Tommaso Falcone
William W. Hurd
Editors

Clinical Reproductive Medicine and Surgery

A Practical Guide

Second Edition

Springer

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The editors would like to dedicate the second edition of this textbook to all enthusiastic students of reproductive medicine, particularly the Fellows, Residents, and new clinicians for whom this book was created.

Preface

We put together the original edition of *Clinical Reproductive Medicine and Surgery* in an attempt to provide a comprehensive, easy-to-read textbook that covered all aspects of an academic clinical practice in reproductive medicine. We received a great deal of favorable feedback from Fellows, Residents, new clinicians, and our target audience. However, it was also commonly noted that our textbook was somewhat expensive and cumbersome in terms of both weight and size.

For our second edition, we have made several noteworthy changes in an attempt to improve upon the original textbook. Although we have tried to keep true to our goal of a comprehensive and readable clinical textbook, we have shortened it significantly to decrease both its price and physical dimensions. To do this, we asked each author to condense their information and combine chapter contents whenever possible. We also removed some of the more esoteric chapters that, although important for certification tests, are of less value in day-to-day clinical practice. We are very pleased with the results, and anticipate that you will find the resulting compendium useful. We hope that it will confirm most of your current clinical practices and serve as a useful introduction to the newest approaches to both common and unusual problems faced by our patients.

Cleveland, OH, USA — Tommaso Falcone
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1 Ovarian Hormones: Structure, Biosynthesis, Function, Mechanism of Action, and Laboratory Diagnosis

Manjula K. Gupta and Su-Ynn Chia

Introduction

The main function of the ovaries, maturation and release of oocytes, is accomplished via the production of several steroidal and nonsteroidal hormones that locally modulate a series of complex events. Peripherally, these hormones act on various target organs, including the uterus, vagina, fallopian tubes, mammary glands, adipose tissue, bones, kidneys, and liver, leading to the female phenotype.

The secretion of the ovarian hormones in turn is precisely regulated by the hypothalamic–pituitary axis. The complex interactions and regulations of the hypothalamic, pituitary, and ovarian hormones are collectively responsible for the regular and predictable ovulatory menstrual cycle and fertility in females.

The Ovary as an Endocrine Organ

A single ovarian follicle is regarded as the basic endocrine/reproductive unit of the ovary. It is composed of one germ cell that is surrounded by a cluster of endocrine cells, which are organized in two layers separated by a basal membrane. The inner layer surrounding the oocyte is composed of granulosa cells, and the outer layer is composed of thecal cells. These two cell types provide the basic machinery that is responsible for producing ovarian hormones. These cells are also differentially regulated by the gonadotropins (i.e., luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and produce distinctly different steroid hormones). These facts led to the development of the "two cell theory." The two cell theory describes the sequence of events that occur during ovarian follicular growth and steroidogenesis. According to this theory, LH primarily stimulates thecal cells to produce androstenedione and testosterone, both C19 steroids. In contrast, FSH primarily stimulates granulosa cells to aromatize these C19 steroids into estrogens [1, 2].

The ovarian production of steroid hormones is regulated both within the ovary, by paracrine (intercellular) and autocrine (intracellular) mechanisms, and by endocrine regulation of FSH secretion by pituitary. Central to this regulation are several nonsteroidal hormones and factors produced by the ovary [3]. This chapter focuses on these aspects of the ovary and discusses the biochemistry, biosynthesis, and actions of both steroidal and peptide ovarian hormones.

Steroidogenesis and Steroid Hormones of the Ovary

The ovary contains multiple distinctive, steroid-producing cells, including stromal cells, theca cells, granulosa cells, and luteinized granulosa cells. Each cell type contains all the enzymes necessary for synthesis of androgens, estrogens, and progesterone. However, the types of hormones produced vary according to the cell type and the expression of steroidogenic enzymes. Other factors that influence which steroid hormone is synthesized in a given cell include the level and expression of gonadotropin receptor and the availability of low-density lipoprotein (LDL) cholesterol. As discussed below, steroid hormone synthesis occurs via one of two pathways: the Δ (delta)^5 (3β (beta)-hydroxysteroid)-pathway or the Δ (delta)^4-(3 ketone-pathway).

Steroidogenesis

The ovary, like the adrenal gland, produces all three classes of steroid hormones from cholesterol—estrogens, progesterone,

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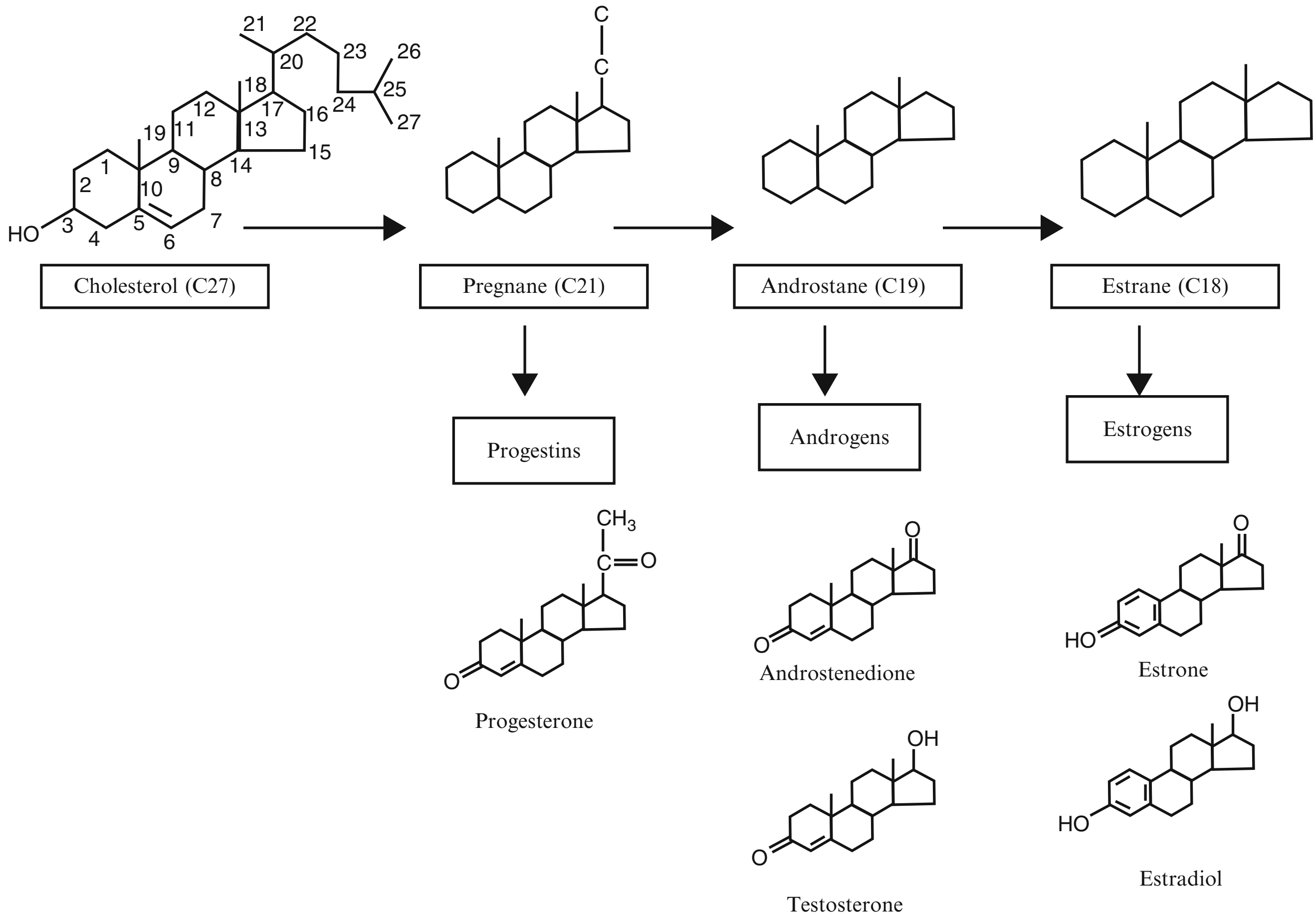

Fig. 1.1 Structures of cholesterol (27 carbons) and of the three major classes of ovarian steroids. The progestins (21 carbons), androgens (19 carbons), and estrogens (18 carbons). Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

and androgens. In contrast to the adrenal gland, the ovary cannot produce glucocorticoid and mineralocorticoid since the ovary lacks enzymes 21-hydroxylase and 11β (beta)-hydroxylase.

The primary source of cholesterol for steroidogenesis in the ovary is derived from the uptake of plasma LDL [4]. The rate-limiting step in steroidogenesis is transfer of cholesterol from the cytosol to the inner membrane of the mitochondria. This is mediated by an LH-induced mitochondrial enzyme called steroidogenic acute regulatory protein (StAR) [5]. The StAR gene is located on chromosome 8p11.2 and codes for a 285-aminoacid precursor-protein of which 25 amino acids are cleaved off after transport to the mitochondria [6]. Nonsense mutations of the StAR gene that result in premature stop codons have been identified as a cause of **congenital lipoid adrenal hyperplasia**, which is characterized by the presence of intracellular lipid deposits that destroy steroidogenesis [6].

Ovary steroid hormones are synthesized in both interstitial and follicular cells. The basic structure of cholesterol is three hexagonal carbon rings and a pentagonal carbon ring to which a side chain is attached (Fig. 1.1). Two important methyl groups are also attached at positions 18 and 19. **Progestins and corticosteroids** (*pregnane* series C21 steroids) are produced by partial cleavage of the side chain (i.e., the desmolase reaction). **Androgens** (*androstane* series C19 steroids) are produced by the total removal of the side chain. **Estrogens** (*estrane* series steroids) are produced by aromatization of one of the three hexagonal carbon rings to a phenolic structure with loss of the 19-methyl group.

The first step in steroidogenesis is the conversion of cholesterol to pregnenolone via hydroxylation at the carbon 20 and 22 positions, which is followed by cleavage of the side chain. From pregnenolone, steroid hormones are produced by one of two general pathways [7]. The pregnenolone (□(delta)5) pathway produces androgens and estrogens (pregnenolone → 17OH-pregnenolone → dehydroepiandrosterone (DHEA) → testosterone → estrogen). The progesterone (Δ(delta)^4) pathway produces androgens and estrogens (pregnenolone → progesterone → 17OH-progesterone → androgen → estrogens) (Fig. 1.2). In the adrenal gland, the Δ (delta)^4 pathway produces mineralocorticoids and glucocorticoids.

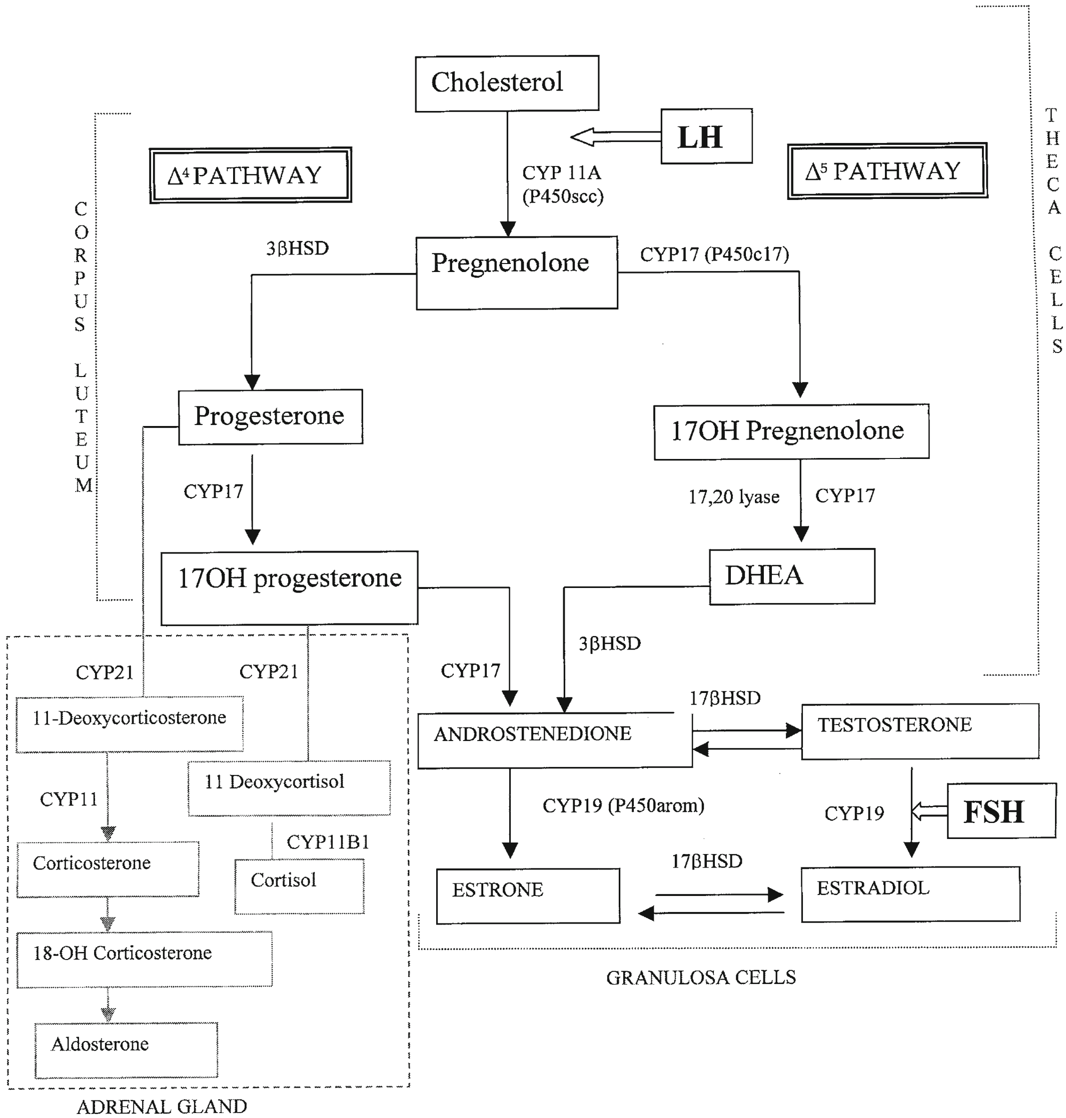

Fig. 1.2 Steroid hormone biosynthesis pathways in the ovary. The Δ (delta)^4-pathway converts pregnenolone to progesterone and is the main pathway in the corpus luteum. The Δ (delta)^5-pathway converts pregnenolone to androgens and, subsequently, estrogens, and is the preferred pathway in the thecal cells. Also shown is the conversion of 17OH progesterone to aldosterone and cortisol as a result of CYP21 and CYP11, which occurs exclusively in adrenal gland. Irreversible reactions are denoted by a *single arrow*, and reversible reactions are denoted by *double arrows*. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

The enzymes involved in the intracellular synthesis of steroid hormones include five hydroxylases, two dehydrogenases, a reductase, and an aromatase. The hydroxylases and aromatase belong to the cytochrome P450 (CYP) supergene family (Table 1.1). These enzymes exist on both the mitochondria and endoplasmic reticulum (ER).

Of these nine enzymes, four key enzymes regulate the main steps of steroidogenesis: (1) CYP 11A (P450scc); this side chain cleavage enzyme catalyzes the conversion of cholesterol to pregnenolone; (2) 3β (beta) HSD, which converts pregnenolone to progesterone; (3) CYP 17 (P450c17), which converts pregnenolone to androgens; and (4) CYP 19 (P450arom), which converts androgens to estrogens. Most reactions are irreversible (denoted by a single arrow in Fig. 1.2). The few reversible reactions (denoted by double arrows) are dependent on cofactor availability (e.g., NADP/NADPH ratio).

The steroid-producing cells of the ovary (granulosa, theca, corpus luteum) contain the full enzymatic complement for steroid hormone synthesis. In the thecal cells, LH also controls 17β (beta) HSD activity and androstenedione production, while CYP19 (P450 aromatase) activity in the granulosa cells is controlled by FSH and, hence, estradiol production. These relationships are the basis for the two-cell, two-gonadotropin system.

Table 1.1 Enzyme reaction and cellular location of steroidogenic enzymes

Enzyme reaction	Gene (enzyme)	Cellular location/ tissue location
Cholesterol side chain cleavage	CYP11A (P450scc)	Mitochondria (theca; granulosa)
17α (alpha)-hydroxylase	CYP17 (P450c17)	ER (theca)
17,20-hydroxylase (lyase)	CYP17 (P450c17)	ER (theca)
Aromatase	CYP19 (P450 arom)	ER (granulosa)
3β (beta)-hydroxysteroid dehydrogenase	3β HSD	ER (theca; granulosa)
17β (beta)-hydroxysteroid dehydrogenase	17β HSD	ER (granulosa)
21-hydroxlase	CYP21 (P450c21)	ER (adrenal)
11β-hydroxylase	CYP11B1 (P450c11)	Mitochondria (adrenal)

Steroid Hormones of the Ovary

On the basis of chemical structure and biological function, the major steroid hormones synthesized and secreted by the ovaries can be classified into three major types: estrogens, progestogens, and androgens.

Estrogens

Physiologic Role

Estrogens are essential in the development and maintenance of the female phenotype, germ cell maturation, and pregnancy. In addition to its reproductive effects, estrogens also have many other nonreproductive systemic effects like bone metabolism/remodeling, nervous system maturation, and endothelial responsiveness [8].

At **puberty**, estrogen stimulates breast development and enlargement and maturation of the uterus, ovaries, and vagina. Estrogen also works in concert with growth hormone (GH) and IGF-I to produce a growth spurt and stimulates the maturation of chondrocytes and osteoblasts, which ultimately leads to epiphyseal fusion [9]. After mid-puberty, estrogen begins to exert a positive feedback on GnRH secretion, leading to the progressive increase of LH and FSH production, culminating in the LH surge, ovulation, and the initiation of the menstrual cycle.

In the **adult female**, estrogen plays a critical role in maintaining the menstrual cycle [10]. The cyclical changes in estradiol, progesterone, and pituitary hormones are illustrated in Fig. 1.3. In the early follicular phase of the menstrual cycle, FSH stimulates granulosa cell aromatase activity, resulting in increased follicular concentrations of estrogen. The rising estrogen level further increases the sensitivity of the follicle to FSH and estrogen by increasing the number of estradiol receptors on the granulosa cells. Follicular growth and antral formation are also promoted by estrogen. This sets up a positive-feedback cycle, which culminates in one dominant follicle producing an exponential rise in estrogen levels. This exerts a negative feedback on FSH so that falling FSH levels contribute to the atresia of other nondominant follicles. The dominant follicle secretes large quantities of estrogen, and estradiol levels must be greater than 200 pg/mL for approximately 50 h in duration before a positive feedback on LH release is achieved [9, 11]. Once the LH surge is initiated, the luteinization of the granulosa cells and progesterone production occurs. In **pregnancy**, estrogen augments uterine blood flow, although it is not required in itself for the maintenance of pregnancy.

In the **central nervous system**, estrogen withdrawal at menopause has been associated with reduced libido, altered mood, and cognitive disturbances. These effects have been attributed to estrogen's ability to modulate the synthesis, release, and metabolism of many neuropeptides and neurotransmitters [12]. Estrogen acts as a serotoninergic agonist by increasing serotonin synthesis in the brain, which may positively influence mood [13]. While prospective observational studies in postmenopausal women have suggested that estrogen replacement therapy might protect against cognitive decline [14] and the development of dementia [15], randomized trials of estrogen for treatment of Alzheimer's disease have shown no evidence of benefit [16–19].

In the **skeletal system**, estrogen antagonizes the effect of parathyroid hormone by directly inhibiting the function of osteoclasts, which decreases the rate of bone resorption and diminishes bone loss. The postmenopausal estrogen/progestin interventions (PEPI) trial was a prospective, placebo-controlled trial designed to study the effects of hormone replacement on bone density in postmenopausal women. After 12 months of treatment with estrogen, bone mineral density at the hip increased by 1.8 % and by 3–5 % at the spine [20]. The Women Health's Initiative (WHI) showed that estrogen reduced the risk of both hip and vertebral fractures by 30–39 % [21].

In the **cardiovascular system**, there is strong evidence that estrogen has a natural vasoprotective role. At a cellular level, estrogen receptors are found on the smooth muscle cells of coronary arteries [22] and the endothelial cells of various sites [23]. Estrogen causes short-term vasodilation by increasing nitric oxide and prostacyclin release in endothelial cells [24]. Several large observational studies, including the Framingham study and the Nurses' Health Study, have shown that cardiovascular incidence rates were lower in premenopausal than postmenopausal women [25]. There was also a significant association between a younger age at menopause and a higher risk of coronary heart disease (CHD) [26]. These studies led to the conviction that estrogen replacement therapy would consequently prevent the progression of atherosclerosis and CHD.

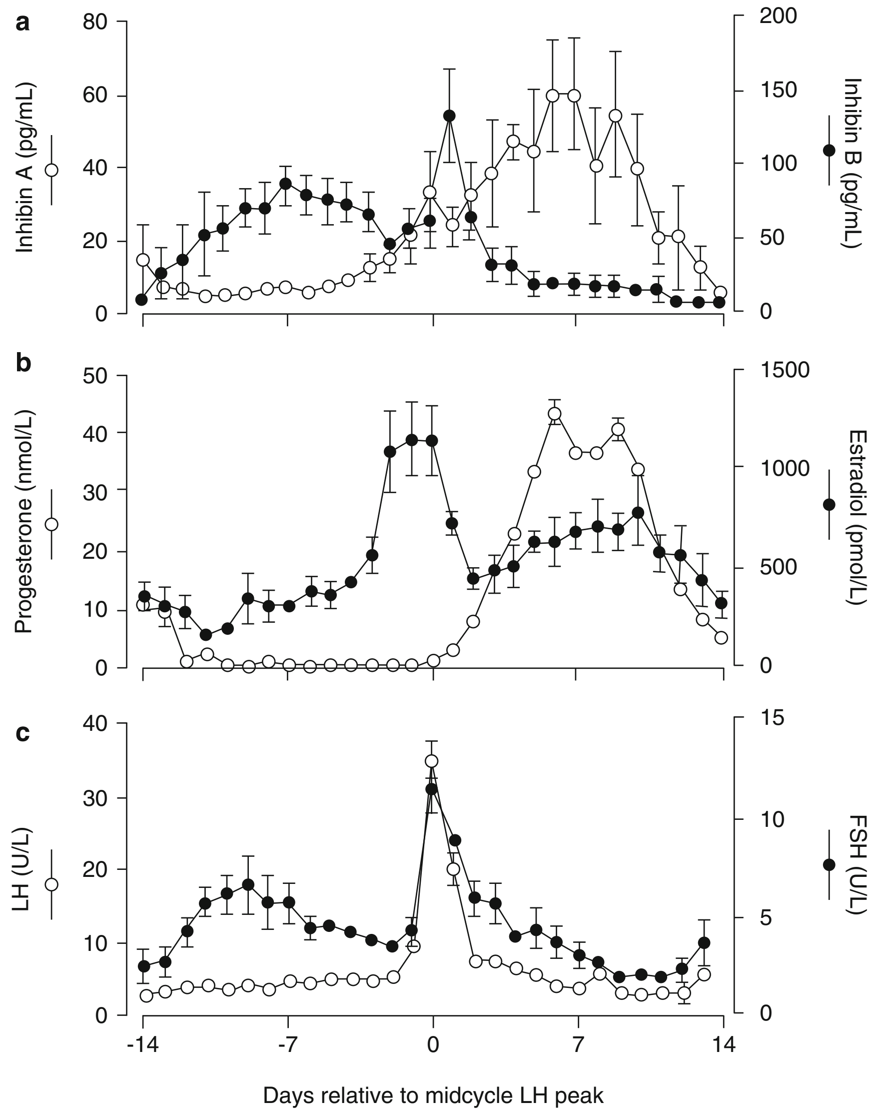

Fig. 1.3 Plasma hormone concentrations (mean) during the female menstrual cycle. Graph (**a**) inhibins; (**b**) progesterone and estradiol; (**c**) LH and FSH. Data from [95]. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Biosynthesis and Metabolism

Estrogens are C_{18} steroids derived from cholesterol (see Fig. 1.1). There are three forms of naturally occurring estrogen: estrone (E1), 17β (beta)-estradiol (E2), and estriol (E3). The main estrogen in premenopausal women is E2, which is almost twofold to fivefold more potent than E1 [27]. The circulating levels of E2 are 2–4 times higher than E1 in premenopausal women and are reduced to one-tenth in postmenopausal women. Estrone is not only a secreted product of the ovary but also derived from the extra-gonadal peripheral aromatization of adrenal androstenedione and its concentrations do not differ with menopausal status, and thus, over time, the premenopausal E2:E1 ratio is reversed [28] In contrast, E3 is not the secretory product of the ovary but is the peripheral metabolite of E1 and E2. Peripheral conversion of androgens to estrogens occurs in skin, muscle, and adipose tissue and in the endometrium [29].

In the **pregnant woman**, the placenta becomes the main source of estrogen, in the form of E3. The placenta is unable to synthesize steroids de novo and depends on circulating precursors from both fetal and maternal steroids. Most of the placental estrogens are derived from fetal androgens (e.g., DHEA sulfate) produced by the fetal adrenal gland [30].

Estradiol is rapidly converted in the liver to E1 by 17β (beta)-hydroxysteroid dehydrogenase. E1 can be further metabolized via three pathways. First, it can be hydroxylated to 16α (alpha)-hydroxyestrone, which is then converted to E3. Estriol is further metabolized by sulfation and glucuronidation, and the conjugates are excreted into the bile or urine. Secondly, estrone can be conjugated to form estrone sulfate, which occurs primarily in the liver. Estrone sulfate is biologically inactive and is present in concentrations that are tenfold to twentyfold higher than E1 or E2 [31]. This can be hydrolyzed by sulfatases present in various tissues to estrone and may serve as a reserve of estrogen in an inactive form. Estrone sulfate may be of some importance in assessing estrogenicity in women and can be detected in serum as well as in urine [32]. Thirdly, E1 can also be metabolized by hydroxylation to form 2-hydroxyestrone and 4-hydroxyestrone, which are known as catechol estrogens. These are then converted to the 2-methoxy and 4-methoxy compounds by catechol-*O*-methyltransferase.

Progesterone

Physiologic Role

Progesterone plays a critical role in reproduction. It inhibits further endometrial proliferation mediated by estrogen and converts the endometrium into a secretory type, preparing it not only to receive the blastocyst for implantation but also to maintain the pregnancy. It inhibits uterine contractions and increases the viscosity of cervical mucus. Progesterone also inhibits the action of prolactin so that lactation occurs only after delivery. It raises the basal body temperature by about 0.5 °C (0.9 °F) and increases the sensitivity of the respiratory center to CO_2, leading to hyperventilation.

During pregnancy, progesterone increases insulin resistance in concert with the production of the other placental counter-regulatory hormones, including placental growth hormone, placental lactogens, placental corticotropin-releasing hormone, and cortisol [33].

Biosynthesis and Metabolism

Progesterone is part of the group of C_{21} steroids, which also includes pregnenolone and 17OH-progesterone. Progesterone is responsible for all the progestational effects, whereas pregnenolone is the precursor for all steroid hormones. 17OH-progesterone has little biologic activity. Progesterone and 17OH-progesterone are mainly produced by the corpus luteum in the luteal phase of the menstrual cycle and by the placenta if pregnancy occurs. Circulating levels of progesterone at concentrations greater than 4–5 ng/mL (12.7–15.9 nmol/L) are indicative of ovulation [34].

Progesterone is rapidly metabolized by the liver and has a half-life of approximately 5 min. It is converted to pregnanediol and conjugated to glucuronic acid in the liver. Pregnanediol glucuronide is excreted in the urine. Pregnanetriol is the main urinary metabolite of 17-OH-progesterone.

Androgens

Physiologic Role

In women, androgens originate as C19 steroids from the adrenals and ovaries. The major androgens produced in the ovary, primarily by the thecal cells and to a lesser degree by the ovarian stroma, include DHEA, androstenedione, and a small amount of testosterone. Both DHEA and androstenedione serve as precursors to estrogen synthesis and have little, if any, androgenic activity. However, these biologically inactive androgens are converted by extraglandular metabolism to biologically active androgens such as testosterone and dihydrotestosterone (DHT). Normally, the levels of these potent androgens are low in females and have no significant physiologic function. Excessive production of androgens by the ovary or adrenals has been implicated as the cause of hirsutism and virilization in women with polycystic ovary syndrome (PCOS) [35]. In contrast, the androgens are of primary importance in the male, of which testosterone and DHT are the most crucial.

Biosynthesis and Metabolism

Androgens are C_{19} steroids derived from cholesterol. The rate-limiting step in androgen synthesis is the conversion of cholesterol to pregnenolone, which is mediated by the action of LH on the ovary and testes. In a normal ovulatory woman, the ovaries secrete about 1–2 mg of androstenedione, 1 mg of DHEA, and about 0.1 mg of testosterone. The majority of (about 0.2 mg) circulating testosterone is derived from peripheral metabolism of DHEA and androstenedione. Overall, testosterone production in women is about 0.3 mg/day; roughly 50 % of this is derived from peripheral conversion, whereas the remaining 50 % is secreted equally by the ovary and the adrenals [36].

Most of the circulating testosterone is metabolized in the liver into androsterone and etiocholanolone, which are conjugated with glucuronic acid or sulfuric acid and excreted in the urine as 17-ketosteroids. Of note, only 20–30 % of the urinary 17-ketosteroids are derived from the testosterone metabolism; the rest originate from the metabolism of adrenal steroids.

Transport of Ovarian Steroid Hormones in Plasma

Steroid hormones are not water soluble and require transport proteins to be carried to their target tissues. Less than 2 % of ovarian steroid hormones are free in the circulation; the remainders are mostly bound to SHBG and albumin [37, 38]. SHBG, a beta globulin of 95 kDa, is synthesized in the liver. Its gene is localized on the short arm of chromosome 17 (p12–13) [39]. It is a homodimer composed of two polypeptide chains and has a single binding site for androgens and estrogens. Dimerization is a necessary step in the binding process [40]. The bound and free fractions appear to exist in a steady state of equilibrium. The amount of free fraction depends on the concentration of steroid hormone and on the levels and binding affinities of the binding proteins.

Of all the steroid hormones, DHT has the highest affinity for SHBG. About 98 % of testosterone circulates bound to SHBG (~65 %) and albumin (~33 %). Estradiol is primarily bound not only to albumin (~60 %) but also to SHBG (38 %);

about 2 % circulates as the free fraction [42]. Progesterone, on the other hand, is mainly bound to albumin (~80 %) but also to CBG (~18 %). Only ~0.6 % of progesterone is bound to SHBG and ~2 % exists in the "free" state.

The metabolic clearance of these steroids is inversely related to their binding affinity to SHBG. Thus, conditions that affect levels of SHBG (e.g., pregnancy, oral contraceptives) directly affect the levels of free hormone. Because estrogens increase SHBG synthesis and androgens decrease its synthesis, SHBG levels are twice as high in women compared to men. Several other hormones and other factors are known to influence SHBG levels. Thyroid hormones increase its synthesis and release by the liver [41]. Insulin, IGF-1, and prolactin have been shown to inhibit SHBG production in cultured cells [42]. Furthermore, serum concentrations of SHBG are increased in many disease states, including hyperthyroidism and liver cirrhosis. Certain drugs can also increase serum concentrations, including estrogen, tamoxifen, and phenytoin. Carrier protein levels are decreased by hypothyroidism, obesity, and acromegaly and by administration of exogenous androgens, glucocorticoids, and growth hormones.

For many years, only the free fraction of testosterone was regarded as the biologically active component. However, researchers noted that steroid hormones bind with greater affinity to their specific carrier proteins and with much less affinity to albumin. In addition, studies of tissue delivery in vivo showed that the dissociation of albumin-bound testosterone can occur rapidly in a capillary bed so that the active fraction can be larger than the free fraction measured under equilibrium conditions in vitro [43]. Thus, unconjugated steroids that are bound to albumin may be treated as free and biologically available [44, 45].

As mentioned above, SHBG levels can be influenced by numerous disease states. As such, changes in SHBG concentrations can result in large shifts in the free and SHBG-bound fractions. Hence, measurement of SHBG is of great clinical interest, as it allows more accurate assessment of free hormones. SHBG was initially measured by a technique called saturation analysis, in which specific binding capacity of ^{3}H labeled testosterone is detected [46]. With modifications, this method can also measure the non-SHBG-bound fraction (bioavailable) [47]. Recently, specific nonisotopic two-site immunoassays for SHBG have become available and are used in most clinical laboratories.

Measurement of Steroid Hormones in Circulation

The technique responsible for the accurate measurement of low concentrations of various steroid hormones and metabolites is competitive inhibition immunoassay or radioimmunoassay (RIA), which was originally described in 1960 by Yalow and Berson [48]. However, the development of steroid immunoassays presented several problems. First, they are not immunogenic and have a similar structure—they all have the same cyclopentahaptene nucleus with only minor structural variations—which makes it difficult to generate specific antibodies. Steroids can be made immunogenic via chemical coupling to a carrier protein known as "hapten," and antibodies can be raised by immunization with haptens [49]. However, the site of the steroid where the protein is conjugated has a significant impact on the specificity of the resulting antibody [49]. Antibodies raised to conjugates of BSA coupled at the 19th position show higher specificity than those coupled at 3rd or 17th positions [50]. For accurate clinical interpretation, it is important to know the cross-reactivity data on each antibody that is selected for a given assay. Most commercial assay reagent manufacturers provide cross-reactivity data, but it may not always be reliable and must be evaluated in clinical labs performing the assay [51].

Second, the high-affinity binding proteins such as SHBG in the serum compete with the antibody and thus interfere with the measurement of steroid molecules by RIA. This makes direct measurement difficult and necessitates a preassay extraction procedure with organic solvents and often a chromatographic separation of the steroid. Alternatively, the use of certain chemicals, such as 8-anilinonaphthelene sulfonic acid (8-ANS), can inhibit the binding of steroids to proteins, which allows the direct measurement of steroid hormones without the extraction step. Direct assays are fast and can be automated. Several automated platforms for measuring E2, progesterone, and testosterone are commercially available and are used in most clinical laboratories. These assays, however, have a low sensitivity [52]; therefore, they may not be the best choice for clinical applications that require the ability to measure very low hormone concentrations, such as estradiol measurement in children and in men (<100 pg/mL) [53], testosterone measurement in children and women (<1.5 ng/mL) [54], or progesterone measurement during ovarian stimulation (<1 ng/mL) [55]. To overcome this problem, serum can be extracted with hexane-ethyl acetate (3:2 by volume), dried, and reconstituted in steroid-free serum, which can then be assayed in the automated platform [56].

Gas chromatography combined with mass spectrometry (GC-MS) addresses many of the shortcomings of immunoassays and is considered more reliable and accurate. However, this technique requires multiple steps that include solvent extraction, chromatographic fractionation, and chemical dramatization before instrumental analysis, and it is often less sensitive than some immunoassays. This technique has now been superseded by liquid chromatography combined with tandem mass spectrometry detection (LC-MS/MS). This newer technique has a higher sensitivity and throughput than GC-MS and is considered a reference methodology [57]. The technique has been used to simultaneously measure

Table 1.2 Assay techniques and clinical applications of ovarian steroid hormones

Hormone	Assay techniques	Clinical application
Estradiol	Radioimmunoassay	Evaluation of ovarian function
	Nonisotopic-immunoassays	Assessment of menopausal status
	Mass spectrometry	Monitoring ovulation induction and IVF cycle
Estrone sulfate	Radioimmunoassay	Marker of estrogenicity in women on HRT
Progesterone	Radioimmunoassay	Marker for ovulation
	Nonisotopic-immunoassays	Detection of luteal phase defects
	Mass spectrometry	Marker for threatened abortion in early pregnancy
Total testosterone	Radioimmunoassay	Evaluation of patients with hirsutism
	Nonisotopic-immunoassays	Male infertility
	Mass spectrometry	
Free testosterone	Equilibrium dialysis	Evaluation of patients with hirsutism
	Diafiltration	PCOS

estradiol and estrone in human plasma with no cross reactivity [58]. These methods are recommended as reference methods and can be used to standardize and validate immunoassays, which provide the simplicity and rapid throughput needed for clinical use.

Estrogens

Measurement of E2 is important in the assessment of female reproductive function and is measured by immunoassays in most labs. It can be used as an aid in the diagnosis of infertility and oligomenorrhea and to determine menopausal status (Table 1.2). In addition, measurement of E2 is widely used to monitor ovulation induction and in in vitro fertilization protocols [59, 60]. Estrone levels are of limited clinical value in nonpregnant women because their levels closely parallel those of estradiol, except in postmenopausal women, in whom estrone becomes the main form of circulating estrogen. Also, as mentioned above, a specific radioimmunoassay for the measurement of estrone sulfate has been described and made available commercially. Because E1 sulfate has a large circulating pool, it can serve as a marker of estrogenicity, especially in women on estrogen replacement therapy, where E2 measurements are of little value due to variable cross reactivity of conjugated estrogens in the E2 assays [61]. In pregnant women, estriol is the main form of estrogen produced, and the amount of estrogen secreted increases from microgram quantities to milligram quantities.

Progesterone

Although GC-MS has been recommended as the reference method for the measurement of progesterone [62], immunoassays using steroid-specific antibodies are again the preferred mode of measurement in most clinical laboratories [63, 64]. Both RIA and nonisotopic immunoassays for progesterone are available for commercial use. Progesterone measurement is routinely used to detect ovulation and luteal phase defects (see Table 1.2) [65]. In the follicular phase, progesterone levels are low (<5 nmol/L or 1.5 ng/mL). In the luteal phase, they range between 9 and 7 nmol/L (3–25 ng/mL). As noted above, progesterone is required to maintain pregnancy, and progesterone measurement in early pregnancy can be valuable in the diagnosis of defective or threatened abortion [66].

Androgens

The measurement of androgens, including androstenedione and total testosterone or DHT, can also be accomplished using immunoassays [67]. Direct measurement of androstenedione and testosterone without extraction is now possible with new commercial assays. However, some commercial assays demonstrate high variability, which is greatest in samples from females [68]. Total testosterone in hirsute women overlaps significantly with levels seen in normal women, and measurement of free testosterone correlates better with disease [69]. Free testosterone has been measured by equilibrium dialysis, which is a time-consuming and difficult technique for most clinical laboratories to perform. An ultrafiltration technique can be used instead, which depends on MPS-1 centrifugal gel filtration devices and correlates well with the equilibrium dialysis method [69, 70] as well as with GC-MS [71]. Direct, single-step, non-extraction immunoassay methods using ^{125}I-labeled testosterone analog have been developed commercially and are used in a number of labs. The accuracy and validity of this direct assay has been questioned [72, 73]. Alternatively, an indirect parameter of free testosterone—FAI—can be calculated as a ratio of testosterone to SHBG [74]. FAI is a better discriminator of hirsutism than either total testosterone or SHBG levels [73, 74]. When bound with albumin and CBG, testosterone dissociates more quickly than when bound with SHBG. This loosely bound testosterone may be biologically available through dissociation during capillary transit. Cumming and Wall provided evidence for this hypothesis and suggested that this non-SHBG bound bioavailable testosterone is a marker of hyperandrogenism [47].

Saliva Measurements

SHBG is either undetectable or minimally present in saliva. Thus, this biological fluid may reflect the free fraction of plasma steroids. Therefore, measurement of steroid hormones in saliva has attracted considerable attention [75, 76]. The ease of noninvasive collection combined with the simplicity of measurement makes salivary measurement a promising and attractive alternative to plasma steroids. In the future, salivary assays may become useful adjuncts to those performed in plasma.

Peptide Hormones and Intraovarian Growth Factors of Ovary

The role of gonadotropins and gonadal steroids in the process of folliculogenesis in the ovary is well recognized. However, multiple other phenomena within the ovary suggest the presence of other intraovarian factors that may "fine tune" the effects of gonadotropins and gonadal steroids. For example, the initiation and arrest of meiosis and the different rates of follicular growth leading to the selection of a dominant follicle point toward the existence of an intraovarian modulatory system. The concept of a gonadal factor with endocrine action at the pituitary level can be traced back more than 70 years when the first moiety was identified and named inhibin for its inhibitory effect on the pituitary [77]. Since then, there has been an explosion of information regarding multiple and potential intraovarian regulators and their physiology, biochemistry, and biosynthesis as well as the identification of their receptors. Some of these compounds, which have been the subject of intense investigation, include peptide hormones/growth factors, cytokines, and neuropeptides. These factors may act in either an endocrine, autocrine, or paracrine fashion.

Peptide Hormones of the Ovary

The first water-soluble peptide hormone in testicular extracts that exerted selective inhibitory activity at the pituitary level was described in 1932 and termed "inhibin" [77]. It was not until 1985 (almost 50 years later) that inhibin was isolated and characterized [78, 79]. This was followed by the identification and characterization of two other related peptide factors (i.e., activin and follistatin) [80, 81]. In the 1940s another testicular product that was responsible for the regression of Müllerian ducts in male fetus was described as anti-Müllerian inhibitory substance (MIS) and later named anti-Müllerian hormone (AMH). With the cloning of inhibin α (alpha) and β (beta) subunit genes [82] and of AMH [83], it was recognized that inhibin and activin along with AMH belonged to the TGFβ superfamily of growth and differentiation factors. Although the roles of inhibin and activin on folliculogenesis are of particular clinical interest and have been extensively studied [3, 84, 85], the role of AMH on ovarian function is still evolving. Another TGFβ (beta) family member, follistatin, a glycoprotein, which is structurally distinct but functionally closely linked to inhibin and activin, is also discussed here. The site of production and areas of clinical interest of these peptide hormones are listed in Table 1.3.

Inhibins

The primary sources of inhibin production are the granulosa cells of the ovary and the Sertoli cells of the testis. Inhibin is also produced during pregnancy by the fetus, placenta, decidua, and fetal membranes. The main role of inhibin is to selectively suppress the production of FSH by the pituitary [86]. This is achieved by modulating FSH biosynthesis through two main mechanisms: by reducing steady-state FSH mRNA in pituitary gonadotropes [87] and decreasing the stability of FSH mRNA [88].

Inhibin is a 32 kDa glycoprotein heterodimer consisting of two subunits, α (alpha) and β (beta), linked by disulphide bonds [89]. There is not only a common α (alpha) subunit but also two types of β (beta) subunits known as β $(beta)_A$ or β $(beta)_B$. Thus, the two isoforms of inhibin are denoted

Table 1.3 Role of inhibins and activins in reproduction

Hormone	Site of production	Circulating levels	Clinical areas of interest/applications
Inhibins			
Inhibin A	Granulosa cells	↑ Luteal phase	Reflect dominant follicle growth
	Antral/dominant follicle	↑ Pregnancy	Prenatal screening for Down's syndrome
		↑ Ovarian tumors	Marker for certain ovarian tumors
Inhibin B	Granulosa cells	↑ Follicular phase	Reflects total output of growing follicles
	Small developing follicle	↑ Ovarian tumors	Predict response to ovulation induction/IVF
		↓ Perimenopause	Marker for certain ovarian tumors
			Marker for the onset of menopause
Activins	Granulosa cells	↑In luteal phase	Inhibits progesterone production by CL
	Pituitary/brain	↑Spontaneous labor	May prevent premature luteinization
	Adrenal cortex		May predict preterm delivery
			May relate to lack of follicle development
AMH	Granulosa cells of growing follicles	↓ Mulleriun ducts	Marker of ovarian reserve
		↓ Primordial follicle recruitment	Predict response to controlled ovarian hyperstimulation
		↓FSH response of growing follicles	Predict fertility outcome of assisted reproductive technology
Follistatin	Granulosa cells	↓ PCOS	May relate to lack of follicle development
	Antral follicles	↑PCOS	

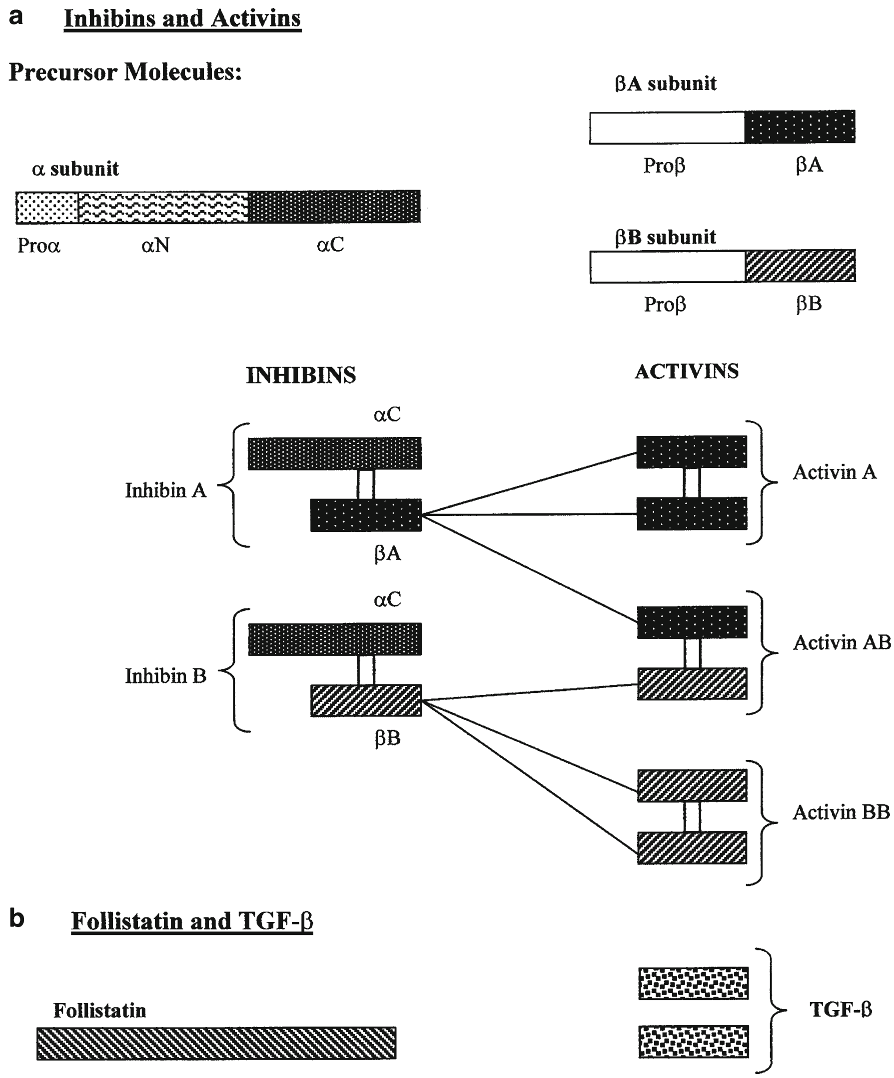

Fig. 1.4 Activins, inhibins, follistatin, and TGFβ (beta). A schematic representation of the formation of different dimeric proteins from three basic subunits, α (alpha), β (beta)$_A$, and β (beta)$_B$. Inhibins are heterodimers of α (alpha)and β (beta) subunits (α-βA, α-βB); while activins are homodimers of the β subunits (βAβA, βAβB, βBβB). Links between the units represent disulfide bridges *Pro* α, *Prepro alpha;* α*N, N-terminal alpha;* α*C, C-terminal alpha*. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

inhibin A and inhibin B. As shown in Fig. 1.4, each subunit derives from a separate precursor molecule called prepro α (alpha) (364 amino-acid residues), prepro β (beta)$_A$ (424 amino acid residues), and prepro β (beta)$_B$ (407 residues). These are processed by proteolytic cleavage to yield the mature forms [82]. In addition to the fully mature forms (αβ (alpha beta) dimers, Mr ~ 32,000), larger forms of dimeric inhibins with amino-terminally extended α (alpha) and/or β (beta) subunits have been identified in follicular fluid, which also possess FSH-suppressing bioactivity [90]. Furthermore, monomeric forms of both α (alpha) and β (beta) subunits and certain fragments (α (alpha)N and proα (alpha) N-α (alpha) C) generated during subunit processing are present in follicular fluid and have intrinsic biological activities distinct from classical inhibin-like bioactivity [91, 92].

