rectovaginal septum and incomplete endometriosis excision during previous operations. There is limited evidence that malignant transformation often occurs against the background of estrogen monotherapy in menopausal women. According to this fact, special attention is nowadays paid to discussing the issue of the ineligibility of prescribing this regimen of hormonal therapy. Thus, there may be a possible benefit in delaying the start of MHT, by leaving time for residual endometriotic tissue to regress before beginning an estrogen therapy [13].

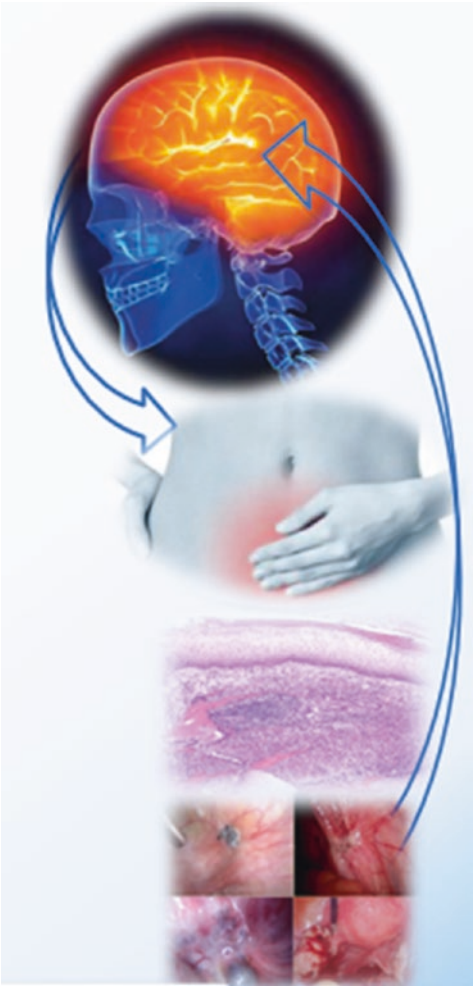
Several cases of malignant transformation of endometriosis in women receiving tamoxifen for breast cancer have also been described. Anyway in each case of severe recurrent endometriosis, the possibility of hormonal therapy should be considered with caution since every relapse may be associated with pain, the need for surgical treatment, and even malignant transformation of residual endometriosis.

At the same time, prolonged treatment with aGnRH and subsequent surgery can probably reduce ovarian reserve and cause development of premature/early menopause. This means that the risks associated with estrogen deficiency such as cardiovascular disease, cognitive decline, Alzheimer's disease, and early death will increase, and therefore it is necessary to carry out MHT. The European Menopause and Andropause Society (EMAS, 2010) recommends to use a continuous combined treatment regimen, regardless of whether a hysterectomy was performed or not. This will help reduce the risk of endometriosis recurrence and malignant transformation of remaining lesions. Herbal medicines should be used with caution since their effectiveness has not been adequately studied, and estrogen-like components in these drugs may have unpredictable effects in menopausal women [13].

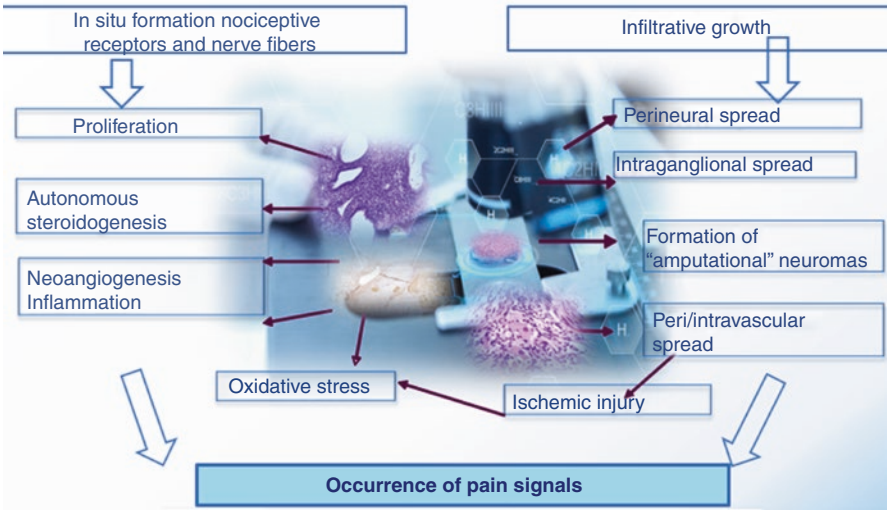
15.11 Biomarkers and Research Directions in Endometriosis

The importance of stem cells in endometriosis development is investigated in common with the theory of retrograde menstruation of Sampson and independently as, for example, hematogenous metastasis of stem cells. In the interesting study of Gargett, it was detected that endometrial samples of patients who underwent bone marrow transplantation contain endometrial cells with an HLA type of donor. It indicates their migration and differentiation after bone marrow transplantation [63, 64].





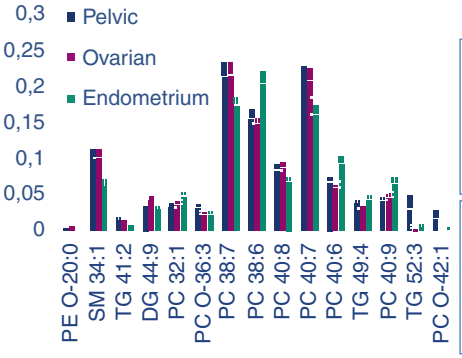
Causes of pain associated with endometriosis are also a subject of many studies. Research group from the Research Center for Obstetrics, Gynecology, and Perinatology revealed the destruction of the ganglion in 10 (27%) patients with endometriosis and the formation of ganglioneuroma in 2 (6%) patients, resulting in "phantom pain." Authors also demonstrate differences in the expression of PGP 9.5, NFs, NGF, and NGFRp75 in the endometriotic lesions and adjacent tissue in painful and painless endometriosis irrespective of the localization of heterotopies. All these molecular features denote a manifestation of remodeling of nerve fibers and nerve endings in endometriotic lesions. Furthermore, PGP9.5, NGF, and NGFRp75 ensure nerve fiber neoformation and pain syndrome in endometriosis [65]. Moreover 65% of women with adenomyosis/retrocervical endometriosis showed changes in the activity of the limbic-reticular complex, and 70% women with endometriomas had changes in trophotropic synchronizing structures of the brain stem.



According to the World Endometriosis Research Foundation, the search for biomarkers for minimally invasive and noninvasive diagnostics of endometriosis is one of the top research priorities [66, 67].

Currently, numerous scientific institutes from all over the world are looking for the potential biomarkers of endometriosis in blood samples, urine, endometrium, and cervical mucus of patients. Scientists consider a wide range of markers: glycoproteins, cytokines, neuronal proteins, growth factors, metabolites, transcriptomic data, single-nucleotide polymorphisms, and microRNAs. In the literature, more than 120 markers of endometriosis have been described, but these data have not yet been converted into an effective diagnostic test [68–72].

Research group from the National Medical Research Center for Obstetrics, Gynecology, and Perinatology named after Kulakov identified five classes of lipids, which levels were significantly different in eutopic and ectopic endometrium of patients with endometriosis. Investigated lipids include phosphatidylcholines (PC 32: 1, PC O-36: 3, PC 38: 7, PC 38: 6, PC 40: 8, PC 40: 7, PC 40: 6, PC O-42: 1), phosphoethanolamine (PE O-20: 0), sphingomyelin (SM 34: 1), diglyceride (DG 44: 9), and triglycerides (TG 41: 2, TG 49: 4, TG 52: 3) [73].