Because the circulation contains multiple molecular forms of inhibin, it can be difficult to accurately measure inhibin levels using conventional RIAs. Also, conventional inhibin bioassays based on FSH suppression or release by cultured pituitary cells lack specificity due to FSH regulating activities of follistatin and activins. The development of two site immunoassays utilizing αβ (alpha beta) dimer-specific antibodies overcame these problems and allowed specific measurement of the two forms of inhibin dimers (A and B) [93]. With use of these novel two-site assays, inhibin levels are measured throughout the normal menstrual cycle [94].

Synthesis of the two isoforms of inhibin differs during the different phases of the menstrual cycle (see Fig. 1.3). Inhibin B levels are highest during the luteal–follicular transition and the early follicular phase, and studies on its presence in follicular fluid and basal granulosa cell secretion suggest that it is secreted by small developing antral follicles [95]. In contrast, inhibin A levels in the early and mid-follicular phase reflect the sum of FSH- and LH-stimulated inhibin A secretion from all antral follicles. Levels of inhibin A during the late follicular phase, however, mainly reflect secretion from the dominant follicle. Hence, inhibin A and B levels can be used as markers of follicular development. Inhibins have also been investigated as prognostic markers for women undergoing assisted reproductive technologies (ART). In particular, it was suggested that measuring inhibin B levels during the early stages of FSH stimulation for ovulation induction could predict the number of oocytes retrieved and may be useful in monitoring ovarian stimulation for in vitro fertilization. However, because there is a large overlap between normal and subnormal ovarian responses in terms of inhibin B levels, it may be just as effective to obtain Day 3 FSH or perform a clomiphene challenge test [96, 97]. Women in early perimenopause show significant decrease in inhibin B (no significant change in inhibin A and E2), which correlates with a mild increase in FSH levels. This perimenopausal decrease in inhibin B precedes inhibin A, suggesting that inhibin B may serve as a sensitive marker for the onset of menopause [98]. Studies investigating the role of inhibins in the pathophysiology of PCOS show conflicting results. Serum inhibin B levels in early follicular phase show significant increase in some studies or no change in others [99, 100]. Future studies are needed to confirm its role in PCOS.

During pregnancy, inhibin A is produced primarily by the fetoplacental unit, whereas inhibin B levels remain low throughout the pregnancy [101]. Because there is a twofold elevation of circulating inhibin A levels in the second trimester of Down's syndrome pregnancies, it is a clinically useful and important test [102]. When added to alpha-fetoprotein, maternal age and β (beta)-HCG (quad screen), the detection rate for Down's syndrome increased from 53 to 75 % [103].

Inhibin A has also recently been used as a tumor marker for ovarian sex cord tumors. This heterogenous group of tumors accounts for 7 % of all malignant primary ovarian neoplasms. They are composed of granulosa cells, thecal cells, Sertoli cells, Leydig cells, and other nonspecific stromal cells. It is important to distinguish this group of tumors from carcinomas and sarcomas, because the former are low-grade tumors with a better prognosis. Inhibin A and its α (alpha)-subunit have been found to be a sensitive immunohistochemical marker of most ovarian sex cord-stromal tumors [104]. Inhibin B, however, has been found to be elevated in sex cord-stromal and epithelial tumors and, hence, is of limited value in differentiating between the two entities. In addition, low levels of inhibin A in the cyst fluid of epithelial ovarian tumors has recently been reported to be associated with a worse prognosis [105].

Inhibin B has been found to be the predominant inhibin secreted in males and is produced by the Sertoli cells of the testes. It also has a negative-feedback role on FSH from the pituitary, and its production is regulated by spermatogenesis. Inhibin B levels correlate with sperm count and testicular volume [106, 107] but cannot distinguish between spermatidic arrest and obstructive azoospermia—a condition in which sperm counts are normal [108]. As such, it is unlikely to replace testicular biopsy in the evaluation of male infertility.

Activins

Activins are made up of dimers of the inhibin β (beta) subunit ($\beta_A\beta_A$, $\beta_A\beta_B$, or $\beta_B\beta_B$) and have a molecular weight of ~25 kDa (see Fig. 1.4) [109]. It is predominantly produced by the granulosa cells of the ovary. Activin/inhibin mRNA and protein have also been detected in extra-gonadal sources, including the placental trophoblast and decidua, testes, adrenal cortex, brain, spinal cord, and anterior pituitary. This implies that activin has diverse physiologic roles that are not confined to the reproductive system.

Acting either alone or with FSH, activin exerts an autocrine effect on granulosa cells to promote and maintain granulosa cell differentiation. It promotes FSH receptor expression on small undifferentiated granulosa cells [110], enhances their response to FSH and LH, and, hence, increases aromatase activity and estrogen production [111]. This may explain how small preantral follicles progress from a gonadotropin-independent to a gonadotropin-dependent stage of development. Having acquired FSH receptors, further growth and differentiation of granulosa cells to a preovulatory stage would be driven by activin acting in concert with FSH. Activin also inhibits both spontaneous and LH/hCG-induced increases in progesterone output by human follicles [111], implying that it plays a role in delaying the onset of premature luteinization.

Activin also has a paracrine effect on thecal steroidogenesis by inhibiting thecal androgen output. It has been proposed that at the earlier stages of follicular development, when the androgen requirements are low, thecal androgen synthesis is kept in check due to the relative excess of activin over inhibin and follistatin. However, as dominant follicles approach preovulatory status, increasing granulosa cell expression of inhibin and follistatin upregulates thecal androgen synthesis and ensures that the granulosa cells receive an adequate supply of aromatase substrate for conversion to estradiol. Activin stimulates pituitary FSH production, acting as a functional antagonist to inhibin (Fig. 1.5) [80]. It achieves this effect by increasing FSH mRNA synthesis as well as by increasing the stability of produced mRNA. Its actions are intimately modulated by intrapituitary concentrations of follistatin, which bind to activin to limit its bioavailability.

Free activin levels as measured by competitive protein binding assay, using follistatin as binding protein, showed very little change during the menstrual cycle [112]. However, activin levels were elevated throughout the cycle in older

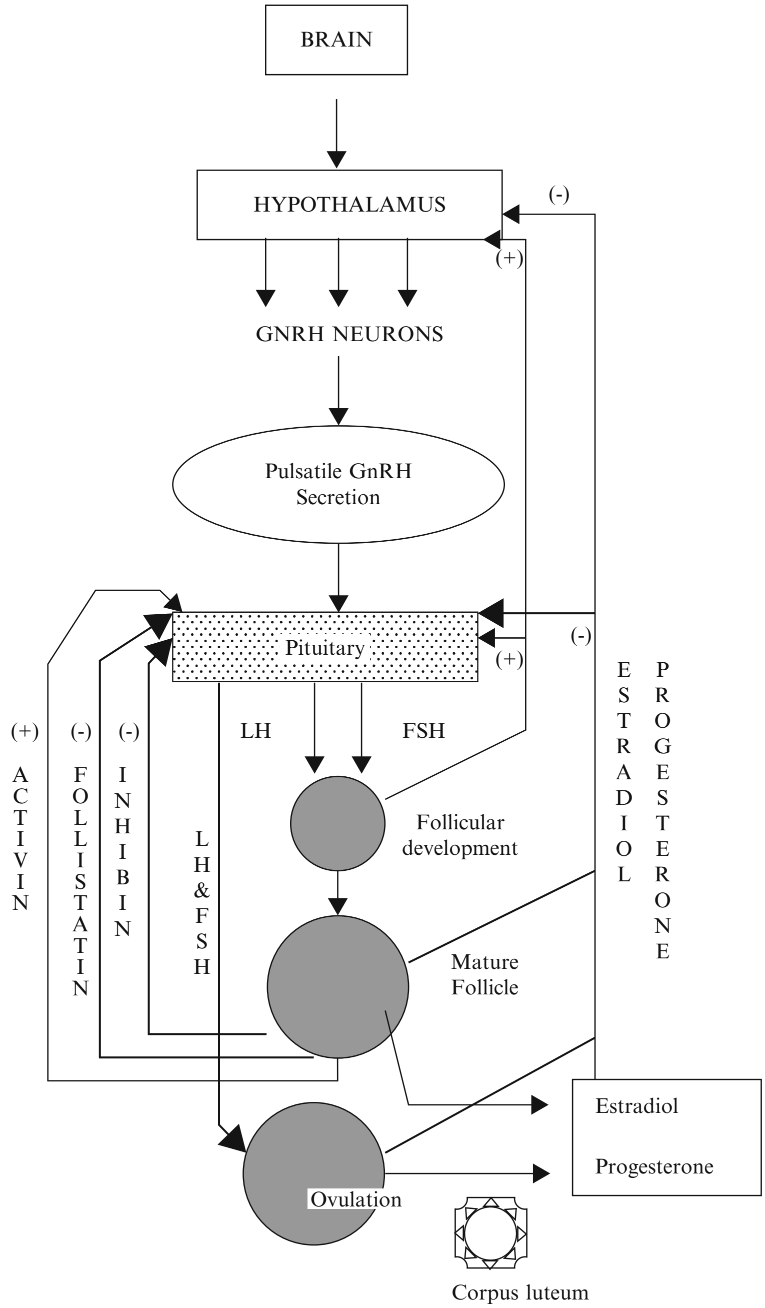

Fig. 1.5 A diagrammatic presentation of the hypothalamic–pituitary–ovarian axis. Developed follicles secrete steroid hormones (estradiol and progesterone) and peptide hormones (inhibin, activin, and follistatin) all collectively control secretion of gonadotropins. Estradiol and progesterone, depending on concentration, have either positive or negative feedback and can alter the frequency and/or amplitudes of pulses both at the level of the hypothalamus and pituitary. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

versus younger women, suggesting it may play an endocrine role in maintaining FSH elevation in reproductive aging [113]. Lower levels of activin are detected in PCOS patients with a simultaneous increase in inhibins and follistatin, suggesting that an imbalance in these hormones may contribute to an abnormal LH to FSH ratio [100, 114].

Anti-Müllerian Hormone

AMH, also known as Müllerian inhibiting substance, was initially recognized for its role in male sex differentiation. AMH produced by the fetal Sertoli cells prevents the development of müllerian ducts and other related structures resulting in the normal development of male reproductive tract. Absence of AMH results in the development of normal female phenotype [115]. AMH is a dimeric glycoprotein hormone that, like inhibins and activins, also belongs to the TGFβ (beta) superfamily. In the ovary AMH is produced by the granulosa cells from preantral and antral follicles and shows restricted expression only to the growing follicles, until they have reached the size and differentiation state at which they are selected for dominance by the action of pituitary FSH [116].

AMH is secreted in circulation and can be measured in serum. The first sensitive ELISA assay of AMH capable of detecting low level (~2.0 ng/mL) was described almost a decade ago. At present, two commercial ultrasensitive sandwich ELISA assays are available that have sensitivity approaching <0.1 ng/mL. Data generated with these assays have shown that serum AMH levels correlate and are proportional to the number of small antral follicles [117, 118]. AMH levels decrease with age and are undetectable in postmenopausal women and in women with premature ovarian failure [119]. Recently, age-specific serum AMH levels in women between the ages of 24 and 50 years are determined, showing steady decrease with increasing age [120, 121]. AMH has been evaluated by several groups as a potential novel clinical marker of ovarian reserve, such as in cancer patients, and of response to gonadotropins [122, 123]. AMH levels reflect the ovarian follicular pool, and the decrease in the number of small growing follicles is followed by the decrease in circulating AMH levels.

AMH levels are increased in women with PCOS as compared to normal controls likely due to the increased expression/synthesis and secretion of AMH by granulosa cells and are not effected by FSH administration [124, 125] but decrease during metformin treatment [126]. AMH measurement has been suggested as a sensitive diagnostic marker for PCOS and possibly as a substitute to follicle count, which is the current diagnostic criterion for PCOS.

Furthermore, in the last few years several large prospective studies have been published reporting extremely interesting new data on the possible clinical application of AMH measurement in the prediction of quantitative and qualitative ovarian response in ART [127, 128].

Follistatin

Follistatin, a single-chain polypeptide (315 amino acids), is produced in the ovary by the granulosa cells in antral follicles as well as by luteinized granulosa cells, which are under the positive regulation of FSH. It modulates the function of granulosa cells in favor of luteinization or atresia by neutralizing the effects of activin. It may also directly modulate progesterone metabolism by granulosa cells [129].

Follistatin functions as the binding protein for activin, thereby neutralizing it. Most of its biology is explained by its antagonism with activin. Follistatin exists in two forms; as full-length FS (FS 315) in the circulation and as processed isoform (FS 288) in follicular fluid [130] and the pituitary. It is part of an intrapituitary negative-feedback loop in which activin promotes FSH biosynthesis and the increased expression of follistatin limits its bioavailability for binding to the activin receptor on target cell membranes. It has been shown that the processed isoform of follistatin, FS 288, binds to cell-surface heparin sulfate proteoglycans with a higher affinity than FS 315 [131]. Since proteoglycans are anchored to cell membranes, this suggests that this would limit follistatin diffusion from the site of release, leading to high local concentrations of follistatin being maintained. The membrane-anchored follistatin would be able to compete with activin receptors on nearby cells, thus modulating the biologic effects of activin. Once bound to activin, follistatin is able to accelerate the endocytotic internalization and lysosomal degradation of activin by pituitary cells [132].

Follistatin levels as measured by sensitive and specific two-site enzyme immunoassay are significantly higher in women with PCOS in comparison to controls. Higher follistatin levels combined with lower activin levels in these patients suggest its role in the lack of pre-ovular follicle development and FSH suppression [100].

Growth Factors/Cytokines as Intraovarian Regulators

Certain growth factors have been implicated in cellular communications within the ovary including insulin-like growth factors (IGFs: IGF-I and IGF-II), epidermal growth factor (EGF), transforming growth factor α (TGFα (alpha)), basic fibroblast growth factor (bFGF), cytokines such as interleukins (IL-1 and IL-6) and tumor necrosis factor (TNFα (alpha)). Growth factors and cytokines that are important as intraovarian regulators are outlined in Table 1.4 and are discussed here.

Insulin-Like Growth Factors

IGF-I and IGF-II promote cellular mitosis and differentiation in a variety of systems and play an important role in modulating folliculogenesis in an autocrine/paracrine fashion [133–135]. IGFs consist of two single-chain polypeptide growth factors that are structurally and functionally similar to proinsulin. The IGF autocrine/paracrine system includes IGFs, their specific receptors in target cells, and a family of IGF-binding proteins (IGFBPs) that regulate their bioavailability. Both IGF-I and IGF-II are produced in the ovary and augment the effects of the gonadotropins, although the main IGF in human follicles is IGF-II [136].

In small antral follicles, both IGF-I and IGF-II are expressed but restricted to thecal cells. In dominant follicles, however, no IGF-I mRNA has been detected and IGF-II expression is restricted to granulosa cells only. IGF-I receptors are expressed in granulosa cells only, and IGF-II receptors are expressed in both cell types [134]. Thus, in small antral follicles IGF-1 functions as an autocrine regulator in thecal cells and as a paracrine regulator in granulosa cells; in the dominant follicle, IGF-1 functions mainly as a paracrine regulator [137, 138] and IGF-II acts as an autocrine factor. This suggests that IGF-II has an important role in coordinating differential follicular development within the ovary.

Table 1.4 Autocrine and paracrine growth factors and cell-cell signaling in the ovary

Factor/hormone	Site of production	Site of action (type)	Function
TGFα (alpha) and EGF	Theca cells	Theca (autocrine) granulosa (paracrine)	Growth stimulation
Interleukin-1	Granulosa cells	Granulosa (autocrine)	Cellular differentiation
Interleukin-6	Granulosa cells	Theca, granulosa	
Interleukin-8	Theca cells	Theca, granulosa (auto/paracrine)	
TGFβ	Theca cells Granulosa cells	Granulosa, theca (auto/paracrine)	Growth inhibition Cell differentiation
IGF-I IGF-II	Granulosa cells Theca cells	Granulosa, oocyte, and theca (auto/paracrine)	Growth stimulation Cell differentiation
Bone morphogenic proteins (BMPs)	Theca cells	Granulosa (paracrine)	Cell differentiation
Inhibins	Granulosa cells	Oocyte, theca, and granulosa (auto/paracrine)	Cell differentiation
Activins	Granulosa cells	Granulosa (autocrine)	Cell differentiation
AMH	Granulosa cells of growing follicles	Granulosa cells Theca cells (auto/paracrine)	Growth inhibition Cell differentiation
Follistatin	Granulosa cells Antral follicles	Granulosa cells (autocrine)	Growth inhibition

Epidermal Growth Factor/Transforming Growth Factor α and Basic Fibroblast Growth Factor

A number of other peptide growth factors have been implicated as regulators of follicular development and steroidogenesis (see Table 1.4) [139]. These include EGF, TGFα (alpha), and bFGF. EGF is a single polypeptide chain of 53 amino acids with three disulphide bonds and has mitogenic effects in a variety of ectodermal and mesodermal tissues. TGFα (alpha) is a 50 amino-acid peptide with 30–40 % homology with EGF. The EGF receptor is a 170-kDa glycoprotein with tyrosine kinase activity. TGFα (alpha) binds to EGF receptors with the same affinity as EGF.

The presence of EGF/TGFα (alpha) and bFGF as well as their receptors has been shown both at the protein and mRNA levels in the preovulatory follicles [140] and corpus luteum [141, 142]. Furthermore, studies have shown the presence of TGFα (alpha) [143] and bFGF [144] mRNAs in follicular cells and that the TGFα (alpha) message is upregulated by FSH in vivo. FSH plus TGFα (alpha) or FSH plus EGF resulted in significantly elevated progesterone and 20α (alpha)-hydroxyprogesterone levels in granulosa cells in culture [145]. The presence of TGFα message in cultured granulosa cells and the fact that it mediates its action via binding to EGF receptor all point to its autocrine role in granulosa cell differentiation, follicle development, and selection. bFGF has been shown to be mitogenic for granulosa cells and to cause an inhibitory action on granulosa cell differentiation and thecal cell steroidogenesis [146, 147]. It also has potent angiogenic activity [148].

Cytokines

Cytokines primarily produced by white blood cells modulate various cellular functions. The cytokines in the ovary are secreted both by the immune cells that are recruited from the circulation in the ovarian stroma as well as by the thecal and granulosa cells. A number of cytokines have been linked to modulate ovarian function and include interleukins (IL-1 and IL-6) and TNFα (alpha). Both IL-1 and IL-6 have been found in significant amounts in follicular fluid [149, 150]. Granulosa cells accounted for the majority of immunostaining for IL-1 and IL-6 in follicular aspirates, which suggests that these cytokines are produced in granulosa cells [150, 151] and that they affect granulosa function [152]. During folliculogenesis, IL-1 promotes proliferation and suppresses differentiation. In the ovulatory process, it promotes ovulation by increasing production of chemokines, steroids, eicosanoids, and vasoactive substances [153]. IL-6 demonstrates inhibitory effects on both E2 and progesterone secretion by FSH-stimulated granulosa cells [154]. Elevated IL-6 levels during genital infections may provide a possible link to reproductive dysfunction. TNFα (alpha) expression has also been detected in granulosa cells of human antral and atretic follicles by immunohistochemistry [155]. Also, in vitro treatment with TNFα (alpha) enhanced steroidogenesis in both healthy and atretic follicles [156], suggesting that TNFα (alpha) has a paracrine and/or autocrine role. Nonetheless, the physiological implications of these actions remain unclear and require further investigation.

Neuropeptides

Some evidence suggests that an independent ovarian-central nervous system axis exists [157]. Electrical stimulation of the hypothalamus in hypophysectomized and adrenalectomized rats produced a change in ovarian steroidal synthesis that was independent of changes in ovarian blood flow [158]. In addition, murine thecal cells can produce androgens under adrenergic stimulation [159]. Adrenergic innervation of the ovary acts primarily on the thecal–interstitial cells through β (beta)$_2$ receptors, synergizing with the effects of gonadotropins in the production of ovarian androgens [159]. This may in turn play a role in the regulation of estrogen production by granulosa cells, thereby influencing follicular recruitment and selection.

Mechanisms of Action of Hormones

Hormones exert their biologic effects by interacting with the high-affinity receptors expressed on target cells, which in turn trigger one or more effector systems within the cell. The high affinity, specificity, and the receptor expression level together define the nature and degree of biologic response of a hormone. All receptors have at least two functional domains: a recognition domain and a signal-generating domain. The recognition domain binds to the hormone, and the second domain generates a signal that couples hormone recognition to some intracellular function. This coupling of hormone binding to signal transduction, or "receptor-effector coupling," provides the first step in the amplification of a hormonal response and distinguishes the target cell receptor from the plasma carrier proteins that bind hormone but do not generate a signal.

Just on the basis of the location of the hormone receptor (i.e., intracellular/nuclear or cell surface), two distinct mechanisms of hormone actions can be classified. These mechanisms further differ by the nature of the signal transduction pathway or second messenger responsible to mediate hormone action (Table 1.5). Examples of nuclear receptors include steroid hormones that are lipophilic and pass through the cell membrane to interact with receptors located either within the cytoplasm or the nucleus. This in turn affects gene transcription within the nuclear compartment. Polypeptide hormones (i.e., LH, FSH, HCG, GnRH, inhibins, and activins) and growth factors that are hydrophilic interact with cell surface receptors that are located on the plasma membrane at the cell surface. They trigger a plethora of signaling activity in the membrane and cytoplasmic compartments as well as exert parallel effects on the transcriptional apparatus in the nuclear compartment. These cell surface receptors can be further classified based on the second messenger into four major subgroups as listed in Table 1.5.

Table 1.5 Classification of receptors for steroid and peptide hormones

Hormones that bind to intracellular/nuclear receptors	Hormones that bind to cell surface receptors
Type 1 receptor: classical steroid hormone receptors	*Seven transmembrane domain receptors*
Requires ligand binding for activation	1. Second messenger is cAMP
Homodimerization pattern	LH and FSH
Estrogen receptor	2. Second messenger is calcium and/or phosphati-dylinositols
Progesterone receptor	GnRH and TRH
Androgen receptor	3. Second messenger is cGMP
Glucocorticoid receptor	Nitric oxide and atrial natriuretic factor
Mineralocorticoid receptor	*Single transmembrane domain receptors*
Type 2 receptors	1. Intrinsic kinase activity
Able to bind DNA in the absence of ligand, exerting repressive effect	a. Second messenger is tyrosine kinase
Hetero-dimerization pattern	IGF, Insulin, and EGF
Thyroid hormone	b. Second messenger is serine kinase
Retinoic acid receptor	TGFβ (beta) super family
Vitamin D receptor	Activin, inhibins, and AMH
Retinoid X receptor	2. Acquired kinase activity by interaction with transducer molecules
Orphan receptors	Growth hormone
Ligand unknown	Prolactin

Steroid Hormone Action

Nuclear Receptors Superfamily

Steroid hormone nuclear receptors (estrogen receptor [ER], progesterone receptor [PR], and androgen receptors [AR]) are ligand inducible transcription factors that regulate the expression of target genes involved in reproduction and metabolism. They belong to the superfamily of nuclear hormone receptors and share many structural and functional features [160]. Other members of the superfamily include receptors for glucocorticoids (GR), mineralocorticoids (MR), thyroid hormones (TR), 1.25-dihydroxy vitamin D_3 (VDR), retinoic acid (RAR), and an ever-increasing number of "orphan" receptors, which show structural similarity but for which ligands are not known. Within this nuclear receptor superfamily, two main groups have been identified based on the differences in their functional and recognition characteristics [161]:

- *Type 1* ("*steroid*" *or* "*classical*") *receptor subfamily*. This includes the ER, PR, AR, GR, and MR. These receptors cannot bind to DNA in the absence of ligand, and thus remain functionally silent. They exist as cytoplasmic/nuclear, multimeric complexes that are in association with heat shock proteins (e.g., HSP 90, HSP 70, and HSP 56). The association of the ligand with the receptor and the dissociation of the HSPs are required for the activation of the receptor.
- *Type 2 receptors subfamily*. This includes the TR, RAR, VDR, and the retinoid X receptor (RXR). In the absence of ligand, these receptors can bind to DNA and exert the repressive effect or silence their respective promoters. Unlike steroid receptors, type 2 receptors bind constitutively to response elements and are capable of forming

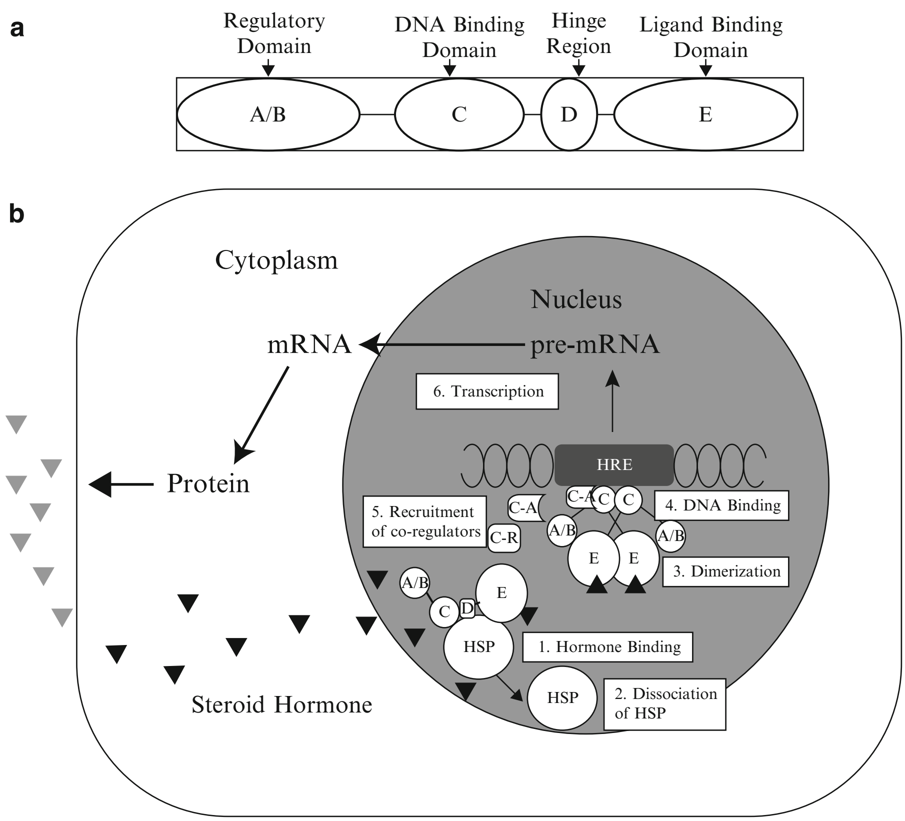

Fig. 1.6 Basic structure and general mechanism of action for cytoplasmic/nuclear steroid hormone receptors. (**a**) Basic structure of steroid hormone receptor. (**b**) Mechanism of action involving steps 1 through 6 as described in the text. *HSP90* heat shock protein; *HRE* hormone response element; *C-A* coactivators. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

heterodimers with RXR. These interactions may serve to modulate the amplitude of the transcriptional response to the ligand.

Most of the early knowledge about steroid hormone mechanism of action has been derived from in vitro and in vivo binding studies that used radiolabeled estradiol as a ligand [162]. The role of ER in the regulation of breast cancer growth has been well studied and was the first steroid receptor to be discovered in the early 1960s. This led to the subsequent elucidation of a general pathway for steroid hormones action. In general, nuclear receptors share a common protein architecture consisting of five functional domains [163], as illustrated in Fig. 1.6a and described below.

Amino-Terminal Transactivation Domain (A/B Region)

This is the most variable domain in terms of sequence and length in the family of nuclear receptors. It can range in size from 20 amino acids in the VDR to 600 amino acids in the MR. It usually contains a transactivation function (TAF-1), which interacts with other core transcriptional machinery (e.g., coactivators) to activate target gene transcription.

Central DNA-Binding Domain (C Region)

This region is essential for activation of transcription. It encodes two zinc finger motifs and has a very high degree of homology between all types of nuclear receptors. Hormone binding to the receptor induces specific conformational changes in this region, allowing receptor binding to the hormone responsive element (HRE) of the target gene. The amino acid sequence that lies between the first and second zinc fingers (i.e., the recognition helix) is responsible for establishing specific contact with the DNA. The second zinc finger stabilizes the contact and increases the affinity of the receptor for the DNA.

Hinge Region (D Region)

As the designation hinge indicates, this region is the site of rotation that allows the protein to alter its conformation after ligand binding. It provides the localization signal that is important for moving the receptor to the nucleus in the absence of ligand and contains a nuclear localization domain (GR, PR) and/or a transactivation domain (TR, GR).

Carboxy-Terminal Ligand-Binding Domain (E Region)

This region is responsible for binding of the relevant ligand and is responsible for receptor dimerization or heterodimerization. It also contains the binding site for HSPs and has a transactivation function (TAF-2) that can drive transcriptional activity. Unlike TAF-2, its transcriptional activity depends on hormone binding. Conformational change that occurs after ligand binding is responsible for interaction with coactivators or corepressors.

Cellular Mechanism of Action of Steroid Hormone

As mentioned before, the steroid hormone nuclear receptors are known as ligand-dependent transcription factors. Binding with their ligands is a necessary step for their function as transcriptional regulators. Unliganded receptors may be localized to either the cytoplasm (e.g., GR) or the nucleus (e.g., ER, PR, and TR). For most steroid hormones, the unliganded receptors exist in the cell nucleus as large molecular weight oligomers (~300 K; 7–10S sedimentation rate) [164] and can be isolated in cytosolic fraction from cells or tissues disrupted in hypotonic media. The oligomers are formed by non-covalent association of a monomeric receptor protein with a dimer of heat shock protein (HSP 90, 70, or 56) [165].

The general features of the mechanism of action of these hormones are depicted in Fig. 1.6b. Steroid hormones that freely diffuse through the cell membrane bind to the specific receptors in the nucleus. Ligand binding to receptor initiates the receptor transformation or so called **"activation" process**. During this process the receptor undergoes conformational changes that primarily occur as a result of its dissociation from HSP, which exposes the DNA binding site. Nuclear translocation and **dimerization** of the activated receptor then occurs. Most evidence suggests that this process is thermodynamically irreversible. The hormone receptor complex then binds to a specific region of DNA, the **hormone response element (HRE)**, which is located upstream of the gene. The first HRE was identified for GR. Later the HREs for PR, AR, ER, and MR were shown to be similar to that of GR and are palindromic (inverted-repeat) DNA sequence of 15 bp [166]. This interaction leads to the recruitment of a host of ancillary factors known as coregulators (coactivators or corepressors), creating a transcriptionally permissive or nonpermissive environment at the promoter, as well as communicating with other general transcription factors and RNA polymerase II. Coactivators function as adaptors in a signal transduction pathway. It is the binding of these coregulators that modulates the resulting transcription (i.e., the activation and inactivation of specific genes). Hormone antagonists, for example, induce a different conformation in the TAF-2 that hinders the coactivator-binding site and recruits a corepressor instead and inhibit the gene expression. The availability of these coregulators in different tissues plays an important role in defining the biological response to both steroid hormone agonists and antagonists [167].

The biologic activity of a hormone is determined by at least four factors intimately related to the structure of the hormone receptor in question. The first factor is the affinity of the hormone for the hormone-binding domain of the receptor. The second factor is the differential expression of receptor subtypes in the target tissue, altering the response to the same hormone. The third factor is the conformational shape of the ligand–receptor complex and its consequent effects on dimerization and the modulation of adaptor proteins. The last factor is the differential expression of target tissue adaptor proteins and phosphorylation. A higher concentration of coactivators or corepressors in the target tissue can affect the cellular response of that tissue to the same ligand. Phosphorylation of the receptor by protein kinases increases the transcriptional activity of the receptor.

The Estrogen Receptor

Structure and Function

The structure of ER (now known as ER α (alpha)) was reported in 1986 [168]. It consists of five components or domains that are divided in to six regions referred to as A–F (Fig. 1.7), instead of five regions seen in most steroid receptors. The F region is a C-terminal segment of 42 amino acids that influences the conformational changes that occur after estrogen/antiestrogen binding. Thus, it modulates the level of transcriptional activities, most likely via affecting the interaction with coregulator proteins. It has a molecular weight of 66,000 and contains 595 amino acids. Estrogen receptor mRNA is 6.8 kb and contains eight exons derived from a gene located on long arm of chromosome 6. More recently, a second form of ER has been discovered and named ERβ (beta), which is encoded by a gene located on chromosome 14 [169] and is in close proximity to the genes that are related to Alzheimer's disease [170].

The two receptors show a high degree of homology in the DNA-binding domain (97 %) and ligand-binding domain (59 %) but less so to hinge (30 %), regulatory (17 %), and F regions (17.9 %) [170, 171] (see Fig. 1.11). Hence, the binding characteristics of these two receptors are similar, although they differ significantly in their ability to activate gene transcription by regulatory domain TAF-1, which is minimal or absent in ERβ (beta). Both ERα (alpha) and ERβ (beta) are required for normal ovarian function as shown by specific receptor knockout studies in mice [172]. ERα (alpha) is primarily responsible for estrogenic effects in other tissues, including the uterus.

17β (beta)-estradiol binds to the estrogen receptor with a much higher affinity than estrone or estriol. In addition, the binding of estradiol to its receptor and its subsequent activation also enhances *cooperativity*, meaning that the action of estradiol binding to one site increases the affinity for it to bind to another site, enabling the receptors to respond to small

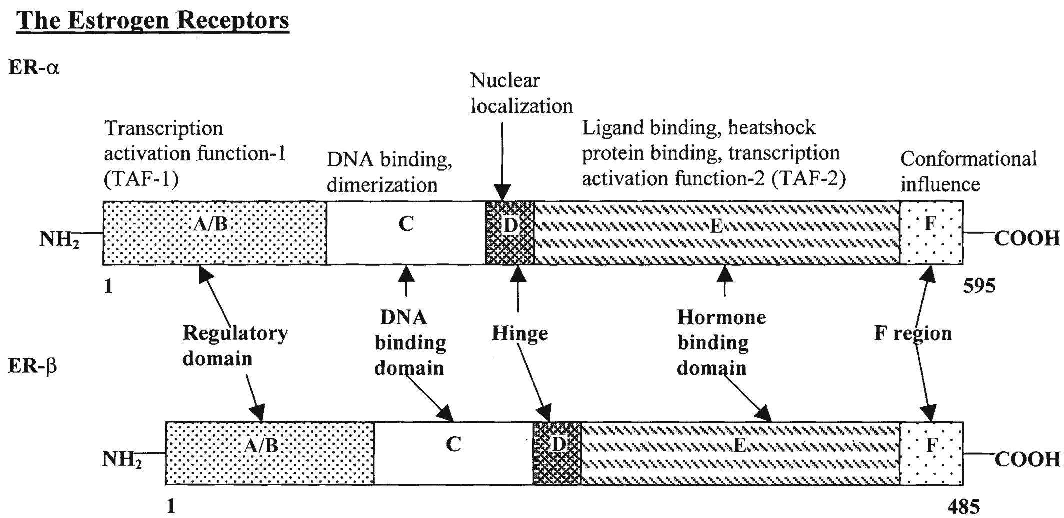

Fig. 1.7 A schematic diagram of two estrogen receptor (ER) isoforms. Different domains (A–F) and their corresponding functions are illustrated. *ERα (alpha)* estrogen receptor-α; *ERβ (beta)* estrogen receptor-β. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

changes in the hormone concentration. Estrogen's relatively long duration of action is also due in part to the high affinity state achieved by its receptor. On the other hand, clomiphene exerts its antiestrogenic effects by *negative cooperativity*, preventing the transition of the estrogen receptor from its low-affinity state to its high-affinity state.

The two receptors (ERα (alpha) and ERβ (beta)) are differentially expressed in different tissues, leading to the differences in the response to the same hormone subtypes [173]. The α (alpha) receptors are predominantly expressed in breast cancer tissue, ovarian stroma, and endometrium. ERβ (beta) receptors, on the other hand, are expressed in several nonclassic target tissues, including the kidney, intestinal mucosa, lung, bone, brain, endothelial cells, and the prostate gland. 17β (beta)-estradiol and estrone have a higher affinity for α (alpha) receptors and thus exert their effects predominantly on target tissue with α (alpha)-receptor expression. Phytoestrogens such as genistein and coumestrol, on the other hand, bind predominantly to β (beta) receptors [174] and would be expected to exert their effects on target tissues expressing these receptors.

The conformation change of the ligand-binding domain also differs in both α (alpha) and β (beta) estrogen receptors, depending on which ligand has been bound to the receptor [175]. This distinct conformational change is the major factor that determines the receptor's ability to interact with coactivators or repressors. For example, estradiol activates transcription when it binds to ERα (alpha) but inhibits transcription when bound to ERβ (beta). Raloxifene and tamoxifen, on the other hand, inhibit transcription when forming complexes with ERα (alpha) and activate transcription when bound to ERβ (beta).

The differential expression of target tissue adaptor proteins and phosphorylation also affect gene transcription. A higher concentration of coactivators or corepressors in the target tissue can affect the cellular response of that tissue to the same ligand. Phosphorylation of the receptor by protein kinases increases the transcriptional activity of the receptor. For example, growth factors such as EGF and IGF-1 can stimulate protein kinase phosphorylation, activating the estrogen receptor—even in the absence of estrogen.

Somatic mutations in ERα (alpha) are described and may be associated with certain disease states. A nonsense mutation (premature stop codon) in ERα (alpha) has been described in a patient with decreased bone mineral density, increased bone turnover, and incomplete closure of bone epiphysis, which demonstrates its role in bone growth and homeostasis [176]. ER mutations have also been detected in patients with breast cancer. Such mutations include exon 5-deletion within the LBD leading to constitutively active receptor and exon 7 deletion displaying dominant negative activity and inhibition of ER function [177].

Antiestrogens and ER

Compounds with antiestrogenic activity can be classified into two categories: one with pure antiestrogenic activity and others with both agonist and antagonist properties. Tamoxifen—an antiestrogen that is used both as chemopreventive agent and as hormonal therapeutic agent for breast cancer—inhibits ER action [178].

Paradoxically, tamoxifen acts as an estrogen in uterine tissue, and this tissue-specific estrogenic effect is the reason why prolonged tamoxifen therapy increases the risk of uterine

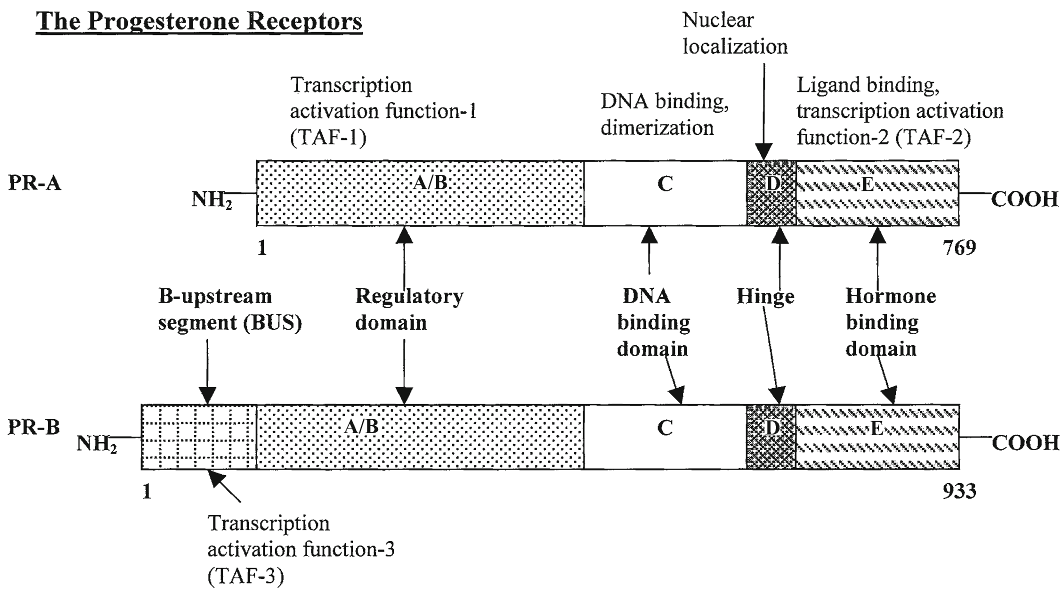

Fig. 1.8 A schematic diagram of progesterone receptors isoforms. Different domains (A–E) and their corresponding functions are shown. *PR-A* progesterone receptor-A; *PR-B* progesterone receptor-B. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

cancer [179]. Raloxifene, a related benzothiophene analog, retains its antiestrogenic effect in breast and uterine tissue. Both tamoxifen and raloxifene have estrogen-like effects on nonreproductive tissues such as bone and heart and lung tissue [180].

Tamoxifen acts by competing with estrogen for receptor binding. As estrogen-binding affinity is of higher magnitude than tamoxifen, severalfold higher concentrations of tamoxifen are required to inhibit estrogen action. The agonistic or antagonistic effect of tamoxifen is determined by the presence of different promoter elements in the specific cell type [181]. Estrogen binding to receptors activates both transcription domains (i.e., TAF-1 and TAF-2). Tamoxifen's agonistic activity is due to activation of TAF-1, and its antagonistic activity is due to its ability to inhibit the estrogen-dependent activation of TAF-2. The ligand-binding sites for estrogen and antiestrogen are not identical, and tamoxifen binding on receptors induces conformational changes that alter interaction with estrogen-associated proteins and modulate transcriptional activity [182]. Tamoxifen also activates ER-mediated induction of promoters that are regulated by TAF-1 site, which explains why it has estrogenic effects on the endometrium—a tissue with significant TAF-1 transcription function. In other cell types, such as those in the breast, TAF-1 has weak transcriptional activity. Hence, antiestrogens have no effect on TAF-1-mediated transcription [183]. Raloxifene, on the other hand, may activate estrogen-responsive genes through a response element that is distinct from ERE [184].

Pure antiestrogens are derivatives of estradiol that have a long hydrophobic side chain at position 7. Examples of pure antiestrogens include ICI 164,384 and ICI 182,780 (Fulvestrant). Binding of pure antiestrogens may sterically interfere with the dimerization process and thus inhibit DNA binding. Furthermore, these compounds increase the rate of receptor degradation and also inhibit ER-mediated transcription by preferential binding to corepressors, which contribute to their antiestrogenic activity [185].

Progesterone Receptor

Structure and Function

Like ER, there are two major forms of PR: PR-A and PR-B receptors, which are derived from the same gene. PR-A and PR-B are identical except that PR-B contains an additional 164 amino acids sequence at the N-terminal end, which is referred to as the B-upstream segment (BUS) (Fig. 1.8). PR-A has a molecular weight of 94 kDa and contains 768 amino acids and PR-B is 114 kDa with 933 amino acids. The two forms derive from two distinct estrogen-regulated promoters [186]. The transcription function domain (TAF-1) in PR is located in the 91 amino acid segment of the regulatory region, and TAF-2 is located in the hormone-binding domain. In PR-B, the BUS contains a third activation domain TAF-3, which can synergize the actions of other TAFs or autonomously activate transcription [187]. TAF-3 recruits and allows a separate subset of coactivators to bind with PR-B that do not interact efficiently with PR-A. Thus PR-A and PR-B display different transactivation properties that are both cell-specific and target gene promoter specific [188]. The two isoforms

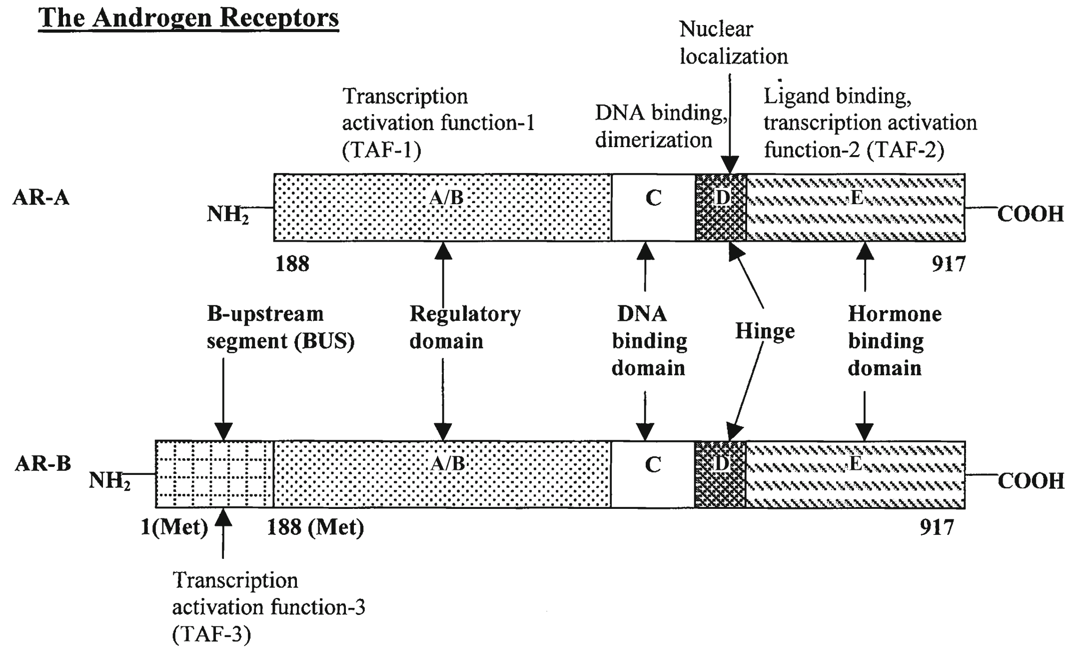

Fig. 1.9 A schematic diagram of androgen receptor isoforms. The androgen receptor is very similar to the progesterone receptor and exists in a shorter A form and a full-length B form. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

PR-A and PR-B have distinct cellular localization, and in the absence of ligand binding, PR-A is predominantly localized in the nucleus and PR-B is present in the cytoplasm [189].

The role of PR isoforms is not yet fully elucidated. Selective ablation of PR-A expression in mice resulted in severe abnormalities in ovarian and uterine function that lead to infertility but did not affect progesterone responses in the mammary gland or thymus [190]. In contrast PR-B ablation does not affect ovarian, uterine, and thymic responses to progesterone and manifests as reduced mammary ductal morphogenesis. Thus, PR-A is essential for female fertility, and in the absence of PR-A, PR-B functions in a tissue-specific manner and mediates some of the PR actions in the mammary gland [190]. However, transgenic mice caring an extra copy of PR-A gene have abnormal mammary gland development, indicating that overexpression of PR-A may have physiologic significance.

The relative levels of the two isoforms differ in the endometrium during the menstrual cycle [191]. In the uterus, progesterone action downregulates cell cycle arrest proteins but upregulates growth factors and their receptors and other regulators [192] and is also essential for the initiation and maintenance of pregnancy. Progesterone antagonist RU 486 (generic name mifepristone) initially was synthesized as a GR antagonist [193], but later was found to display marked anti-progesterone activity [194]. It binds to GR with threefold higher affinity than dexamethasone and to PR with a fivefold higher affinity than natural progesterone [195]. Unlike progesterone, the RU486-PR complex inhibits transcription because it has a slightly different conformational change in the TAF-2 domain [196]. If implantation occurs, it downregulates progesterone-induced genes and results in decidual necrosis and detachment of the conception products [181, 195].