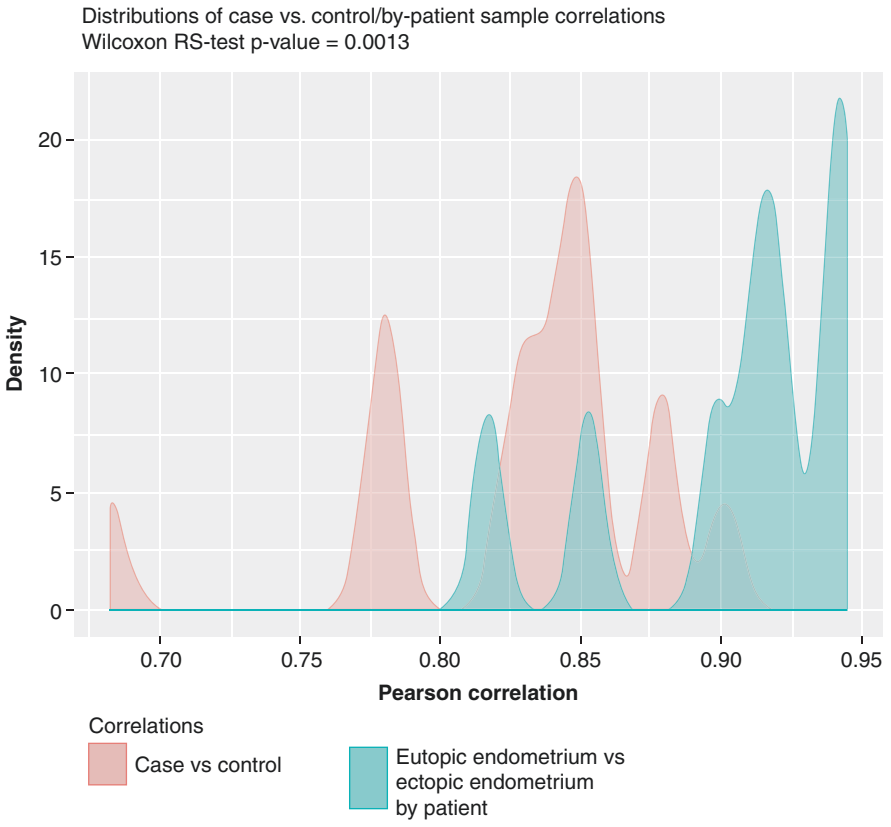
Mass spectrometry-technique that ionizes chemical species and sorts the ions based on their mass-to-charge ratio

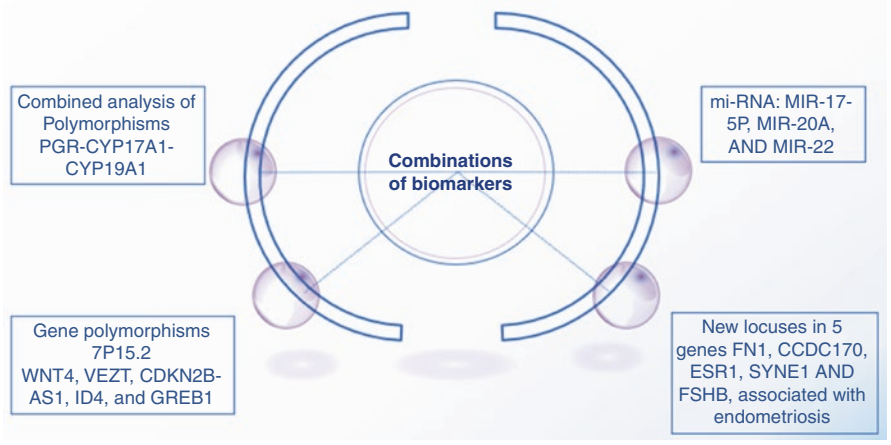
Increasing of the Sphinhomilines and phosphatidylcholines levels in the ectopic endometrium is an important factor explaning the pathogenesis of the disease