Androgen Receptor

Structure and Function

The androgen receptor gene was cloned in 1988 and was localized on human X chromosome between the centromere and q13 [197]. Like PR, AR also exists in two forms: a full-length B form and a shorter A form (molecular weights ~110 and 87 kDa); both are encoded by the single gene [198]. The 87 kDa isoform (AR-A) contains the intact C terminus but lacks 188 amino acid residues in the N terminus of the 110 kDa isoform (AR-B) (Fig. 1.9). The ratio of AR-B–AR-A in genital skin fibroblasts from healthy subjects is 10:1. It is not known whether there are functional differences between these isoforms [198]. The DNA-binding domain or TAF-2 of AR is similar to TAF-2 regions of other steroid hormone receptors (PR, ER, GR, and MR) but is related most closely to PR [199]. Progesterone shows crossreactivity with androgen receptors, to a degree that becomes clinically relevant only at pharmacologic doses.

In most tissues, testosterone is converted to DHT via action of enzyme-5α (alpha)-reductase. DHT binds to AR with a higher affinity than testosterone, leading to greater stabilization of the receptor and more efficient signaling, which amplifies the androgen action. Hence, the efficiency of local conversion of testosterone to DHT is an important intracellular step in the androgen response.

A large number of AR mutations have also been described that can alter receptor function [200, 201]; for example, inactivating point mutations in the hormone-binding domain of the androgen receptor can generate different phenotypes that lead to partial to complete androgen insensitivity. A point mutation at residue 689 that substitutes proline to histidine may alter the conformation of LBD, which reduces AR's affinity for DHT and abolishes its ability to transactivate ARE [202]. A serine to proline substitution at residue 865 eliminates androgen binding and transactivation; thus, it also causes complete androgen insensitivity [203]. At residue 807, a substitution of threonine for methionine causes partial androgen insensitivity by reducing—but not abrogating—the androgen binding to AR. However, a valine or arginine substitution at the same site totally abrogates androgen binding and causes complete androgen insensitivity syndrome [204].

Non-genomic Actions of Steroids

Some steroid hormone actions are independent of their classic genomic actions as mediated via nuclear receptors—these are called non-genomic actions [205]. These effects are rapid, occur within seconds, and are not affected by inhibitors of gene transcription like actinomycin D or inhibitors of protein synthesis like cycloheximide. These rapid actions include sodium and calcium ion transport and some neural and cardiovascular effects [206]. The messenger and effector system vary with the steroid and the cell type. Thus far, studies have suggested that specific binding sites or receptors are present on the cell membrane and that steroid binding triggers rapid changes in the electrolyte transport system. For example, estrogens have been shown to modulate some cardiovascular effects by inducing Ca^{2+} flux and vasodilation in the coronary artery [207]. Furthermore, steroids can activate second messenger pathways, generating second messengers capable of altering gene transcription independently of their classical receptor-mediated gene transcription [208, 209]. Thus, steroid hormones have non-genomic effects as well as genomic effects that are mediated by both classical steroid receptor-mediated pathways and second messenger pathways.

Peptide Hormone/Growth Factor Action

Pituitary gonadotropin signaling plays a key role in follicular growth, ovulation, and luteinization. LH, FSH, and GnRH act via their seven transmembrane domain receptors, also known as G-protein-coupled receptors (GPCRs) because they depend on G-protein transducers to execute their biological effects. However, it is increasingly recognized that their actions are also dependent on their interaction with peptide/growth factor signaling pathways, including IGFs, EGF, and members of TGFβ (beta) family (Fig. 1.10). Understanding these pathways provides insight into how these factors interact and complement the FSH/LH pathway to control follicular growth.

Family of Receptors with Tyrosine Kinase Activity

Insulin, IGFs, and EGF receptors belong to a distinct group of receptors and differ from the GPCRs both structurally and functionally. Unlike the GPCRs, they only span the membrane once and acquire their signaling ability through the activation of tyrosine kinase, which is intrinsic to these individual receptor molecules. Hence, they are known commonly as "tyrosine kinases." Their main ligands include hormones such as insulin and IGF, as well as paracrine and autocrine regulators like platelet-derived growth factor (PDGF), bFGF, and EGF. Thus, via tyrosine phosphorylation, a number of physiological processes like cell proliferation, cell migration, cell differentiation, and apoptosis are mediated by these receptors. This explains why this group of receptors has been the target of much oncogenic research.

All tyrosine kinase receptors have a similar structure: an extracellular domain for ligand binding, a single transmembrane domain, and a cytoplasmic domain. Ligand specificity is determined by the unique amino acid sequences making up the extracellular domain, which determines the three-dimensional conformation of the receptor. The transmembrane domains are heterogenous, whereas the cytoplasmic domains are fairly homologous. They respond to ligand binding by undergoing conformational changes and autophosphorylation. The structure of the IGF-1 receptor is strikingly similar to the insulin receptor with two transmembrane domains linked by disulfide bridges, formed by two α (alpha) and β (beta) subunits [210]. The IGF-1 receptor gene is located on chromosome 15 at bands q25-26 and contains 21 exons [211].

The steps involved in signal transduction by IGF-1/insulin have been extensively reviewed in recent years [212, 213] and are illustrated in Fig. 1.10. The association of the ligand (e.g., insulin) with the receptor's extracellular domain triggers receptor dimerization. This results in the phosphorylation of tyrosine residues on both the receptor and non-receptor substrates. The phosphorylation of receptor tyrosine residues occurs at specific locations, which causes these sites to associate with a variety of accessory proteins that have independent signaling capabilities. These accessory proteins include phospholipase Cγ (gamma) (PLCγ (gamma)), PI3 kinase (PI3K), GTPase-activating protein (GAP), and growth factor receptor-bound protein 2 (GRB2). These interactions are mediated by the presence of highly conserved type 2 *src*

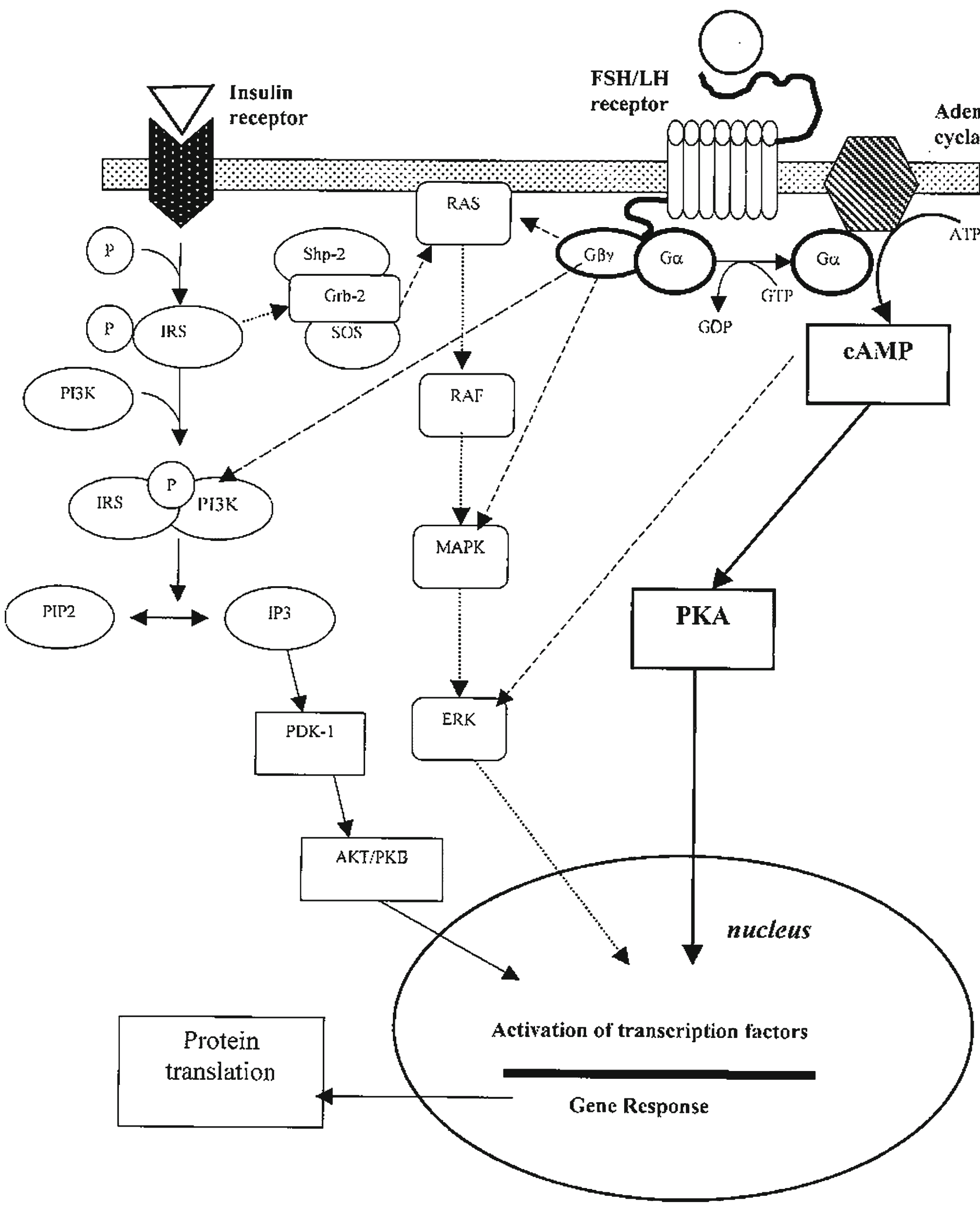

Fig. 1.10 A diagrammatic representation of the interlinked signaling pathways between FSH/LH G-protein-coupled receptors (GPCRs) and insulin/IGF-1 receptor tyrosine kinases in ovarian cells. Activation of GPCR by hormone binding stimulates Gα (alpha) subunit to bind GTP instead of GDP leading to its dissociation from β(beta)/γ(gamma) subunits to activate downstream signaling factors like adenylyl cyclase, which synthesizes second messenger cAMP. Binding of cAMP in turn activates protein kinase A (PKA) leading to DNA binding and downstream cellular response. Also illustrated here is the IGF-1R signaling pathway. IGF-1/insulin binding to the receptor initiates autophosphorylation and tyrosine phosphorylation of insulin receptor substrates (IRS), leading to activation of PI3K and generation of 3-phosphorylated-inositol (IP3) from phosphoinositol (PIP2), which activates PI-dependent protein kinase-1 (PDK-1). PDK-1 in turn activates Akt/protein kinase B (Akt/PKB), leading to biological effects. The activation of IRS also allows the docking and activation of small adaptor molecules with SH-2 domains (e.g., growth factor receptor binding protein-2 (Grb-2) and Shp2). Activated Grb-2 recruits SOS-1, which activates the RAS pathway and gene transcription (main pathways are in *bold*, interlinked signaling pathways are in *broken lines*). *IRSs* insulin receptor substrates; *PI3K* phosphatidylinositol-3-kinase; *SOS-1* son-of-sevenless; *ERK* extracellular signal regulated kinase; *cAMP* cyclic adenosine monophosphate; *FSH* follicle-stimulating hormone; *LH* luteinizing hormone. Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

homology domains (SH2) in each accessory molecule, thus named based on their sequence homology to the *src* proto-oncogene. Each SH2 domain is specific for the amino acids surrounding the phosphotyrosine residues in the receptor molecule. PI3K also produces a second messenger such as phosphatidylinositol 3,4,5-triphosphate (IP3) which in turn activates kinase AKT, also known as protein kinase B (PKB). Proteins phosphorylated by AKT promote cell survival.

While these associations may trigger immediate signaling events, other accessory proteins (e.g., GRB2) may serve to construct the scaffolding for a more complex signaling apparatus, such as that seen in the RAS–RAF–MEK pathway. This pathway recruits multiple other proteins, resulting in the activation of nuclear transcription and protein synthesis (see Fig. 1.10).

Mechanism of Action of Activins, Inhibins, Follistatin, and AMH

Activins and inhibins along with AMH and follistatin are members of the TGFβ (beta) superfamily and use a common general mechanism for signal transduction through serine/threonine-specific protein kinases rather than tyrosine kinases. The mechanisms involved in signal transduction by the serine/threonine kinases has been extensively studied and reviewed in recent years [214, 215]. Briefly, after ligand binding to type II receptor, type I receptors are recruited to form a heterodimeric receptor complex.

One of the receptor kinase phosphorylates and activates the other, which in turn phosphorylates their substrates—the SMAD proteins (the term SMAD is derived from the combination of names of two genes, the *C. elegans* gene called Sma and the Drosophila gene Mad). SMADs are a novel family of signal transducers that can be divided into three groups, including receptor-regulated SMADs (R-SMAD) and a single common SMAD (C-SMAD or SMAD4), and inhibitory SMADS that antagonize signaling. Two distinct signaling pathways (activin/TGFβ (beta) and BMP) are involved based on selective recruitment of SMAD proteins.

In activin/TGFβ (beta) signaling, activated type I receptor (ALK4 and ALK5) phosphorylates ligand-specific R-SMADs (SMAD2 and SMAD3), allowing these proteins to associate with SMAD4. In BMP signaling pathway, activated MPB type 1 receptor (ALK2, ALK3, and ALK6) recruits SMAD 1, 5, or 8. The complex then translocates to the nucleus as transcription cofactor. In the nucleus, the action of SMAD complex is modulated by a variety of transcription factors (DNA-binding proteins) at target DNA promoters, which leads to gene transcription. The steps involved in the signal transduction through serine/threonine-specific protein kinase (activin/TGFβ (beta) family) are depicted in Fig. 1.11.

The activin receptor was first to be cloned, which was later followed by other receptors [216, 217]. They are glycoproteins of approximately 55 kDa and consist of a 500 amino-acid sequence. Two types of activin membrane receptors have been identified: type I (ActR-I) and type II receptors (ActR-II). Each contains an intracytoplasmic serine/threonine kinase domain. Activin directly interacts and binds to the relatively short extracellular region of ActR II when expressed alone or in concert with ActR I. It can also bind to other TGFβ (beta) family members—for example, bone morphogenic proteins (BMPs) 2, 4, and 7 in concert with BMP type I receptor, which suggests that these receptors have the ability to cross talk. Activin binding brings together two type II receptors and two type I receptors, which forms a receptor complex leading to signal transduction.

Inhibin is a heterodimer formed between an inhibin-α (alpha) chain and an activin-β (beta) chain, and its biological activity is opposite that of activin. A separate receptor for inhibin has not been identified. The mechanisms by which inhibin antagonizes activin actions are not fully understood. It has been shown that inhibin is capable of binding to type II activin and BMP receptors through its β (beta) subunit and preventing the recruitment of type I receptor, thus antagonizing the actions of activins [218]. This may be one of the mechanisms by which inhibins antagonize activin actions. Other proposed mechanism relates to the interaction of inhibin with additional binding proteins that increase its binding affinity for type II receptors, thus mediating its antagonistic action. Type III TGFβ (beta) receptor (also known as betaglycan) has been shown to bind inhibin and to enhance its binding affinity for type II activin and BMP receptors [219]. More recently it has been shown that inhibin via its α (alpha) subunit N terminus interacts with activin type-IB receptor (ALK4) and thus blocking activin signaling [220]. The combination of these two mechanisms may account for antagonism of activin-stimulated FSH production.

Follistatins bind and bio-neutralize activin activity, and thus exert their anti-activin activity. Recent structural studies have elucidated the crystal structure of a follistatin: activin complex revealing the ability of follistatin to mask the binding sites of activin for both type 1 and type II receptors [221].

AMH/MIS type II receptor (MISRII) gene has been cloned in 1994; it is mapped to chromosome 12 (13, q12), has a total length of 8.7 kbp, and is divided into 11 exons [222]. However, the type I receptor identity remained unclear and among several type-I candidates, ALK2 has been shown to significantly enhance AMH signaling response via MISII/ALK2 receptor complex [223]. Also, the expression of both MISRII and ALK2 in the embryo and in the adult suggests similar signaling pathway in these reproductive tissues. These findings collectively indicate AMH activates the BMP-like signaling pathway and uses ALK 2 as its type I receptor.

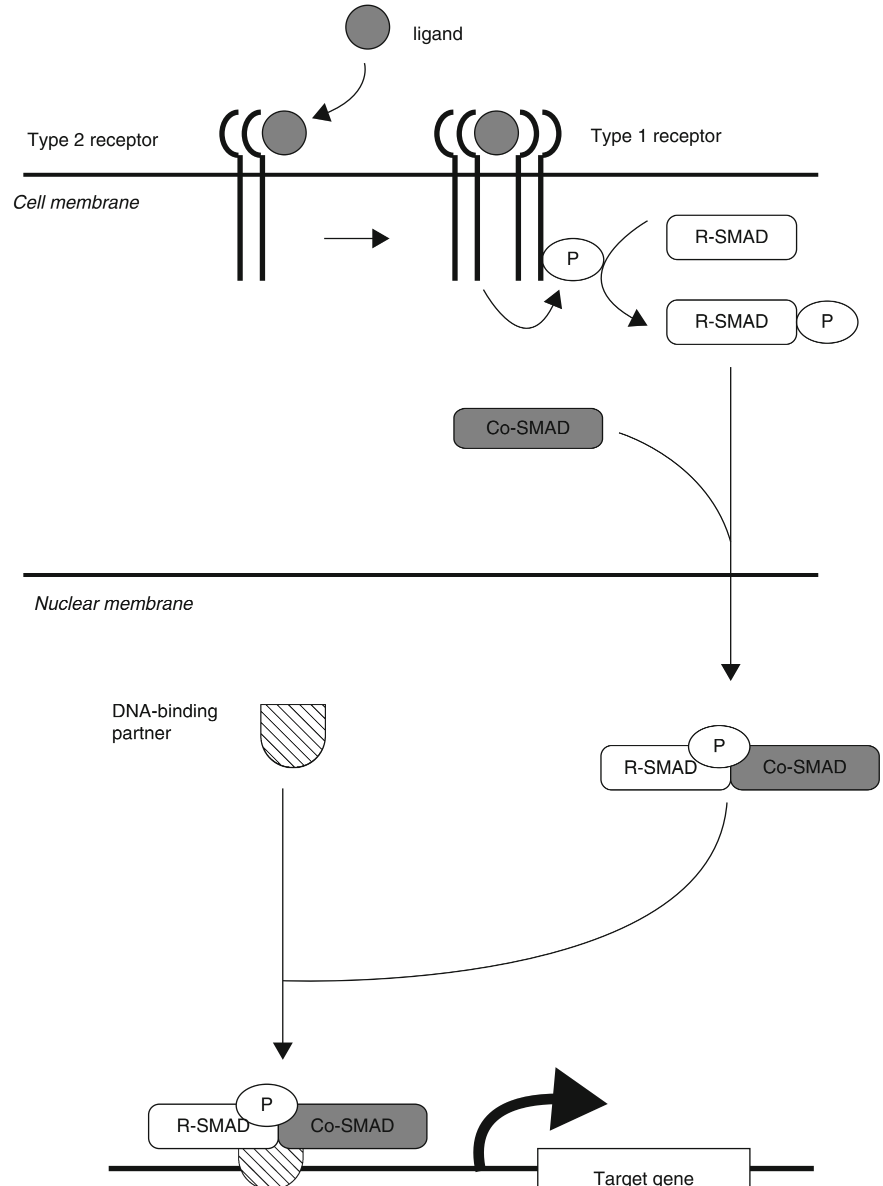

Fig. 1.11 The TGFβ (beta)/activin-signaling pathway. Binding of a TGFβ (beta) family member (e.g., activin) to its type 2 receptor forms a ligand-receptor complex, and activation of the type 1 receptor by phosphorylation. The activated type 1 receptor then phosphorylates receptor-regulated SMAD (R-SMAD). This allows R-SMAD to associate with Co-SMAD and move into the nucleus. In the nucleus, the SMAD complex associates with a DNA-binding partner, such as FAST-1. This complex then binds to specific enhancers in the target gene, activating gene transcription (in the TGFβ (beta)/activin pathway, R-SMAD is formed by SMAD2 and SMAD3. Co-SMAD is formed from SMAD4). Reproduced with permission from Gupta MK, Chia SY. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

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2 Hypothalamic-Pituitary-Ovarian Axis and Control of the Menstrual Cycle

Victor E. Beshay and Bruce R. Carr

Introduction

The menstrual cycle is the result of an orchestra of hormones. It involves the interaction of many endocrine glands as well as a responsive uterus. The menstrual cycle remains a complex process where many aspects are still not well understood. In this chapter we will examine the control of the menstrual cycle through the interaction of the central nervous system, namely, the hypothalamus and pituitary, and the ovaries, resulting in the cyclic and ordered sloughing of the uterine endometrial lining. Key hormones that play a role in the control of the menstrual cycle include gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone (Table 2.1). In addition to these key hormones, there are other peptide and non-peptide hormones that play a role in the menstrual cycle that will also be discussed.

The Menstrual Cycle

The menstrual cycle can be divided into three phases: proliferative (follicular), ovulation, and secretory (luteal). The menstrual cycle is also described based on its length (number of days between onset of menstrual bleeding in one cycle and the onset of bleeding of the next cycle). The median duration of a menstrual cycle is 28 days [1–3]. Most individuals will describe a cycle length of between 25 and 30 days [1–3]. The variability in length of a menstrual cycle is based on the variable length of the follicular phase. The luteal phase is constant in most women and is 14 days in length. Polymenorrhea is described as menstrual cycles that occur at intervals less than 21 days. Conversely, oligomenorrhea is described as menstrual cycles that occur at intervals more than 35 days. During menstruation, blood loss is typically 30 mL [4], and amounts greater than 80 mL (menorrhagia) are considered abnormal [4].

The **proliferative phase** begins at the onset of menses until ovulation takes place. Folliculogenesis takes place during this phase of the menstrual cycle. A dominant follicle is selected from a pool of growing follicles that will be destined to ovulate. The growth of follicles in this stage will depend on pituitary hormones such as FSH. The growth of the follicle also leads to production of estradiol from the layers of granulosa cells surrounding it. Estradiol is responsible for the proliferation of the endometrial lining of the uterus.

Ovulation happens at the peak of follicular growth in response to an LH surge [5]. Prior to ovulation, follicles grow to sizes greater than 20 mm in average diameter [6]. LH is then released in a positive-feedback mechanism from the anterior pituitary due to prolonged exposure to estradiol. For this positive feedback to take place, levels of estradiol above 200 pg/mL for approximately 50 h are necessary [7] (Fig. 2.1). Approximately 12 h after the LH pea, the oocyte is released [8, 9]. In order for the oocyte to release from the follicle, several proteolytic enzymes and prostaglandins are activated, leading to the digestion of the follicle wall collagen [10]. Once an oocyte is released, the fallopian tube is responsible for picking it up where it will await fertilization.

The **secretory phase** starts after ovulation. During this phase, the remaining granulosa cells that are not released with the oocyte during the ovulation process enlarge and acquire lutein (carotenoids), which is yellow in color. These granulosa cells are now called the corpus luteum and predominantly secrete progesterone. Peak progesterone production is noted 1 week after ovulation takes place (see Fig. 2.1). Progesterone is required to convert the endometrial lining of the uterus from a proliferative one into a secretory endometrium in preparation for embryo implantation. The life span of the corpus luteum

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Table 2.1 Major hormones of the hypothalamic-pituitary-ovarian axis[a]

Hormone	Structure	Gene location	Major site(s) of production	Half-life	Serum concentration
GnRH	Decapeptide	8p21–8p11.2	Arcuate nucleus of hypothalamus	2–4 min	N/A
FSH	Glycoprotein with α- and β-subunits	α: 6q12.21 β: 11p13	Gonadotrophs of anterior pituitary	1.5–4 h	5–25 mIU/mL
LH	Glycoprotein with α- and β-subunits	α: 6q12.21 β: 19q12.32	Gonadotrophs of anterior pituitary	20–30 min	5–25 mIU/mL
Estradiol	18 carbon steroid	NA	Granulosa cells	2–3 h	20–400 pg/mL
Progesterone	21 carbon steroid	NA	Theca-lutein cells	5 min	0.1–30 ng/mL
Inhibin	Peptide with α- and β-subunits Inhibin A = α + βA Inhibin B = α + βB	α: 2q33 βA: 2q13 βB: 7p15	Granulosa cells	30–60 min	A: 10–60 B: 10–150 pg/mL

[a]Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

and, hence, progesterone production will depend on continued LH support from the anterior pituitary. If a pregnancy takes place, hCG (human chorionic gonadotropin) of pregnancy will maintain the corpus luteum. However, if a pregnancy fails to happen, luteolysis takes place and the corpus luteum is converted to a white scar called the corpus albicans. The loss of the corpus luteum and the subsequent loss of progesterone leads to the instability of the endometrium and the sloughing of the endometrium, signaling a new menstrual cycle.

Anatomy of the Menstrual Cycle

The initial signals for a menstrual cycle are initiated from the central nervous system. The pertinent endocrine portion of the central nervous system consists of the hypothalamus and the pituitary gland.

The **hypothalamus** consists of only 0.3 % of the total brain, measures 4 cm^3, and weighs approximately 10 g. Despite its small size, it contains many nuclei that are responsible for endocrine regulation, reproduction, metabolism, temperature regulation, emotional responses, and electrolyte balance [11] (Fig. 2.2). The hypothalamus lays beneath the thalamus, hence, the nomenclature. Laterally, it is bordered by the anterior part of the subthalamus, the internal capsule, and the optic tract [11]. The hypothalamus forms the lateral wall and floor of the third ventricle. The median eminence of the hypothalamus extends to the anterior pituitary and contains neurosecretory neurons that affect hormone production from the anterior pituitary. The hypothalamus is comprised of three zones: lateral, medial, and periventricular. Within each zone lie several nuclei, where the arcuate nucleus is pertinent to reproduction. The arcuate nucleus is responsible for the production of GnRH. GnRH is secreted into the portal pituitary circulation, reaching the anterior pituitary to affect FSH and LH release from the anterior pituitary. The hypothalamus also influences thyroid function via TRH (thyrotropin-releasing hormone), adrenal function via CRH (coricotropin-releasing hormone), and growth and metabolic homeostasis via GHRH (growth hormone-releasing hormone) [11].

The **pituitary** gland is a pea-sized gland, also known as the master endocrine gland. It measures 12 × 8 mm and weight approximately 500 mg [11]. It is located beneath the third ventricle and above the sphenoidal sinus in a bony cavity called the sella turcica (see Figs. 2.1 and 2.3). The adult pituitary gland contains two major parts: the adenohypophysis and the neurohypophysis. The neurohypophysis is a diencephalic downgrowth connected with the hypothalamus, while the adenohypophysis is an ectodermal derivative of the stomatodeum [11]. The pituitary gland can also be divided into two major lobes: anterior and posterior. The anterior lobe is equivalent to the adenohypophysis, while the posterior lobe is equivalent to the neurohypophysis. The difference is that the nomenclature of anterior and posterior lobes does not include the infundibulum, which extends from the hypothalamus to the pituitary gland, which contains neural hypophysial connections and is continuous with the median eminence [11]. The anterior pituitary contains several cell types: gonadotropes (responsible for secretion of FSH and LH), thyrotropes (responsible for the secretion of thyroid-stimulating hormone [TSH]), adrenocorticotropes (responsible for the secretion of ACTH), somatomammotropes (responsible for the secretion of GH), and lactotropes (responsible for the secretion of prolactin) (Table 2.2). In addition to these hormones, the anterior pituitary secretes activin, inhibin, and follistatin, which play a role in menstrual cycle regulation. The posterior pituitary lobe contains two cell types that secrete ADH (antidiuretic hormone) and oxytocin. The communication between the hypothalamus and the anterior pituitary is vascular; however, it is a neuronal connection between the hypothalamus and the posterior pituitary.

The gonads in the female consist of the bilateral **ovaries**. The ovaries are located in the pelvis along the sides of the uterus. In reproductive-age women, ovaries measure approximately 2.5 × 3 × 1.5 cm in size. Laterally, the ovary is attached to the pelvic sidewall by the infundibulopelvic ligament, which contains the vascular supply to the ovary (ovarian

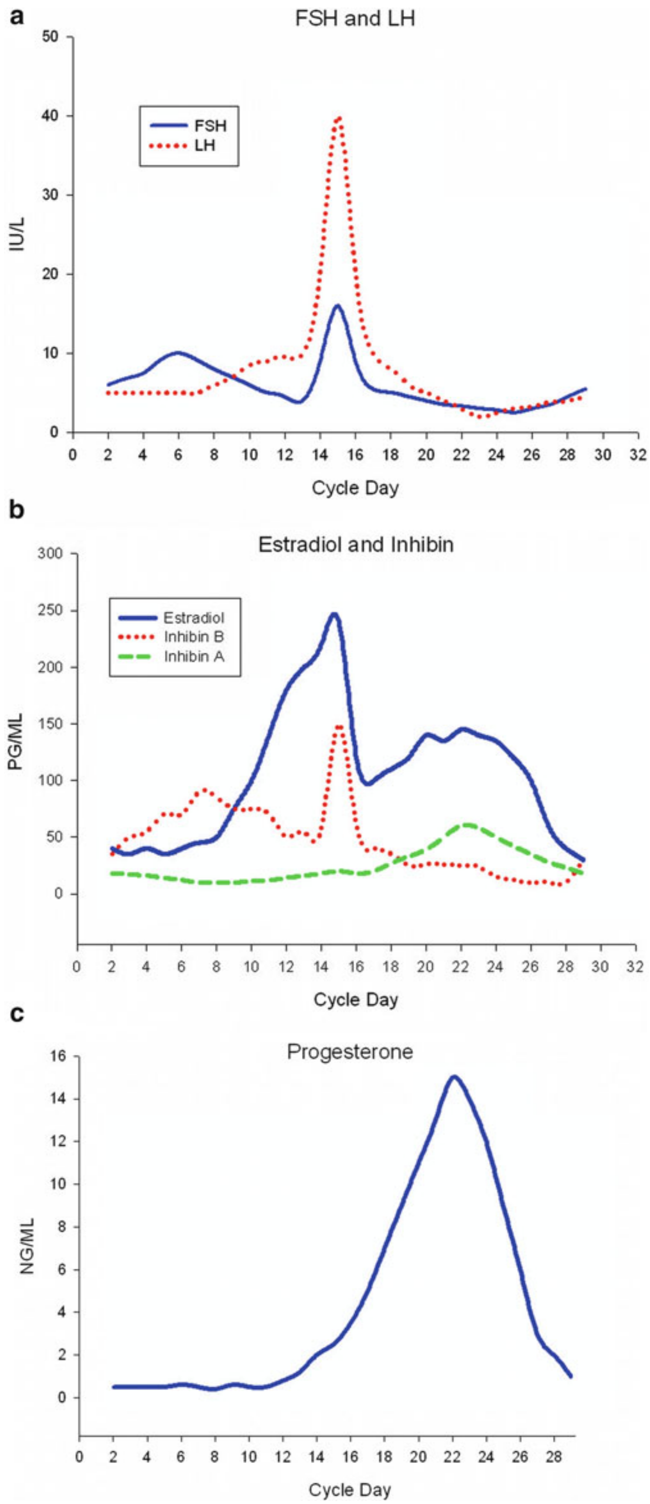

Fig. 2.1 Hormone fluctuations during the menstrual cycle. (**a**) Mean values of FSH and LH throughout the cycle. (**b**) Mean values of estradiol and inhibin. (**c**) Mean values of progesterone during the menstrual cycle. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

artery and vein). The ovary consists of an outer cortex and an inner medulla. The ovarian follicles are found in the cortex, while the medulla mainly contains fibromuscular tissue and vasculature. Each ovarian follicle consists of an oocyte surrounded by layers of granulose and theca cells. These layers will vary depending on the maturation stage of the oocyte contained within the follicle. Within the ovarian cortex, follicles can be found in different stages of development. Earlier stages of follicular development are independent of central nervous system hormone production, while later stages of follicular development will depend on reproductive hormones produced by the central nervous system. The growing ovarian follicle will produce estradiol from the granulose cells (Table 2.3). After ovulation, the remnant cells of the follicle luteinize and start secreting progesterone. The granulosa cells are also responsible for the secretion of inhibin as well as anti-Müllerian hormone (AMH).

The **uterus** is largely a receptive organ to all the steroid hormones that emanate from the endocrine glands. The uterus is a fibromuscular organ that is bordered anteriorly by the urinary bladder and posteriorly by the rectum. The uterus can be divided into two major portions: an upper body (corpus) and a lower cervix. The hollow portion of the uterus contains a mucosal lining called the endometrium. The endometrium contains several layers of cells: the basal layer and the superficial layer. The basal layer is responsible for the regeneration of the endometrial cells. The superficial layers undergo the cyclic changes of the menstrual cycle. The endometrium normally proliferates in response to the rising estradiol levels in the first half of the menstrual cycle and is converted to a secretory layer in response to progesterone produced by the corpus luteum in the second half of the menstrual cycle. If the cycle does not result in a pregnancy, where there is lack of hCG, progesterone production is not maintained by the corpus luteum, and the endometrium becomes unstable and sloughs in preparation for a new cycle and another attempt for pregnancy.

Endocrinology of the Menstrual Cycle

GnRH is a decapeptide synthesized in the hypothalamus and first described in the 1970s by Schally [12–14] and Guillemin [15] for which they received the Nobel prize [14–18] (Fig. 2.4). GnRH neurons can be detected in the fetal hypothalamus as early as 9–10 weeks of gestation [19]. GnRH neurons originate from the olfactory area [20], later migrating to the olfactory placode to rest in the arcuate nucleus of the hypothalamus [21]. The hypothalamic GnRH neurons then send projections to the pituitary. The association of GnRH neurons and the olfactory system can be demonstrated in a condition called Kallman syndrome, where GnRH

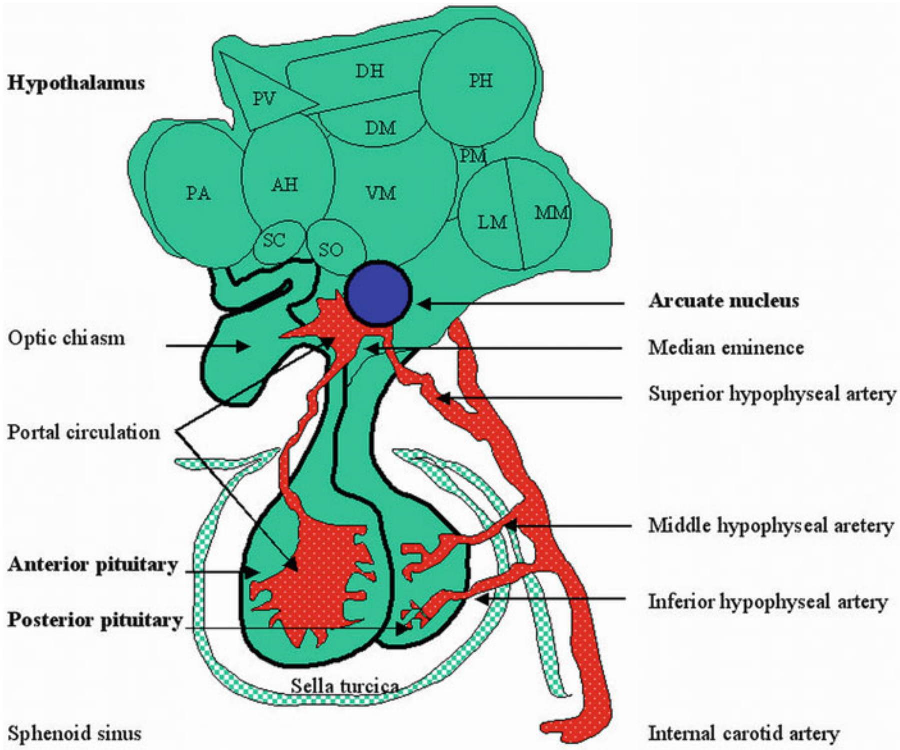

Fig. 2.2 Illustration of the hypothalamus, pituitary, sella turcica, and portal system. The arcuate nucleus is the primary site of GnRH-producing neurons. GnRH is released from the median eminence into the portal system. The blood supply of the pituitary gland derives from the internal carotid arteries. In addition to the arcuate nucleus, the other hypothalamic nuclei are *SO* supraoptic nucleus; *SC* suprachiasmatic nucleus; *PV* paraventricular nucleus; *DM* dorsalmedial nucleus; *VM* ventromedial nucleus; *PH* posterior hypothalamic nucleus; *PM* premamillary nucleus; *LM* lateral mamillary nucleus; *MM* medial mamillary nucleus. The three hypothalamic areas are *PA* preoptic area; *AH* anterior hypothalamic area; *DH* dorsal hypothalamic area. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

deficiency is coupled with anosmia [22]. Pheromones, small airborne molecules secreted by one individual and perceived by another individual, also suggest the common origin of GnRH molecules and the olfactory system. Pheromones may explain why women living or working in close proximity may develop synchrony in their menstrual cycles [23, 24].

To date, three types of GnRH (GnRH-I, GnRH-II, and GnRH-III) have been detected in humans [25, 26]. Many other GnRH types have been described in fish, amphibians, and protochordates [27, 28]. GnRH-I is the classic hypothalamic hormone responsible for the regulation, synthesis, and secretion of the pituitary gonadotropins FSH and LH [29]. GnRH-II was first described in brain tissue and has since been found in many other peripheral tissues, such as the endometrium, breast, and ovaries [30–33]. GnRH-III was first identified in Lamprey in 1993 [34], and Yahalom et al. described the presence of GnRH-III in neurons from the hypothalamus [25]. The role of GnRH-III in humans is unclear.

GnRH-I is synthesized from a larger, 92 amino acid precursor [35]. GnRH-I then travels to the median eminence of the hypothalamus and is released in the portal circulation in a pulsatile fashion. The GnRH-I molecule lifespan is very short, as it is cleaved rapidly with a half-life of 2–4 min. Because of this rapid cleavage, peripheral levels of GnRH-I are difficult and do not correlate well to pituitary action.

GnRH-I acts on the anterior pituitary leading to the synthesis and storage of gonadotropins, movement of the gonadotropins from the reserve pool to a readily released point, and finally the secretion of gonadotropins. For this action to take place appropriately, pulstaile GnRH release is necessary [36, 37]. Continuous GnRH secretion will lead to the suppression of FSH and LH release as well as suppression of FSH and LH gene transcription by the anterior pituitary [38, 39]. This is the basis of use of GnRH agonists such as Lupron for the suppression gonadotropin secretion. The pulse frequency of GnRH will vary depending on the menstrual cycle phase. LH pulse frequency is used to indicate GnRH pulse secretion (Table 2.4).

GnRH-II differs from GnRH-I by three amino acids at positions 5, 7, and 8 [26, 40]. Also, in contrast to GnRH-I, GnRH-II is mainly expressed outside the brain [26, 41, 42], including the human placenta [43]. Similar to GnRH-I release from the hypothalamus, GnRH-II is released from the placenta in a pulsatile fashion [44].

Various factors are believed to play a role in GnRH secretion. Estrogen has been shown to have a positive as

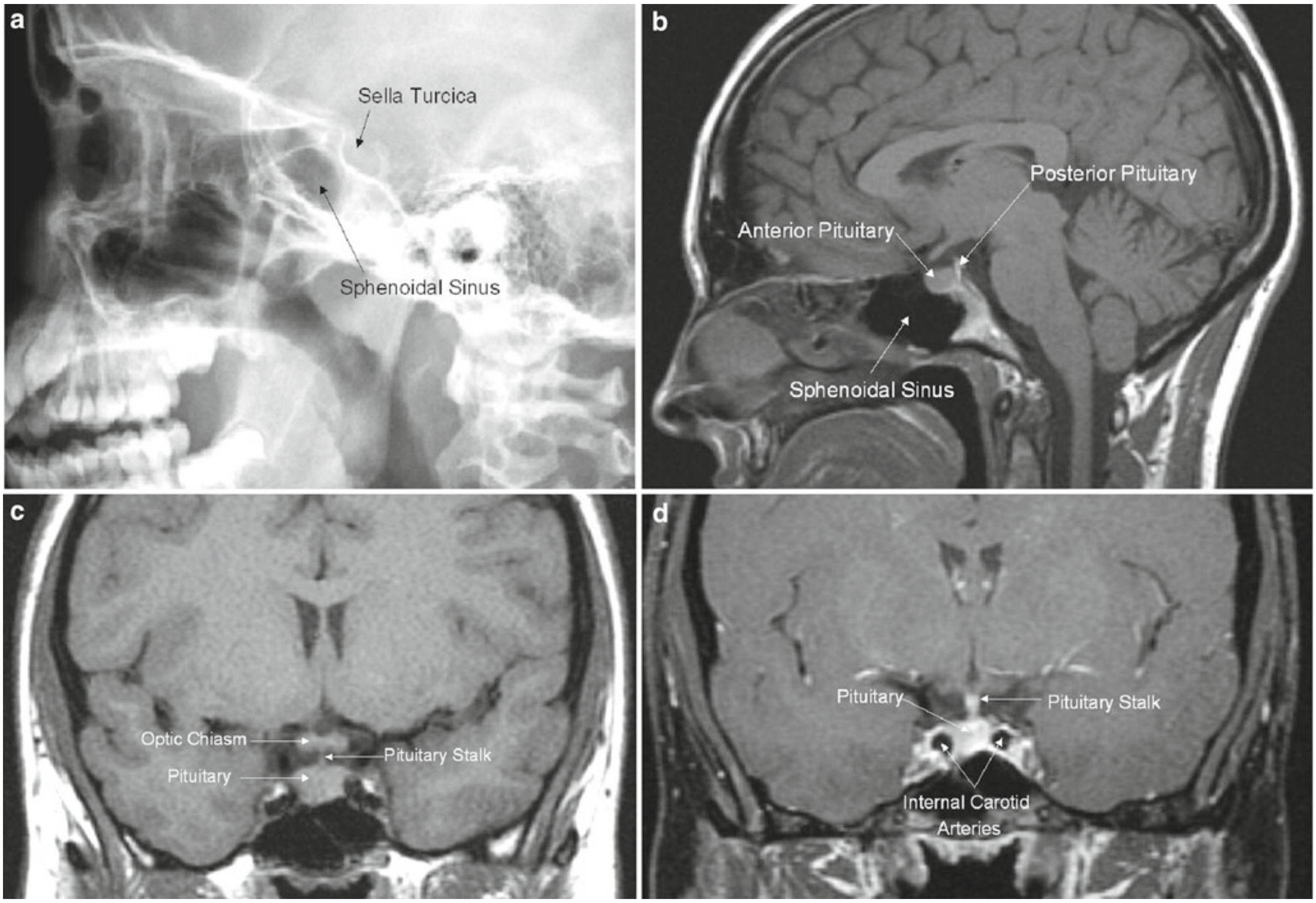

Fig. 2.3 X-ray and T1-weighted MRI images of the pituitary gland. (**a**) Lateral skull film with the sphenoidal sinus and sella turcica. (**b**) Sagittal section demonstrating the relationship between the sphenoidal sinus and the pituitary gland. The normal posterior pituitary is brighter on MRI compared to the anterior pituitary. The sella turcica is not well seen on MRI. (**c**) Coronal section demonstrating the relationship of the pituitary to the optic chiasm and the pituitary stalk. (**d**) Coronal section after gadolinium contrast, demonstrating the close proximity of the pituitary to the internal carotid arteries. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Table 2.2 Major cell types of the anterior pituitary gland[a]

Cell type	Appearance on light microscopy	Cellular frequency (%)	Hormone products
Somatotrophs	Acidophilic	50	Growth hormone
Lactotrophs	Acidophilic	20	Prolactin
Corticotrophs	Basophilic	20	Adrenocorticotrophic hormone (ACTH)
Thyrotrophs	Basophilic	5	Thyroid-stimulating hormone (TSH) and free α-subunit
Gonadotrophs	Basophilic	5	Follicle-stimulating hormone (FSH), luteinizing hormone (LH) and free α-subunit

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Table 2.3 Site of synthesis of major steroidogenic products of the ovary

Cell type	Major steroid hormone products
Theca cells	Androgens (androstenedione, DHEA, testosterone)
Granulosa cells	Estrogens (estradiol, estrone, inhibin, AMH)
Theca-lutein cells	Progestogens (progesterone, 17-hydroxyprogesterone)
Granulosa-lutein cells	Estrogens (estradiol, estrone)

well as a negative effect on GnRH-I secretion. Estrogen suppresses GnRH-I secretion in a negative-feedback fashion [45]. In addition, estrogen has a differential regulation on GnRH-I and GnRH-II mRNA levels. Estrogen increased GnRH-II mRNA levels while it decreased GnRH-I mRNA levels [46]. Progesterone is also noted to play a stimulatory role on GnRH-1 mRNA, which was decreased by the progesterone receptor antagonist RU48 [47]. However, no difference in the expression level of GnRH-II was seen with progesterone or the anti-progestin mifepristone [47].

pyro-Glu[1]-His[2]-Trp[3]-Ser[4]-Tyr[5]-Gly[6]-Leu[7]-Arg[8]-Pro[9]-Gly[10]-NH_2

Fig. 2.4 Structure of GnRH-1. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Table 2.4 Menstrual cycle variation in LH pulse frequency and amplitude[a]

Cycle phase	Mean frequency (min)	Mean amplitude (mIU/mL)
Early follicular	90	6.5
Mid-follicular	50	5
Late follicular	60–70	7
Early luteal	100	15
Mid-luteal	150	12
Late luteal	200	8

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Two types of GnRH receptors have been described in humans: GnRH-I receptor (GnRH-IR) and the GnRH-IIR (GnRH-IIR). The GnRH-IR is a G protein-coupled transmembrane receptor (GPCR) [48, 49]. However, the mammalian GnRH-IR lacks the carboxyl-terminal tail [49, 50]. Activation of the GnRH-IR leads to the activation of phospholipase C, which in turn generated the second messengers inositol triphosphate and diacyl glycerol, stimulating protein kinase, cyclic adenosine monophosphate (cAMP), and release [51] of calcium ions. In addition to the brain, GnRH-IR can be found in the human placenta [52, 53], ovarian follicles [33, 54], in myometrium and leiomyomata [55, 56], as well as human pancreas, liver, heart, skeletal muscle, kidney, placenta, and peripheral blood [57–60]. GnRH-IIR is also a GPCR, but unlike the GnRH I-R, it has a C-terminal cytoplasmic tail [61]. GnRH-IIR can be found the pituitary, placenta, ovary, uterus, prostate, mature sperm, pancreas, small and large intestines, kidney, and liver [26, 33, 62–64].