Grande et al. for the first time carried out a complete proteome profiling of cervical mucus in patients with endometriosis and found a number of differentially expressed proteins. It was shown that the expression of six proteins, four of which are associated with inflammation, is increased in patients with endometriosis and infertility compared with the control group: polymeric immunoglobulin receptor (PIGR), neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinases-1 (TIMP1), fibulin-1 (FBLN1), alpha-1-acid glycoprotein (Orosomukoid-A1AG2), and complement protein C3 (CO3). Expression of nine proteins, such as azurocidin-1 (CAP7), involucrin (INVO), aldolase-A (ALDOA), cysteine-rich secretory protein-3 (CRISP3), heat shock protein-1 (HSPB1), histones (H2A1H, H2A2C, H2A1B), and S10A8 protein, on the contrary, was lower in patients with endometriosis (including proteins associated with congenital immunity). Several proteins (15) were not found in samples of cervical mucus, including endogenous neuromodulator (SLURP1) and protein involved in liquefaction of seminal plasma (KLK13). Despite the data obtained, a larger study is needed with qualitatively selected comparison and control groups [74].

Genetic and transcriptomic studies are also an important part of biomarkers research in endometriosis. Research group from Moscow State University of Medicine and Dentistry calculated pairwise correlations between the following groups of samples: between all the individually taken samples of endometriotic lesions (ectopic endometrium) and endometrial samples of healthy women and between endometrial samples of women with endometriosis and the endometrial tissue of the same women. Authors demonstrated that eutopic and ectopic endometrial samples taken from the same patients showed significantly higher correlation between each other as compared with degree of correlations between these tissues and the endometrial samples of women without endometriosis [75].

Due to the non-specificity of biomarkers for the diagnosis of endometriosis, the current trend in this area is to evaluate the effectiveness of a combination of biomarkers, such as combinations of miRNAs (miR-199a + miR-542-3p, miR-199a + miR-122 + miR-145 + miR-542-3p) [76], interleukins (IL-6 > 12.20 pg/ml + TNF-α > 12.45 pg/ml) [77], oncomarkers, and chemokines (CA-125+/CCR1+/MCP-1) [78, 79].





Unfortunately, these combinations of biomarkers did not demonstrate significant efficacy in the detection of endometriosis as compared to individual biomarkers. In this regard, their further investigation, expansion of study groups, careful selection of the control group, and a qualitative analysis of the results are necessary.

15.12 Prevention

Limited data is available and shows that COCP use is associated with a reduction of the risk of endometriosis [80].

15.13 Conclusion

Nowadays there are a lot of studies from all over the world devoting to different aspects of endometriosis development and management. Endometriosis is certainly a disease beyond the age. Endometriosis affects every tenth woman of reproductive age (15–49 years), the prevalence of endometriosis in postmenopausal women is 2–4%, and about 50–60% of women and adolescents with pelvic pain have endometriosis.

The delay in diagnosis, chronic symptoms, and risk of recurrence of endometriosis lead to a significant decrease in the quality of life of patients in each age group. In adolescents this is due to severe dysmenorrhea and pelvic pain, which causes girls to miss school and other activities; in the reproductive age, chronic pelvic pain, dyspareunia, recurrence of the disease, and, of course, infertility; and in postmenopause, a risk of malignant transformation. This fact makes endometriosis not only a medical but also a socially significant problem. The factors described above necessitate the development of methods for early diagnosis, the selection of effective treatment and the prognosis of relapses, the fertility forecast, and undoubtedly the risk of malignant transformation.

Numerous studies are contributed to the biomarkers research in endometriosis: we have data on potential biomarkers in blood, saliva, urine, endometrium, and cervical mucous. Those biomarkers include various proteins, hormone levels, microRNAs, gene mutations, signaling pathway activation profiles, mass spectrometric profiles, etc. Unfortunately, none of them did not form the basis of noninvasive diagnostic test. Thus the gold standard for the diagnosis of endometriosis is still laparoscopy with subsequent histological verification. This complicates timely establishment of a diagnosis, especially in adolescents, because laparoscopy is a surgical intervention and should not be used as a screening test.

The abovementioned data dictate the need to change generally accepted perception of endometriosis as an “adult disease” and to expand biomarkers research that will help to speed up the diagnosis of this enigmatic disease at the early stages and to choose the most correct treatment approach in a timely manner.

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Selective Progesterone Receptor Modulators for Contraception with Added Health Benefits

16

Kristina Gemzell-Danielsson and Marielle E. Meurice

16.1 Introduction

There are estimated to be 214 million women who do not have access to contraception in developing countries [1]. Contraception as a medical intervention has immense benefit to public health—protecting reproductive freedom, preventing maternal death, and impacting the health of children [2]. There exist a multitude of options, all with different efficacies, risks, and benefits as well as efforts needed for correct use. LARC (long-acting reversible contraception), which include the intrauterine devices (IUDs) and implants, are methods in which the user has to do very little to follow recommendations for usage, but the method is highly effective. Globally, about one fourth of the reproductive age women who are married or in a union use LARC [3], with Sweden's rate estimated to be 24.3% [4]. The CHOICE project from the United States found that following education and after removing the barrier of cost, 67% of women chose LARC methods [5].

Innovation in contraception has the potential for widespread benefits on a population and individual level. Research on selective progesterone receptor modulators (SPRMs) is a promising development in contraception, and studies indicate there are additional applications and health benefits to women taking them [6, 7]. This chapter is an overview of SPRMs which introduces the mechanism of action, applications, health benefits, and future directions of this group of medications.

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16.2 Selective Progesterone Receptor Modulators (SPRMs)

16.2.1 The Role of Progesterone in Female Reproduction and Menstrual Cycle

Progesterone plays an essential role in reproductive physiology, affecting tissues of the breasts, uterus, and ovaries as well as the endocrine system via the hypothalamus-pituitary axis. Within the uterus, it plays a role in creating changes seen in the endometrium during the secretory phase, promoting implantation, and helping to maintain pregnancy by suppressing contraction of myometrium and softening of the cervix. Progesterone also plays a role in regulating ovulation and facilitating the LH surge [6–8].

16.2.2 Development of SPRMs and Basic Mechanism of Progesterone Receptors (PRs)

Given progesterone's important role in reproduction and the menstrual cycle, its properties have been utilized to create medications that modulate progesterone receptors. Mifepristone (RU-486) is a 19-norsteroid that binds and antagonizes the progesterone receptor, which can act directly on the target tissue (e.g., the dominant follicle or endometrium) but also can act via the pituitary by affecting gonadotropin release [9]. Since the discovery of this drug, which was the first PR antagonist, many other steroid-derived compounds have been developed that modulate progesterone receptors—which are called selective progesterone receptor modulators (SPRMs). It has been hypothesized that further discovery and production of other SPRMs has been delayed due to the negative connotation of mifepristone with abortion, despite not necessarily being related to mixed action of SPRMs [6, 7].

Progesterone receptors (PRs) exist in two nuclear isoforms, A and B, whose downstream effects are mediated by ligand-activated transcription factors. Progesterone binds the receptors and causes a conformational change that allows for binding to DNA. Additionally, the receptors dimerize and dissociate from heat shock proteins. The dimerized receptor binds DNA at progesterone-binding elements. Co-regulators play a large role in determining whether antagonistic or agonistic activity occurs by influencing transcription (Fig. 16.1). Intracellular pathway and ratios of isoforms also play a role in regulation, the details of which are beyond the scope of this introductory chapter [6].

PRs are found in the endometrium, with their prevalence depending on the menstrual cycle. PRB are more prevalent in the follicular phase, whereas PRA are increased in both the follicular and luteal phase. SPRMs act on these receptors and modulate their activity, leading to antagonism or agonism depending on the medication and dose [6]. Progesterone receptor modulators can exhibit both agonism and antagonism, by recruiting both coactivators and corepressors. Examples of SPRMs that have or are being developed for clinical usage include mifepristone, ulipristal acetate, asoprisnil, onapristone, and vilaprisan. Potential application during the