GnRH analogues have been developed by changes made to the amino acid sequence of the GnRH molecule. These changes result in the extension of the GnRH half-life as well as its biologic activity. There are two major groups of GnRH analogues: GnRH agonists and GnRH antagonists (Table 2.5) In the case of GnRH agonist use, the continuous activation of the GnRH receptor results in desensitization due to a conformational change of the receptor, uncoupling from G proteins, internalization of the receptor as well as reduced synthesis of the receptor [65, 66]. Prior to the desensitization by GnRH agonists, there is an initial flare where there is increased gonadotropin secretion. Desensitization then takes place 7–14 days later. Unlike GnRH agonists, GnRH antagonists do not cause a flare effect upon initial administration; instead, GnRH antagonists cause an immediate suppression of gonadotropin secretion that is rapid and is reversible [67]. Currently,

Table 2.5 Properties of commercially available GnRH agonists[a]

	Structure and substitutions at positions 6 and 10	Half-life	Relative potency	Route of administration
GnRH	Native decapeptide	2–4 min	1	IV, SC
Naferelin	Decapeptide 6: Nal for Gly	3–4 h	200	Intranasal
Triptorelin	Decapeptide 6: Trp for Gly	3–4 h	36–144	SC, IM depot
Leuprolide	Nonapeptide 6: Leu for Gly 10: NHEt for Gly	1.5 h	50–80	SC, IM depot
Buserelin	Nonapeptide 6: Ser(O^{t}Bu) for Gly 10: NHEt for Gly	1.5 h	20–40	SC, intranasal
Goserelin	Decapeptide 6: Ser(O^{t}Bu) for Gly 10: AzaGly for Gly	4.5 h	50–100	SC implant
Histrelin	Decapeptide 6: DHis for Gly 10: AzaGly for Gly	50 min	100	SC

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GnRH analogues are available in injectable form in the treatment of many reproductive conditions, such as precocious puberty, endometriosis, and uterine leiomyomata; they are also being used in in vitro fertilization treatment cycles. Oral forms of GnRH analogues are under investigation. Elagolix is an orally active GnRH antagonist under investigation for use in reproductive conditions [68, 69].

GnRH acts on the anterior pituitary to secrete gonadotropins: FSH and LH. **FSH** is a glycoprotein dimer consisting of two subunits: α (alpha)- and β (beta)-subunits. The α-subunit is common in FSH and LH as well as TSH and hCG. The β-subunit is distinct and hormone-specific, which allows the differential function of each hormone. The α-subunit consists of 92 amino acids, while the FSH β-subunit consists of 118 amino acids and five sialic acid residues. Sialic acid residues are responsible for the half-life of the hormone, where the higher the sialic acid content the longer the half-life of that molecule [70]. FSH has a half-life of several hours. The addition of sialic acid to urinary obtained or recombinant FSH products leads to their longer half-life. The rate-limiting step in gonadotropin production is the availability of β-subunits. In addition to GnRH stimulation of FSH β-subunit synthesis, FSH β-subunit synthesis is dependent on the presence of activin [71, 72].

FSH starts to rise a few days prior to the onset of menses and is responsible for the recruitment of a cohort of ovarian follicles as well as a selection of the dominant follicle (see Fig. 2.1). FSH induces granulosa cell growth and activates aromatase activity, which converts androgens into estrogens.

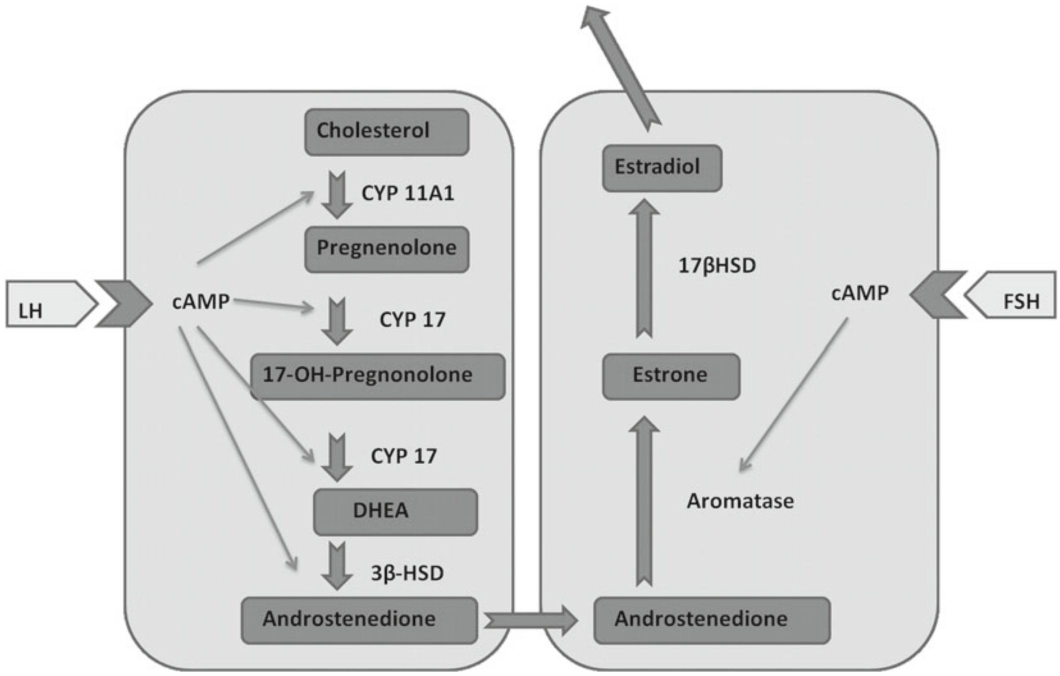

Fig. 2.5 The two-cell theory of ovarian steroidogenesis. Binding of luteinizing hormone (LH) to its receptor on ovarian theca cells stimulates the conversion of cholesterol to androstenedione. Binding of follicle-stimulating hormone (FSH) to its receptor on ovarian granulosa cells stimulates the aromatization of androgens to estrogens. *cAMP* cyclic adenosine monophosphate; *CYP11A1* side-chain cleavage enzyme; *CYP17* 17-hydroxylase; *HSD* hydroxysteroid dehydrogenase; *17-OH pregnenolone* 17-hydroxy pregnenolone

FSH levels then start to decline owing to estrogen and inhibin B production by the growing follicular granulosa cells. Despite this drop in the FSH level, the dominant follicle continues to grow as it acquires the highest concentration of FSH receptors (secondary to increase in surrounding granulosa cell number), making it more resistant to the drop in FSH level [73]. In addition, the drop in FSH level causes a higher androgenic microenvironment in the nondominant follicles. FSH then declines after ovulation of the dominant follicle.

LH is also a glycoprotein dimer consisting of two subunits: α (alpha)- and β (beta)-subunits. The β-subunit of LH consists of 121 amino acids and one to two sialic acid residues, giving it its shorter half-life of approximately 20 min. Because of this shorter half-life, LH needs to be rapidly synthesized and typically has pulses higher in amplitude than FSH. As with FSH, LH also starts to rise prior to the onset of menses. The LH increase throughout the follicular phase of the cycle is gradual. Immediately prior to ovulation, LH surges in response to estradiol production by the dominant follicle in a positive-feedback fashion. LH levels then decline in the secretory phase of the cycle (see Fig. 2.1).

FSH and LH receptors both belong to the GPCR family. FSH receptors exist exclusively on the membrane of granulosa cells, while LH receptors are found on membranes of theca cells. In the presence of estradiol, FSH induces LH receptors on granulosa cells. LH receptor activity primarily stimulates androstenedione production from theca cells, which is transported to neighboring granulosa cells, aromatized to estrone, and eventually converted to estradiol. This is the basis of the two-cell theory of the ovary (Fig. 2.5).

Endogenous **opiates (opioids)** are naturally occurring narcotics produced by the brain. There are three classes of opiates: enkephalin, endorphin, and dynorphin. Endorphin levels increase throughout the menstrual cycle; they are at their lowest at the time of menses and at their highest in the luteal phase. Sex steroids appear to play a role in endorphin secretion. Estradiol has been shown to increase endorphin secretion, while the sequential addition of progesterone to estradiol showed a higher endorphin secretion in ovariectomized monkeys [74]. An increase in endorphin release has been shown to decrease LH pulse frequency [75], while opioid receptors blockers, such as naltrexone, have been shown to increase LH pulse frequency [76]. The suppression of gonadotropin secretion by endogenous opiates is secondary to suppression of hypothalamic GnRH release [77]; thus, opiates appear to play a role in hypothalamic amenorrhea (Table 2.6). Treatment of women with hypothalamic amenorrhea with opioid receptor antagonists appears to correct the problem, causing a return of ovulation and menstrual cyclicity [78, 79]. It is also believed that stress-related amenorrhea is the result of GnRH suppression by endogenous opiates. Women suffering from stress-related amenorrhea demon-

Table 2.6 Neurotransmitter effects on GnRH release[a]

Neurotransmitter	Effect
Dopamine	Inhibits GnRH release
Endorphin	Inhibits GnRH release
Serotonin	Inhibits GnRH release
Norepinephrine, epinephrine	Stimulates GnRH release

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strate higher hypothalamic corticotropin-releasing hormone. Proopiomelanocortin, the precursor to endorphins, is controlled mainly by corticotropin-releasing hormone [74]. In addition, hypothalamic amenorrhea that develops in athletes may also be secondary to opioid rise during exercise [80, 81].

Ovarian peptide hormones such as inhibin, activin, and AMH also play a role in the menstrual cycle by modulating central nervous system hormone release. Inhibin, activin, and AMH all belong to the transforming growth factor-β (beta) superfamily (TGF-β) of ligands.

Inhibin is a polypeptide mainly secreted by granulosa cells, but has also been found in pituitary gonadotropes [82, 83]. Inhibin is comprised of a α (alpha)- and β (beta)-subunits. Two forms of inhibin have been identified: inhibin-A and inhibin-B, each containing an identical α-subunit but a unique β-subunit. Inhibin-A is predominantly secreted in the luteal phase of the menstrual cycle, while inhibin-B is predominantly secreted in the follicular phase of the menstrual cycle [84]. Inhibin is released by granulosa cells in response to FSH [85] and selectively inhibits FSH secretion from the anterior pituitary [86], thus creating a negative-feedback loop (see Fig. 2.1).

In contrast, **activin**, which is also secreted by the granulosa cells, augments the secretion of FSH by enhancing GnRH receptor formation [87, 88]. The effects of activin are blocked by inhibin and follistatin [89].

Follistatin is a peptide secreted by pituitary gonadotropes [90]. Follistatin inhibits FSH synthesis and secretion by sequestering activin [91, 92]. Inhibin inhibits follistatin production, while activin stimulates its production.

AMH is a product of the granulosa cells of small antral and pre-antral follicles and is reflective of their quantity [93]. It may be reflective of the ovarian reserve which is often a clinical term for the size of the primordial follicle pool. Although the role of AMH has been well described for causing Müllerian duct regression in the male fetus, its role in females in the post-fetal life period has not been well defined. It is believed that AMH, through a paracrine effect in the ovary, inhibits FSH-stimulated follicle growth, contributing to the emergence of the dominant follicle [74]. The relationship among AMH, the follicular pool, and recruitment throughout the reproductive life cycle is complex and is dependent on the stage of sexual development [94]. Clinically AMH has been used in the prediction of ovarian reserve in women undergoing fertility evaluation and treatment [95]. However, the dichotomy of poor reserve vs. normal reserve is not evident [95]. AMH levels are elevated in patients with polycystic ovary syndrome and decreased in women exposed to antineoplastic drugs.

Leptin is a protein cytokine secreted by adipocytes. It consists of 167 amino acids and is secreted by adipose tissue, reflecting amounts of body fat [96]. Leptin's most significant role is energy homeostasis. It is regulated by many factors, such as obesity, glucose, and insulin, which promote its secretion, whereas fasting, androgens, and thyroid hormone inhibit its secretion. Its role in reproduction is not well understood. As mentioned earlier, CRH is increased in stress-related amenorrhea and is also increased in weight-loss amenorrhea. It is not understood why CRH increases. The reduction in leptin level in these clinical scenarios may play a role in this CRH increase in the brain [97]. Leptin has also been shown to indirectly affect pituitary FSH and LH secretion in gonadotropin-stimulated fertility treatment cycles [98].

Estrogens are 18-carbon steroid hormones and include estrone (E1), estradiol (E2), and estriol (E3). The most potent estrogen is **estradiol** and is the product of the ovary. Estrone is mainly the product of peripheral androstenedione conversion. Estrone is also generated in the liver via 17 β (beta)-hydroxysteroid dehydrogenase conversion of estradiol. Estriol is the principal estrogen formed by the placenta during pregnancy. Serum estradiol levels rise during the follicular phase of the menstrual cycle and are in parallel to the growth of the follicle. Estradiol is mainly found bound in the bloodstream to carrier proteins. Albumin carries approximately 60 % of estradiol, while sex hormone-binding globulin binds 38 % of estradiol, with 2 % remaining as free in the bloodstream. This free hormone is active and capable of entering target cells. In the early follicular phase, serum estradiol levels do not exceed 50 pg/mL. At peak follicular growth, the level rises to approximately 200–250 pg/mL. Estradiol levels drop with ovulation, but a second rise is seen in the mid-luteal phase, reflecting estrogen secretion from the corpus luteum (see Fig. 2.1). Circulating estrogens are conjugated in the liver to form sulfates and glucuronides; 80 % are excreted in the urine and the remaining 20 % in bile.

There are two known estrogen receptors: estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β) [99, 100]. Both receptors contain DNA-binding and hormone-binding domains, a hinge region, and a transcriptional activation function (TAF) domain. Estrogen will enter any cell, but only cells containing the estrogen receptor will respond. The receptor is typically nuclear in location, but can be shuttled to the cytoplasm via a process called nucleocytoplasmic shuttling [74]. Once estrogen binds to its receptor, activation of gene transcription then takes place.

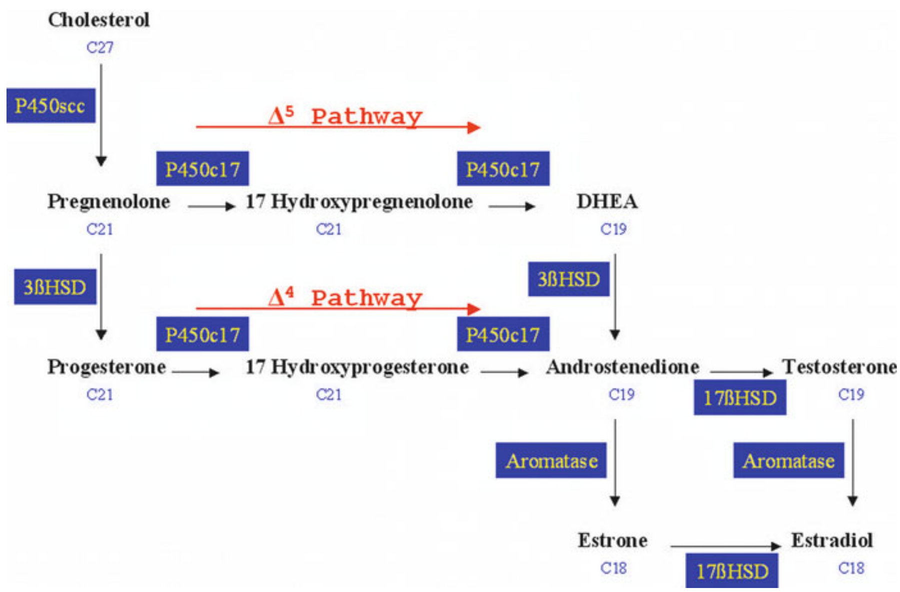

Fig. 2.6 The Δ(delta)[5] and Δ(delta)[4] pathways. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone via side-chain cleavage (P450scc). In the follicular phase, pregnenolone is preferentially converted to androstenedione via the Δ[5] pathway involving 17-hydroxypregnenlone and dehydroepiandrosterone (DHEA). In contrast, the corpus luteum preferentially converts pregnenolone to progesterone (Δ[4] pathway) via 3β(beta) hydroxysteroid dehydrogenase (3βHSD). Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

It is also known that estradiol has a negative-feedback effect on FSH secretion. This negative-feedback effect is the direct effect of estradiol coupled to its receptor, causing repression of FSH-β (beta) subunit transcription [101].

Similar to estrogen, **progesterone** is a steroid hormone. Progesterone is a 21-carbon molecule and is the main steroid of the corpus luteum. In the follicular phase, progesterone levels are typically <2 ng/mL. Progesterone reaches its peak in the mid-luteal phase, with levels exceeding 5 ng/mL (see Fig. 2.1). The majority of progesterone in the bloodstream is bound to albumin (80 %) and corticosteroid-binding globulin (18 %). A very small amount of progesterone is bound to SHBG (0.5 %). The remaining progesterone is free in the circulation. The liver is responsible for clearing progesterone from the circulation by converting progesterone to pregnanediol, which is conjugated to glucuronic acid and excreted in the urine.

Similar to estrogen, there are several progesterone receptors: progesterone receptor-A (PR-A), progesterone receptor-B (PR-B), and progesterone receptor-C (PR-C). PR-B is the positive regulator of progesterone effects, while PR-A and PR-C antagonize PR-B.

At high concentrations, progesterone inhibits FSH and LH secretion through effects on both the hypothalamus and pituitary [102]. The presence of progesterone in the luteal phase also causes the decline in GnRH pulse frequency in the hypothalamus. At low concentrations, progesterone can stimulate LH release only after exposure to estrogen and progesterone [103]. Progesterone also causes a depletion of estrogen receptors, which is the mechanism of protection against endometrial hyperplasia by progesterone.

Androgens are the major products of theca cells. Androgens are 19-carbon steroids and include: androstenedione, testosterone, and dehydroepiandrosterone (DHEA). The principal secreted androgen by theca cells is androstenedione. Most of the testosterone is the product of peripheral conversion of androstenedione through the actions of 17 β-hydroxysteroid dehydrogenase. Under the effect of FSH, androstenedione and testosterone are then further aromatized in granulosa cells and converted to estrogens (Fig. 2.6).

The androgen receptor exists in a full-length B form and a shorter A form [104]. Androgens and progestins can cross-react to their receptor but only when present in high concentration.

In preovulatory follicles, the preferred steroid pathway for androgen and estrogen synthesis is the Δ(delta)[5] pathway, which involves the conversion of pregnenolone to 17-hydroxypregnenolone. In the theca cell, 17-hydroxypregnonolone is converted to androgens. Due to the lack of ability of theca cells to metabolize androgens, they are carried to the neighboring granulosa cells for aromatization (see Figs. 2.5 and 2.6). In contrast, in the corpus luteum the preferred pathway is the Δ(delta)[4] pathway of steroidogenesis, which deals with the conversion of pregnenolone to progesterone. The rate-limiting step in steroidogenesis is the side-chain cleavage of cholesterol to pregnenolone. In the ovary, this step is regulated by LH. LH stimulation leads to increased cAMP production and increased low-density lipoprotein (LDL) receptor mRNA and subsequent increased LDL intake. LDL is the major form of cholesterol used for steroidogenesis. cAMP-activated steroidogenic acute regulatory protein (StAR) causes an increase in the transport of cholesterol across the mitochondrial membrane, where side-chain cleavage can take place [105]. From there, all the remaining ovarian hormones can be produced.

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Female and Male Gametogenesis

3

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Introduction

Oogenesis is an area that has long been of interest in medicine, as well as biology, economics, sociology, and public policy. Almost four centuries ago, the English physician William Harvey (1578–1657) wrote *ex ovo omnia*—"all that is alive comes from the egg."

During a women's reproductive life span only 300–400 of the nearly 1–2 million oocytes present in her ovaries at birth are ovulated. The process of oogenesis begins with migratory primordial germ cells (PGCs). It results in the production of meiotically competent oocytes containing the correct genetic material, proteins, mRNA transcripts, and organelles that are necessary to create a viable embryo. This is a tightly controlled process involving not only ovarian paracrine factors but also signaling from gonadotropins secreted by the pituitary.

The contribution of the male to the biology of reproduction is to produce a genetically intact spermatozoa that will fertilize an oocyte. The end product of male gametogenesis, the mature spermatozoa, is designed for one purpose: to deliver the male contribution of the genetic makeup to the embryo. The biology of gamete production is different in males compared to females. Gamete production in females is intimately part of the endocrine responsibility of the ovary. If there are no gametes, then hormone production is drastically curtailed. Depletion of oocytes implies depletion of the major hormones of the ovary. In the male this is not the case. Androgen production will proceed normally without a single spermatozoa in the testes.

This chapter presents basic aspects of human ovarian follicle growth, oogenesis, and some of the regulatory mechanisms involved [1], as well as some of the basic structural morphology of the testes and the process of development to obtain mature spermatozoa.

Structure of the Ovary

The ovary, which contains the germ cells, is the main reproductive organ in the female. It also functions as an endocrine organ, secreting estrogen and progesterone in response to gonadotropin and paracrine signaling. Ovaries exist as a pair of glands, approximately the size of almonds, on either side of the uterus. Within the abdominal cavity, ovaries are found closest to the lateral wall of the pelvis, attached to the back portion of the broad ligament of the uterus [2]. This area is known as the ovarian fossa and is surrounded by the external iliac vessels, the umbilical artery, and the ureter [2, 3].

The ovary comprises several different layers and types of tissues, shown in Fig. 3.1. The innermost layer is the medulla, which houses the blood vessels essential to supporting the ovary. To the outside of this is the ovarian cortex, which is made up of follicles and stromal tissue. The outermost layer of the ovary consists of a thin layer of epithelial cells. Known as *the germinal epithelium*, this layer produces thousands of primordial follicles during fetal growth [4]. Underlying the germinal epithelium is a strong connective tissue layer known as the tunica albuginea (TA). Ovum production and oocyte maturation occur within the cortex of the ovary [5]. As primordial follicles are recruited and develop, they move closer to the outer edge of the ovary, eventually bursting through the surface during ovulation [3].

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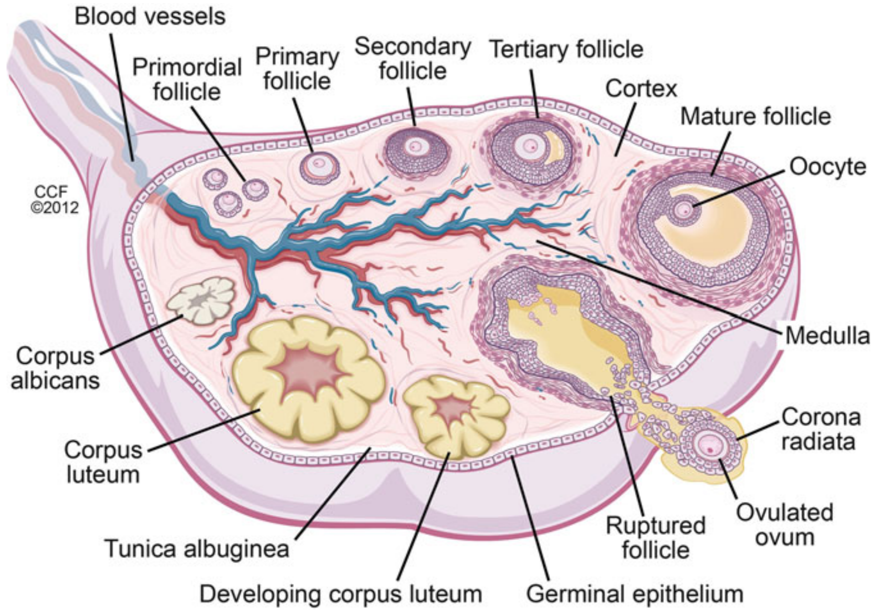

Fig. 3.1 This schematic of the ovary depicts the developing follicle and oocyte in the ovarian cortex. The outermost layer comprises a thin layer of epithelial cells known as the germinal epithelium, which gives rise to primordial oocytes during fetal growth. Just below this is a strong connective tissue layer known as the tunica albuginea. The medulla, located at the center of the ovary, houses blood vessels and ligaments that are vital to the survival and function of the ovary

Overview of Oogenesis

In humans, oogenesis begins approximately 3 weeks after fertilization. PGCs arise from the yolk sac and migrate via amoeboid movements, through the hindgut, to the genital ridge [6–9]. PGCs undergo rapid mitotic division during this migration. The genital ridge, formed at around 3.5–4.5 weeks gestation, is composed of mesenchymal cells overlaid with coelomic epithelial cells. Upon arrival, the PGCs give rise to oogonia or germ stem cells (GSCs) that continue to proliferate to further expand the germ cell pool. The number of oogonia increases from 600,000 by the eighth week of gestation to over 10 times that number by 20 weeks. At around 7 weeks' gestation, these cells form the primitive medullary cords and the sex cords, respectively.

Follicle formation begins at around week 16–18 of fetal life. Oogonia are enveloped by somatic epithelial cells derived from genital ridge mesenchymal cells, forming primordial follicles. The oogonia then cease mitotic activity and enter meiosis [8, 10, 11].

Once meiosis has been initiated, the oogonial germ cell is termed a primary oocyte. The surrounding mesenchymal somatic cells secrete the follicle's basement membrane and give rise to the granulosa cell layer. By 4–5 months' gestation, the ovary has its maximum number of oocytes, between 5 and 8 million. This number decreases dramatically to 1–2 million at birth and less than 500,000 by puberty [12]. The primordial follicles containing these immature oocytes remain essentially dormant. Through the next 35–40 years of a women's reproductive life span, a small number of follicles are steadily released into the growing pool [13–15].

Oocyte Development and Meiosis

Oogenesis is the process by which the mature ovum is differentiated. It is not clear whether the signals controlling germline entry into meiosis are cell-autonomous or dependent on the mesonephric somatic cells. Meiosis is unique to germ cells. It results in the production of daughter cells with haploid DNA content as a result of a two-step division. During oogenesis, cell division is unequal. The result is a single ovum, with excess genetic material extruded as polar bodies [16]. This is quite unlike spermatogenesis, where meiosis results in four identical haploid daughter cells [17].

The four main stages of meiosis are prophase, metaphase, anaphase, and telophase (Fig. 3.2). DNA replication, chromosome pairing, and recombination, steps that are integral to sexual reproduction, all occur during prophase. Prophase can be subdivided into four stages, leptotene, zygotene, pachytene, and diplotene. DNA replication is finalized in preleptotene, while in leptotene, sister chromatids search for their homologous counterparts. Interaction between homologous chromosomes is facilitated by the formation of recombination nodules. In zygotene, homologous chromosomes pair and begin to synapse. The synapses are maintained by the synaptonemal complex. The crossing over and recombination of chromosomes occur in the pachytene stage, prior to the formation of ovarian follicles. Synapsis is completed in pachytene, and by the diplotene stage, homologous chromosomes are held together mainly at sites of chiasmata. Oocytes in primordial follicles are arrested at the diplotene stage of the first meiotic prophase [1, 18].

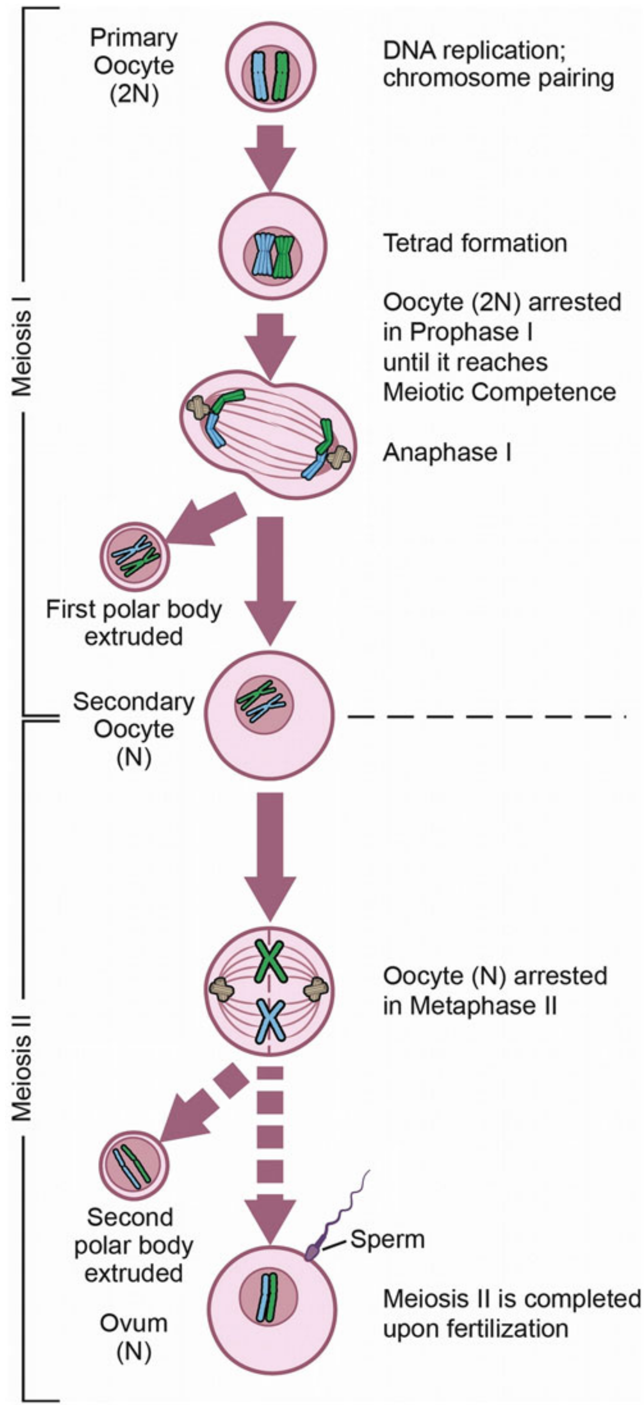

Fig. 3.2 The stages of meiosis I and II leading to the ovulation of a haploid ovum are depicted. Unlike with spermatogenesis, meiosis during oogenesis results in disproportionate cell division with the production of a single ovum with extraneous genetic material being extruded in the first and second polar bodies. During this division, the majority of the cytoplasm, containing important proteins, organelles, and growth factors, remains within the oocyte

The nucleus in the prophase oocyte is called the germinal vesicle. Ooplasmic factors prevent the resumption of meiosis in the prophase oocyte until it reaches a specific diameter and stage [13, 19–22]. This stage, referred to as "meiotic competence," occurs in the antral follicle. Once meiosis resumes, there is rapid progression through the metaphase, anaphase, and telophase stages of the first meiotic division. The oocyte then arrests at metaphase 2 of the second meiosis until sperm entry. Oocyte morphology at different stages of maturity is shown in Fig. 3.3.

Follicle Development

Folliculogenesis within the ovary is a very complex process with a high rate of follicle loss. Follicles periodically leave the resting primordial pool to join the growing pool but, in the absence of follicle-stimulating hormone (FSH), undergo atresia. After puberty, once the hypothalamus-pituitary-ovarian axis has been activated during the follicular phase, elevated FSH levels rescue the growing cohort of follicles. The ovarian paracrine signaling induces the continued growth of follicles from this cohort in a process called *initial recruitment* [23]. The recruited growing follicles, known as primary follicles, will subsequently grow into secondary and antral follicles. Figure 3.4 illustrates the different stages of follicle development. Ultimately, a single follicle will be selected from this cohort to become the dominant follicle. It will release a mature oocyte after exposure to increased levels of LH (luteinizing hormone). Almost 90 % of follicles undergo apoptosis or programmed cell death without ever becoming meiotically competent [24].

Critical to this process is the interaction between the somatic cell components and the oocyte itself. Follicle growth from the primordial to the preovulatory stage can be divided into two distinct stages based on responsiveness to the gonadotropins, FSH and LH. The first stage is relatively slow in humans, taking over 120 days [13, 25] and is not directly dependent on gonadotropin levels. Key growth mediators at this early stage may include TGF-β(beta), activin, bone morphogenetic proteins (BMPs), anti-Müllerian hormone (AMH), insulin, estrogen, and androgens. Follicle and oocyte diameters increase, follicles growing in size from 30 to 40 μm in primordial follicles to 100–200 μm in pre-antral follicles (see Fig. 3.4). The single layer of squamous granulosa cells present in the primordial follicles starts to proliferate and the oocyte becomes surrounded by several layers of cuboidal granulosa cells. Precursor thecal cells are recruited from surrounding stroma and a basement membrane forms around the follicle.

The second stage of follicular growth is far more rapid. The follicle is now responsive to the gonadotropins, FSH and LH. Granulosa cell secretions result in the formation of a fluid-filled cavity or antrum. During the early antral stage of follicle development, follicle size increases from 200 μm to

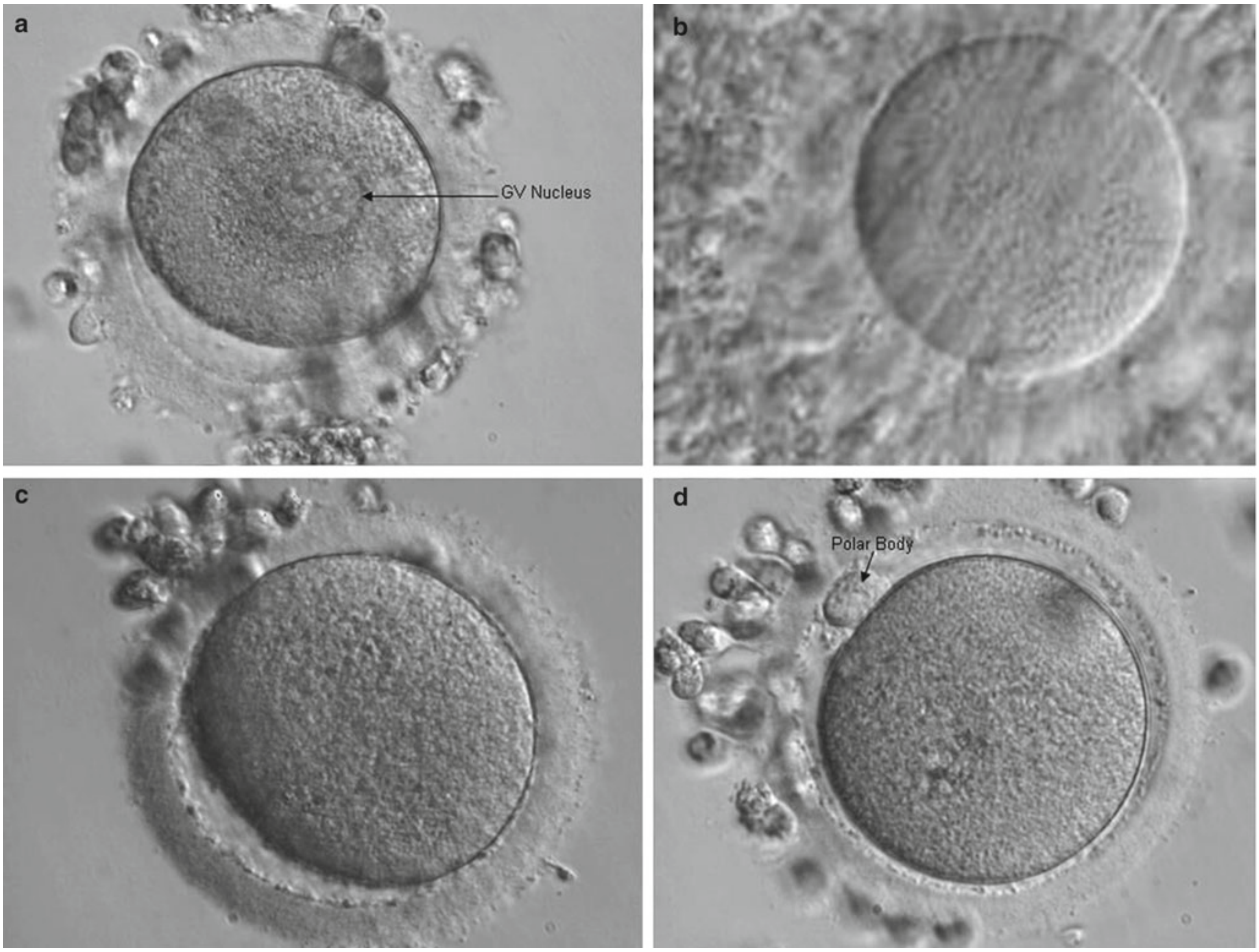

Fig. 3.3 Various stages of oocyte maturation: (**a**) immature oocyte in prophase I of meiosis I; (**b**) metaphase I oocyte; (**c**) cumulus:oocyte complex exhibiting morphology typically associated with mature oocytes at the time of ovulation; (**d**) mature metaphase II oocyte

2–5 mm. Follicle size increases to 20 mm by the time of oocyte ovulation.

The formation of a fluid-filled antrum and synthesis of steroid hormones mark the transition to the antral stage of follicle development. During this stage and with the influence of FSH, granulosa cells differentiate and become capable of aromatizing androgen, secreted by thecal cells, into estrogen. The local estrogenic environment, combined with high FSH levels, promotes further granulosa cell proliferation and an increase in FSH receptors. This, in turn, makes the follicles even more sensitive to FSH. The negative feedback of rising estrogen levels on the hypothalamus-pituitary axis limits FSH secretion. Thus, only follicles with increased FSH receptors will be able to continue to develop, while other follicles in the cohort will undergo atresia. It is through this mechanism that a single dominant follicle is selected. Continued growth of the selected follicle occurs despite the midcycle fall in FSH concentrations as a result of granulosa cells now acquiring LH receptors [26–28]. While granulosa cells of the early antral follicle are only responsive to FSH, late antral stage follicles become responsive to both FSH and LH and continue to secrete high levels of estradiol [29, 30]. The layers of specialized granulosa cells bordering the oocyte are known as cumulus cells, which are also called *corona radiata*. These cells not only support cytoplasmic maturation, but are pivotal in maintenance of meiotic arrest and induction of ovulation. The preovulatory follicle, also known as a Graafian follicle, measures over 18 mm in size, and oocyte diameter is close to its final size of about 120 μm [31]. The multilayer follicle is enclosed in a basement membrane that separates it from the underlying vascularized thecal cell layer.

Oocyte Growth

Coordinated growth of this diplotene-arrested oocyte and follicle is dependent on bi-directional communication between the oocyte and the surrounding granulosa cells [32, 33]. This communication occurs via gap junctions connecting the

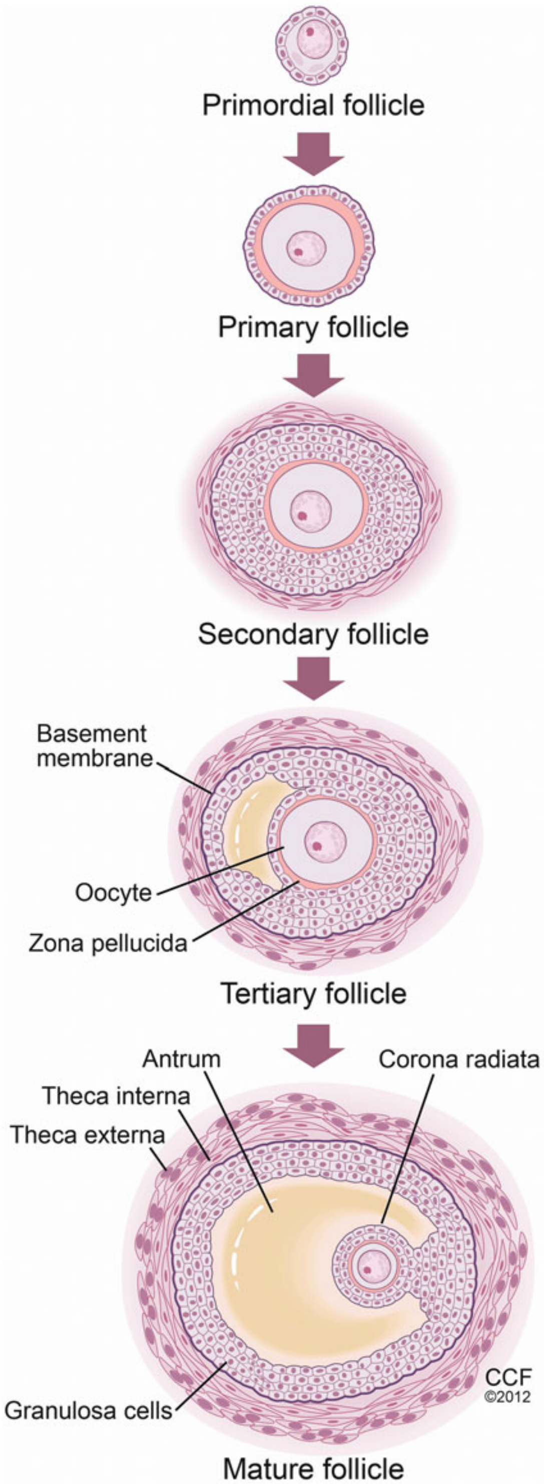

Fig. 3.4 Depicted is a sequential illustration of folliculogenesis within the human ovary. The process begins with the oocyte enclosed within single layer of granulosa cells known as the primordial follicle and ends with a fully developed multilayered follicle with antrum containing a secondary oocyte

granulosa cells and the oocyte [33–39]. These membrane channels enable the sharing of small essential molecules, including inorganic ions, second messengers, small metabolites, and secreted paracrine factors that allow growth of both cell types [39–42].

The oocyte is unable to transport certain amino acids, carry out biosynthesis of cholesterol, or undergo glycolysis without a supply of the necessary factors by the granulosa cells. Similarly, evidence suggests that granulosa cell proliferation as well as select other metabolic processes require oocyte-derived secretions [43–46]. In vitro studies on cultured follicles demonstrate that severing of gap junctions and intercellular communications triggers premature ovulation and eventual degeneration of the released oocyte [47].

Gap junctions are comprised of a variety of connexin proteins [37]. The basic structure of connexins consists of four membrane-spanning domains, two extracellular loops, a cytoplasmic loop, and cytoplasm N-and C-termini. Different connexins contain different properties, providing increased complexity in the regulation of designated molecules. Gene knock-out experiments in the mouse model have identified specific gap junction proteins and their critical role in folliculogenesis. Absence of connexin-37 interferes with antral follicle formation [48, 49], while follicles in mice lacking the gene for connexin-43 arrest in the early pre-antral stage and are unable to produce meiotically competent oocytes [50]. Phosphorylation and several different protein kinases appear also to be associated with the activation and regulation of gap junctions [51, 52].

The oocyte is metabolically active from its early growth stage, synthesizing the maternal RNA pool necessary to support early embryonic events after fertilization. Oocytes synthesize over 400 different proteins. Shortly after follicle activation and entry into the growing pool, the oocyte secretes a thick glycoprotein coat [53, 54]. This matrix coat, known as the zona pellucida, encircles the oocyte and is composed of three zona pellucida proteins, ZP1, ZP2, and ZP3. Thickness of the zona pellucida increases as the oocyte grows, reaching about 15 μm. The zona pellucida plays an important role in protecting the oocyte/embryo as it traverses the reproductive tract. The zona mediates sperm binding, confers species specificity, and serves as a block to polyspermic fertilization. Release of cortical granules by the oocyte cytoplasm at the time of fertilization results in a hardening of the zona and deters additional sperm from penetrating into the oocyte.

Resumption of Meiosis and Ovulation

Meiotic progression of the oocyte is highly dependent on a delicate balance between factors keeping the oocyte in meiotic rest and factors promoting oocyte maturation [55–61]. Cyclic AMP is one of the intracellular signaling molecules that keep the oocyte in meiotic arrest. Cyclic AMP, produced by granulosa cells, is transported via gap junctions to the oocytes. As long as the cAMP threshold is maintained, meiosis is inhibited.

Meiotic competence is also linked to oocyte size, presumably because increasing volume corresponds to increasing cytoplasmic accumulation of synthesized proteins. Oocytes that measure less than 70–80 μm have lower rates of meiotic competence compared to 100 μm fully grown follicles, which are usually able to resume meiosis. Activation of maturation or M-phase promoting factor (MPF) is required to resume meiosis [62, 63]. MPF levels increase with oocyte growth and eventually reach a threshold, at which point the oocyte becomes *meiotically competent*. MPF is composed of a regulatory unit, cyclin B, and its protein kinase CDK1 (also called $p34^{cdc2}$).

The onset of the LH surge triggers a cascade of events culminating in the ovulation of the oocyte from the Graafian follicle and initiation of oocyte maturation. LH induces a shift in steroidogenesis by granulosa cells to progesterone [64]. Cumulus cell expansion just prior to ovulation results in the severing of cumulus: oocyte connections, thus reducing intracellular cAMP levels in the oocyte levels and therein reducing its inhibitory influence on meiosis. Subsequent MPF activation drives the oocyte towards meiosis, starting first with breakdown of the germinal vesicle (GVBD). Neosynthesis of cyclin B may be a rate-limiting step, accounting for the observed lag time between resumption of meiosis and GVBD, as well as transition from metaphase I to metaphase II.

After the breakdown of the germinal vesicle, bivalents start to become organized and then align at the metaphase plate, forming a meiotic spindle. The oocyte remains arrested at the metaphase II stage until sperm penetration. An intracellular Ca^{2+} signal triggered by either the binding of sperm to oocyte receptors or else the release of a soluble sperm-derived factor during oocyte: sperm fusion initiates the destruction of endogenous cyclin [63]. The oocyte is now able to complete meiosis, with chromosome segregation beginning at the metaphase-anaphase transition. Defective chromosome segregation at this stage can lead to aneuploidy in the resulting egg and embryo.

Table 3.1 Human oogenesis and folliculogenesis beginning with the primordial germ cells (key modulators and their points of action are shown)

Developmental event	Key regulators
Primordial germ cells	
• 3 wk pc	BMP4, BMP8b
• Formation	c-KIT, SCF
• Migration and proliferation	
• Colonization of the forming gonad	
• Post-migratory survival	
Oogonia	
• Proliferation by mitosis	
Oocyte meiotic prophase 1	
• Preleptotene: replication of DNA	
• Leptotene: start of homologous pairing	
• Zygotene	
◦ Pairing of homologs	
◦ Synapsis	
• Pachytene	
◦ Crossing over	
◦ Recombination	
◦ DNA repair	
• Diplotene: meiotic arrest	cAMP
Primordial follicle	Activins, AMH, TGF β, BMPs, insulin, estrogen, androgens, c-KIT
• 16–18 wk pc	
• Diplotene-arrested oocyte	
• Single flat granulose cell layer	
Primary follicle	Activins, AMH
• Cuboidal granulose cell layer	
Pre-antral secondary follicle	Activins, Inhibins, AMH
• Late folliculogenesis	
• Granulosa proliferation	GDF-9, BMP-15
• Theca precursor formation	
Antral/Graffian follicle	Inhibins, AMH, cAMP, FSH/FSH receptor, LH/LH receptor
• Formation of antrum	
• Formation of preovulatory follicles and corpus luteum	
Cumulus expansion	LH signaling, PGE2
Meiotic resumption	MPF
Ovulation	COX-2, LH
Oocyte maturation and fertilization	Ca^{2+}

wk pc weeks post coitum; *AMH* anti-Müllerian hormone; *BMP* bone morphogenetic protein; *GDF* growth differentiation factor; *FSH* follicle-stimulating hormone; *LH* luteinizing hormone; *SCF* stem cell factor; *COX-2* cyclooxygenase isoform; *PGE2* prostaglandin E(2)

Additional Regulatory Mechanisms

Clear maintenance of the primordial follicle pool, follicle recruitment, follicle atresia and selection of a dominant follicle, combined with controlling oocyte maturation, and synchronous growth of the follicular unit, is a complex process. In this section, we discuss a few of the most important ovarian paracrine factors involved in the coordination of events during folliculogenesis (Table 3.1).

cAMP

As mentioned earlier, FSH and LH, working in concert, are two of the most important hormones involved in folliculogenesis. An important component of this system is the second messenger cAMP, which amplifies the signal induced by FSH and LH. This amplification allows for a much larger response to the FSH and LH hormones than they are able to create alone [65]. Two important sets of enzymes control intracellular cAMP homeostasis: adenyl cyclases, which generate cAMP, and the phosphodiesterases (PDEs), which break down cAMP. Binding of FSH and LH to their respective receptors, along with adenyl cyclase, activates the generation of cAMP from ATP in follicular granulose cells. The cAMP molecules activate a cascade of protein kinases, dissociating their catalytic component, which then works to phosphorylate transcription factors. These factors bind to DNA upstream of genes at positions known as cAMP response elements, regulating various follicular events such as the growth of the dominant follicle [66]. In the oocyte, a high concentration of this second messenger prevents meiosis.

TGF-β Growth Factor Family

Several key regulators involved in folliculogenesis belong to the transforming growth factor-β (TGF-β) family, many of which utilize gap junctions for communications. Important members of this group include AMH, growth differentiation factor-9 (GDF-9), BMPs, activin, and inhibin.

Anti-Müllerian Hormone

AMH is secreted by granulosa cells when a woman is reproductively active. In studies utilizing the mouse model, AMH has been shown to have two important roles during folliculogenesis. First, AMH inhibits the recruitment of additional primordial follicles when there is already a growing cohort of follicles. By this means, AMH prevents women from speeding too quickly through their oocyte reserve. Second, AMH decreases the response of these growing follicles to FSH. Studies in humans have suggested that each follicle has a unique FSH threshold that must be met in order to proceed to the preovulatory stage. AMH may influence how responsive a follicle is to the FSH surge during cyclic recruitment [67].

AMH has also been used as a measure of a woman's ovarian reserve [68]. AMH levels decrease along with the size of a woman's follicle pool. Concomitantly, lower AMH levels fail to adequately inhibit the primordial pool, and as a result there is an increase in the rate of depletion of the follicular reserve [69].

Growth Differentiation Factor-9 (GDF-9) and Bone Morphogenetic Proteins

BMPs play a variety of roles in oogenesis and are produced by a variety of cells. While little is known about specific BMP function in the human ovary, results from numerous animal studies have contributed to our understanding of the biological activities of BMPs during follicular development. Evidence from the rat model proposes that BMPs influence the primordial-primary transition as well as the subsequent transition to a secondary follicle. It has also been suggested that a drop in BMP levels may be indicative of dominant follicle selection [70, 71]. In the rat, thecal cells of secondary follicles have expressed BMP-4 and BMP-7, while their respective receptors expressed on granulosa cells [71]. They serve to modulate the actions of FSH on the synthesis of the essential steroids estradiol and progesterone [72].

Oocyte paracrine signaling is responsible for activating pathways involved in regulating cumulus cell differentiation. BMP-15 and GDF-9 are two factors secreted by the oocyte to control its local microenvironment and ultimately oocyte quality [45]. BMP-15 works alongside GDF-9 to activate signaling pathways responsible for the regulation of cumulus cell differentiation and maintenance of their phenotype [45]. The absence of these oocyte-specific factors has been demonstrated to result in sterility [73, 74]. During in vitro culture of ovarian cortical slices, GDF-9 supplementation was observed to increase the number of secondary oocytes present after 7 days of growth and also enhanced follicle density after 14 days of culture. GDF-9 may also serve to prevent atresia [75].

Activin and Inhibin

Activin and inhibin are produced by granulosa cells in the follicle and work in an antagonistic relationship. Activin, produced by many different tissues, including the gonad and anterior pituitary, stimulates the release of FSH by acting as a transcription factor activator of the FSHβ-subunit gene [76]. Meanwhile, inhibin hinders the secretion of FSH from the pituitary, balancing the actions of activin. While it is believed that inhibin plays an important role in the production of steroids and gonadotropins, little else is known about its role in the recruitment and differentiation of thecal cells [77].

c-Kit and Kit Ligand

Another important signaling pathway involved in the regulation of primordial follicles involves the c-Kit receptor and its granulosa cell produced ligand (frequently referred to as KL, stem cell factor or SCF) [78]. Little is known about the specific role of this receptor/ligand combination in the early development of follicles. The presence of the mRNA encoding for c-Kit and KL has been detected in early antral follicles present in human ovarian tissue, oocytes, and granulosa cells [78]. Much of what is known about c-Kit comes from research in the mouse model. Here, c-Kit and KL play a role in PGC survival, activation, migration, proliferation of granulosa cells, recruitment of theca cells, maintenance of meiotic competence, and the development of follicles [79].

Future Directions and Challenges

Increasing our understanding of the complex regulation of ovarian folliculogenesis and the interactions between the oocyte and granulosa-thecal cell layers has contributed significantly to the advancement of infertility treatment. Medications and ovarian stimulation regimens have been designed which permit the manipulation of a women's menstrual cycle, resulting in the production of multiple mature competent oocytes. This, combined with advances in our ability to treat male factor infertility, has dramatically altered pregnancy outcomes with in vitro fertilization over the last 2 decades.

One hurdle that has been impossible to overcome has been poor ovarian reserve. The continuous loss and eventual elimination of a woman's follicle pool through accelerated atresia is considered to be the impetus leading to menopause. This is based on the belief set forth over 50 years ago that oogenesis cannot occur in the adult ovary and so, at birth, the female ovary contains a finite oocyte pool [80]. Recently, exciting data have emerged that question this basic established dogma [81, 82]. The intriguing study, first presented in 2004 by Johnson and colleagues, questions the concept of a "non-renewing oocyte pool" [83]. An increasing body of evidence indicates that oogenesis may in fact occur in the adult mammalian ovary [82, 84, 85]. The potential existence of germ cells in the adult ovary and the development of techniques to manipulate such cells may open up new avenues for fertility treatment [86].

Another challenge in reproductive medicine has been fertility preservation for young women diagnosed with cancer. Radiation and chemotherapy during cancer treatment can result in fertility loss through damage to the ovarian follicle reserve. Cryopreservation of ovarian tissue offers hope to patients, but how best to use this tissue to restore fertility is still problematic. Ovarian tissue transplant after the patient is in remission has been met with limited success. Additionally, the possibility of reintroducing the cancer always remains. An alternate solution is the isolation of ovarian follicles from cryopreserved tissue, followed by in vitro maturation of the follicles [87]. The long time interval necessary for human follicle growth from the primordial to the preovulatory stage (~120 days) and the intricate signaling mechanisms necessary for proper follicle growth are quite difficult to mimic in vitro. Maintenance of the spheroid follicle architecture during prolonged growth in conventional 2D culture systems is not possible. Moreover, as the follicle flattens, oocyte:granulosa cell connections are disrupted and critical bi-directional communications between the oocyte and its surrounding somatic cells are lost. Design of 3D follicle culture models has therefore been the focus of much research [87–94].

The challenge of maturing human follicles in vitro and creating competent oocytes may take years to accomplish and will be fueled by our growing understanding of the complex interactions between the oocyte and its supporting granulosa and thecal cell components. The successful culture of human follicles in vitro will ultimately herald a new age in reproductive medicine and the treatment of infertility.

Organization of the Testis

The testes are ellipsoid in shape, measuring 2.5×4 cm in diameter and engulfed by a capsule (tunica albuginea) of strong connective tissue [95]. Along its posterior border, the testis is loosely connected to the epididymis, which gives rise to the vas deferens at its lower pole [96]. The testis has two main functions: it produces hormones, in particular testosterone, and it produces the male gamete—the spermatozoa. The spermatozoa express unique antigens that are not formed until puberty. The blood–testis barrier develops as these autoantigens develop. The blood–testis barrier makes the testis an immunologically privileged site. The testis is incompletely divided into a series of lobules. Most of the volume of the testis is made up of seminiferous tubules, which are looped or blind-ended and packed in connective tissue within the confines of the fibrous septae (see Fig. 3.5). The fibrous septae divide the parenchyma into about 370 conical lobules consisting of the seminiferous tubules and the intertubular tissue. The seminiferous tubules are separated by groups of Leydig cells, blood vessels, lymphatics, and nerves. The seminiferous tubules are the site of sperm production. The wall of each tubule is made up of myoid cells of limited contractility and also of fibrous tissue. Each seminiferous tubule is about 180 μm in diameter, the height of the germinal epithelium measures 80 μm, and the thickness of the peritubular tissue is about 8 μm. The germinal epithelium consists of cells at different stages of development located within the invaginations of Sertoli cells, namely, spermatogonia, primary and secondary spermatocytes, and spermatids. Both ends of the seminiferous tubules open into the spaces of the rete testis. The fluid secreted by the seminiferous tubules is collected in the rete testis and delivered in the ductal system of the epididymis.

Supporting Cells

The supporting cells of the testes refer to cells that are part of the cellular development that leads to a mature sperm. They are, however, extremely important to sperm production, and spermatogenesis would be impossible without them. The two most important cells are the Leydig and Sertoli cells.

Leydig Cells

The Leydig cells are irregularly shaped cells with granular cytoplasm present individually or more often in groups within the connective tissue [97, 98]. Leydig cells are the prime source of the male sex hormone testosterone. The pituitary hormone, luteinizing hormone (LH), acts on Leydig cells to stimulate the production of testosterone. This acts as

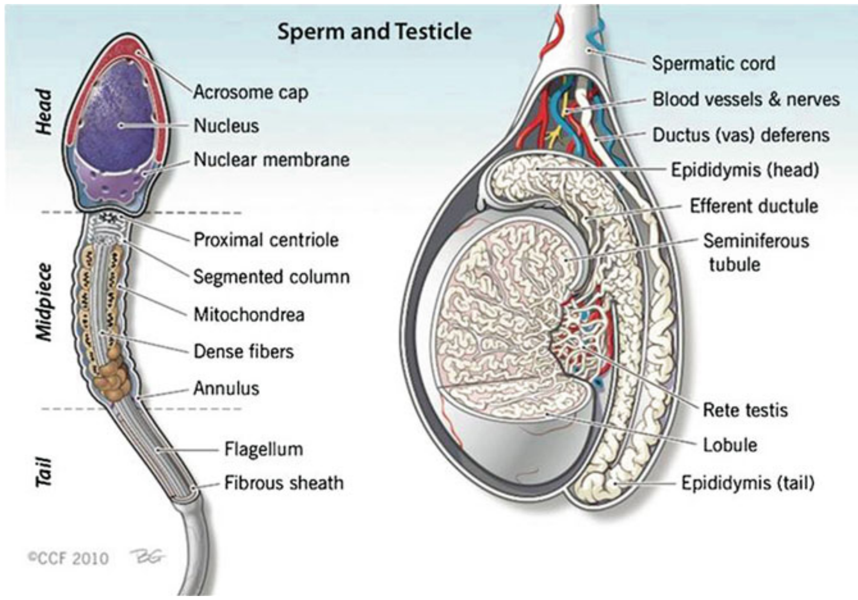

Fig. 3.5 The human testis and the epididymis. The testis shows the tunica vaginalis and tunica albuginea, seminiferous tubule septae, rete testis, and the overlying head, body, and tail of the epididymis. To the *left* is a diagrammatic representation of a fully mature spermatozoon (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

a negative "feedback" on the pituitary to suppress or modulate further LH secretion. Compared with the testosterone levels in the blood, the intratesticular concentration of testosterone is many times higher, especially near the basement membrane of the seminiferous tubule.

The Sertoli Cell

The seminiferous tubules are lined with highly specialized Sertoli cells that rest on the tubular basement membrane and extend into the lumen with a complex ramification of cytoplasm (see Fig. 3.6). Spermatozoa are produced at puberty, but are not recognized by the immune system that develops during the first year of life. The seminiferous tubule space is divided into basal (basement membrane) and luminal (lumen) compartments by strong intercellular junctional complexes called "tight junctions." These anatomic arrangements, complemented by closely aligned myoid cells that surround the seminiferous tubule, form the basis for the blood–testis barrier. The blood–testis barrier provides a microenvironment for spermatogenesis to occur in an immunologically privileged site. Sertoli cells serve as "nurse" cells for spermatogenesis, nourishing germ cells as they develop. These also participate in germ cell phagocytosis. Multiple sites of communication exist between Sertoli cells and developing germ cells for the maintenance of spermatogenesis within an appropriate hormonal milieu. FSH binds to the high-affinity FSH receptors found on Sertoli cells signaling the secretion of androgen-binding protein. High levels of androgens are also present within the seminiferous tubule.

The two most important hormones secreted by the Sertoli cells are AMH and inhibin. AMH is a critical component of embryonic development and is involved in the regression of the Müllerian ducts. Inhibin is a key macromolecule in pituitary FSH regulation. Some of the functions of the Sertoli cell are (1) maintenance of integrity of seminiferous epithelium, (2) compartmentalization of seminiferous epithelium, (3) secretion of fluid to form tubular lumen to transport sperm within the duct, (4) participation in spermiation, (5) phagocytosis and elimination of cytoplasm, (6) delivery of nutrients to germ cells, (7) steroidogenesis and steroid metabolism, (8) movement of cells within the epithelium, (9) secretion of inhibin and androgen-binding protein, (10) regulation of spermatogenic cycle, and (11) providing a target for hormones LH, FSH, and testosterone receptors present on Sertoli cells.

Spermatogenesis

The process of differentiation of a spermatogonium into a spermatid is known as spermatogenesis. It is a complex, temporal event whereby primitive, totipotent stem cells divide to either renew them or produce daughter cells that become into a specialized testicular spermatozoa over a span of weeks (see Fig. 3.7). Spermatogenesis involves both mitotic and meiotic proliferation as well as extensive cell remodeling. Spermatogenesis can be divided into three major phases:

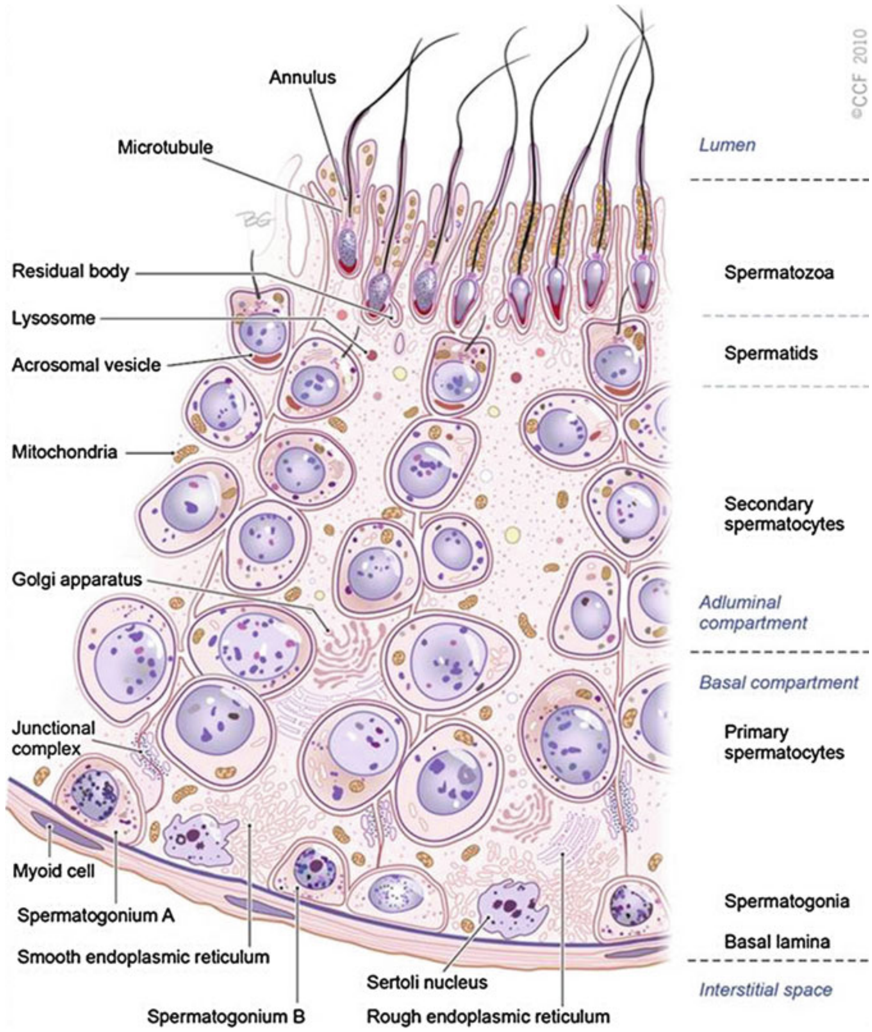

Fig. 3.6 Section of the germinal epithelium in the seminiferous tubule. Sertoli cells divide the germinal epithelium into a basal and adluminal compartment, via the Sertoli cell. Spermatozoa are released into the lumen (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

(1) proliferation and differentiation of spermatogonia, (2) meiosis, and (3) spermiogenesis, a complex metamorphosis that transforms round spermatids arising from the final division of meiosis into a complex structure called the *spermatozoon*. In humans, the process of spermatogenesis starts at puberty and continues throughout the entire life span of the individual. It takes place in the lumen of seminiferous tubules. In fact, 90 % of the testis volume is determined by the seminiferous tubules and their constituent germ cells at various stages of development. Once the gonocytes have differentiated into fetal spermatogonia, an active process of mitotic replication is initiated very early in the embryonic development. This appears to be under FSH control and develops the baseline number of precursor cells of the testicle.

Within the seminiferous tubule, germ cells are arranged in a highly ordered sequence from the basement membrane to the lumen. Spermatogonia lie directly on the basement membrane, followed by primary spermatocytes, secondary spermatocytes, and spermatids as they progress toward the tubule lumen. The tight junction barrier supports spermatogonia and early spermatocytes within the basal compartment and all subsequent germ cells within the luminal compartment.

Spermatogenesis can also be disturbed by a number of external factors, including nutrition, therapeutic drugs, increased scrotal temperature, and X-radiation.

Types of Spermatogonia

Spermatogonia represent a population of cells that divide by mitosis, providing a renewing stem cell population as well as spermatogonia that are committed to enter the meiotic process. Germ cells are staged by their morphologic appearance; there are dark type A (A_{dark}), pale type A (A_{pale}), and type B spermatogonia; primary spermatocytes (preleptotene, leptotene, zygotene, and pachytene); secondary spermatocytes;

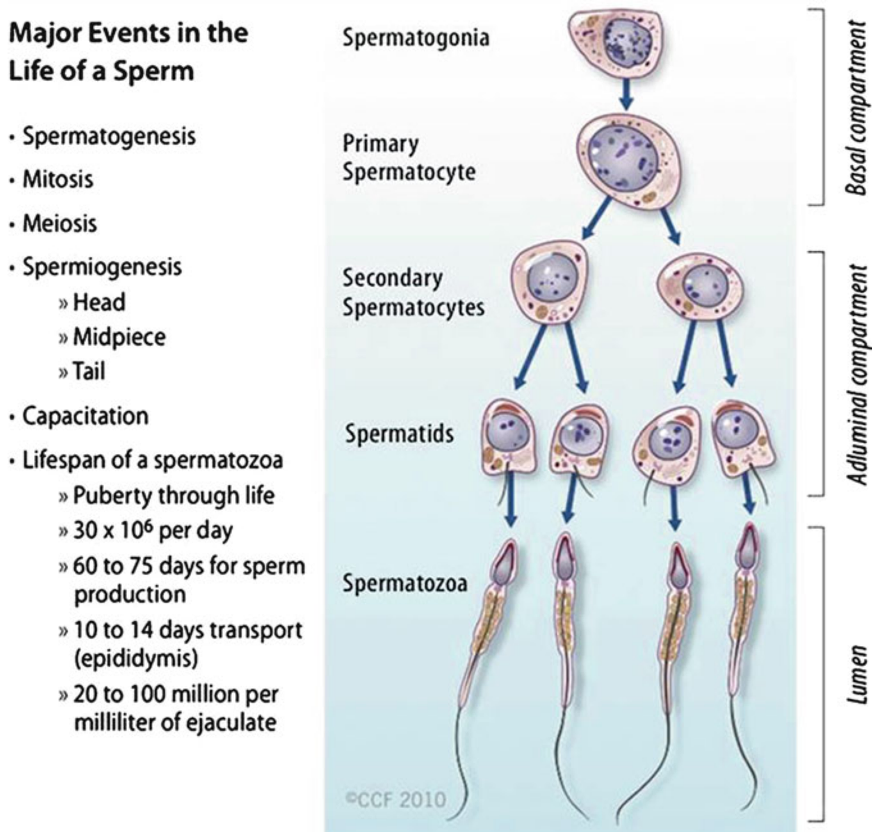

Fig. 3.7 A diagrammatic representation of major events in the life of a sperm involving spermatogenesis, spermiogenesis, and spermiation during which the developing germ cells undergo mitotic and meiotic division to reduce the chromosome content (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

and spermatids (Sa, Sb, Sc, Sd_1, and Sd_2). Other proliferative spermatogonia include A_{paired} (A_{pr}), resulting from dividing $A_{isolated}$ (A_{is}), and subsequently dividing to form $A_{aligned}$ (A_{al}). Differentiated spermatogonia include type A1, A2, A3, A4, intermediate, and type B, each a result of the cellular division of the previous type. In humans, four spermatogonial cell types have been identified; these are A_{long}, A_{dark}, A_{pale}, and B [99, 100]. In the rat, type $A_{isolated}$ (A_{is}) is believed to be the stem cell [101]; however, it is not clear which human type A spermatogonia is the stem cell. Some investigators have proposed that the type A_{dark} spermatogonia represent the reserve or nonproliferative spermatogonial population that gives rise to A_{pale} [100]. Type B spermatogonia possess considerably more chromatin within the inner nuclear envelope than intermediate or type A spermatogonia (see Fig. 3.8). Type B spermatogonia represent the cells that differentiate and enter into the process of meiosis, where they are called primary spermatocytes [100]. They are the differential precursors to preleptotene spermatocytes. This last mitotic division helps maintain a pool of stem cells so that the process can continue indefinitely.

Spermatogonia do not separate completely after meiosis but remain joined by intercellular bridges, which persist throughout all stages of spermatogenesis and are thought to facilitate biochemical interactions, allowing synchrony of germ cell maturation [102].

Spermatocytogenesis

The purpose of spermatogenesis is to produce genetic material necessary for the replication of the species through mitosis and meiosis. Spermatocytogenesis takes place in the basal compartment. Primary spermatocytes enter the first meiotic division to form secondary spermatocytes. The prophase of the first meiotic division is very long; thus, the primary spermatocyte has the longest life span. Secondary spermatocytes undergo the second meiotic division to produce spermatids. Secondary spermatocytes are short-lived (1.1–1.7 days).

Mitosis

Mitosis involves proliferation and maintenance of spermatogonia. It is a precise, well-orchestrated sequence of events involving duplication of the genetic material (chromosomes), breakdown of the nuclear envelope, and equal

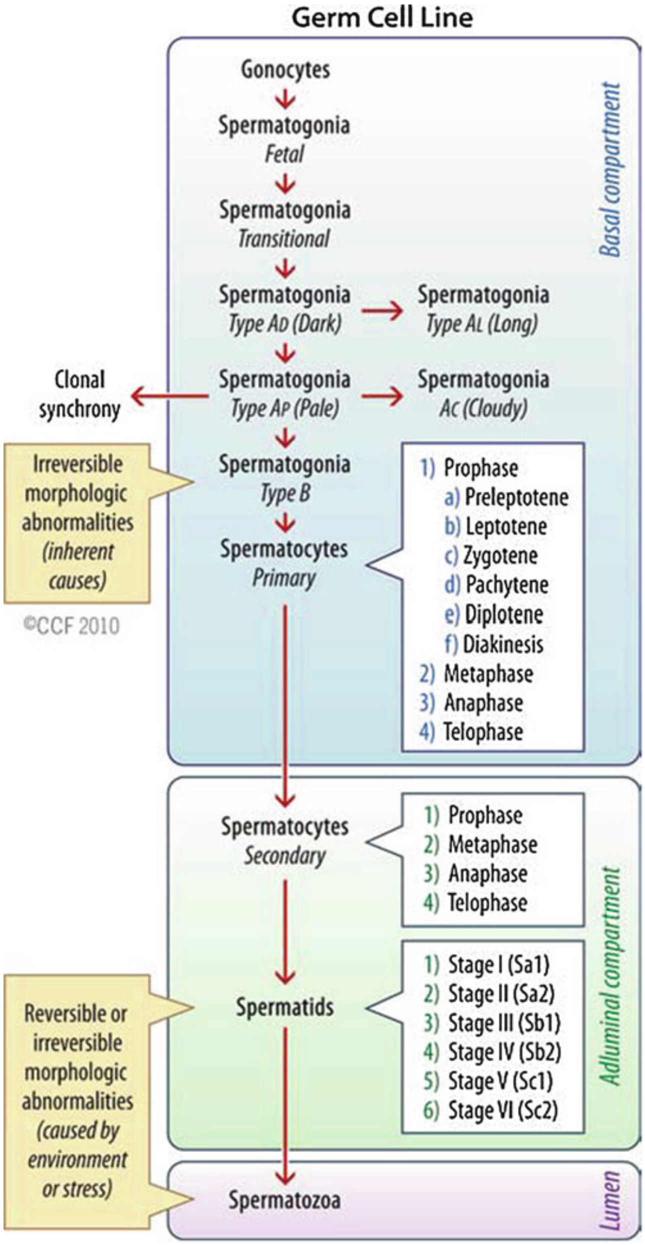

Fig. 3.8 Schematic representation of the development of a diploid undifferentiated germ cell into a fully functional haploid spermatozoon along the basal to the adluminal compartment and final release into the lumen. Different steps in the development of primary, secondary, and spermatid stages are also shown and the irreversible and reversible morphological abnormalities that may occur during various stages of spermatogenesis (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

division of the chromosomes and cytoplasm into two daughter cells [103]. DNA is also spatially organized into loop domains on which specific regulatory proteins interact during cellular replication [103–105]. The mitotic phase involves spermatogonia (types A and B) and primary spermatocytes (spermatocytes I). Developing germ cells interconnected by intracellular bridges produce the primary spermatocyte through a series of mitotic divisions. Once the baseline number of spermatogonia is established after puberty, the mitotic component will proceed in order to continue to provide precursor cells and to start the process of differentiation and maturation.

Meiosis

Meiosis is a complex process with specific regulatory mechanisms of its own [106]. The process commences when type B spermatogonia lose their contact with the basement membrane to form preleptotene primary spermatocytes. Thus, each primary spermatocyte can theoretically yield four spermatids, although fewer actually result, because some germ cells are lost due to the complexity of meiosis. The primary spermatocytes are the largest germ cells of the germinal epithelium. Meiosis is characterized by prophase, metaphase, anaphase, and telophase. In this, two successive cell divisions yield four haploid spermatids from one diploid primary spermatocyte. As a consequence, the daughter cells contain only half of the chromosome content of the parent cell. After the first meiotic division (reduction division), each daughter cell contains one partner of the homologous chromosome pair, and they are called *secondary spermatocytes*. These cells rapidly enter the second meiotic division (equational division), in which the chromatids then separate at the centromere to yield haploid early round spermatids. Meiosis assures genetic diversity and involves primary and secondary spermatocytes, which give rise to spermatids.

The meiotic phase involves primary spermatocytes until spermatids are formed; during this process, chromosome pairing, crossover, and genetic exchange are accomplished until a new genome is determined. In turn, a post-meiotic phase involving spermatids all the way up to spermatozoa develops, ending in the formation of the specialized cell.

Spermiogenesis

Spermiogenesis is a process during which the morphologic changes occur during the differentiation of the spermatid into the spermatozoon. It begins once the process of meiosis is completed. Six different stages have been described in the process of spermatid maturation in humans, as S_{a-1} and S_{a-2}, S_{b-1} and S_{b-2}, and S_{c-1} and S_{c-2} (see Fig. 3.9). Each of these stages can be identified by the morphologic characteristics. During the S_{a-1} stage, both the Golgi complex and mitochondria are well developed and differentiated, the acrosomal vesicle appears, the chromatid body develops in one pole of the cell opposite from the acrosomal vesicle, and the proximal centriole and the axial filament appear. During S_{b-1} and

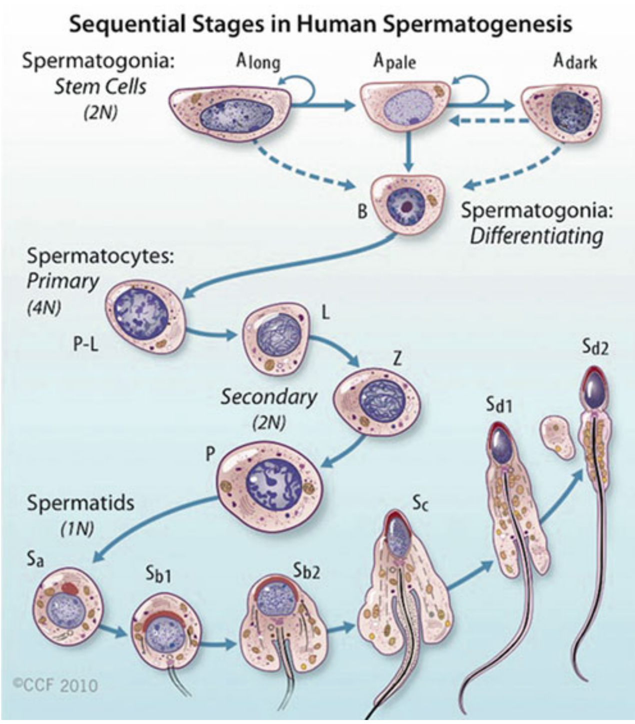

Fig. 3.9 Differentiation of a human diploid germ cell into a fully functional spermatozoon (All Rights Reserved Sperm Chromatin, ed. Zini A and Agarwal A, Biological and Clinical applications in Male Infertility and Assisted Reproduction, Springer Science + Business Media 2011)

$S_{b\text{-}2}$ stages, acrosome formation is completed, the intermediate piece is formed, and the tail develops. This process is completed during the S_c stages.

Nuclear Development

During spermatogenesis, a number of changes occur to the nucleus and its contents. The nucleus of the spermatozoa elongates and flattens from step 1 to step 8 of spermiogenesis [107], giving the head its characteristic oval shape. The necessity of this compaction has been debated, but it is widely believed to facilitate the penetration of the oocyte and to help optimize the swimming capability of the spermatozoa [108].

The compaction of the nucleus includes modification of the chromatin material. During the last post-meiotic phase of spermiogenesis, the histone molecules, around which the DNA is organized, are converted into transitional proteins, which are then converted to protamines [109]. Protamines contain large amounts of cysteine, which aid in the formation of protamine disulfide bonds as the sperm matures in the epididymis [110–112]. Within 2–4 h of fertilization, the protamines in the chromatin of the spermatozoa are replaced by histones from the oocyte.

Spermiation

The process whereby a mature spermatid frees itself from the Sertoli cell and enters the lumen of the tubule as a spermatozoon is known as *spermiation*. The spermatids originating from the same spermatogonia remain connected by bridges to facilitate the transport of cytoplasmic products. Spermiation involves the active participation of the Sertoli cell. This may also involve actual cell movement as the spermatids advance towards the lumen of the seminiferous tubules [112]. The mature spermatids close their intracellular bridges, disconnect their contact to the germinal epithelium, and become free cells called *spermatozoa*. Portions of the cytoplasm of the Sertoli cell known as *cytoplasmic droplet* may remain as part of the spermatozoon during the process of spermiation. This is a morphological feature present on the immature sperm in semen [113].

The Cycle or Wave of Seminiferous Epithelium

A cycle of spermatogenesis involves the division of primitive spermatogonial stem cells into subsequent germ cell types through the process of meiosis. Type A spermatogonial divisions occur at a shorter time interval than the entire process of spermatogenesis. Therefore, at any given time, several cycles of spermatogenesis coexist within the germinal epithelium. In humans, spermatocyte maturation takes 25.3 days, spermiogenesis 21.6 days, and the total estimated time for spermatogenesis is 74 days. Spermatogenesis is not random throughout the seminiferous epithelium. Germ cells are localized in spatial units referred to as *stages* and represent consistent associations of germ cell steps [114, 115]. In rodent spermatogenesis, one stage can be found in a cross-section of seminiferous tubule.

Each stage is recognized by development of the acrosome, meiotic divisions and shape of the nucleus, and release of the sperm into lumen of the seminiferous tubule. The cycle of spermatogenesis can be identified for each species, but the duration of the cycle varies for each species [100]. The stages of spermatogenesis are sequentially arranged along the length of the tubule. This arrangement of the stages of spermatogenesis is such that it results in a "wave of spermatogenesis" along the tubule. This wave is in space but the cycle is in time [115]. Along the length of the seminiferous tubule there are only certain cross-sections where spermatozoa are released. In the rat, all stages are involved in spermatogenesis, but spermatozoa are released only in stage VIII.

The stages of spermatogenesis are organized spatially as well as in time [115]. Thus, a position in the tubule that is occupied by cells comprising stage I will become stage II, followed by stage III, until the cycle repeats. In humans, the duration of the cycle is 16 days, and the progression from spermatogonia to spermatozoa takes 70 days, or four and a half cycles of the seminiferous cycle. During spermatogenesis, cytoplasmic bridges link cohorts of germ cells that are at the same point in development, and these cells pass through the process together. Groups of such cells at different stages can be observed histologically on cross-section, and many germ cell cohorts are seen only in association with certain other germ cells. This has led to the description of six stages of the seminiferous tubule epithelium in men. To add another level of complexity, the steps of the spermatogenic cycle within the space of seminiferous tubules demonstrate a specific spatial organization, termed *spermatogenic waves*. In humans, this wave appears to describe a spiral cellular arrangement as one progresses down the tubule. This spatial arrangement probably exists to ensure that sperm production is a continuous rather than a pulsatile process.

Efficiency of Spermatogenesis

Spermatogenic efficiency varies between different species but appears to be relatively constant in man. The time for the differentiation of a spermatogonium into a mature spermatid is estimated to be 70 ± 4 days [116]. In comparison to animals, the spermatogenetic efficiency in man is poor. The daily rate of spermatozoa production is calculated at 3–4 million per gram of testicular tissue [117]. A higher number of spermatozoa should be expected in the ejaculate than the 20 million/mL described by the World Health Organization manual in 1999 [118] and the 15 million/mL in 2010 [119]. A majority of the cells developed (>75 %) are lost as a result of apoptosis or degeneration; of the remaining, more than half are abnormal. Therefore, only about 12 % of the spermatogenetic potential is available for reproduction [120]. An age-related reduction in daily sperm production in men, which is associated with a loss of Sertoli cells, is also seen. This may result from an increase in germ cell degeneration during prophase of meiosis or from loss of primary spermatocytes. There is also a reduction in the number of Leydig cells, non-Leydig interstitial cells, myoid cells, and Sertoli cells.

Structure of the Spermatozoa

Spermatozoa are highly specialized and condensed cells that neither grow nor divide. A spermatozoon consists of the head, which contains the paternal material (DNA) and the acrosome, the neck, and the tail, which provides motility. The spermatozoon is endowed with a large nucleus but lacks the large cytoplasm characteristic of most body cells. Men are unique in the morphologic heterogeneity of the ejaculate.

Head

The head of the spermatozoa is ovular, measuring about 4.0–5.5 μm in length and 2.5–3.5 μm in width. The normal length-to-width ratio is about 1.50–1.70 [118]. Under bright field illumination, the most commonly observed aberrations include head shape/size defects, including large, small, tapering, piriform, amorphous, vacuolated (>20 % of the head surface occupied by unstained vacuolar areas), and double heads, or any combination [118].

The head also contains the acrosome, which is a cap-like structure represented by the Golgi complex and covers about two thirds of the anterior head area [118]. The apical thickening seen in many other species is missing; however, the acrosome shows a uniform thickness/thinning toward the equatorial segment and covers about 40–70 % of the sperm head. When observed under the scanning electron microscope, a furrow that completely encircles the head (i.e., acrosomal and post-acrosomal regions) divides the sperm head unequally. An equatorial segment that is followed by the post-acrosomal region is not very clearly visible on scanning electron microscope. The maximal thickness and width of the spermatozoon is seen in the post-acrosomal region.

During fertilization of the egg, the fusion of the outer acrosomal membrane with the plasma membrane at multiple sites releases the acrosomal enzymes at the time of the acrosome reaction. The anterior half of the head is devoid of the plasma and outer acrosomal membrane and is covered only by the inner acrosomal membrane. The posterior region of the sperm head is covered by a single membrane called the *postnuclear cap*. The overlap of the acrosome and the postnuclear cap results in an equatorial segment which does not participate in the acrosome reaction.

Neck

This forms a junction between head and tail. It is fragile and the presence of decapitated spermatozoa is a common abnormality.

Tail

The sperm tail is 40–50 μm long and arises at the spermatid stage. The tail contains the motility apparatus of the spermatozoa and propels by waves generated in the neck region and pass along the tail like a whiplash. It is formed during spermiogenesis due to the differentiation of the centriole into three parts, which can be clearly observed under scanning electron microscopy: the midpiece, the main or principle piece, and the endpiece. The mitochondria are organized helically around the midpiece. The mitochondrial sheath of the midpiece is relatively short but slightly longer than the combined length of the head and neck. An axial core comprising of two central fibrils is surrounded by a concentric ring of nine double fibrils, which continue to the end of the tail. The additional outer ring comprises nine coarse fibrils. The principle piece, the longest part of the tail, provides most of the propellant machinery. The coarse nine fibrils of the outer ring diminish in thickness and finally disappear, leaving only the inner fibrils in the axial core for most of the length of the principle piece. The fibrils of the principle piece are surrounded by a fibrous tail sheath, which consist of branching and anastomosing semicircular strands or ribs held together by their attachment to two bands that run lengthwise along opposite sides of the tail. The tail terminates in the endpiece with a length of 4–10 μm and a diameter of less than 1 μm. The small diameter is due to the absence of the outer fibrous sheath and a distal fading of the microtubules.

The tail can be clearly observed under bright field illumination. Common tail abnormalities include tail absence, bent tails, distended or irregular/bent midpiece, abnormally thin midpiece (no mitochondrial sheath), and short, multiple, hairpin, and broken tails, tails of irregular width, coiling tails with terminal droplets, or any of these combinations [118].

Regulation of Spermatogenesis

The spermatogenic process is maintained by different intrinsic and extrinsic influences.

Intrinsic Regulation

Leydig cells secrete hormone (testosterone), neurotransmitters (neuroendocrine substances) and growth factors to neighboring Leydig cells, blood vessels, lamina propria of the seminiferous tubules, and Sertoli cells [97, 120, 121]. They help maintain the nutrition of the Sertoli cells and the cells of the peritubular tissue and influence the contractility of myofibroblasts, thereby regulating the peristaltic movements of seminiferous tubules and transportation of the spermatozoa. Leydig cells also help in the regulation of blood flow in the intertubular microvasculature [95]. In addition, different growth factors are delivered from Sertoli cells and various germ cells participating in a complicated regulation of cell functions and developmental processes of germ cells. Altogether these factors represent an independent intratesticular regulation of spermatogenesis.

Extrinsic Influences

The local regulation of spermatogenesis is controlled by the hypothalamus and hypophysis. Pulsatile secretion of gonadotropin-releasing hormone of the hypothalamus initiates the release of LH from the hypophysis; in response, the Leydig cells produce testosterone. Testosterone not only influences spermatogenesis, but is also distributed throughout the body. It thus provides feedback to the hypophysis that regulates the secretory activity of Leydig cells. Stimulation of Sertoli cells by FSH is necessary for maturation of the germ cells. Complete qualitative spermatogenesis requires both FSH and LH. The interaction between the endocrine and paracrine mechanisms determines the functions within the testis [122]. Inhibin secreted by Sertoli cells functions in the feedback mechanism directed to the hypophysis. These extratesticular influences are necessary for the regulation of intratesticular functions. Thus, both growth and differentiation of testicular germ cells involves a series of complex interactions both between somatic and germinal elements [123, 124].

The Epididymis

The epididymis lies along the dorsolateral border of each testis. It is made up of the efferent ductules, which emanate from the rete testis, and the epididymal ducts. The epididymis opens into the vas deferens, which then passes through the inguinal canal into the peritoneal cavity and opens into the urethra adjacent to the prostate. It is divided into three functionally distinct regions: head, body, and tail or caput epididymis, corpus epididymis, and cauda epididymis. Their functions can be described simplistically and increasing the concentration, maturation, and storage of the spermatozoa.

Much of the testicular fluid that transports spermatozoa from the seminiferous tubules is resorbed in the caput, increasing the concentration of the spermatozoa by 10- to 100-fold. The epididymal epithelium secretes the epididymal plasma in which the spermatozoa are suspended. As the newly developed spermatozoa pass through these regions of the epididymis, many changes occur, including alterations in net surface charge, membrane protein composition, immunoreactivity, phospholipid and fatty acid content, and adenylate cyclase activity. Many of these changes are thought to improve the structural integrity of the sperm membrane and also increase the fertilization ability of the spermatozoa. The capacities for protein secretion and storage within the epididymis are known to be extremely sensitive to temperature and reproductive hormone levels, including estrogens.

As many as half of the spermatozoa released from the testis die and disintegrate within the epididymis and are resorbed by the epididymal epithelium. The remaining mature spermatozoa are stored in the cauda epididymis, which contain about 70 % of all spermatozoa present in the male tract. The capacity for sperm storage decreases distally; spermatozoa in the vas deferens may only be motile for a few days. The environment of the cauda epididymis is adapted for storage in laboratory animals. In humans, it is not a perfect storage organ, and the spermatozoa do not remain in a viable state indefinitely. After prolonged sexual activity, caudal spermatozoa first lose their fertilizing ability, followed by their motility and then their vitality; they finally disintegrate. Unless these older, senescent spermatozoa are eliminated from the male tract at regular intervals, their relative contribution to the next ejaculate(s) increases, thus reducing semen quality, even though such ejaculates do have a high sperm concentration. The transit time of sperm through the fine tubules of the epididymis is thought to be 10–15 days in humans.

As spermatozoa traverse the epididymis, they are exposed to a continuously changing milieu of the luminal fluid derived from the rete testis and modified by the secretory and absorptive activity of the epididymal epithelium. In nonhuman mammals, there is compelling evidence that the epididymal epithelium does provide essential factors for sperm maturation [125, 126]. In humans, most of the information is obtained from treatment of pathologic cases rather than from normal fertile men. Both epididymal maturation and capacitation are necessary before fertilization. The epididymis is limited to a storage role because spermatozoa that have never passed through the epididymis and that have been obtained from the efferent ductules in men with congenital absence of vas deferens can fertilize the human oocyte in vitro and result in pregnancy with live birth (as well as with intracytoplasmic sperm injection with sperm obtained after testicular biopsy). A direct involvement of the epididymis comes from the results of epididymovasostomy bypass operations for epididymal obstruction. However, these results are not consistent, whereas significantly reduced fertility was reported in anastomosis of vas deferens to the proximal 10 mm of the duct compared to anastomosis of the more distal region of the tract [127]; others have reported pregnancies in anastomosis of the vas deferens to the efferent ductules [128] or from spermatozoa retrieved from the efferent ductules and proximal caput regions of men with congenital absence of vas deferens and used for successful in vitro fertilization [129]. More direct evidence of epididymal involvement comes from experiments in which immature epididymal sperm recovered from fertile men were incubated in the presence of human epithelial cell cultures [130]. These spermatozoa showed improved motility and a significant increase in their capacity to attach to human zona in vitro.

Sperm Entry into Cervical Mucus

At the moment of ejaculation, spermatozoa from the cauda epididymis are mixed with secretions of the various accessory glands in a specific sequence and deposited around the external cervical os and in the posterior fornix of the vagina. Spermatozoa in the first fraction of the ejaculate have significantly better motility and survival than the later fractions. The majority of the spermatozoa penetrate cervical mucus within 15–20 min of ejaculation [130]. The ability to migrate across the semen–mucus interphase is highly dependent on the specific movement pattern of the spermatozoa [131]. At the time of sperm penetration into the cervical mucus, further selection of the spermatozoa occurs based on the differential motility of the normal vs. abnormal spermatozoa. This is further modified once the "vanguard" spermatozoon is within the cervical mucus [132]. The receptivity of the cervical mucus to the penetration by the spermatozoa is cyclic, increasing over a period of about 4 days before ovulation and decreasing rapidly after ovulation. Maximum receptivity is seen the day before and on the day of the LH peak [133]. Spermatozoa enter the uterine cavity from the internal cervical os by virtue of their own motility. From here the spermatozoa traverse to the site of fertilization in the ampulla of the fallopian tube or the oviduct.

Capacitation and Acrosome Reaction

Animal studies in rats and rabbits indicate that spermatozoa that are stored in the female tract are unable to penetrate the ova. They have to spend time in the female tract before they acquire this ability. Capacitation is a series of cellular or physiological changes that spermatozoa must undergo in order to fertilize [134]. It represents a change in the molecular organization of the intact sperm plasma membrane that is characterized by the ability to undergo the acrosome reaction, to bind to the zona pellucida, and to acquire hypermotility.

Capacitation involves the removal of seminal plasma factors that coat the surface of the sperm; modification of the surface charge; modification of the sperm membrane and of the sterols, lipids, and glycoproteins and the outer acrosomal membrane lying immediately under it. It also involves an increase in intracellular-free calcium [135]. Changes in sperm metabolism, increase in 3′,5′-cyclic monophosphate, and activation of acrosomal enzymes are believed to be components of capacitation. Sperm capacitation may be initiated in vivo during migration through cervical mucus [136]. Capacitation may be an evolutionary consequence of the development of a storage system for inactive sperm in the caudal epididymis.

Capacitation can also be achieved by culture medium supplemented with appropriate substrates for energy and in the presence of protein or biological fluid such as serum or follicular fluid. Usually it takes about 2 h for sperm to undergo capacitation in vitro. Further modifications occur when capacitated sperm reach the vicinity of the oocyte.

The acrosome reaction confers the ability to penetrate the zona pellucida and also confers the fusogenic state in the plasmalemma overlying the nonreactive equatorial segment, which is needed for interaction with the oolemma. There are distinct fusion points between the outer acrosomal membrane and the plasma membrane. The fusion begins posteriorly around the anterior border of the equatorial segment, which is always excluded from the reaction. The changes, termed *acrosome reaction*, prepare the sperm to fuse with the egg membrane. The removal of cholesterol from the surface membrane prepares the sperm membrane for the acrosome reaction [137]. In addition, D-mannose-binding lectins are also involved in the binding of human sperm to the zona pellucida [138].

Thus, these series of changes are necessary to transform the stem cells into fully mature, functional spermatozoa equipped to fertilize the egg.

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4 Normal Puberty and Pubertal Disorders

M. Jonathon Solnik and Joseph S. Sanfilippo

Normal Puberty

The activation of the hypothalamic-pituitary-ovarian (HPO) axis represents the commencement of reproductive life in the adolescent female. This period of change allows for a pivotal entry point towards establishing longitudinal care with the clinician. A thorough understanding of the appropriate timing of events and an awareness of the stressors that frequently complement these changes is then essential for the pediatrician, gynecologist, or primary care provider.

In the higher cortical centers, from the arcuate nucleus of the hypothalamus, gonadotropin-releasing hormone (GnRH) is synthesized and released [1]. Through its effect on the anterior pituitary, GnRH regulates the synthesis, storage, and release of the pituitary gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). These hormone levels approach that of an adult in the fetal circulation by mid-gestation. However, with increasing maternal steroid hormone production towards term, gonadotropin levels decline. Shortly after delivery, as the maternal source of estrogen is withdrawn, gonadotropin levels are noted to increase as a result of the release from the negative-feedback circuit [2].

This sequence of events demonstrates the functional capability of the HPO axis early in development and results in follicular growth in the prepubertal ovary and an increase in circulating estradiol. This effective and exquisitely sensitive negative-feedback system, often referred to as the gonadostat, develops rapidly. In the years preceding puberty, gonadotropin levels remain low in response to suppression by low levels of circulating estrogen (10 pg/mL).

It is thought that the two primary inhibitory influences on the pulsatile release of GnRH and the downregulation of the HPO axis during childhood are the (1) intrinsic central nervous system (CNS) inhibition via γ(gamma)-aminobutyric acid (GABA) and the (2) negative-feedback system driven by ovarian steroid hormones [3, 4]. With continued maturation of the CNS after birth, a more profound internal inhibitory effect can be noted in reference to GnRH-secreting neurons. In premature infants with less developed neuronal pathways, pituitary gonadotropins are higher than in term counterparts, presumably due to a weaker inhibitory influence [5]. The presence of a nonsteroidal regulator of these pathways is further substantiated by the ability of patients with gonadal agenesis to secrete moderate levels of gonadotropins in response to GnRH [6].