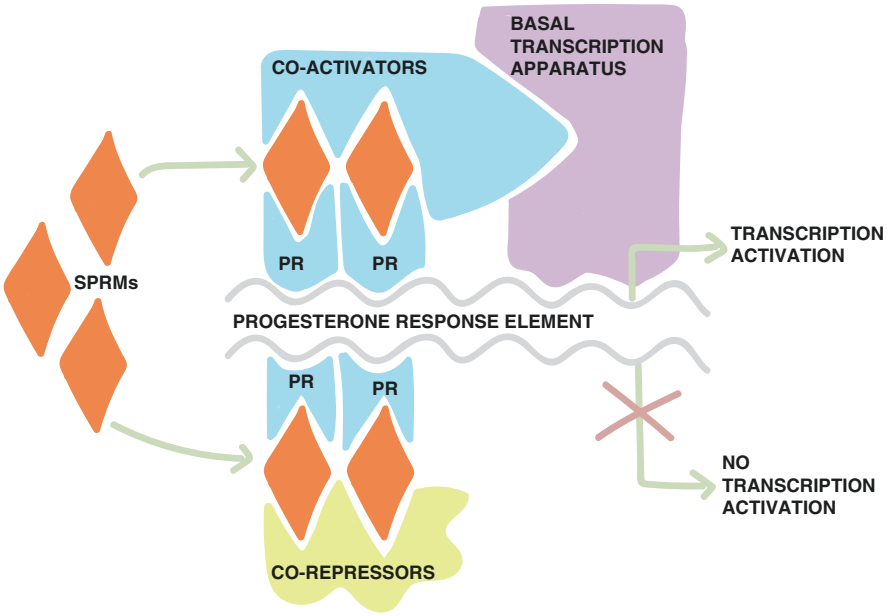

Fig. 16.1 Selective progesterone receptor modulator (SPRM) mechanism. (Figure modified from Berger et al., Thesis, Karolinska Institutet, 2017)

menstrual cycle and pregnancy includes contraception, contragestion, interruption of pregnancy, adjuvant to late pregnancy termination, and labor induction.

16.2.3 Effects on Nonpregnant Female Reproductive System

The effects of SPRMs have been studied on pregnant and nonpregnant women. In regard to the cervix, mifepristone was shown to be effective in softening the cervix in a study done in 1990 [10], but this result was not recreated in a study done in 2004 [11].

16.2.4 Effects in Pregnant Female Reproductive System

Progesterone allows the endometrium to become hospitable to an embryo and keeps the uterus quiescent by preventing contractions. The hormone is initially secreted by the corpus luteum until the placenta develops and takes over progesterone production. Mifepristone, which antagonizes the effects of progesterone, binds to the receptor with high affinity and during the first trimester will lead to contractions and changes in the endometrium that cause an abortion. Additionally, cervical dilation occurs, which allows the pregnancy to pass [12]. It can also be used as a labor induction method as well [13].

16.2.5 Contraception

Progesterone receptor modulators have been shown to be effective for contraception in different forms in several preliminary studies. Mifepristone has been shown to be a suitable medication for contraception through the different mechanisms described above and various applications. If given in the follicular phase, after selection of the dominant follicle, a low dose may temporarily inhibit follicular development and delay ovulation, while a higher dose will result in prevention of further follicle development and inhibition of ovulation. If given immediately after ovulation once-a-month on cycle day LH + 2 it blocks the secretory changes that are normally seen in the endometrium while not disrupting the normal ovulatory and menstrual cycle [14]. A study of mifepristone as endometrial contraception was done that showed a single 200 mg dose immediately post-ovulatory was highly effective and the only side effect noted was occasional irregular bleeding [15]. When 0.5 mg of mifepristone was used daily in an attempt to inhibit endometrial receptivity while not affecting ovulation, the rates of pregnancy was less than if no method was used but not adequate for use as a contraception alone [16]. Alternative approaches that resulted in anovulation and amenorrhea were also studied. In a study with 2 or 5 mg of mifepristone administered daily, very high levels of efficacy in contraception were seen [17]. Weekly doses with anovulation and amenorrhea were also investigated and found to be highly effective in pregnancy prevention [18]. Ulipristal acetate was studied in a similar way with continuous dosing, again showing amenorrhea and anovulation.