Onset of Puberty

Pulsatile secretion of GnRH from the arcuate nucleus of the hypothalamus leads to gonadarche, documented by profound increases in sex steroid hormone production [1]. Early pubertal changes are temporally associated with an increase in GnRH pulse frequency, primarily during the sleep cycle [7]. As menarche approaches, GnRH pulses increase in amplitude and can be detected throughout the day, similar to that of an adult [8, 9].

Both genetic and environmental effects may play a role with the initiation of pubertal development. It has been suggested that appropriate weight gain and percent body fat are required for these events to occur [10]. This concept is substatiated by data from adolescent females who suffer from chronic illness, malnutrition, or have low body mass indices due to vigorous exercise. These young girls frequently experience delays in sexual maturation and may present with primary amenorrhea, resulting from hypothalamic hypogonadism.

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Accordingly, normal menstrual cycles resume with reversal of their nutritional status [11]. Investigators who followed healthy females throughout puberty found that body composition did not change prior to, but rather along with, the increase in GnRH secretion [12].

Plasma concentrations of leptin, an adipocyte-derived hormone, correlate well with body composition and have been shown to rise throughout puberty in female patients [13]. Specific leptin deficiencies have been shown to prevent sexual maturation, which can then be triggered by restoring normal levels [14]. Nevertheless, the role of leptin in pubertal development has not been clearly elucidated. The concept of intrauterine growth restriction, imprinting, and subsequent developmental disorders follows a common thread, since early exposure to a spendthrift, "low-calorie" environment may have a contrary effect in childhood, as suggested by the Barker hypothesis, resulting in early onset menarche and adrenarche [15–17].

Another molecule that may play a role in the reversal of the HPO downregulation is neuropeptide Y (NPY). Circulating levels are regulated by steroid hormones as well as nutritional status, with a net influence in gonadotropin synthesis through an alteration in GnRH pulsatility and pituitary response to GnRH [18]. Increased levels of NPY have been documented in females with eating disorders such as anorexia nervosa and bulimia [19], representing another possible correlation with percent body fat and reproductive potential.

Kisspeptin is a strong stimulator of the HPO axis, acting through GnRH neuronal activity, and may be a key player in early pubertal development [20]. Although the exact mechanisms on the gonadotropic axis are not well-defined, receptor mutations have been identified in women with precocious puberty, and when administered to women with hypothalamic amenorrhea, kisspeptin agonists have successfully stimulated gonadotropin secretion.

Characteristics of Sexual Development

The predictable and ordered series of events, which have historically been referred to as the standard for sexual development and somatic growth, were initially described by Tanner and Marshall more than 30 years ago [21] (Fig. 4.1). Although the activation of the HPO axis results in normal onset of sexual development, alternate sources of steriod hormone production may signal abnormally early development in adolescent girls. Such agents include organic pesticides, soy-based products, and shampoos containing placental extract. Investigators have suggested several possible pathways by which these agents influence development, including direct activation of the HPO axis and steroid hormone-like activity [22–24]. These publications raise the notion of endocrine-disrupting chemicals, and although there is little doubt that persistent exposure may adversely affect developmental pathways and promote disease progression, the association with pubertal development remains tentative and weakly causative from an epidemiological perspective.

Thelarche

The first sign of development in the majority of white females is breast budding. According to Tanner and Marshall, this initial event occurs between 8 and 13 years of age in most females, with a mean of 10.6 years. The transition period from stage II to stage V breast development may last 4.2 years [21].

Adrenarche

Pubic hair growth typically occurs after thelarche, but may occur concomitantly, with the activation of the hypothalamic-pituitary axis. Although adrenarche may also present before breast development in a normally maturing female, adult hair distribution should not be detected at this early stage, as it may represent an excess of androgen production. Accordingly, breast maturation should not be so advanced in the absence of pubic hair development, a potential sign of androgen insensitivity syndrome. Adrenarche typically occurs between the ages of 11 and 12, with adult hair distribution by age 14. Androgen levels change without a corresponding change in ACTH and cortisol secretion throughout life. So the means by which adrenal androgens are produced is not as clearly delineated and appears to occur independent of the hypothalamic-pituitary axis.

Growth Spurt

The growth spurt (peak growth velocity), during which adolescents achieve approximately 20% of their adult final height, occurs with the onset of puberty [21]. Peak growth velocity (2–3 cm/year) precedes menarche and typically occurs earlier in girls than in boys. Rapid growth of the extremities occurs first, followed by a gradual lengthening within the vertebral column. The timing of the growth spurt varies according to ethnicity.

Menarche

According to Tanner, girls in the United Kingdom in 1969 had their first menses at the average age of 13.5 years, with a range of 9–16 years [21]. The mean age of menarche for a Caucasian adolescent in the United States is approximately 12.7 years. At the time of menarche, most have achieved Tanner stage IV breast development, and the interval from initial breast development to menarche is 2.3 years [21].

There seems to have been a decline in the average age of menarche in the first half of the twentieth century, in part due to the improvement in general health and nutrition [25]. Nonetheless, few reports have documented any further changes since the mid-twentieth century.

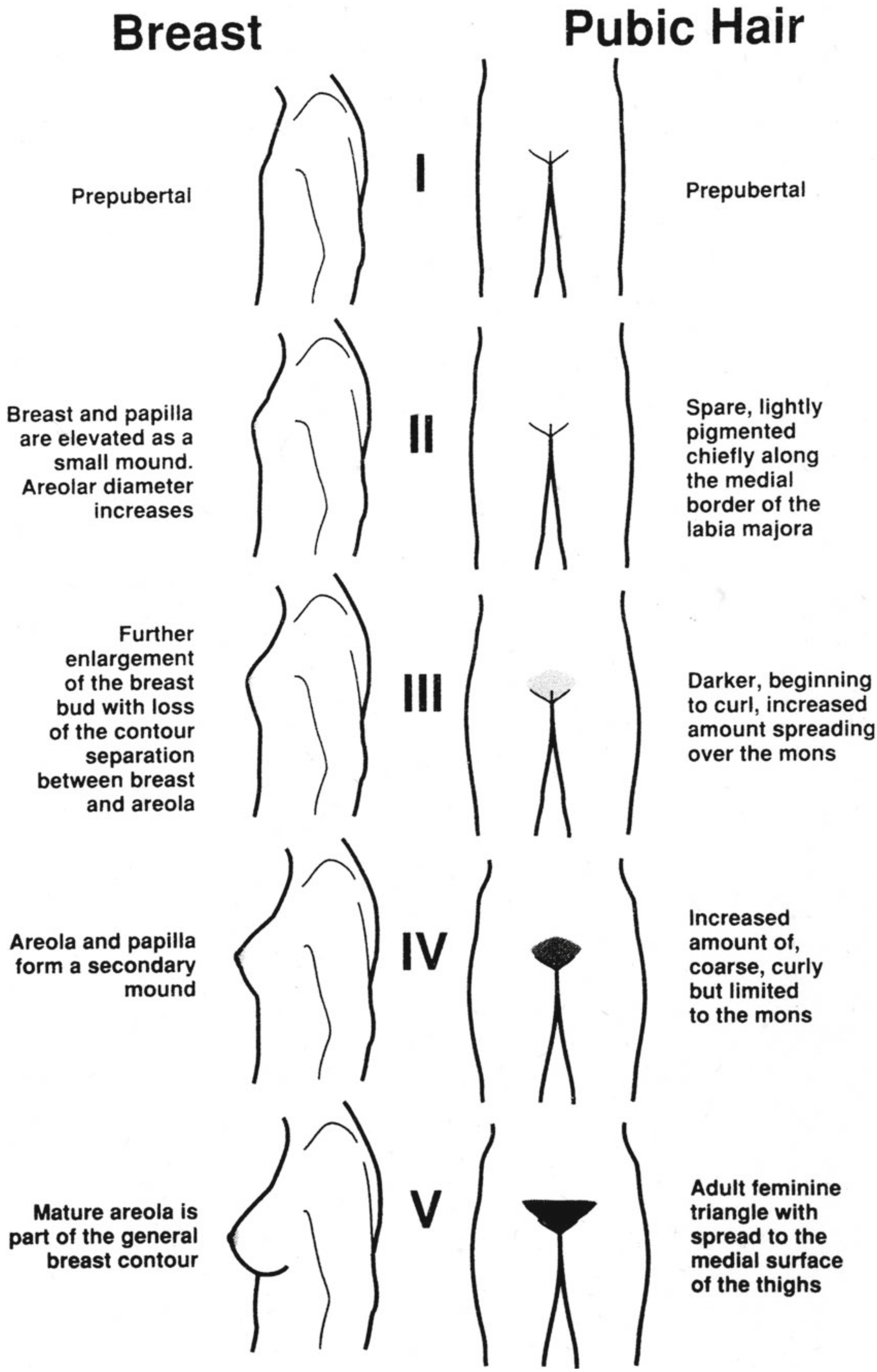

Fig. 4.1 Timing of events of puberty. 1969 data from a study of British schoolchildren. 1997 data from a study of American schoolchildren. Reproduced with permission from Solnik JM, Sanfilippo JS. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [21]

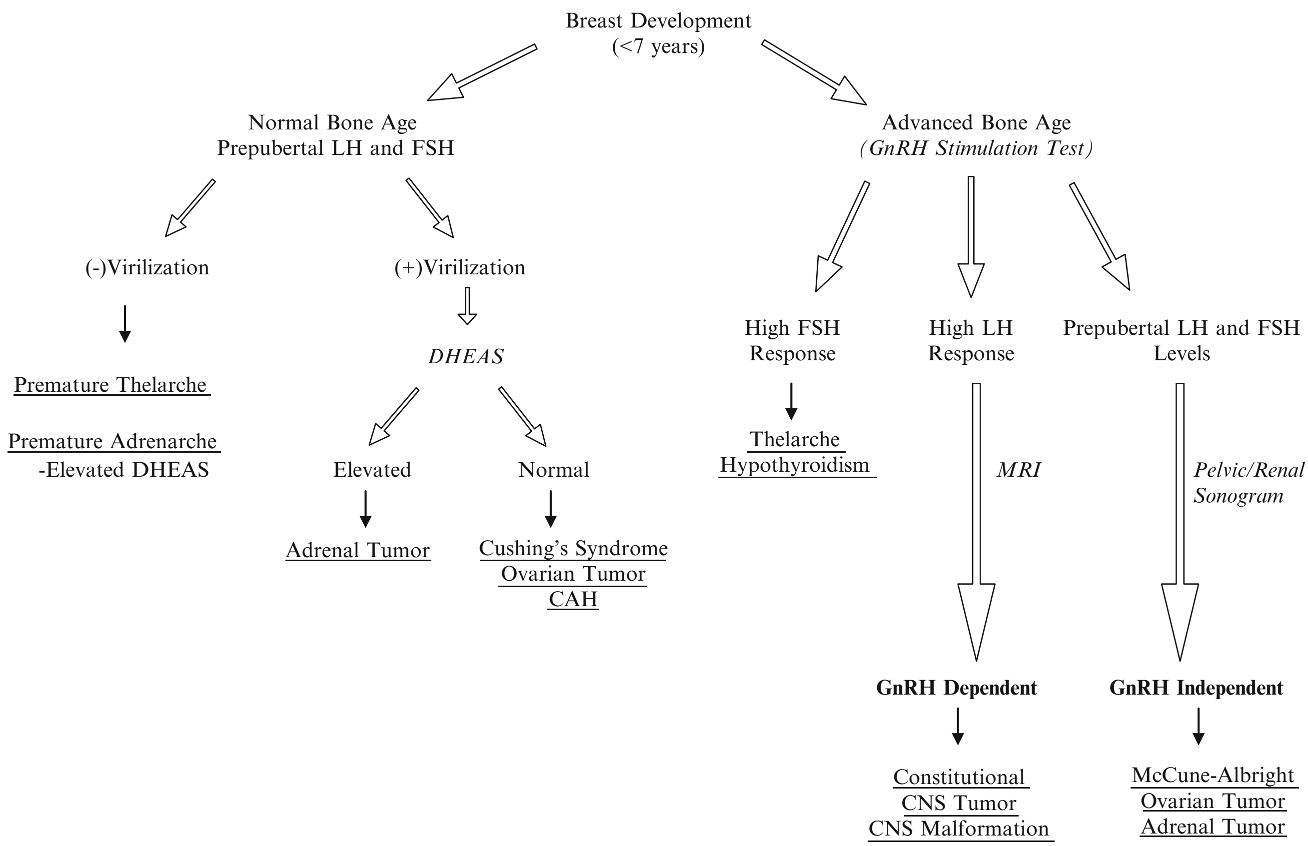

Fig. 4.2 Evaluation of central, peripheral, and incomplete precocious puberty

There is good evidence that African-American girls have an earlier onset of puberty compared to Caucasian girls [26, 27]. This was well demonstrated by the Pediatric Research in Office Settings (PROS) study published by Herman-Giddens in 1999 [26]. This multicenter, cross-sectional study evaluated over 17,000 female patients between the ages of 3 and 12 years of age [26]. On average, African-American females show early signs of puberty up to 1.5 years earlier than their Caucasian counterparts. By 7 years of age, 27.2 % of African-American girls and 6.7 % of Caucasian girls showed breast or pubic hair development. Menarche was achieved almost a year earlier. The mean age for onset of breast development was 8.87 in African-American girls and 9.96 years in Caucasian girls. At each consecutive stage of development, African-Americans were more advanced per year than Caucasians. Girls of other ethnic backgrounds may also have a characteristic difference in onset of pubertal maturation. However, only Caucasian and African-American girls were included in this study.

The PROS was the first large publication to address current and demographically relevant standards for assessing normal and abnormal onset of puberty. Updated guidelines have since been proposed and recommend a formal evaluation for precocious puberty be initiated in African-American girls who present before the age of 6 and Caucasians who present before the age of 7. Although this provocative investigation has drawn much criticism, it does invite us to reconsider the current standards (Fig. 4.2).

Precocious Puberty

One challenge each clinician faces is when to initiate the assessment of a child suspected of having precocious puberty. Historical accounts from the nineteenth century report a relative later age of onset of menstruation (16–17 years), presumably due to malnutrition. The definition of precocious puberty since remained stable, such that any female who presented prior to 8 years of age was observed, if not evaluated, for progression of sexual growth [21]. As referred to earlier, the traditional definition was challenged by Herman-Giddens, who strongly suggested that normal pubertal development may begin as early as 6 years of age [26]. Causes of precocious puberty are listed in Table 4.1.

Effects of Precocious Puberty on Adult Height

Low levels of estrogens have been shown to promote bone growth, as is manifest by rapid growth velocity during the

Table 4.1 Causes of precocious puberty

Central precocious puberty (GnRH-dependent)
• Idipathic
• CNS tumors
◦ Craniopharyngiomas
◦ Trauma
◦ Infection
◦ Primary hypothyroidism
• Syndromes associated with elevation of gonadotropins
◦ Silver's syndrome (dwarf-like characteristics)
Peripheral precocious puberty (GnRH-independent)
• Exogenous steroids (estrogens)
• Ovarian tumors
◦ Granulosa cell
◦ Adrenal
◦ Functional cyst
• McCune-Albright syndrome
Heterosexual precocious puberty
• Exogenous steroids (androgens)
• Adrenal and ovarian androgen-producing tumors

growth spurt. Conversely, high levels promote closure of the epiphyseal plates. Girls who present early in the course of precocious puberty are generally taller than their age-respective cohorts due to increased levels of steroids and the actions of IGF-I. This growth is premature and limited, so that the final height in untreated patients will likely be less than 155 cm [28]. As a result, by the time most adolescents achieve menarche, they have likely reached their final height. Notwithstanding the apparent risk for short stature, a significant number of untreated patients with idiopathic disease will likely attain relatively normal adult height, greater than the third percentile [28]. Some specialists in the field believe that the diagnosis of precocious puberty cannot be assigned unless symptoms are also associated with an accelerated growth spurt.

The First Visit

The first gynecologic encounter represents a critical event and possible first pseudo-adult exposure to health care, which can have either a positive or negative influence on her own care needs. A thorough examination should begin with a thorough intake, including age and order of onset of symptoms, progression of secondary sexual characteristics, and a linear growth for at least 6 months.

Psychosocial history should be obtained focusing on relationships with peers, authority figures, parents, teachers, coaches, as well as siblings. A nutrition history focused on fad diets, fast food, athleticism, and overt eating disorders should be assessed. Significant weight changes may also help direct the clinician towards a more appropriate list of differentials.

Vaginal bleeding, for example, although not the typical initial presenting symptom of precocity, may signal poor hygiene, neglect, or abuse and should be investigated promptly. Height and weight should be charted on a linear growth curve and followed over the course of years to watch for trends and rate of change. Predicted final height has traditionally been based on the methodology described by Bayley and Pinneau [29]. Target height (cm) considers genetic potential and is calculated from the averages of the height of the child's parents:

- Female: [father's height + mother's height + 13]/2
- Male: [father's height − 13 + mother's height]/2

The most important and least invasive portion of the physical examination is a visual exam of her axilla, breast, and external genitalia. Only if there is suspicion of a pelvic mass or significant pathology should a pelvic or recto-abdominal exam be performed. Cervical cytology is no longer indicated in this group of girls, nor is testing for sexually transmitted infection, unless there is suspicion for abuse. A vaginal smear in an estrogenized system will reveal increased numbers of superficial squamous cells. An estrogen-secreting tumor may be suspected when greater than 40% of the cells are superficial and when rapid increase in height is noted.

Physical findings suggestive of central precocious puberty (CPP) include Tanner stage II breast development with darkening of the areola, labial fullness with a dullness of the vaginal mucosa, and leukorrhea. Coarse pubic hair, acne, oily skin, clitoromegaly, and deepening of the voice are signs of androgen production, which may occur in the setting of heterosexual development and should likewise be investigated. Tall stature and adult-type body odor are other indications for the evaluation of precocious puberty. A complete neurological exam, psychological evaluation, and skin assessment should be performed initially and with subsequent visits as well. Simple findings such as elevated blood pressure, suggestive of non-classic congenital adrenal hyperplasia (CAH), or skin changes consistent with café-au-lait spots are most helpful and easy to notice.

Central Precocious Puberty

CPP is more frequently noted among girls, with an incidence of 1:5,000–1:10,000 [30]. It results from the premature activation of the hypothalamic GnRH neurons. Approximately 70–95 % of such cases are idiopathic in nature; however, other potential etiologies must first be considered, since the level of urgency and need for management of individual causes will vary [31, 32]. For a full list of etiologies, see Table 4.1.

Laboratory Findings

Baseline gonadotropin levels in the pubertal range with a predominant LH response are suggestive of CPP. Random daytime levels may be of less use in early central pubertal development because the initial increase in pulsatility occurs at night. To help distinguish CPP from GnRH-independent forms of precocious puberty, a GnRH stimulation test should be performed. To accomplish this, 100 μg of GnRH (gonadorelin acetate) is administered intravenously, and gonadotropin levels are drawn at baseline and at 20, 40, and 60 min following. One of the earliest signs of physiologic puberty is the nocturnal, pulsatile secretion of GnRH with a subsequent increase in serum LH. There is a corresponding rise in LH for each pulse of GnRH secreted. These same events occur with early onset, and an LH:FSH ratio >1 would be expected. Serum estradiol levels would be detected in the pubertal range as well. In order to maintain the diagnosis of CPP, androgen (DHEA, DHEA-S, testosterone) and 17-hydroxyprogesterone (17-OHP) levels should be drawn.

Imaging Studies

Imaging studies play a key role in the evaluation of children with precocious puberty, because a rapid increase in growth and bone age are typically seen in children with rapidly increasing levels of sex steroid hormones. Linear growth and skeletal maturation are often a more accurate assessment of pubertal development than progression of secondary sexual characteristics.

Bone age is typically evaluated by radiographic plain films of the left hand and wrist. This is a simple and noninvasive test that is well tolerated by most children. Bone age advance over chronological age is diagnostic for precocious puberty, and a disparity of greater than 2 years is more suspicious for a progressive disorder [33]. Given the higher prevalence of CNS abnormalities, especially in girls who present with particularly early onset or who have a known history of childhood seizures, neuroimaging is always indicated to rule out space-occupying lesions, malignant neoplasms, and other CNS anomalies, even in the absence of neurological complaints.

Pelvic ultrasound, however, is typically one of the easiest and most useful studies since it provides a good picture of ovarian function (developing follicles capable of producing estradiol, increased cortical volume suggestive of excess androgen production) or neoplastic processes. Ultrasound may also demonstrate subsequent steriod hormone influence on other reproductive organs. A diagnostic approach to precocious puberty is given in Fig. 4.2.

Treatment

The ultimate therapeutic goal is to suppress the HPO axis and return the hormonal environment to that of the prepubertal state (serum estradiol <10 pg/mL). Most important is the normalization of linear growth velocity and bone maturation. The outcome for patients with CPP can vary significantly, which further limits our ability to predict who will benefit most from therapy.

Hypothalamic Suppression

Initial attempts to achieve such a degree of hypothalamic suppression included the use of progestins; however, these were unsuccessful at limiting progressive changes and their use has since been abandoned [34]. The most commonly used GnRH analogs to treat CPP in the United States are leuprolide, nafarelin, and histrelin. Children with precocious puberty generally require higher doses to achieve suppression, which can be monitored with serum estradiol levels and GnRH stimulation tests. In order to improve compliance, subcutaneous formulations can be used. Early treatment protocols using long-acting GnRH agonists reported significant regression of secondary sexual characteristics and overall improvement in final height compared to nonrandomized controls [35]. A few randomized series have, however, been published that addressed the effect of GnRH agonists on final height in girls who presented with early or slowly progressive puberty [36, 37]. They confirmed results from previous observational and non-randomized reports that documented very little effect of hypothalamic suppression on improving final height in patients presenting at a later age. Children presenting with either "early puberty" or advanced "slowly progressive puberty" were likely to achieve reasonable adult height without hypothalamic suppression.

One theory that may help explain impaired growth during GnRH analog therapy in this group is early growth plate senescence related to estrogen exposure prior to onset of treatment [38]. So it may be this rate-limiting step, patients presenting beyond the window of opportunity, that limits final height.

Significant consideration should be given to promptly initiating therapy in girls presenting early with advanced bone age, as they will likely benefit most from GnRH agonist therapy [39–42]. Adan et al. suggested the following as risk factors for decreased stature and appropriate indications for therapy, especially at an earlier age of onset: (1) predicted adult height below 155 cm (may include those with a predicted height over 155 cm if the LH/FSH ratio is consistent with CPP) and (2) bone age advance over chronological age greater than 2 years [42]. Hormonal monitoring of therapy can be performed with the GnRH stimulation test at 3, 6, and 12 months after initiation, with annual follow-up thereafter.

Although the optimal time of discontinuing therapy remains unclear, many recommend that suppression stop at a bone age of 12–12.5 years. Other elements to consider include the total duration of therapy and growth velocity over the months preceding. Routine evaluation of secondary sexual characteristics, weight, and sonographic measurements of pelvic structures should be performed on an ongoing basis as well. Bone mineral density may be affected by prolonged use of GnRH agonists, so attention to bone health should not be omitted.

Recombinant Growth Hormone

Some children with precocious puberty will have early closure of their epiphyseal plates despite the use of GnRH analogs. As a result, these girls will grow up to be short adults without further intervention. The use of growth hormone as an adjunct to GnRH agonists in girls with precocious puberty has been evaluated by several observational and randomized series and has been found to improve final height prognosis [43]. Although the use of growth hormone in certain patients is frequently prescribed among pediatric endocrinologists, the studies evaluating efficacy are troubled by obstacles quite similar to those seen with the analysis of GnRH agonists on final height. It is important to be aware that growth hormone has not yet been approved by the U.S. Food and Drug Administration for treatment of girls with short stature as a result of precocious puberty

GnRH-Independent Precocious Puberty

When precocious puberty occurs independent of pituitary gonadotropins, the source of estrogen production must be established. One common source is surreptitious ingestion of exogenous hormones, such as those found in oral contraceptive pills or anabolic steroids. Other less common sources include primary hypothyroidism. However, the most common origin of GnRH-independent estrogen production is frequently the ovary itself.

Autonomous Ovarian Estrogen Production

Ovarian tumors are uncommon but important childhood neoplasms that present with precocious puberty in approximately 10 % of cases [44]. Granulosa cell tumors are the most common estrogen-producing neoplasms detected. However, other tumors, such as thecal cell tumors, gonadoblastomas, teratomas, cystadenomas, and ovarian cancers, may be responsible. Intra-abdominal masses are often palpable, but imaging with sonography or magnetic resonance imaging may help characterize the tumor, and surgical exploration is generally warranted.

Laboratory criteria that help distinguish these processes from a central source include low baseline gonadotropin levels and a prepubertal response to the GnRH stimulation test. Similar to CPP, estradiol levels will be high and bone age advanced (see Fig. 4.2). Treatment is based on surgical extirpation of the source, which results in regression of pubertal changes.

McCune-Albright Syndrome

McCune-Albright syndrome, also known as polyostotic fibrous dysplasia, is a genetic disease affecting the bones and pigmentation of the skin. The hallmark of McCune-Albright syndrome in girls is precocious puberty, and this condition accounts for approximately 5% of all girls with precocious puberty. These patients have estrogen-producing ovarian follicular cysts that develop independent of gonadal hormone stimulation, a condition termed autonomous follicle development.

Children with this rare disorder also have fibrous dysplasia in their bones, which leads to fractures, deformities, and X-rays abnormalities. Facial bone deformities may result in appropriate concerns for cosmesis. In addition, these children have cafe-au-lait spots, which are light tan birth marks. McCune-Albright syndrome is often associated with several other endocrinopathies, including hyperthyroidism, acromegaly, pituitary adenomas, and adrenal hyperplasia [45].

Treatment

In contrast to girls with CPP, girls with McCune-Albright syndrome exhibit a lack of GnRH pulsatility, gonadotropin levels are low, and estradiol is produced from autonomous follicle development. Treatment protocols for McCune-Albright syndrome are aimed at inhibiting peripheral estradiol production with aromatase inhibitors or blocking the effects at the receptor level with selective estrogen receptor modulators (SERMs).

Aromatase inhibitors offer several theoretical benefits for the treatment of McCune-Albright syndrome. Unfortunately, results from studies evaluating testolactone have been inconclusive [46–48].

It has been suggested that continuous estrogen exposure from a peripheral source may secondarily induce the HPO axis, such that a central component may occur simultaneously [49]. These findings help to explain the lack of therapeutic benefit of aromatase inhibitors in certain patients with McCune-Albright syndrome. Evaluation and management of these complicated patients should then be based on algorithms used for CPP.

The SERM, tamoxifen, was studied in a prospective, multicenter trial, for the 12-month treatment of 25 girls with McCune-Albright syndrome. This treatment decreased the incidence of vaginal bleeding and also decreased bone velocity and bone maturation [50]. Other causes of precocious puberty can be found in Table 4.1.

Premature Thelarche

Early breast development in the absence of other signs of sexual maturity is typically a benign, self-limited event. Initial laboratory evaluation will reveal prepubertal gonadotropin levels and normal bone age. GnRH stimulation will result in a predominant FSH response. Continued observation is nonetheless mandatory, and breast development, which may be unilateral or bilateral, will likely regress, but may persist until normal onset of puberty.

Premature Adrenarche

Adult pubic hair growth before the age of 6 may result from an abnormal adrenal secretory response, which promotes androgen production (17-hydroxypregnenolone, DHEA, and DHEA-S). Like premature thelarche, the diagnosis can only be made after longitudinal evaluation, in the absence of other signs of sexual development. Although mild increases in bone age may occur, no treatment is necessary, as these children will likely achieve normal adult height [51].

A fasting 17-OHP level is generally sufficient to rule out nonclassic CAH unless there is significant bone age advance. Only patients with conclusively high levels of 17-OHP warrant treatment. There is evidence to suggest that girls with premature adrenarche may be at risk for developing polycystic ovarian syndrome (PCOS).

Delayed Puberty and Primary Amenorrhea

Delayed puberty in girls is defined as lack of thelarche by 13 years of age or when more than 4 years pass between thelarche and menarche. Primary amenorrhea is diagnosed when girls who have developed secondary sexual characteristics do not reach menarche by 16 years of age.

Most girls with delayed puberty have normal ovaries (eugondal) and their sexual development is constitutionally delayed. Hypogonadism, which can occur, can be the result of ovarian failure, termed hypergonadotropic hypogonadism, or because normal ovaries are not stimulated to secrete hormones, referred to as hypogonatotropic hypogonadism.

Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism is the single most common etiology of pubertal delay. The essential condition required to make this diagnosis is elevated gonadotropins, both FSH and LH.

Turner syndrome is the most commonly (1:2,000 live born females) diagnosed condition within this subset. Karyotype may reveal 45,X or a mosaic, which may occur in up to 40–50 % of patients with gonadal dysgenesis. DNA analysis is crucial, because the presence of the Y chromosome places patients at risk for gonadal neoplasias such as gonadoblastoma and dysgerminoma. Mixed gonadal dysgenesis, 45,X/46,XY (the most common karyotype), is also representative of the abnormal sex chromosome group.

Several forms of primary and secondary ovarian failure with normal sex chromosomes also exist. Pure gonadal dysgenesis, Swyer's syndrome, typically presents with delayed puberty. Chemotherapy and/or radiation therapy may result in gonadal dysfunction and delayed development in an otherwise genetically and phenotypically normal female.

Other causes of ovarian failure include autoimmune oophoritis, galactosemia, gonadotrophin-resistant ovary syndrome, steroidogenesis enzyme deficiency, infection, or gonadotropin receptor gene mutation. Autoimmune disorders associated with hypergonadotropic hypogonadism include Hashimoto's thyroiditis and Addison's disease. Patients with 17α(alpha)-hydroxylase deficiency present with adrenal insufficiency, hypertension, and lack of sex steroids, including androgens.

Hypogonadotropic Hypogonadism

This condition results from a failure of the hypothalamic-pituitary-ovarian (HPO) axis and deficiency of GnRH. The most common cause of this is constitutional delay, a diagnosis of exclusion. Bone age and height age are delayed, unlike that seen in hypothyroidism where bone age is delayed more than height age. Unfortunately, the only way to discern constitutional delay from idiopathic hypogonadotropic hypogonadism is by longitudinal observation.

Central etiologies of hypogonadotropic hypogonadism include brain tumors, Kallmann syndrome, hypothyroidism, and chronic disease states (Crohn's disease, Cushing's disease, anorexia nervosa, or malnutrition).

Primary Amenorrhea with Otherwise Normal Sexual Development

Genetically normal patients with a normally functioning HPO axis who present with primary amenorrhea typically have anomalies of the genital outflow tract, such as imperforate hymen or vaginal septum (Table 4.2). One of the most common causes of primary amenorrhea in these patients is Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome. This condition is characterized by a blind vaginal pouch in an otherwise normally sexually developed adolescent and results from the failure of development of the Müllerian (paramesonephric) duct system in genotypic females.

Androgen insensitivity syndrome is another common cause of primary amenorrhea. Androgen insensitivity syndrome, previously termed testicular feminization, is the result of an abnormal androgen receptor. This maternal X-linked recessive disease occurs in individuals with XY genotypes and normal but partially or completely undescended testicles that produce testosterone. Because of the abnormal androgen receptors,

Table 4.2 Causes of primary amenorrhea

Hypogonadism
Hypergonadotropic hypogonadism
Abnormal sex chromosomes
Turner syndrome
Normal sex chromosomes
46,XX gonadal dysgenesis
46,XY gonadal dysgenesis
Pseudo-ovarian failure
Hypogonadotropic hypogonadism
Congenital abnormalities
GnRH deficiency
Gene mutations
Constitutional delay
Acquired abnormalities
Endocrine disorders
Pituitary tumors
Systemic disorders
Eugonadism
Anatomic abnormalities
CAUV
Imperforate hymen
Transverse vaginal septum
Intersex disorders
Androgen insensitivity
PCOS

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CAUV congenital absence of the uterus and vagina; *PCOS* polycystic ovary syndrome

high levels of circulating testosterone result in appropriately timed puberty in females who appear phenotypically normal. A harbinger is sparse or absent pubic hair.

Primary amenorrhea or delayed menarche is frequently associated with hyperandrogenia, secondary to either PCOS or adult-onset CAH. These patients have otherwise normal puberty, but will also present with signs of androgen excess ranging from hirsutism and acne to virilization.

Evaluation

A complete evaluation should be undertaken for any adolescent who meets the standard criteria for delayed puberty or primary amenorrhea:

- Lack of any pubertal development by 13 years of age
- When more than 4 years have passed between thelarche and menarche
- No menses by 16 years of age with secondary sex characteristic

Extensive neonatal and family history, including ages of pubertal development and attainment of final height extending to members beyond the nuclear family, is helpful. History pertinent to information of prior exposure to exogenous steroid hormones or chemotherapy must also be elicited. Review of systems to evaluate for chronic illnesses and pattern of exercise and diet may uncover the diagnosis.

Physical examination and height and weight plotted on a growth chart should be completed as well as blood pressure, thyroid exam, Tanner staging, and abdominal exam. A complete neurological evaluation, including assessing the ability to smell, should also be performed.

Hypergonadotropic hypogonadism patients often present with short stature. Patients with Turner syndrome (45,XO), the most common presentation of hypergonadotropic hypogonadism, may present during infancy with lymphedema or during childhood with typical features such as short stature, webbed neck, and shortened fourth metacarpals. Cardiovascular anomalies and renal abnormalities such as aortic coarctation, bicuspid aortic valves, and horseshoe kidney can be determined with imaging studies.

Patients with mixed gonadal dysgenesis (mosaic XY/XO) may present phenotypically similar to those with Turner's syndrome; however, virilization or ambiguous genitalia may also be evident in the presence of the Y chromosome. Patients with 46,XX complete gonadal dysgenesis are normal to tall in stature and are most commonly phenotypically female. Sexual infantilism with lack of Müllerian structures and 46,XY karyotype is consistent with Swyer's syndrome.

Hypogonadotropic hypogonadism can be seen in adolescents who are extremely athletic or malnourished. Minimal body fat from either cause is associated with reversible hypothalamic dysfunction. Patients with CNS tumors may present with persistent headaches or visual field defects. Marked centripetal obesity and moon facies are typical of Cushing's syndrome. Anosmia along with hypothalamic hypogonadism is consistent with the diagnosis of Kallmann's syndrome (isolated GnRH deficiency). A prolactinoma may present with hyperprolactinemia and galactorrhea.

Adolescents with primary amenorrhea who have normal development of other secondary sexual characteristics are usually of normal stature. Pelvic or recto-abdominal examination is performed in these patients to exclude anatomic abnormalities of the reproductive tract. Examples include vaginal septum and the blind vaginal punch associated with MRKH and androgen insensitivity syndrome.

Imaging

Bone age can be assessed similarly to adolescents presenting with precocious puberty. Stature, consequently, will be decreased in patients with hypergonadotropic hypogonadism, with the exception of pure gonadal dysgenesis (46,XX).

If gonadotropin levels are low with delayed puberty, then a central cause must be determined. Likewise, an elevated prolactin suggests a pituitary or hypothalamic problem. In these cases, MRI scan of the brain and pituitary gland is indicated to exclude abnormality of the hypothalamic-pituitary axis such as pituitary or hypothalamic tumors.

Adolescents with primary amenorrhea but otherwise normal sexual development require pelvic ultrasound to evaluate the internal reproductive organs and detect the presence of a fluid collection consistent with hematocolpos related to a vaginal septum. Abdominal and pelvic MRI is helpful in evaluating for renal or skeletal anomalies in patients with vaginal agenesis.

Treatment of Delayed Puberty

Specific Therapy

Therapy for hypogonadotropic disorders is focused on treating the primary etiology whenever possible. If an intracranial lesion compresses the pituitary stalk, then surgical therapy is indicated. If prolactinoma is the cause, then bromocriptine becomes the first line of therapy. Medical therapy generally restores menses and fertility, and although surgical treatment may portend good results initially, there is a high incidence of recurring hyperprolactinemia. Surgery may therefore be postponed unless the condition is refractory to medical management.

Treatment for competitive athletes and patients with anorexia nervosa becomes somewhat more challenging. Since body weight with total body fat of at least 12–14 % has been associated with return of menses, many clinicians feel that patients should be encouraged to change their lifestyle and improve their diet before introducing pharmaceutical management. Whereas patients with idiopathic or irreversible gonadal failure who have not demonstrated sexual development begin therapy at 14–15 year of age, therapy may be initiated later for these patients.

Estrogen Therapy

If the cause of delayed puberty is determined to be irreversible or idiopathic (i.e., constitutional delayed), sex steroid hormone replacement is indicated. The goal of therapy involves induction of breast development, bone growth, and menses. Hormone replacement for patients with ovarian failure is important not only to induce pubertal development, but also to decrease the risk of subsequent osteoporosis and cardiovascular disease due to prolonged hypoestrogenic state.

Timing is quite important when starting hormone replacement. In most instances, therapy is initiated when patients present with delayed puberty during their early teenage years. However, some Turner's syndrome patients will be referred for evaluation during childhood. If these patients begin estrogen therapy too early, their potential growth may be limited by epiphyseal closure.

Hormone replacement for treatment of delayed puberty is begun with low-dose estrogens, typically 0.3 mg conjugated estrogens for 6–12 months. The main goal is to induce normal breast development, as too high of an estrogen dose can result in the development of tuberous breasts [52]. Subsequent goals include regulation of menses and maintenance of bone mass. This can be achieved by increasing the dosage of estrogen slowly after the first year until menstruation occurs. Progestin therapy (e.g., medroxyprogesterone acetate [5–10 mg] at end of estrogen cycle) is initiated approximately 3 months after the increase in dosage of estrogen, typically when breakthrough bleeding occurs. The most common formulation includes continuous estrogen therapy with sequential progestins given orally in the latter part of the cycle to create regular menses. Alternative forms include transdermal estrogen replacement and micronized progestins that have less negative impact on lipid profiles. Gonadotropins have also been used to induce ovulation, but are costly and more difficult to administer, especially in the adolescent patient population.

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Fertilization and Implantation

5

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Introduction

Infertility is a common problem with significant psychological, economic, and medical implications. It is estimated to affect one in seven couples [1]. There are approximately 70 million infertile couples worldwide, most of whom reside in developing countries [2]. In the United States, the CDC estimated that of married women ages 15–44, 1.5 million (6 %) are infertile, and of all women ages 15–44 years old, 7.4 million have used infertility services [3]. Two of the most common mechanisms of infertility are failure of fertilization and failure of implantation. Fertilization is the union of a sperm and an oocyte, while implantation is the attachment of a developed blastocyst to the uterine endometrium. Although the majority of our knowledge on this subject relies on other mammalian fertilization models, with advancing technology and a deeper understanding of gamete biology, transport, and implantation, our knowledge and available treatment options and medical care for infertile couples have significantly improved.

In this chapter, we will summarize the current evidence on molecular and cellular interactions that are essential for successful fertilization and implantation and their relevance to current clinical reproductive medicine.

Sperm Transport in the Male Reproductive Tract

A mature and healthy spermatozoon is essential for reproductive success. The male gonads, commonly referred to as testes, have two important functions for reproduction. The first is to produce a constant supply of spermatogenic stem cells and mature them through meiosis into spermatozoa, a process called spermatogenesis. The second is to produce sex hormones, which have diverse metabolic and reproductive functions essential for spermatogenesis and male endocrinology. Even though these functions are accomplished in two distinct anatomical compartments of the testes, the interstitial compartment and seminiferous tubules, respectively, they are very reliant on each other.

Seminiferous tubules are the most abundant anatomical component of the testicle. Each testicle contains approximately 360 m of seminiferous tubules. This convoluted tubular structure is lined by Sertoli cells, which act to nourish and aid the process of spermatogenesis. In between the Sertoli cells, germline stem cells undergo differentiation into spermatogonia and ultimately mature into spermatozoa. In order to protect spermatogenesis, Sertoli cells form tight junctions between each other called the blood-testis barrier. This barrier helps regulate the entry of hormones and nutrients, and most importantly, prevents an immune response to the developing spermatozoon [4].

The interstitial compartment of the testes contains Leydig cells, blood vessels, myofibroblastic cells, and nerves, which all contribute to spermatogenesis via hormone production and control of the local environment. Leydig cells produce the majority of the androgens needed for spermatogenesis and male reproductive function.

It is well known that ultimate control of spermatogenesis and production of gonad-derived steroid hormones comes from the anterior pituitary gonadotropins LH and FSH. Luteinizing hormone (LH), secreted by the anterior pituitary gland, stimulates Leydig cells to produce androgens. Intratesticular testosterone exerts its effect through binding to testosterone receptors found in Sertoli, Leydig, and peritubular cells.

The other gonadotropin, follicle-stimulating hormone (FSH), stimulates Sertoli cells to produce androgen-binding protein, which binds androgen and optimizes local androgen levels that are essential for spermatogenesis. Other secretory functions of the Sertoli cell include, but are not limited to,

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production of aromatase, which converts androgens to estrogens, and release of inhibin/activin, which regulate the release of FSH.

Spermatogenesis takes approximately 72 days. After production in the seminiferous tubules, spermatozoa are transported to the rete testis, efferent ducts, caput epididymis, corpus epididymis, and finally to the cauda epididymis, where they are stored until ejaculation [5]. The epididymis is not merely a storage site for spermatozoa; in fact, it is the site where spermatozoa undergo physiological modifications that result in the acquisition of progressive motility and the ability to undergo capacitation [6]. Spermatozoa in the epididymis contain free sulfhydryl groups rather than disulfide bonds, and the oxidation of those free groups helps stabilize the sperm structures [7]. Spermatozoa are able to become motile in the epididymis, but their motility is suppressed by acidification of the epididymal lumen [8]. Also, activation of cannabinoid receptors on the sperm surface helps to keep spermatozoa in an immotile state [9]. Additionally, various secretory proteins in the epididymal lumen may contribute to sperm maturation [10]. Human spermatozoa seem to rely on glycolysis for ATP production [11] and the activity of glycolytic enzymes is modified during epididymal maturation [7].

With ejaculation, the spermatozoa are rapidly transported from the epididymis through the ductus deferens where they are mixed with seminal vesicle and prostatic secretions. In fact, only 5 % of the ejaculate volume is composed of spermatozoa. The bulk of the ejaculate is composed of secretions from the seminal vesicle (70 %) and prostatic secretions (25 %). Less than 1 % of the ejaculate volume comes from the bulbouretral glands.

Seminal vesicle secretions have an alkaline nature and contain rich nutritional substances, which serve as an initial energy source for spermatozoa, and proteins responsible for the "coagulum" formation, which is important to stabilize the deposited sperm in the female reproductive tract. An important component of prostatic secretions is a prostate-derived serine protease, prostate-specific antigen (PSA), responsible for liquefaction of the coagulum so that the sperm can swim freely once in the vaginal vault.

Components of ejaculate differ among individuals, as well as within a single individual. Initially, prostatic, non-coagulable secretions are followed by the sperm-rich non-coagulable component. Subsequently, seminal vesicle secretions predominate, which results in coagulation of the ejaculate. The initial non-coagulable spermatozoa have the advantage of entering the female reproductive tract earlier than the spermatozoa that are trapped in the coagulum. An average ejaculate contains 200–500 million spermatozoa, most of which are mature and motile [12].

In addition to spermatogenesis, penile anatomy is also important for reproductive success. Various anatomical abnormalities like penile curvature and uncorrected urethral openings can cause male factor infertility.

Sperm Transport in the Female Reproductive Tract

After ejaculation, mature spermatozoa are deposited near the external cervical os, or in the anterior vaginal fornix. The ejaculate coagulates within a minute and forms a loose gel in humans rather than a compact gel that is observed in rodents. The principal proteins involved in coagulation are semenogelin I and semenogelin II, which are secreted from seminal vesicles [13]. The gel formation minimizes the back-flow of deposited sperm into the vagina and protects spermatozoa against the harsh vaginal environment, yet a median of 35 % of spermatozoa are still lost through retrograde flow down and out of the vagina [14]. The coagulate is then enzymatically digested in about 30–60 min. PSA is the main enzyme that is involved in this digestion. Although the alkaline nature of the seminal plasma protects the spermatozoa from the acidic vaginal environment, this protection is only transient. Spermatozoa have to leave the coagulant quickly to escape from inactivation or immune attack; they are only able to remain motile in the vagina for a few hours. Within a few minutes of vaginal deposition, human sperm begins to leave the seminal pool and enter the cervical canal [15]. The amount of sperm that transverse the cervix depends on multiple factors, including sperm concentration, morphology, and motility. The remaining spermatozoa that do not enter the female reproductive tract are either inactivated by the acidic environment or phagocytized.

As the spermatozoa enter the cervical canal, they encounter the cervical mucus. At the time of ovulation, under the influence of estrogen, the cervical mucus is highly hydrated and is optimal for spermatozoa transport [16]. In fact, coitus on the day of maximal mucus hydration is more closely related with pregnancy success than using other indicators such as basal body temperature [17]. If the conception does not occur during this period, then, under the effect of progesterone, the cervical mucus gets thicker and creates an unfavorable environment for passage of spermatozoa.

Cervical mucus acts as a selective gate for sperm transport. Cervical mucus also acts as a barrier to abnormal sperm transport, selecting for the more vigorous and motile sperm [16, 18]. Additionally, due to flow of uterine secretions, cervical mucus is aligned to form a microarchitecture in cervical mucosal grooves. The microarchitecture is thought to guide spermatozoa to the uterus [19].

Like the vagina, the cervix also contains immunologic barriers such as immunoglobulins, the complement system, and neutrophils, that together act to combat entry of microorganisms. However, with the aid of the seminal plasma proteins which coat spermatozoa against immune attack, highly motile spermatozoa can escape this barrier without difficulty.

As sperm enter the uterus, they are able to quickly transverse the cavity. Uterine smooth muscle contractions, which are directed caudally, increase in intensity during the late follicular phase [20]. The smooth muscle contractions appear to be limited to the layer of myometrium directly beneath the endometrial layer [21]. Thus, it appears that both active flagellar beating and uterine contractions aid in transportation through the uterine cavity. It was also suggested that these contractions could draw watery cervical mucus into the uterus. As the uterine lumen is small in volume and cervical mucus is plentiful during the peri-ovulatory phase, this would easily drag the spermatozoa through the uterine lumen [22].

The final part of the sperm transport is the passage through the uterotubal junction. In most mammals, it is narrow and may be filled with mucus. Although mucus has been shown in the uterotubal junction in humans, it does not appear to be a rate-limiting factor for sperm transport [15]. Only a few spermatozoa traverse the oviduct at any given time and move towards the ampulla, the most common site of fertilization. Movement is facilitated by oviduct contractions and fluid flow.

Sperm Capacitation

Although mature, spermatozoa are not able to fertilize an oocyte immediately after spermatogenesis. A series of molecular and physiological events that begin in the cervix and occur in the female reproductive tract give the spermatozoa the ability to fertilize the oocyte; this process is known as capacitation [23]. Capacitation encompasses plasma membrane reorganization, ion permeability regulation, membrane hyperpolarization, cholesterol loss, and changes in the phosphorylation state of many proteins [24]. Capacitation normally takes place within the female reproductive tract; however, it can be mimicked in the laboratory by incubation of sperm in a defined medium containing bicarbonate, a cholesterol acceptor like albumin, calcium, and an energy source such as pyruvate, glucose, or lactate [7].

Spermatozoa have two principle structural compartments, the head and the tail, which behave relatively independent from each other during capacitation. However, some studies suggested that they are functionally related, and as capacitation starts in the tail (hyperactivation), it subsequently triggers capacitation in the other compartment, the sperm head (acrosomal reaction) [7].

Hyperactivation, which occurs in the tail (or flagellum), increases the speed, velocity, and rate of flagellum beating when compared to spermatozoa prior to capacitation. The spermatozoa exhibit asymmetrical flagellar beats with increased amplitude of principal flagellar bend [25]. This characteristic allows the spermatozoa to more easily navigate through the viscous oviduct fluid and penetrate the outer layers of the oocyte, namely, the cumulus oophorus and corona radiata. Hyperactivation requires an alkaline environment and increased intracytoplasmic calcium levels. Calcium enters into the spermatozoa from both the external milieu and release from intracellular stores [26]. In addition to calcium alterations, changes in membrane permeability to potassium, sodium, protons, bicarbonate, and chloride contribute to sperm capacitation [7].

The spermatozoon head, where the "acrosomal reaction" occurs, is further divided into two parts: the acrosomal region and nucleus. The acrosomal region contains various enzymes that play a critical role in penetrating the zona pellucida and fusion with the oocyte, whereas the nucleus carries the paternal genetic code. The acrosomal reaction is the last step of capacitation and occurs when the spermatozoon approaches the oocyte, normally in the ampulla of the oviduct. It is described as acrosomal exocytosis and is a prerequisite for fertilization because it allows the sperm to penetrate the oocyte zona pellucida. The acrosome contains various hydrolytic enzymes like proteases, arylsulfatases, phosphatases, phospholipases, hyaluronidase, and acrosin. Calcium is obligatory for acrosomal exocytosis. There are various theories on the source of calcium increase needed for acrosomal exocytosis. One theory is that the depletion of calcium from the acrosome activates Ca^{++} channels, which allows the entry of Ca^{++} from the surrounding medium [27]. Other theories suggest that the exposure of the sperm head to the zona pellucida proteins or progesterone releases calcium stores, which are found in the redundant nuclear envelope at the posterior end of the sperm head. The increase in calcium starts from the head-tail junction and the calcium wave propagates towards the head [26]. An increase in calcium levels leads to a concomitant increase in cAMP levels, with the release of the vesicular fusion proteins; the acrosome completely discharges its enzymatic contents to penetrate the zona pellucida and fuses with the oocyte plasma membrane. The fertilization process will be discussed further, after a discussion of the oocyte.

An understanding of the capacitation process has useful clinical applications in some couples with infertility. Since the fertilizable life of a sperm is decreased once it has been capacitated, with evaluation of acrosomal status and in vitro capacitation, the timing of conception can be precisely controlled and aid in the treatment infertility.

Oocyte Development

Early Follicular Development

During early embryonic development, at the seventh week of gestation, gonadal stem cells derived from the yolk sac endoderm migrate to the gonadal ridges. After this migration, primordial germ cells undergo mitosis and substantially increase in number becoming "oogonium." During embryonic development, oogonium form nests and are not initially surrounded with somatic cells. These oogonial cells are then individually surrounded with flat pre-granulosa cells, forming primordial follicles. The process of forming oogonium from primordial germ cells continues until around the third trimester. Concomitantly, around the 11th week of gestation, oogonia begin to enter their first meiotic division to become primary oocytes, but this division gets arrested at the dictyotene stage. Primary oocytes will stay arrested at the dictyate stage of prophase I until postnatal life when the female enters menarche. With each menses/ovulation, only a few primary oocytes will continue to develop while the others remain arrested. The number of primary oocytes is highest in the 20th gestational week [28], estimated to be around 7 million, and steadily decreases thereafter. It was previously believed that no oocytes were produced in reproductive-age women; however, recent data suggest the possibility of oogonial stem cells, which can give rise to new oocyte-like structures in the adult [29–32]. Further studies are needed to understand their biology and contribution to the "oocyte pool." This pool of available oocytes is the ultimate determinant of menopausal age under physiologic conditions. Depletion of the oocyte reserve starts in utero and continues thereafter [33, 34]. At the time of puberty, there are on average 200,000 primary oocytes remaining in the ovary [35].

Follicles provide support for the oocyte, and folliculogenesis occurs concomitantly with oocyte development. Initially, primordial follicles (immature oocytes surrounded by flat granulosa cells) develop and reach their maximal numbers around the same time as the peak in primary oocytes, around 20 weeks gestational age. These follicles will either continue to develop or spontaneously regress or undergo apoptosis, with only a fraction remaining by puberty [36]. Primordial follicles, also known as pre-antral follicles, are not responsive to gonadotropins and therefore rely on other factors for their development. The factors that initiate follicular development prior to attainment of gonadotropin sensitivity have not been fully determined; however, kit ligand, LIF, EGF, KGF, BMP-4, AMH, and bFGF have been shown to contribute to this process [37–42]. As early follicular development is independent of gonadotropin stimulation, this stage of follicular development can occur before puberty, as well as during reproductive ages. However, they spontaneously regress or undergo apoptosis [36]. Only after the development of antrum, the follicle becomes responsive to the gonadotropins [37]. With puberty, maturation of the hypothalamic pituitary axis and pulsatile release of FSH and LH, antral follicles continue their development until either ovulation or atresia [43, 44]. With puberty, maturation of the hypothalamic pituitary axis and pulsatile release of FSH and LH allow antral follicle development to progress until either ovulation or atresia [44].

Cumulus Cells and Oocyte Interactions During Ovulation

Follicles contain both an oocyte and a number of cells surrounding it, including an inner layer of cumulus and outer layer of granulosa cells. The oocyte actively regulates adjacent cumulus cell (CC)/granulosa cell (GC) metabolism and creates the optimal environment for its own development. The oocyte-CC interaction is achieved by direct contact via gap junctions and by a paracrine effect of oocyte-secreted factors (OSF). Because cumulus cells lie closer to the oocyte than granulosa cells, the oocyte regulates the adjacent CC cells more than the distant granulosa cells. Two distinct factors that have been determined as OSF are growth differentiation factor 9 (GDF9) and BMP-15 [45].

The effect of OSF on granulosa and cumulus cells can be summarized as follows:

1. OSF increases DNA synthesis in both CC and GC and increases cell proliferation
2. Inhibition of CC luteinization
3. Inhibition of CC apoptosis
4. Regulation of CC metabolism
5. Promotion of CC mucification and expansion

In conclusion, the oocyte tightly controls the adjacent microenvironment for its optimal development. Under the effect of OSF, CC/GC are transformed into supportive cells for oocyte development. CC/GC have different physiological properties than mural granulosa cells, which are not affected by the OSF. Mural granulosa cells express FSH receptors and later in follicular development they are involved in steroid hormone secretion, follicular expansion, and finally, ovulation.

Late Follicular Development and Oocyte Pickup

In the follicular phase, under the influence of hypothalamic GnRH pulse frequency, anterior pituitary gonadotrophs release FSH. FSH binds to its receptors on the primary follicular granulosa cells and induces proliferation. Under continuous FSH stimulus, pre-antral follicles escape from follicular atresia and continue to develop. A dominant follicle is then selected and continues to grow and develop under continued FSH and eventually LH stimulus, while other follicles begin to undergo atresia. After the LH surge in midcycle, the oocyte of the dominant follicle completes its first meiotic division (it had been arrested in meiosis prophase I since initial development in gestation) and shortly thereafter is expelled from the ovary. At this stage, the oocyte is surrounded by a thick glycoprotein layer, the zone pellucida, and overlying granulosa cells, which altogether form the cumulus oophorus complex. The oocyte and granulosa cells are functionally connected through gap junctions, which are thought to play an important role in local regulation of the oocyte. Shortly after ovulation, the cumulus oophorus complex is taken up by the infundibular part of the fallopian tubes. The infundibula contain fimbriae that are finger-like projections that constantly sweep the ovarian surface. The fimbriae guide the ovulated COC into the fallopian tube. Myometrial contractions together with tubal epithelial ciliary beatings are thought to contribute to this process. Within minutes, the cumulus oophorus oocyte complex can be found in the ampullary region of the tube.

During oocyte transport, the spermatozoa are moving up the fallopian tubes to meet the cumulus oophorus oocyte complex. Unlike spermatozoa which maintain fertilizing capability for days, the oocyte loses its capability to become fertilized after 12 h in the female reproductive tract. The differential timing of oocyte and sperm viability demonstrates the clinical importance of proper timing intercourse to assure sperm availability at ovulation.

Fertilization

Sperm Penetration through the Cumulus Oophorus

To fertilize the oocyte, the capacitated sperm has to pass through the cumulus oophorus, a specialized layer of cuboidal granulosa cells that surround the oocyte (Fig. 5.1). These cells are formed by follicular cells, which are adherent to the oocyte prior to ovulation and originate from the squamous granulosa cells present at the primordial stage of follicular development. These cumulus cells are attached to each other with an extracellular matrix that is mainly composed of hyaluronic acid, heparin sulfate, and chondroitin sulfate [46]. Although cumulus-free oocytes surrounded only by a zona pellucida are able to induce an acrosomal reaction, cumulus cells seem to foster the reaction before the sperm reach the zona pellucida [47]. Sperm hyperactivated motility also helps penetration through this initial barrier.

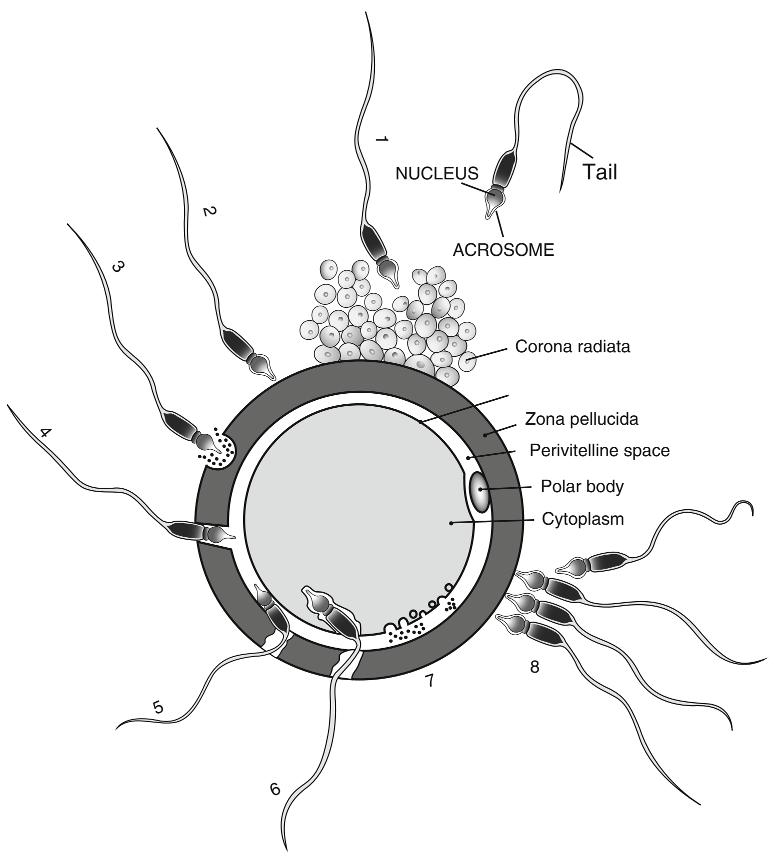

Fig. 5.1 Fertilization process. (*1*) Sperm penetration of cumulus cells, (*2*) attachment to zona, (*3*) exocytosis of acrosomal contents, (*4*) penetration to the zona pellucida, (*5*) entry into perivitelline space, (*6*) binding and fusion with the egg plasma membrane, (*7*) cortical reaction, and (*8*) block to polyspermy. Reproduced with permission from Esfandiari N. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Structure of Zona Pellucida and Sperm Penetration

The sperm to zona pellucida binding is a species-specific process. This concept is the basis of the “hamster zona binding test.” Human sperm cannot bind to hamster eggs with an intact zona pellucida, which led to the thought that the zona pellucida contains species-specific receptors. Human sperm can only bind to hamster eggs after this glycoprotein layer is removed. Although there are some species–-species exceptions, the zona pellucida is an important barrier between interspecies fertilization.

With the aid of electron microscopy and advanced molecular techniques, our understanding of the zonal structure has increased. There are three major glycoproteins that compose the zona pellucida and have distinct roles in this structure: ZP1, ZP2, and ZP3 [48]. The ZP2 and ZP3 proteins form a filamentous structure that is then cross-linked with ZP1 proteins [49, 50].

In a classical model, ZP3 binds sperm and initiates the acrosomal reaction (see Fig. 5.1). Mutagenesis of O-glycosylation sites of ZP3 has been shown to decrease sperm receptor activity, suggesting that ZP3 serves as the sperm receptor in zona pellucida [51]. As sperm binds to ZP3, the outer acrosomal membrane fuses with the sperm plasma membrane that subsequently causes membrane blebs and results in the releasing of acrosomal enzymes that lyse the zona pellucida. This reaction exposes the inner acrosomal membrane that can bind to ZP2. Eventually, sperm penetrate the zona pellucida and enter into the perivitelline space. Various other models have shown that this acrosomal reaction could occur when sperm encounter cumulus cells [52]. However, as mentioned above, some oocytes do not have cumulus cells and can still be fertilized.

Cortical Reaction to Block Polyspermy

As the spermatozoa enter the perivitelline space, they initiate the cortical reaction (see Fig. 5.1). Release of proteolytic enzymes from egg cortical granules causes cleavage of ZP2, with subsequent dissociation of ZP2 from ZP3 [53]. Thus, after the cortical reaction, sperm can no longer bind to ZP3. Additionally, cleaved ZP2 cannot bind a spermatozoon that had previously undergone an acrosomal reaction. In conclusion, neither a sperm with an intact acrosome nor a sperm that has undergone an acrosomal reaction would be able to bind to the zona pellucida after the cortical reaction [54]. This is the principal mechanism preventing polyspermy.

Although through murine models we have learned an impressive amount about the process of fertilization, there are still many questions that need to be answered and further research on the exact mechanism of sperm binding is needed.

Sperm-Oocyte Membrane Fusion

After a spermatozoon penetrates the zona pellucida and enters the perivitelline space, the oocyte membrane and the spermatozoon membrane unite (see Fig. 5.1). At this stage, the spermatozoon has already undergone an acrosomal reaction, which exposed the inner acrosomal membrane and modified the membrane composition of both equatorial and post-acrosomal regions of the spermatozoon. The fertilizing spermatozoon binds to the microvillar region of the oocyte membrane with its equatorial segment [55]. Sperm tail movement decreases or stops within a few seconds of sperm-oocyte fusion [56]. Subsequently, the posterior region of the sperm head and the tail are incorporated into the egg. Unfortunately, the details of molecular interactions in sperm-egg fusion are not fully known. Initially, ADAM family members that are found on the sperm membrane, specifically fertilin and cyritestin, gained much attention. However, gene knockout studies questioned their fundamental roles in sperm-egg fusion. Currently, cyritestin, fertilin α(alpha), fertilin β(beta), CRISP1, izumo proteins, α(alpha)6β(beta)1 integrin, GPI-anchored proteins, CD151, CD9, and CD81 on the plasma membrane are thought to be involved in sperm-oocyte membrane fusion and are the subjects of ongoing research [57].

Oocyte Activation

Mammalian oocytes become arrested at the metaphase of the second meiotic division. After sperm-oocyte fusion, the oocyte continues meiotic division, releases cortical granules, progresses cell cycle, forms its pronucleus, and recruits maternal mRNA that are all essential for gamete formation [58]. These morphologic and biochemical changes that occur in the oocyte are collectively called “oocyte activation.” Another important hallmark of oocyte activation is calcium oscillations. It has been shown that injecting calcium into mice oocytes is enough to trigger embryo development up to the blastocyst stage [59]. In a mammalian oocyte, the calcium oscillations are a direct result of inositol triphosphate-mediated calcium release. Sperm-derived phospholipase-zeta (PLC-ζ(zeta)) is also responsible for oocyte activation [60]. Another protein that has been shown to activate oocytes is post-acrosomal sheath WW domain-binding protein. Its exact signaling mechanism is not clearly known, but it presumably acts through calcium signaling [61]. Regardless of the signaling pathway, oocyte activation is essential for pronucleus formation and subsequent embryo formation.

Oocyte activation clearly has clinical importance. A deficiency in oocyte activation was regarded as the principle cause of fertilization failure or low fertilization rate after ICSI. Recently it has been suggested that PLC-ζ(zeta) could be used as an alternative oocyte-activating agent, similar to other artificial oocyte activators [62].

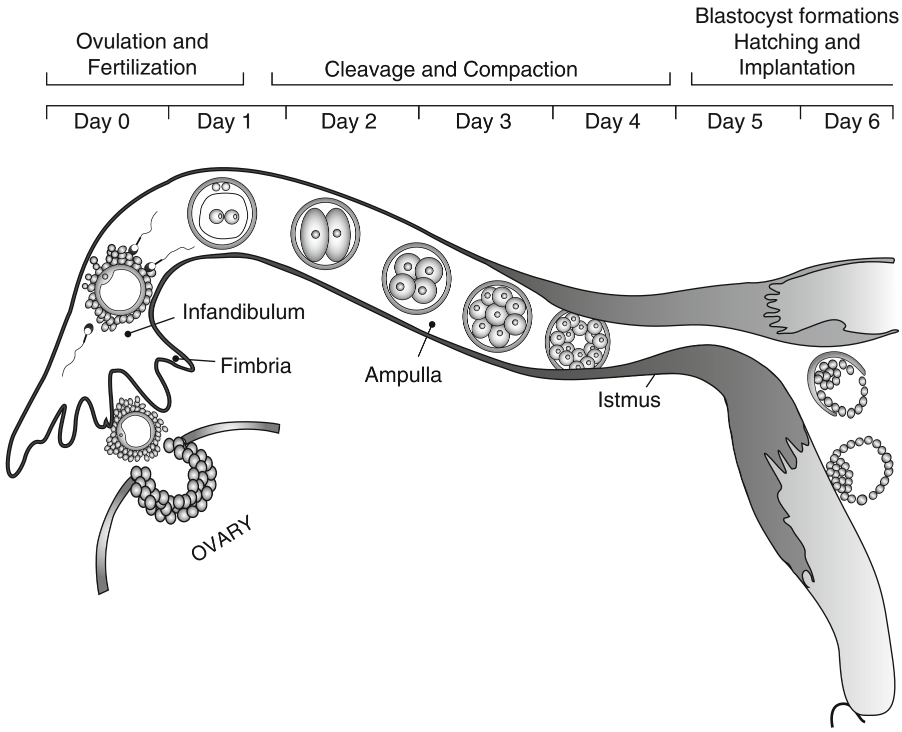

Fig. 5.2 Schematic drawing showing the major events from ovulation to the implantation of blastocyst during the first week of human life. Reproduced with permission from Esfandiari N. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Male Pronucleus Formation and Genomic Union

The final step of fertilization is the union of sperm and egg pronuclei, producing a diploid cell, the zygote. Dynactin, nucleoporins, vimentin, dynein, and microtubules are involved in bringing the two pronuclei together. It was proposed that a nuclear pore complex is inserted into the nuclear envelopes of the newly forming pronuclei. Dynactin and vimentin filaments are then incorporated into this nuclear pore complex. Formation of the complex probably starts after egg activation. The sperm aster then extends the microtubule "plus ends" away from the male pronucleus, some of which reach the female pronuclear envelope. With the aid of the dynactin-dynein motor complex, the two pronuclei are apposed [63]. Subsequently, the two nuclear envelopes disappear and the DNA undergoes replication. Homologous chromosomes are paired and aligned on the newly formed mitotic spindle. Eventually, the zygote is ready to undergo its first mitotic division.

Early Embryonic Development

In mammals, the zygote undergoes mitotic division (known as cleavage) as it travels through the fallopian tube, and eventually develops into a blastocyst once in the uterus (Fig. 5.2). The symmetrical cell divisions and cleavage create a ball of totipotent cells (blastomeres) that are still enclosed in the zone pellucida. When the zygote is approximately 16 cells, blastomeres form a closely packed group of cells with a smooth outer surface. This early developmental event is called compaction. The smooth surface is created by the formation of adherens and tight junctions between the blastomeres. At this time, two types of polarity originate in the zygote. The first type of polarity is cellular polarity. Cellular polarity occurs as the formation of microvilli on the external surface of the outer blastomeres separate from the basolateral surface [64]. The second type of polarity is developmental polarity. Developmental polarity is represented by the ability of the blastomeres in the internal compartment, the inner cell mass, to remain pluripotent, whereas the outer blastomeres begin to form trophoblast cells as they continue to divide [65]. This begins formation of the blastocyst and typically occurs around day 5 of fertilization. As cleavage continues, outer blastomeres express tight junction proteins, including ZO-1 and uvomorulin, gap junction proteins such as Connexin-43, and differentially position Na-K ATPase pumps selectively along the apical-basolateral axis. The outer blastomeres have a highly restricted developmental fate, eventually becoming the cells of trophoectoderm. The polarized expression of Na-K-ATPase in trophoectoderm creates a trans-trophoectoderm sodium gradient, which drives the osmotic accumulation of water into the nascent blastocoelic cavity. Growth factors like TGF-α(alpha) and EGF increase expression of Na-K-ATPase, which subsequently stimulate further expansion of the blastocoelic cavity (blastocoel). Meanwhile, the inner blastomeres continue to divide, and with the expansion of the blastocoel, they create a cluster of cells that impend into blastocoel. This totipotent cell cluster is commonly called the inner cell mass. The inner cell mass will eventually give rise to the embryo and extraembryonic tissues [65]. The outer layer of blastomeres, which have developed into trophoblasts/trophoectoderm, eventually give rise to the placenta. It is at this point that the developing embryo is called a "blastocyst."

Although difficult to completely exclude, this initial exponential division and formation of the blastocyst seems to be relatively independent of maternal contribution.

Around day 6 after ovulation, the embryo/blastocyst reaches the uterine cavity and is initially still covered with zona pellucida. For proper implantation, it must shed the zona pellucida. Trophoblast-derived trypsine-like enzymes, strypsin and plasmin, are thought to lyse the zona pellucida, allowing the embryo to hatch from the zona pellucida and begin to attach to the uterine endometrium [66, 67].

Trophoblastic Development and Invasion

The blastocyst is lined with a layer of trophoectoderm, which, as stated above, will give rise to the placenta. Although the inner cell mass is destined to produce embryonic and extraembryonic tissues, it stimulates trophoectodermal growth. In vitro, the removal of the ICM causes maturation of the trophoblastic cells, inducing them to turn into trophoblastic giant cells that are unable to invade the endometrium. For proper endometrial attachment, the blastocyst should remain attached to the trophoectoderm cells that are adjacent to the inner cell mass. Attachment of trophoblastic cells remote from the ICM has been associated with abnormal placental shape and eccentric insertion of the umbilical cord [68].

Prior to blastocyst attachment, for a successful pregnancy in the window of implantation, the uterine epithelium has to retract its cilia and express pinopodes. If all necessary molecular events occur, the blastocyst is firmly attached to the uterine epithelium around 6–7 days postconception.

The trophoblastic cells in contact with the inner cell mass start to proliferate and invade the uterine epithelium. As they invade, they fuse with each other and form multinucleated giant trophoblastic cells, known as syncytiotrophoblasts. An inner layer of mononucleated trophoblastic cells also develops called cytotrophoblasts. With fusion, the multinucleated syncytiotrophoblastic cells cannot proliferate, so the cytotrophoblastic cells function as a reservoir. Throughout the pregnancy, cytotrophoblastic cells divide and replenish the mature syncytiotrophoblastic cells. In addition to replenishing syncytiotrophoblasts, cytotrophoblasts give rise to various other cell types of the placenta, which are discussed below [69].

The fusion kinetics of cytotrophoblasts changes during pregnancy. In early pregnancy, two mononuclear cytotrophoblasts fuse to become a syncytiotrophoblast. However, later in the pregnancy, cytotrophoblasts fuse with already formed syncytiotrophoblasts [69]. Here we will discuss the process of early embryo development; the process of implantation will be discussed in more detail below.

Around 14 days postconception, cytotrophoblastic cells invade beyond the syncytiotrophoblastic cell layer and come into contact with maternal decidual cells. They form a column of cells with a proliferating core, and as the cells proliferate, more mature cells are passively pushed towards the maternal decidua [70]. More immature cells have α(alpha)6β(beta)4 integrin on their surface, which help bind basal membrane components like collagen IV and laminin. However, as they move further in the column and become closer to maternal decidual cells, they change their expression of surface integrins (integrin α1/β1, α5/β1, or α-v/β3/5), which helps them attach to the maternal extracellular matrix [68, 71].

In addition to adhesion molecules, trophoblasts secrete variety of enzymes that regulate invasion. MMP-2 and MMP-9 degrade collagen IV, which is the main collagen component of the basement membrane, and are therefore regarded as key enzymes in the implantation process, enabling the invasion of the trophoblast cells through the decidua and into the maternal vasculature [72, 73]. Tissue inhibitor of matrix metalloproteinases (TIMP), particularly TIMP-1, TIMP-2, and TIMP-3, were also detected in the trophoblastic cells and decidual tissues. TIMPs are normally inhibitory metalloproteinases and their regulation through trophoblastic and decidual cytokines control MMP activity [74–76]. Other lytic enzymes involved in extracellular matrix degradation are urokinase and tissue-type plasminogen activator (uPA and tPA, respectively). Both uPA and tPA are produced by trophoblasts, and their activity is controlled by plasminogen activator inhibitors (PAI) [76]. Another trophoblast protein, adrenomedullin, decreases PAI levels and subsequently increases plasminogen activators. Additionally, adrenomedullin increases trophoblastic proliferation [77].

Trophoblasts also secrete proangiogenic factors, which stimulate new vessel formation during invasion. Neovascularization is essential for the growth and maintenance of the developing embryo. VEGF, PDGF, and PAF are the main angiogenic factors that have been shown to be secreted by trophoblasts. TGF-β(beta) and TNF-α(alpha), which are present in decidua, further stimulate trophoblastic secretion of these angiogenic factors [78].

Complex molecular interactions take place between the decidua and trophoblasts to regulate trophoblastic invasion. In addition to those factors mentioned above, cytokines like

EGF, HB-EGF, IGFBP-1, LIF, IL-1 and hormones like hCG and progesterone have also been shown to regulate trophoblast invasion [76].

A number of other important types of trophoblast cells are involved in implantation—namely, extravillous, endovascular, and endoglandular. Small extravillous trophoblasts invade maternal decidua up to the inner one-third of uterine myometrium and reach the maternal spiral arteries. EVTs replace the spiral arteries' tunica media, which contains mainly the smooth muscle, and transform the spiral arteries into low resistance vessels that are no longer reactive to maternal vasomotor substances. This transformation aims to allow adequate maternal exchange with the developing fetus, particularly in the second trimester when maternal blood flow increases to the uterus to support the developing fetus. Apart from replacing the smooth muscle, endovascular trophoblasts, a subset of EVTs, replace the intimal layer of the spiral arteries [79]. Disturbances in this remodeling can result in IUGR and preeclampsia. Lastly, endoglandular trophoblasts invade the uterine glands, orient them towards the intervillous space, and replace the uterine epithelial cells.

Despite all these early trophoblastic changes, free transfer of maternal blood is only established towards the end of the first trimester. The large number of endovascular trophoblasts plugs the distal segments of the spiral arteries during initial invasion. Rather than maternal blood, the intervillous space ultimately contains glandular secretion products and maternal plasma filtrate, which are responsible for intrauterine nutrition, up until approximately 10 weeks gestation [68]. The reasoning behind the initial spiral artery plugging is believed that it helps keep a low oxygen environment and thus decrease free-radical formation during early embryogenesis.

After 10 weeks, the trophoblastic plugs dissolve and maternal blood contributes to intervillous fluid, which provides the appropriate amount of nutrients and oxygen for the developing fetus. These carefully regulated interactions between the invading trophoblasts and the maternal decidua eventually create a functional placenta, which is the main organ of nutrition, respiration, metabolite excretion, and hormone production in the developing fetus.

Implantation

Implantation can be divided into three stages: apposition, adhesion, and invasion [80]. Apposition is the initial adhesion of the blastocyst to the endometrial surface. Apposition is unstable and with uterine flushing the blastocyst can be detached from the endometrial surface. Apposition is followed by the adhesion stage, when a stronger connection is established between the embryo and endometrium. Finally, in the invasion stage, trophoblastic cells invade the endometrium [81].

Endometrial Receptivity

Successful implantation requires a properly developed blastocyst, a receptive endometrium, and series of molecular interactions. A non-receptive endometrium is one important cause of implantation failure, and it is essential to understand the basic molecular interactions involved in the process. Under the influence of estradiol, the endometrium proliferates and reaches a critical thickness to support implantation. After ovulation, in response to progesterone, the endometrium differentiates and becomes receptive to the newly hatched blastocyst.

Implantation occurs around 6 days after ovulation, ranging between 6 to 12 days [82]. The ideal time for implantation is thought to be around day 7 to day 9 after the LH surge and is called the "window of implantation" [83]. This period is characterized by structural and secretory changes in endometrial cells, providing the most favorable conditions for successful blastocyst implantation. The endometrium increases in thickness, becomes more vascularized, and glands become tortuous and increase their secretions rich in cholesterol, fat-soluble vitamins, lipid, and protein. These secretions will serve as an energy source for the embryo, which has no connection to uterine vessels at this point in development. In addition, there is a decrease in uterine fluid content to allow greater contact between the embryo and endometrium. These drastic changes in the uterine environment are mostly the result of progesterone stimulating the differentiation of endometrial cells into decidual cells. Decidual cells contain more intracellular lipids and glycogen deposits than endometrial cells, which cause them to get a polygonal shape as opposed to more rounded endometrial cells.

Pinopodes

One characteristic feature of receptive endometrium is the presence of pinopodes on the apical surface of endometrial cells. Pinopodes are bleb-like protrusions into the uterine lumen and are found in large numbers between days 19 and 21 in an idealized 28-day menstrual cycle. Although they are expressed throughout the mid- and late secretory phase, they show different morphological features. This suggests that their morphology, rather than their presence, is important for successful implantation [84]. Pinopodes can be assessed with electron microscopy, and their presence and morphology is a good marker of endometrial receptivity [83]. Blastocyst attachment has been shown to occur preferentially on the top of pinopodes, which suggests that receptors necessary for attachment are located on the pinopode surface [85].

Pinopode development has been associated with progesterone [84], HOXA-10, LIF [86], and aVβ3 [87]. HOXA-10, a homeobox gene, is necessary for blastocyst implantation, endometrial stromal cell proliferation, and epithelial cell morphogenesis [84]. Blocking HOXA-10 expression greatly decreases the number of pinopodes.

Selectins

Selectins are glycoproteins that have a glycosylated extracellular domain, single spanning transmembrane domain, and a cytoplasmic tail. There are three distinct selectins: P selectin, L selectin, and E selectin. Selectins are commonly known for their role in initial leukocyte attachment and subsequent rolling on the endothelial surface. In addition to leukocytes, however, selectins are thought to be responsible for the initial blastocyst-endometrium attachment.

Strong L selectin expression has been shown on the blastocyst surface, whereas on the maternal site, its ligands, namely MECA-79 and HECA-452, are up-regulated during the window of implantation [88]. Although L selectin is found on both luminal and glandular epithelium, expression of L selectin is higher on the luminal epithelium [89]. Initial trophoblast attachment to endometrium is thought to occur with trophoblastic L selectin and endometrial oligosaccharide interactions [90].

Integrins

Integrins are transmembrane glycoproteins composed of noncovalently linked α(alpha)- and β(beta)-subunits. Each subunit has an extracellular, intracellular, and transmembrane domain. The intracellular domains are linked to the cytoplasmic cytoskeleton and intracellular signaling pathways [85]. They are paired to compose integrin heterodimers; 24 functionally distinct integrins have been identified [91]. Among various other functions, they are mainly involved in cell-to-cell and cell-to-extracellular matrix interactions. Among the many different types of integrins that are expressed constitutively in the endometrium, α1β1, α4β1, αvβ3 are co-expressed between days 20 and 24 in the menstrual cycle. β(beta)3 intergrin deserves special attention among other subunits, because its expression starts at cycle day 19 and increases thereafter [92]. Moreover, it is mainly expressed on the endometrial luminal surface, which suggests that αvβ3 integrin, and its endometrial ligand osteopontin, might serve as a receptor for embryonic attachment [92, 93]. Various studies showed that the αvβ3 integrin is regulated in both a hormonal and paracrine manner. For example, estrogens down-regulate integrin expression, but increasing levels of progesterone in the luteal phase counteract the estrogen effect. Rather than a direct effect, progesterone increases epidermal growth factor and heparin binding growth factor in the uterine stroma, which results in increased αvβ3 levels [92]. The embryo is also actively involved in the β3 subunit regulation, probably with the embryonic IL-1 system [85].

Additionally, HOXA-10 increases the expression of the β3 subunit in endometrial cells [94]. This subunit is the rate-limiting step in αvβ3 integrin production. Considering the important role of αvβ3 integrin in the implantation process, it's not surprising that it is used as a clinical marker of endometrial receptivity [85, 95].

Mucins

Mucins are heavily glycosylated proteins. Carbohydrates constitute 50–90 % of their molecular weight. To date, 18 mammalian mucin genes have been identified [96]. Mainly mucin-1 (MUC1) and to lesser extent mucin-6 (MUC6) are expressed in the human endometrium. They are found on the luminal surface of the epithelial cells in the reproductive tract. Their proposed physiological role in the reproductive tract is to trap bacteria and viruses and expel them. They are resistant to digestive enzymes. Their extracellular portion can be cleaved, and those cleaved molecules can join via sulfide bonds to create a mucin gel. Altogether, mucins produce a formidable barrier in microbial defense. Estrogens increase mucin production. Progesterone has no independent effect on mucin production; yet by counteracting the effects of estrogens, the net effect of progesterone is to decrease mucin levels. Cytokines, particularly TNF-α, have also been shown to be involved in mucin regulation.

Although they provide an important barrier in microbial defense, mucins also constitute a barrier against blastocyst implantation. Mucins extend their projections well beyond endometrial surface receptors, thereby hindering blastocyst access to them [97]. At the site of implantation, mucins' extracellular domain needs to be cleaved. The sheddase family of enzymes, particularly TACE/ADAM17 and MT1-MMP, has been suggested to play a role in this cleavage process [98]. The blastocyst, through the action of secreted cytokines, upregulates sheddases that cleave mucins in endometrium [97].

Interestingly, during the implantation period, mucin production is increased [99]. It seems to be a paradoxical phenomenon; however, two possible explanations have been suggested. First, after sexual intercourse, the ejaculate may introduce microbial pathogens into the endometrium and the increased mucin levels may act as an additional protective barrier [100]. Second, as the blastocyst is actively involved in sheddase induction, it has to be competent to do so. Mucins may be a protective mechanism against the attachment of unhealthy embryos that would otherwise have resulted in pregnancy failure [97]. Consistent with this view, women with recurrent pregnancy failure have decreased mucin levels compared to a fertile control group [101].

To summarize, mucins prevent embryo attachment and need to be cleaved at the site of embryonic attachment. This process involves a series of interactions that requires a healthy embryo as well as a functional endometrium.

Cytokines

Cytokines are soluble proteins that have a variety of functions in inflammation, the menstrual cycle, ovulation, and implantation. A disturbance in the normal expression or action of several cytokines results in implantation failure and abnormal placental development in humans. Of known importance are members of the gp130 family, such as LIF, IL-1, IL-11, and IL-15 system [102].

Leukemia Inhibitory Factor

A member of the gp130 cytokines, LIF acts through its surface receptor complex, LIF receptor (LIFR), and the gp130 receptor chain. Binding of LIF to LIFR results in heterodimerization with gp130 and subsequent activation of downstream signaling pathways that include the JAK/STAT, MAP kinase, and PI3 kinase pathways [85]. Other members of the gp130 cytokine family, including oncostatin M, ciliary neurotrophic factor, cardiotrophin-1, IL-6, and IL-11, can also bind to the LIFR [102].

LIF was the first cytokine shown to be critical for implantation in mice [103]. Wild-type mice embryos failed to implant in the endometrium of homozygous LIF mutant female mice, and the implantation failure was reversed after LIF supplementation [104].

LIF mRNA is expressed between menstrual cycle days 18 and 28 in fertile women [105], and it is expressed by both glandular and luminal epithelium [106]. Among many LIF regulators, progesterone is probably responsible for endometrial LIF induction. When treated with a selective progesterone receptor modulator, mifepristone, decreased levels of LIF are observed in endometrium [107]. In addition to progesterone, IL-1α, TNF, PDGF, TGF-β1, and HB-EGF stimulate LIF expression in cultured endometrial stromal cells. The embryo secretes hCG, IGF-1, and IGF-2 that also increases LIF levels [108].

LIF protein expression is maximal in uterine flushings in the midlate secretory phase of the menstrual cycle at the time of expected implantation. Considering the ease of performing uterine flushings, LIF has been suggested as a marker of uterine receptivity [109, 110]. In women with recurrent implantation failure, LIF levels are lower than in controls, emphasizing the importance of LIF in successful implantation [110, 111]. rhLIF has also been suggested to improve endometrial receptivity in recurrent implantation failure patients; however, the efficacy has not been demonstrated in clinical trials [112].

Interleukins

IL-1 is one of the key regulatory mediators of the inflammatory response. IL-1α, IL-1β, and the IL-1 receptor antagonist are members of the IL-1 cytokine family. Stromal cells, glandular cells, and macrophages are the reservoir of IL-1 in the endometrium. In vitro, treating endometrial cells with IL-1 increases integrin β3 expression in the epithelial cells [113]. IL-1α knockout mice are, however, fertile, suggesting redundancy in the effects these interleukins have in implantation. IL-1 receptor antagonist expression is decreased during the implantation window. It is possible that down-regulation of IL-1 antagonist works synergistically with IL-1 to affect implantation [114]. Exogenous IL-1 receptor antagonist treatment during implantation can block blastocyst implantation [115]. Overall, the IL-1 system is clearly involved in implantation; however, its exact role in implantation remains unclear.

IL-6 is involved in many immune interactions, and it has been also suggested to play a role in implantation. Endometrial IL-6 mRNA expression increases during the mid- to late secretory phase and decreases in the late secretory phase. Strong immunoreactivity has been observed in uterine glandular and luminal epithelium during the window of implantation [116]. While controversial, IL-6-deficient mice appear to have reduced fertility and decreased implantation rates [117, 118]. The IL-6 receptor is found on the surface of the blastocyst, and IL-6 is probably involved in paracrine/autocrine interactions in the window of implantation. Decreased levels of mid-secretory IL-6 mRNA are found in patients with recurrent spontaneous abortions, also supporting this hypothesis [119].

Another cytokine that has gained attention is IL-11. IL-11 has anti-inflammatory activities, and it is expressed in endometrial glandular and luminal epithelium. Estrogen, progesterone, and local factors increase IL-11 levels. IL-11 advances progesterone-induced decidualization of human endometrial stromal cells. IL-11 and its receptor IL-11R were immunolocalized to decidualized stromal cells in the mid-late secretory phase epithelium. They were also shown on the trophoblastic cells, suggesting a role in normal placentation [102]. Additionally, inadequate IL-11 signaling was found to result in dysregulation in trophoblastic invasion [120].

Prostaglandins

Prostaglandins (PGs) are lipid mediators of inflammation, and they have a variety of functions in inflammation, menstrual cycle regulation, ovulation, embryo attachment, trophoblastic invasion, and labor. Prostaglandins, leukotrienes, and tromboxanes are members of the eicosanoid family. They are produced from membrane lipids by phospholipase A2 (PLA2) and cyclooxygenase (COX) enzymes. To date, three isoforms of COX enzymes have been discovered: COX-1, COX-2, and COX-3. COX-1 is constitutive and expressed under normal physiological functions, whereas COX-2 is involved mainly in inflammatory responses. COX-3 is expressed in the human brain and is responsible for fever and response to pain.

Murine studies have shown the importance of prostaglandins (PGs) in implantation. Lack of either PLA2 or COX2 in mice leads to defective PG synthesis; PLA2 knockout mice show pregnancy failure [121]. COX expression is maximal in the menstrual and proliferative phases [85]. Among many regulators, IL-1 deserves further consideration. IL-1 increases

COX enzymes and PG production, resulting in increased endometrial integrin levels that are essential for blastocyst implantation [85].

Although prostaglandins' role in the menstrual cycle and pathophysiology in endometriosis is well known, their role in human blastocyst attachment and subsequent invasion needs to be explored further.

HOX Genes

Homeobox (HOX) genes are highly conserved genes that are involved in embryonic development as well as endometrial growth, differentiation, and receptivity [122]. Both estrogen and progesterone increase HOXA10 and HOXA11 expression. Additionally, HOXA10 and HOXA11 expression reach the highest levels during the window of implantation [123]. Wild-type mice embryos cannot implant to the uteri of HOXA10 or HOXA11 knockout mice. These findings suggest that HOXA10 and HOXA11 play an essential role in endometrial receptivity [124]. In parallel with these findings, pinopodes, β3 integrin, and insulin-like growth factor binding protein were shown to be regulated by HOX genes [81]. As discussed above, these genes are among the few proven to be essential for endometrial receptivity. In humans, there are no documented HOXA10 or HOXA11 mutations. However, in various gynecologic disorders such as endometriosis, PCOS, hydrosalpinx, and uterine fibroids, endometrial HOXA10 and HOXA11 mRNA levels are reduced [125–127]. These findings demonstrate that HOX genes contribute to the defective endometrial receptivity that is observed in those disorders.

Immune Response to Trophoblast Invasion: Trophoblast–Leukocyte Interactions

As mentioned above, to achieve a successful pregnancy, the blastocyst must be able to attach to the endometrial decidua without complication. The blastocyst has to invade the endometrium and maternal blood vessels in order to ensure adequate blood supply for nutrients and gas exchange. However, because a blastocyst receives half of its genome from the father and the other half from the mother, it is treated as a semiallogenic by the maternal immune system. Therefore, alterations in the reactivity of the maternal immune system must occur at the maternal-fetal interface.

As the blastocyst attaches to the uterine epithelium, the trophoectoderm differentiates into two layers, as previously mentioned, an outer syncytiotrophoblast and an inner cytotrophoblast layer. Two weeks after implantation, the cytotrophoblast layer protrudes through the syncytiotrophoblasts and forms cytotrophoblastic buds. The buds then differentiate into both villious trophoblasts and extravillous trophoblasts. Villous trophoblasts cover the chorionic villi, which forms the main interface for gas and nutrient exchange between the fetus and mother, and as discussed above, extravillous trophoblasts invade and remodel the spiral arteries.

Maternal and fetal cells are in direct contact during this invasion process, and the immune response deserves a detailed explanation. Maternal leukocytes reside in the uterine endometrium, and it has been estimated that approximately 40 % of the decidua is leukocytes. Fortunately, trophoblasts have a distinct MHC expression profile. They do not express the most common HLA antigens such as HLA-A and HLA-B, and even with potent stimulators like IFN-α, they do not express MHC class II antigens recognized by certain leukocytes. The dominant MHC types expressed on trophoblasts are HLA-C, HLA-G, and HLA-E.

Villous syncytiotrophoblasts line blood-filled lacunae and are in direct contact with maternal blood. They do not express MHC-I antigens and are therefore protected from T-cell-mediated responses. Interstitial trophoblasts invade the decidua and express HLA-C, HLA-G, and HLA-E. Endovascular trophoblasts that line the maternal spiral arteries express HLA-C, HLA-G, and HLA-E. The expression of HLA-G and HLA-E on this cell population confers protection from maternal immune rejection.

Leukocytes are normally found in endometrium and actually 40 % of the decidua consists of leukocytes. In endometrial infection, there are a multiple types of leukocytes that are found in the endometrial lining, and they all act through different mechanisms. B-cells respond to antigenic stimulation and produce antibody secreting plasma cells.

Additionally, macrophages can be found and represent approximately 20 % of the endometrial leukocytes; they can recognize and respond to HLA-G antigens [128].

T cells represent 10 % of the leukocytes found in the endometrium. They require an MHC-II antigen presentation for immune response and, as trophoblasts do not express MHC-II antigens, they cannot directly stimulate T-cell responses. This is how villous syncytiotrophoblasts, which line the maternal blood-filled lacunae, are protected from the maternal T cells. However, maternal endometrial dendritic cells and macrophages can process paternally derived antigens by migrating to lymph nodes where they can initiate an immune response.

Interestingly, antibodies against paternal HLA antigens can be found during pregnancy; however, they are likely formed during birth due to fetal cells crossing the placenta. Fortunately, these antibodies are mainly against HLA-A and HLA-B. As these HLA types are not expressed by the trophoblasts, the presence of these antibodies is not correlated with pregnancy success [79].

Natural killer T (NKT) cells are a subset of T cells that have an immunomodulatory role in infection through cytokine production. Invading trophoblasts are protected from blood NK cells through multiple different mechanisms.

Villous syncytiotrophoblasts are likely protected by the absence of NK-activating ligands on the syncytiotrophoblastic surface. Similarly, endovascular and interstitial trophoblasts, which line maternal spiral arteries and decidua, respectively, express HLA-C, HLA-G, and HLA-E. The expression of HLA-G and HLA-E on these cell populations confers protection from blood NK cells.

Uterine NK cells (uNK) are among the most studied endometrial leukocyte type and are known to be involved in endometrial renewal, differentiation, and breakdown in menstrual cycle. Although their exact role in implantation is unknown, their dysregulation has been shown to be associated with recurrent pregnancy loss, preeclampsia, and implantation failure. Their origin is still unknown, yet they are thought to arise from in utero proliferation and differentiation of CD34+ stem cells. They are found in deeper layers of decidua and are not shed during menstruation. Another alternative to their origin is recruitment from CD56+ cells in the blood into the endometrium. Regardless of their origin, their quantity correlates with maternal progesterone levels. Additionally, they are found to accumulate in large numbers at the site of implantation. Their close proximity to trophoblasts suggests that they may be involved in regulating trophoblastic invasion [79]. In addition, lower uNK counts in the endometrium have been correlated with decreased IVF-ET success [129]. In summary, although dysregulation of uNK cells and their cytokine production profile was shown to be related with recurrent pregnancy loss, preeclampsia, and implantation failure, their exact role in implantation is not known [130].

Clinical Relevance

Infertility is classically defined as the failure of a couple to conceive after 12 months of frequent intercourse in women under 35 years, and after 6 months in women over the age of 35 [131]. Infertility can be due to male factors, female factors, or both. The availability of ICSI/IVF has resulted in pregnancy rates in couples with male factor infertility that are comparable to those without male factor infertility [132].

Key steps for successful fertilization are the oocyte quality and appropriate oocyte maturation. As our understanding of oocyte biology has increased, we are able to mimic endogenous oocyte developmental steps in vitro. One such success in reproductive medicine is improving in vitro maturation (IVM), in which immature oocytes are collected and then maturated for in vitro fertilization. This technique provides an invaluable opportunity for many infertile patients. Additionally, IVM and IVF provide an opportunity for fertility preservation, including use in patients undergoing gonadotoxic chemotherapy for various cancers [133].

Defective uterine receptivity is a significant cause of ART failure [134]. Therefore, it is essential to correctly assess the endometrial receptivity state for successful implantation. Among many others, pinopodes and αvβ3 integrin have been suggested as candidate biomarkers that reflect the window of implantation [135]. However, there is currently insufficient evidence from adequately powered prospective clinical trials to validate these markers. Patients with endometriosis, fibroids, PCOS, and hydrosalpinx frequently present with infertility due at least in part to defective endometrial receptivity. Recognizing and treating the underlying etiology can at least partly improve endometrial receptivity in these patients.