As estrogen is not affected, there is theoretical concern for the effects of unopposed estrogen on the endometrium, but hyperplasia was not detected [19]. A novel approach using a highly specific progesterone antagonist, ZK230211, as intrauterine contraception was conducted. This model was a promising study for future application of use in clinical trials [20]. Clinical trials have also been conducted on SPRM delivered by contraceptive vaginal ring for contraception [21].

The (side) effect of SPRMs on the endometrium is an important consideration giving this medication over the long term. Specifically, novel histological changes have been seen with SPRMs referred to as PRM-associated endometrial changes (PAEC) described as cystically dilated glands with mitotic and secretory features [22, 23]. The main concern would be that PAEC might be mistaken for hyperplasia, malignancy, or atypia. However, there are no reports of malignancy or atypia associated with PAEC, and PAEC is considered a benign condition [22].

16.2.6 Emergency Contraception

Emergency contraception is another important application of SPRMs. At low doses, used for emergency contraception, mifepristone (10 or 25 mg) or UPA (30 mg) blocks or delays follicular development and ovulation and does not inhibit implantation [24, 25], whereas at higher doses or continuing administration, the effect on the endometrium will contribute to the contraceptive effects [26].

16.2.7 Added Health Benefit of PRM

The ability to modulate progesterone receptors has a huge potential to impact women's health. In addition to abortion and contraception, these drugs may be applicable to the treatment of endometriosis, leiomyoma, abnormal uterine bleeding, menopausal symptoms, and fertility treatments. Even non-gynecologic benefits may be observed with potential roles in Cushing's syndrome, depression, and the treatment of steroid-producing tumors, all of which may be influenced by progesterone [8].

16.2.8 Leiomyoma

A Cochrane review of SPRMs and fibroids has concluded that short-term use of SPRMs improves quality of life, decreases bleeding, and leads to higher rates of amenorrhea when compared with placebo. The medication used in the 14 RCTs included in this review included mifepristone, ulipristal acetate, and asoprisnil [27]. The PEARL III trial used a repeated 3-month course of oral UPA 10 mg taken daily, which controlled pain and bleeding, decreased fibroid size, and improved quality of life [28]. Another small study looked at mifepristone and fibroids and found almost 50% decrease in fibroid size with daily dosing. The new agent, vilaprisan, is a SPRM that has stronger antagonism that UPA has been shown to be highly effective in reducing fibroid-related heavy menstrual bleeding and to reduce fibroid volume [29].

16.2.9 Endometriosis

Endometriosis is another disease that shows promising results from treatment with SPRMs. Animal models were used to assess onapristone and ZK 136 799 effects on endometriosis. They found that both anti-progesterones decreased the ectopic endometrial foci [30]. A primate study compared mifepristone and GnRH agonist treatment and found that both decreased endometriosis but that mifepristone did not interfere with estrogen activity and therefore had a better side effect profile for bone mineral density [31]. Mifepristone then was shown to have symptomatic clinical benefit in several small clinic trials [32–34]. A Cochrane review concluded that there was moderate evidence in the use of mifepristone for dysmenorrhea [35].

16.2.10 Breast

Breast tissue proliferation is influenced by estrogen and progesterone. The role of SPRMS has been explored using 50 mcg of mifepristone vs. placebo over a 3-month time period. In this study, mifepristone blocked breast epithelial cell proliferation in pre-menopausal women, indicating its use for other indications may also be beneficial for breast health [36, 37].

16.3 Conclusions

SPRMs have already had an impact on women's health, particularly in the realm of emergency contraception and treatment of symptoms related to uterine fibroids. Future research is needed to fully understand the mechanism and potential for use as endometrial contraception and application to technologies such as IUD and CVR. Other potential health benefits for women are emerging, and PRMs also show promise for treatment of endometriosis and potential prevention of breast disease. Further research is needed on the long-term effects on the endometrium and safety of continued use.

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