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6 Reproductive Imaging

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Introduction

Several different techniques are available to image the female reproductive tract: pelvic sonography, saline infusion sonography (SIS), hysterosalpingo-contrast sonography (HyCoSy), a hysterosalpingogram (HSG), or a magnetic resonance imaging (MRI) procedure. The optimal imaging method should be diagnostically accurate, cost-effective, minimally invasive, and reliable. Depending on the disease process or anatomic variant to be depicted, one imaging modality may be better suited than another, or more than one diagnostic test may be needed. Each procedure has advantages and disadvantages and can be the superior modality in certain specific clinical settings; Table 6.1 summarizes these findings. Laparoscopy and hysteroscopy are minimally invasive means to directly visualize the pelvic structures. An important advantage of these means of direct visualization is the ability to perform therapeutic interventions. Reproductive imaging can include the following modalities: Pelvic sonography, SIS, HyCoSy, HSG, and MRI.

Pelvic Ultrasonography

Both transabdominal and transvaginal sonography are safe, noninvasive, and readily available in most office settings. Sonography can provide an easily accessible image of the uterus, ovaries, and other pelvic structures (pelvic kidney, appendix, or adnexal masses).

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Principles

Ultrasound images are obtained by emitting pulses of high-frequency sound and measuring the echoes that are reflected back to the transducer from interfaces between tissue structures of different impedance. The echoes are then transformed into real-time dynamic images of these structures. Most probes are curvilinear or convex, providing a wider field of view within a compact probe design. Most ultrasound images are obtained using *B-mode* (brightness) or *gray-scale*, displaying images in two dimensions. Ultrasound can also be used in the following modes: (1) *M-mode* to analyze cardiac motion, (2) *color flow Doppler ultrasonography* to measure the speed and direction of blood flow, or (3) *three-dimensional* (*3D*) *ultrasonography*, in which multiple B-mode images are combined into a 3D image that displays volume. Ultrasound frequencies in gynecology usually range between 3 and 7.5 MHz. Lower frequencies penetrate tissue more deeply with poorer resolution [1]. Conversely, higher frequencies penetrate tissue less deeply but give better resolution.

Technical Considerations

Ultrasound can be performed via transabdominal or transvaginal approach, with the latter being preferred since the probe is closer to the pelvic organs and allows better resolution of these structures. Large uterine or ovarian masses extending out of the pelvis, however, can be missed on transvaginal scanning; therefore, both approaches may be needed. The transabdominal approach is an option in a virginal patient and requires a full bladder to provide an acoustic window in order to fully delineate the pelvic structures. No prophylactic antibiotics or special analgesics are required for pelvic ultrasonography. A pelvic ultrasound is considered basically risk-free in nonpregnant patients. In pregnant patients, ultrasounds should only be performed if indicated

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Table 6.1 Advantages and disadvantages of different imaging modalities

Imaging modality	Advantages	Disadvantages
TVS	Easily available Relatively inexpensive	Limited information on tubal or endometrial pathology Limited in obese individuals
SIS	Assessment of endometrial pathology	Limited information on tubal pathology
HyCoSy	Visualization of ovaries, tubes, and endometrial lining	Patient discomfort Limited in obese individuals Requires trained staff and equipment
HSG	Better visualization of the entire fallopian tube Possible increase in pregnancy rate Not limited by body habitus	More invasive procedure Radiation exposure Risk of sensitivity to contrast Not available in all facilities
MRI	Better method to assess leiomyomata or uterine anomalies Not limited by body habitus	Expensive Not available in all facilities

[2]. However no studies have shown any abnormalities in children after prenatal ultrasounds [3].

Transvaginal probes should be disinfected after use and covered to prevent the transmission of infections between patients. Of note, the leakage rate when using condoms as vaginal probe covers is 0.9–2 %, and as high as 8–81 % when using commercial probe covers [4]. Agents available for high-level disinfection include glutaraldehyde, stabilized hydrogen peroxide (6 %), orthophalaldehyde, peracetic acid, and peracetic acid-hydrogen peroxide [5], but compatibility of the probe and disinfectant needs to be confirmed per manufacturer's instructions.

Pelvic ultrasound is best performed in the early follicular phase, when the endometrium is thin and endometrial pathology can be better visualized [6]. Heavy bleeding should not be present, because blood clots can be misinterpreted as polyps or adhesions [7]. A small amount of blood, however, can delineate the endometrial-myometrial interface (spontaneous sonohysterogram [8]).

Evaluation of the pelvis should proceed in the following systematic fashion, examining each area with respect to the following parameters:

1. Uterus: Measurement of length, height, and width in longitudinal and transverse axes; size, number, and location of any leiomyomata, position and configuration of the uterus, thickness and appearance of endometrial lining, any uterine malformations, or cervical abnormalities.
2. Ovaries: Measurement of length, height, and width in longitudinal and transverse axes, number of antral follicles measuring between 2 and 9 mm, size and characteristics of any larger ovarian masses.
3. Posterior cul de sac: Presence of any free fluid.
4. Fallopian tubes: Normal fallopian tubes are rarely seen on pelvic ultrasound. A hypoechoic tubular or tortuous structure is suggestive of a hydrosalpinx [9], particularly if the "waist sign" is observed. The "waist sign" refers to diametrically opposed indentations in the wall of a cystic collection [10].

Limitations

Visualization of pelvic structures can be difficult when using a transabdominal approach on an obese individual. Overlying bowel can also interfere with visualization on both transabdominal and transvaginal ultrasound studies. There can be considerable variability in the quality and diagnostic capability of sonography dependent upon the experience and expertise of the ultrasonographer. Of note, it is often easier for a clinician to detect abnormalities while performing or observing a real-time dynamic scan rather than when reviewing previously acquired static images. Tubal patency cannot be assessed with standard ultrasonography. If endometrial pathology is suspected, saline infusion sonogram should be utilized.

Indications

Indications for pelvic ultrasonography include the following: (1) management of pelvic masses, (2) evaluation for ovarian torsion, (3) abnormal uterine bleeding, (4) uterine leiomyomata, (5) pelvic pain, (6) recurrent pregnancy loss, or (7) foreign bodies in the uterus. Ultrasonography is widely used for an infertility evaluation and includes (1) monitoring of follicle maturation (Fig. 6.1), (2) assessment of endometrial thickness (Fig. 6.2), (3) transvaginal oocyte aspiration, (4) ultrasound-guided embryo transfer [11], or (5) detection of hydrosalpinges (Fig. 6.3). Different publications have shown sensitivity of 86 % [12] and specificity of 99.6 % [13] for detecting a hydrosalpinx on transvaginal ultrasonography.

For the detection and classification of congenital uterine anomalies, a number of studies have demonstrated that 3D sonography is a reasonable alternative to a HSG or MRI procedure [14, 15]. 3D sonography allows one to visualize the external uterine contour as well as the internal morphology in the coronal plane. Bocca et al. [14] performed a prospective

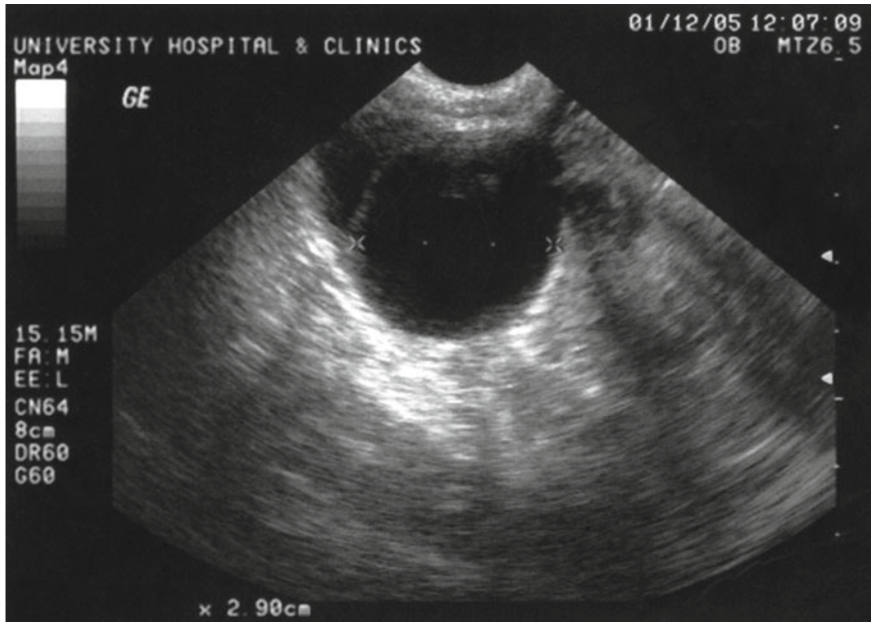

Fig. 6.1 Cystic structure representing a dominant ovarian follicle. Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 30.13 from 1st ed)

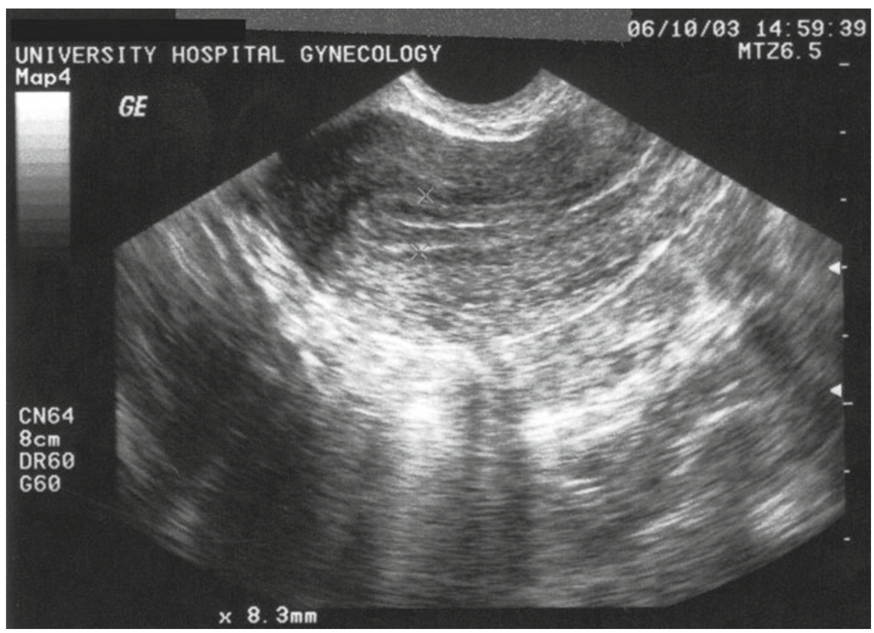

Fig. 6.2 Trilaminar appearance of the endometrial echo. Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 30.12 from 1st ed)

blinded study with 101 females who underwent routine HSG and 3D sonography as compared to surgical findings. The authors found 30 congenital anomalies (arcuate, unicornuate, bicornuate, septate uteri as well as uterine didelphys). Of the 30 congenital anomalies, all were correctly identified with 3D sonography, compared to only 10 correctly identified with HSG. Caliskan et al. [16] found that uterine anomalies visualized on 3D sonography are easier to interpret in the luteal phase compared to the follicular phase secondary to increased thickness and echogenicity of the endometrium. Ghi et al. [17] performed a prospective study on 284 nulliparous patients with at least three consecutive miscarriages who underwent 3D sonography. If the 3D sonography demonstrated a normal external and internal uterine contour, patients would undergo an office hysteroscopy. If the 3D sonography was abnormal, they underwent a concurrent hysteroscopy and laparoscopy. All 230 patients with negative 3D sonography results exhibited a normal uterine cavity at the time of office hysteroscopy. In the group with the abnormal 3D sonography, the presence of a Müllerian anom-

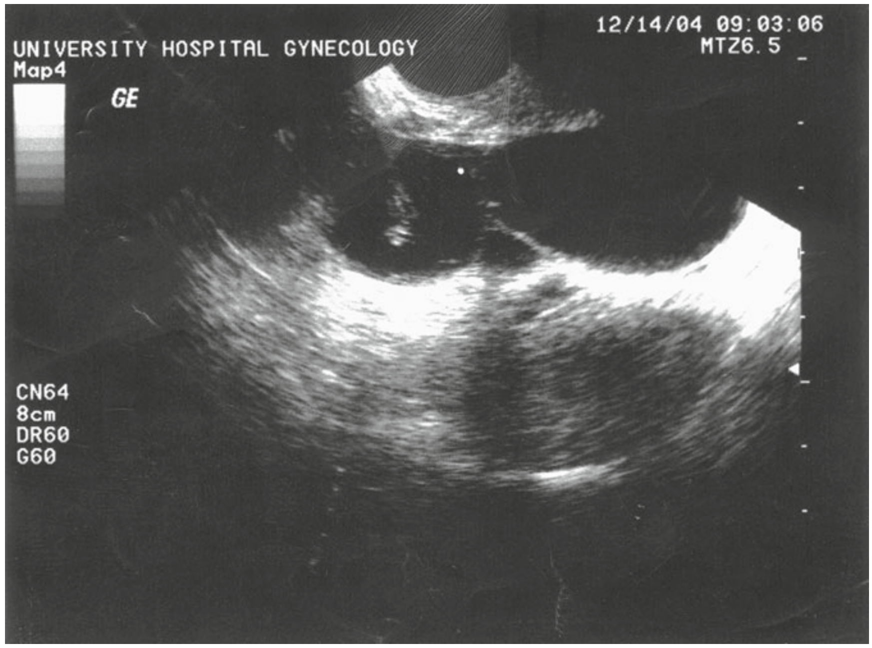

Fig. 6.3 Hydrosalpinx. A hypoechoic tubular structure. Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 30.3 from 1st ed)

aly was correct in 52 out of 54 patients. 3D sonography in this study had a positive predictive value of 96.3 % and a negative predictive value of 100 %.

Currently only limited information is available to compare 3D sonography with MRI in the diagnosis of Müllerian anomalies. Bermejo et al. [15] found a concordance rate (kappa index = 0.880) between the two imaging modalities; however, only 65 of the 286 females undergoing 3D sonography also had an MRI procedure.

The above studies suggest 3D sonography is an accurate and noninvasive tool to detect congenital uterine anomalies, with the advantages of less cost, lower morbidity, shorter examination time, and the availability to perform this procedure in the office setting.

Saline Infusion Sonography or Sonohysterography

Principles

Transvaginal ultrasonography alone has limited usefulness for demonstrating pathology in the endometrial cavity [18]. SIS enhances the ability to visualize lesions projecting into the uterine cavity. During the procedure, the endometrial cavity is filled with saline via a transcervical catheter, as first described by Nannini in 1981 [19]. Deichert et al. reported statistical equivalence among SIS, HSG, and hysteroscopy in regard to the evaluation of intrauterine pathology [20].

Technical Considerations

The SIS procedure should be performed between cycle days 5–10 to avoid menstrual blood being misinterpreted as an intracavitary artifact. A follicular phase study ensures a thin endometrial lining and avoids the possibility of an early pregnancy. Some providers recommend a urine pregnancy test prior to the SIS to decrease the risk of a concurrent pregnancy. If a patient is taking oral contraceptive pills, an SIS can be performed with less concern in regard to an occult conception and can facilitate timing of the procedure. Uterine cramping can occur, which usually responds well to the use of a nonsteroidal anti-inflammatory medication 30 minutes prior to performing the procedure.

Prior to the SIS procedure, informed consent should be obtained for the following possible sequelae: cramping, uterine bleeding, vasovagal reaction, or infection. Using an open-sided speculum, the cervix is cleansed with an antiseptic, and the SIS catheter is placed through the cervix. Different catheters can be used, including a standard size 5- or 7-Fr double-lumen intrauterine HSG catheter, a more rigid Goldstein sonohysterography catheter (Cook Ob/Gyn, Spencer, IN, USA), or a latex-free urethrane H/S Elliptosphere catheter (Ackrad Laboratories, Cranford, NJ, USA), which contains an inflatable balloon (Figs. 6.4 and 6.5). An 8-F pediatric Foley catheter can also be used, but it is more difficult to insert. Next, the speculum is removed and the transvaginal probe replaced. Sterile saline is then slowly injected into the uterine cavity, which leads to separation of the anterior and posterior uterine walls. Usually there is no

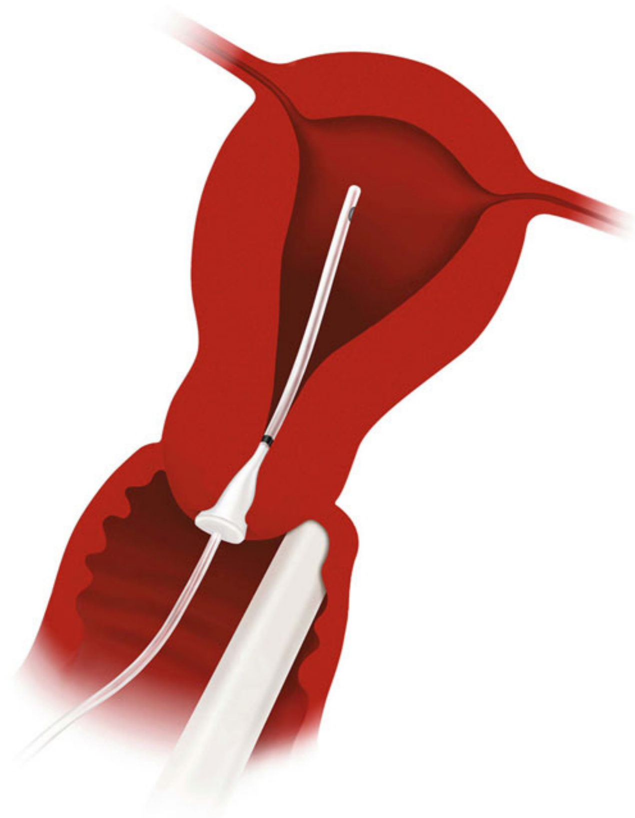

Fig. 6.4 Goldstein sonohysterography catheter (Cook Ob/Gyn, Spencer, IN, USA). Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 30.9 from 1[st] ed)

Fig. 6.5 H/S Elliptosphere catheter with latex-free urethane (Cooper Surgical Inc., Turnbull, CT, USA). Reproduced with permission from Lindheim SR, Uhler ML. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007 Fig. 30.10 from 1[st] ed)

need to insufflate the balloon. The uterus is then scanned in the longitudinal and transverse plane and 3D pictures can be obtained. If one encounters difficulty placing the catheter, a tenaculum can be placed on the cervix for straightening of the cervical canal, and a dilator utilized. If this is unsuccessful, the procedure can be repeated after having the patient take 400 μg of misoprostol orally 12 hours prior to the SIS procedure.

No uniform guidelines exist for antibiotic administration in patients undergoing SIS. For the HSG procedure, the American College of Obstetricians and Gynecologists (ACOG) recommends doxycycline 100 mg orally BID×5 days if the patient has a history of pelvic inflammatory disease or if the procedure demonstrates dilated tubes [21]. There are no studies assessing the rate of post-SIS pelvic infections. One study reported four cases of pelvic infections after diagnostic or operative hysteroscopy that were successfully treated with antibiotics [22]. The decision to prophylactically treat a patient undergoing an SIS procedure is left to the provider, with some arguing to use the same criteria as outlined by ACOG for the HSG procedure.

Limitations

SIS should not be performed in a patient with documented intrauterine gestation, pelvic infection, or unexplained pelvic tenderness [23]. If a patient has a history of a known hydrosalpinx, some would defer the SIS procedure for the concern of a post-procedure infection. SIS can indirectly assess tubal patency by documentation of fluid in the posterior cul de sac; however, it cannot differentiate between laterality.

Indications

SIS can detect focal intrauterine lesions, such as polyps (Fig. 6.6), submucosal uterine leiomyomata, or endometrial hyperplasia [24]. The incidence of polyps and submucosal leiomyomata in symptomatic premenopausal women is 33 % and 21 %, respectively [25]. The SIS procedure is as accurate as hysteroscopy for the detection of focally growing lesions, with sensitivities for both procedures of approximately 96 % [26]. Histologic tissue evaluation is required whenever intrauterine pathology is discovered, and blind endometrial biopsy may miss the pathology [27]. SIS in conjunction with 3D sonography can also help clarify any uterine malformations, such as septate vs. bicornuate uterus, by depicting the outer uterine contour.

Uterine leiomyomas are classified into three categories based on their location within the uterus, according to the European Society of Hysteroscopy Classification of Submucosal Fibroids [28]:

Class 1: Complete submucosal involvement with no myometrial involvement (intracavitary)

Class 2: Submucosal component involving less than half of the myometrium

Class 3: Submucosal involvement with an intramural component of greater than 50 %

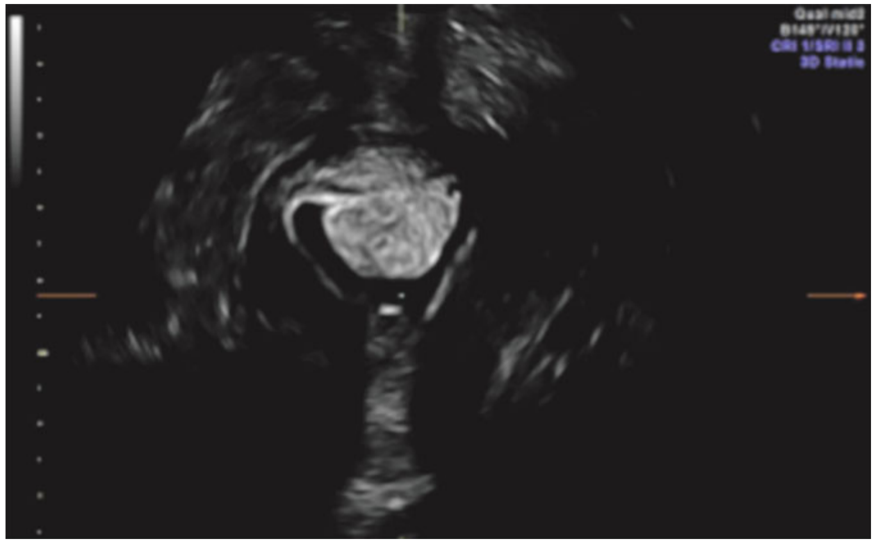

Fig. 6.6 Large endometrial polyp. Image provided by Steven Nakajima, MD, University of Louisville, Louisville, KY

Hysterosalpingo-Contrast Sonography

Principles

The HyCoSy can be the most comprehensive tool to evaluate the pelvic organs when compared to transvaginal sonogram and SIS. In addition to gathering information about the uterus and the ovaries, the HyCoSy can also evaluate patency of the fallopian tubes, which are difficult to visualize on regular ultrasound if they are anatomically normal [29]. The HyCoSy uses contrast media that helps to visualize the structure of the fallopian tubes while transvaginal ultrasound is performed concomitantly. When compared to HSG, there is no exposure to radiation.

Technical Considerations

The HyCoSy procedure is normally conducted after performing an SIS procedure. The timing of the procedure, prerequisites, contraindications, and use of antibiotics are the same as for an SIS procedure. After evaluation of the uterine cavity, the intrauterine balloon is inflated with 3 mL of either fluid or air to occlude the lower uterine segment. For this reason, a Goldstein catheter cannot be used for the HyCoSy procedure. Next, a 20-mL syringe filled with both saline and air can be tilted intermittently to infuse 1–3-mL increments of saline followed by air [26]. Alternatively, a syringe with saline and air can be vigorously shaken immediately prior to infusion [30], or a commercial device is now available that mixes the saline and air prior to the infusion. The mixture of saline and air can then be seen as "scintillations" traveling from the proximal interstitial portion of the tube to the distal fimbria and ovary [31] (Figs. 6.7 and 6.8). If a tube fails to fill with the air/fluid mixture, it can be advantageous to ask the patient to roll slightly to position the tube superiorly. If no proximal or distal scintillations can be seen, this may represent true obstruction vs. spasm in the cornual region. Once the study is completed, the balloon is deflated and all instruments removed from the vagina. The possibility of shoulder pain after the procedure secondary to peritoneal irritation by intra-abdominal air should be discussed with the patient. This common side effect usually resolves after 24 hours.

Different contrast media have been used and evaluated, such as Hyskon (Pharmacia Laboratories, Piscataway, NJ, USA [32]) or Echovist-200 (Schering AG, Berlin, Germany), a galactose microparticle/air microbubble suspension that is not FDA-approved in the United States [33]. Fenzl [34] showed in a prospective randomized trial that using contrast media at body temperature causes less pain. To better characterize the fallopian tubes, Exacoustos et al. have combined 3D imaging with the HyCoSy procedure [35].

Limitations

Visualization of scintillations in the fallopian tubes may be more difficult in obese patients with a BMI greater than 35 kg/m^2, as well as in case of distorted pelvic anatomy secondary to large uterine leiomyomata or adnexal masses [36]. Potential causes for false interpretation of HyCoSy findings include (1) missing distal occlusion when echogenic flow is seen in tube but not over the adjacent ovary, (2) presence of a tubal fistula, and (3) false occluded findings secondary to tubal spasm [26].

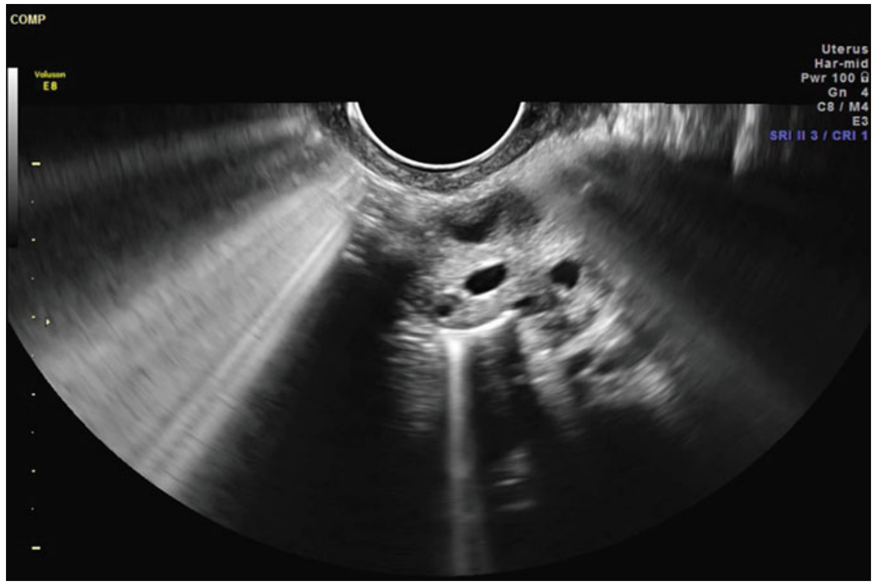

Fig. 6.7 Echogenic contrast identified in the proximal and distal portion of the right fallopian tube. Image provided by Steven Nakajima, MD, University of Louisville, Louisville, KY

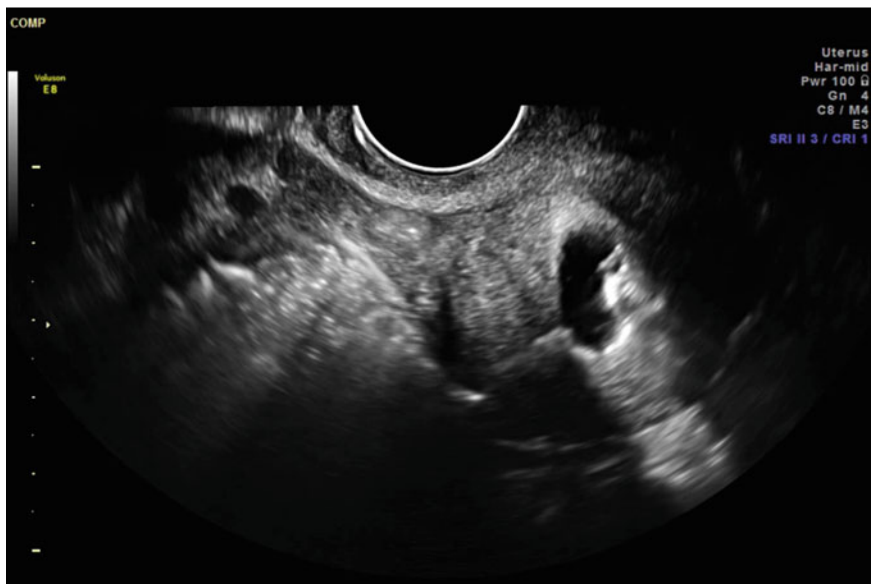

Fig. 6.8 Echogenic contrast identified as scintillations flowing around the left ovary. Image provided by Steven Nakajima, MD, University of Louisville, Louisville, KY

Indications

Indications for HyCoSy are usually fertility-related, since this procedure assesses tubal patency as well as evaluates the uterus. Multiple studies have compared the applicability of HyCoSy in regard to tubal patency with the HSG procedure and laparoscopy with chromopertubation. The concordance rate for these studies was between 72 and 86 % [33, 37], with a 10 % false occlusion rate and 7 % false patency rate of the HyCoSy procedure when compared to laparoscopy. Some studies have evaluated pregnancy rates after laparoscopy, HSG, and HyCoSy and found no statistically significant difference [38, 39], thereby not supporting pregnancy enhancement after HyCoSy. Ayinda et al. [40] found no significant difference between HSG and HyCoSy in regard to frequency or severity of pain at different stages up to 28 days post-procedure and concluded that both tests are equally well tolerated, with HyCoSy avoiding pelvic radiation.

Hysterosalpingogram

Principles

The HSG procedure images the uterine cavity and fallopian tubes by injecting radiographic contrast media through the cervix and taking radiographs of the pelvis (Fig. 6.9). The HSG has a high sensitivity but low specificity for the

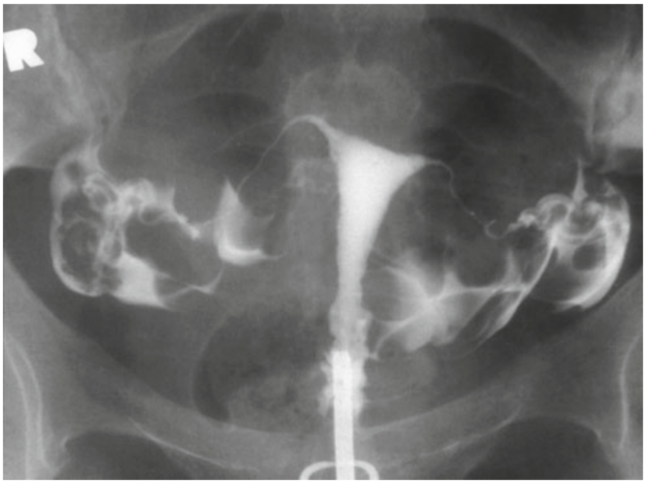

Fig. 6.9 Features of a technically adequate HSG study. The speculum is not obscuring anything. The tenaculum on the cervix has straightened the uterus such that it is perpendicular to the X-ray beam. The tip of the cannula remains below the internal os. Free spill is seen outlining loops of bowel. Reproduced with permission from Goldberg JM. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 29.1 from 1st ed)

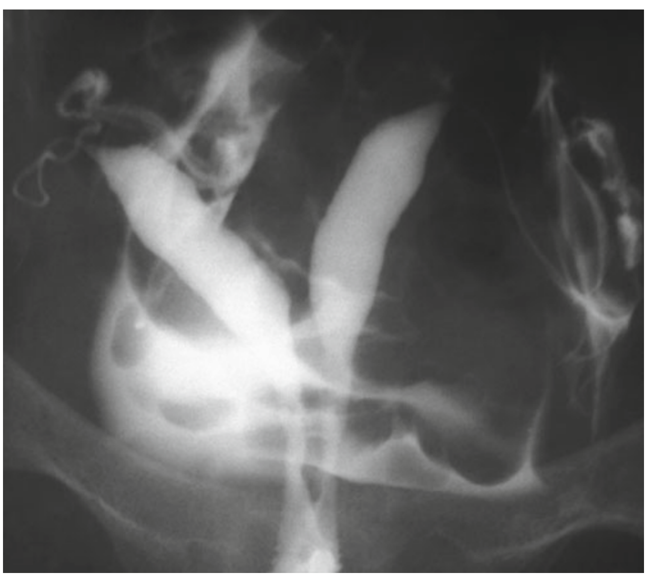

Fig. 6.10 A uterine septum cannot be differentiated from bicornuate uterus by HSG. Reproduced with permission from Goldberg JM. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 29.3 from 1st ed)

diagnosis of uterine cavity abnormalities and is therefore a good screening test [41]. One limitation of the HSG procedure is the differentiation between a septate and bicornuate uterus, since the external fundal contour is not imaged (Fig. 6.10). This obstacle may be overcome by using the uterine push-pull technique to visualize the fundal contour of the uterus [42]. After tubal patency is verified, the uterus is mobilized by movement of the attached tenaculum, dispersing the contrast over the fundus of the uterus and thereby imaging the external fundal contour.

Technical Considerations

The HSG should preferably be performed by a clinician with good knowledge of the female pelvic anatomy. It should be performed during the early follicular phase (cycle days 5–9) to minimize artifacts from blood clots, luteinized endometrium, and to minimize the risk of an occult early pregnancy. A urine pregnancy test can be performed prior to the procedure. Patients usually experience uterine cramping during the HSG, which can be improved by taking a nonsteroidal anti-inflammatory drug (NSAID) 30 minutes prior to the procedure.

Water-soluble media such as iothalamate meglumine 30 or 60 % usually result in better imaging quality. These media don't obscure fine details and they dissipate quickly, making delayed films (1–24 hours later) unnecessary. An oil-soluble media such as Ethiodol can be associated with oil embolism and granuloma formation. However, use of this contrast media may increase post-procedure pregnancy rates [41, 43].

Either a rigid metal cannula or a standard 5- or 7-Fr double-lumen intrauterine HSG catheter can be used. Rigid metal cannulas are inexpensive and reusable and may allow better manipulation of the uterus. Intrauterine HSG catheters are disposable, and the balloon may obscure the lower uterine segment. It is therefore important to only insufflate the balloon if needed and after images of the uterine cavity and lower uterine segment have been obtained.

After informed consent, an open-sided speculum is placed in the vagina and the cervix is cleansed with an antiseptic. Local anesthetic may be used in the form of a gel or spray, and a tenaculum is gently placed on the cervix for uterine traction. It is important to flush the catheter prior to placement in the uterus in order to avoid introducing air bubbles, which could be mistaken for uterine pathology. If cervical stenosis is present, dilation or administration of 400 μg of misoprostol 12 hours prior to the procedure may be necessary. The HSG procedure is combined with fluoroscopy to ensure correct positioning of the pelvic structures prior to the injection of the contrast. If contrast returns from the cervix, the balloon should be inflated to create a seal. Proximal tubal obstruction is usually caused by tubal spasm, and in 60 % of these cases a repeat HSG was normal [44]. If the patient has a history of pelvic infection or if hydrosalpinges are noted (Fig. 6.11), antibiotic coverage in the form of doxycycline 100 mg orally twice a day for 5 days is recommended [21].

Contraindications for performing an HSG are as follows: (1) active pelvic infection, (2) iodine allergy, (3) active uterine bleeding, (4) known or suspected endometrial cancer (for fear of disseminating tumor cells), or (5) pregnancy. If a

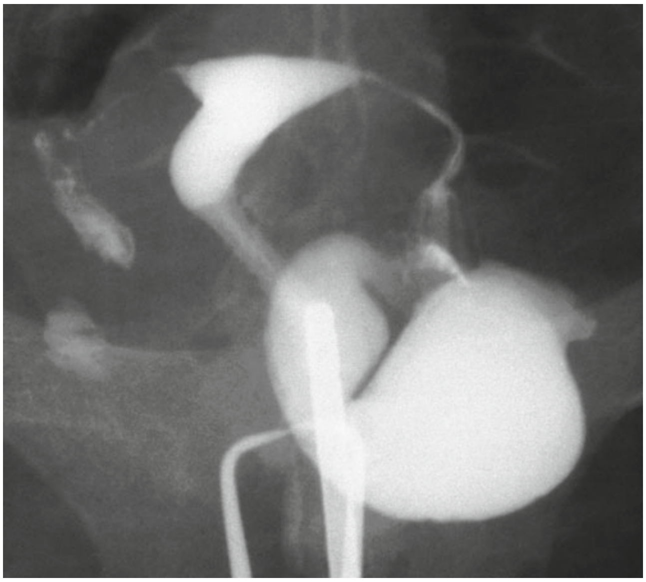

Fig. 6.11 A large left hydrosalpinx. Reproduced with permission from Goldberg JM. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 29.7 from 1st ed)

patient has a known iodine allergy, using a nonionic agent such as Hexabrix (ioxaglate), Isovue 370 (iopamidol), or Omnipaque (iohexol) may decrease the chance of a reaction. Premedication with prednisone, 50 mg orally 13, 7, and 1 hours prior to contrast exposure, and diphenhydramine, 50 mg orally 1 hours prior to exposure, may also be considered [45]. There have been reports of the successful use of gadolinium-based contrast (Magnevist) for HSG in patients who cannot be exposed to iodinated contrast [46]. Some misperceptions exist regarding the history of an "iodine allergy," which is not the same as an allergy to iodinated contrast media. "Iodine allergy" may refer to allergy to topical iodine or to shellfish, and these allergies are not the same as being allergic to iodinated contrast and do not necessarily mean that a patient will react to contrast. The ACR 2011 HSG practice guideline [47] states that a known prior allergic or idiosyncratic reaction to iodinated contrast is a relative contraindication to the procedure and may require premedication as described above [45]. "Non-ionic" contrast does not mean that it is not ionidated. "Non-ionic" contrast has a lower osmolality than ionic contrast, and the risk of an allergic reaction is lower with nonionic agents.

Risks of an HSG include a vasovagal reaction, pelvic infection, radiation exposure, or possibly uterine perforation. Granuloma formation and oil embolism may occur if oil-soluble contrast material is used. A vasovagal reaction with light-headedness, pallor, sweating, bradycardia, and hypotension occurs in less than 5 % of patients [48] and usually resolves while the patient stays in the supine position. A study by Pittaway et al. reported that 1.4 % of patients who had an HSG performed developed pelvic inflammatory disease. All of these patients had tubal dilation [49]. Another study by Stumpf et al. [50] found five risk factors for developing a post-HSG infection, which included (1) history of infertility, (2) previous pelvic surgery for an infection, (3) previous pelvic inflammatory infection, (4) adnexal tenderness at the time of the procedure, and (5) adnexal mass. They suggested that high-risk patients based on these criteria should avoid an HSG and proceed instead with a laparoscopy.

The radiation dose during an HSG depends on a number of factors, including (1) the patient's size, (2) position of the ovaries, (3) the technical equipment used, (4) the distance between the ovaries and the fluoroscopy unit, (5) the duration of fluoroscopy, (6) the number of images acquired, and (7) the degree of image magnification [51]. Only the minimum number of images should be acquired. The average gonadal radiation dose during one completed HSG procedure is estimated to be at most 5 mGy [52] and is considered to be within the margins of safety [53]. Another study by Perisinakis et al. [54] quoted the risk for embryologic anomaly in future pregnancy as 27×10^{-6} and the risk for a fatal cancer as 145×10^{-6}.

Granulomas can occur as a foreign-body reaction in the uterus or tubes in response to oil-soluble contrast and may persist for years. The effects of granulomas on future fertility are unknown [51]. Granulomas rarely form in the normal tube. Therefore, water-soluble media should be used in patients at risk for distal obstruction. An oil embolism can form when oil-soluble contrast intravasates into myometrial veins and lymphatics and is then transported to the lungs via the uterine and ovarian veins [55]. Risk factors for intravasation are tubal obstruction, excessive injection pressure, recent uterine surgery, misplaced cannula, or uterine malformations [51]. If the patient complains of chest pain, cough, light-headedness, or headache, the procedure should be terminated and the patient assessed for contrast intravasation. Overall, there seems to be a provider-directed movement to use predominantly water-soluble contrast media. In February 2010, the manufacture of the oil-based contrast Ethiodol was discontinued, and access to this contrast media is currently limited.

Limitations

Tubal spasm, as mentioned above, can give the false impression of tubal obstruction.

Indications

The HSG procedure is almost exclusively obtained as a diagnostic test of tubal patency in the setting of infertility. Females at risk for tubal obstruction are those with a history of pelvic infection, pelvic surgery, or endometriosis.

Magnetic Resonance Imaging

Principles

The addition of saline infusion hysterosonography and 3D imaging has improved the precision of gynecologic ultrasonography, and it remains the mainstay of reproductive imaging. There are instances, however, in which ultrasound is inconclusive or equivocal. MRI can be used in those situations where the clinical diagnosis is in question and treatment would be affected. MRI technology applies external magnetic fields to align the inherent small magnetic fields of water protons. Radiofrequency electromagnetic pulses are then applied to temporarily alter this alignment. Radiofrequency energy is emitted by the protons as they resume their previous state of alignment. Different types of tissue will recover their original alignment with the external magnetic field at different rates with different time constants. The most common types of images to be encountered in a clinical pelvic MRI are T1- and T2-weighted images. On T1-weighted images, water is darker (low signal intensity) than fat, which appears as bright white (high signal intensity). On T2-weighted images, both fat and fluid are typically of high signal intensity. Some T2-weighted sequences are performed with fat-suppression techniques that cause the fat to be of low signal, thereby accentuating the remaining bright fluid signal [56]. On T1-weighted images, fluid that is of high signal intensity is likely to be hemorrhagic or proteinaceous [57]. MRI has the advantages of multiplanar capabilities, less limitation by uterine and/or patient size, less operator dependence relative to sonography, and no exposure to ionizing radiation.

Technical Considerations

Pelvic MRI is performed per standard radiology protocols. Patients should fast for 4–6 hours to minimize bowel activity and asked to void prior to the procedure. When tubal patency is in question, a new procedure, MR-HSG, has been developed that combines the injection of 10–15 mL of 1:10 gadolinium and normal saline into the uterine cavity while T1-weighted MR images are obtained [58].

Limitations

Patients with pacemakers and other medical or surgical implants should not undergo an MRI procedure. Claustrophobia and patient discomfort are additional concerns that may limit its use. A pregnant patient should not be subjected to MRI imaging unless absolutely necessary and specifically not in the first trimester. Nonetheless, no MRI-associated fetal malformations have been noted to date [59]. Gadolinium contrast use in pregnancy should be avoided since it crosses the placenta and fetal effects are unknown. Gadolinium use is contraindicated in patients with known hypersensitivity [60]. Caution should be exercised if gadolinium is used in women with severe renal failure (stage IV or V) due to the increased risk of subsequent nephrogenic systemic fibrosis [61].

Indications

The MRI procedure is an excellent modality for imaging the pelvic organs when ultrasonography is not adequate or definitive. It is especially good for characterizing congenital (Müllerian) and acquired anomalies of the reproductive tract. Congenital anomalies include uterine agenesis and arcuate, unicornuate, septate, or bicornuate uterus. The most widely accepted classification of Müllerian anomalies was set forth in 1988 by the American Fertility Society (now American Society for Reproductive Medicine), although subtle variations have been found in clinical practice [62]. In a meta-analysis by Chan et al. the reported prevalence of congenital uterine anomalies is about 5.5 % in an unselected population, but in those with recurrent pregnancy loss the rate rises to approximately 13.3 %. An arcuate uterus, which is of no clinical significance, is found in 3.9 % of all women, whereas a septate uterus is found in 2.3 % of an unselected population. In contrast, a septate uterus is present in 5.3 % and 15.4 % of women with recurrent pregnancy loss alone and combined with infertility, respectively. Unification defects, including unicornuate, bicornuate, and uterine didelphys, are found in 0.4, 0.1, and 0.3 % of the unselected population, respectively, and are only mildly elevated in the infertile woman [63].

The MRI procedure can definitely distinguish between a septate (Fig. 6.12) and bicornuate uterus (Fig. 6.13). Numerous studies have examined the sensitivity and specificity of MRI and ultrasound as compared to surgical findings in patients with suspected Müllerian anomalies. In a 1992 study of 24 adult women with uterine anomalies, transvaginal ultrasound had a diagnostic accuracy of 92 %, whereas the accuracy of MRI was 100 % [64]. In the pediatric and adolescent population reported by Santos et al., MRI findings were consistent with surgical findings in 90.9 % of cases, whereas transabdominal ultrasound diagnosis was correct in only 59.1 % [65].

With regard to acquired anomalies, MRI can be helpful in the diagnosis of adenomyosis and in clarifying the exact size, location, and number of leiomyomas (Fig. 6.14).

Multiple prospective studies have examined the diagnostic accuracy of both transvaginal ultrasound and pelvic MRI for adenomyosis (Fig. 6.15), with most showing a nonsignificant

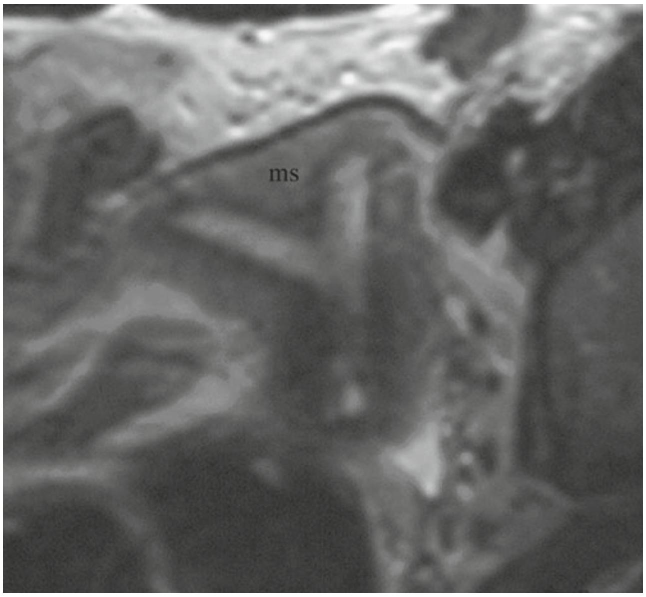

Fig. 6.12 Septate uterus. This is best appreciated on oblique coronal T2-weighted image through the uterine canal demonstrating myometrial septum (ms). Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 31.9 from 1st ed)

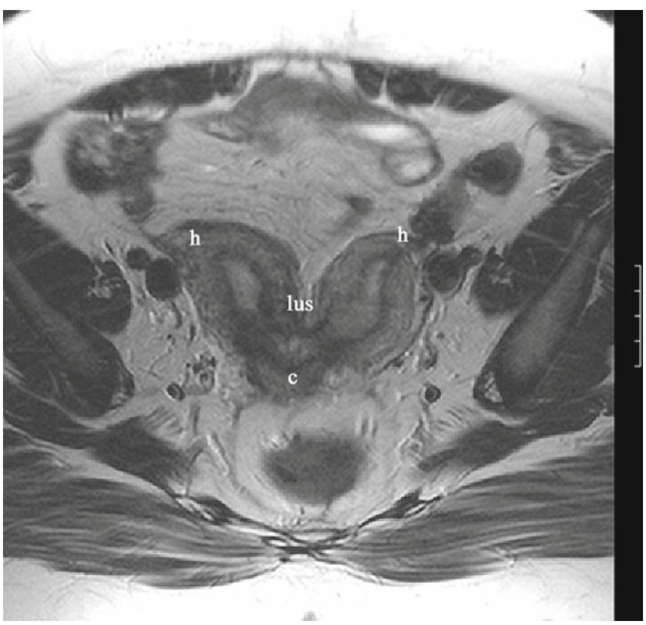

Fig. 6.13 Bicornuate uterus. Coronal image through the plane of the uterus demonstrating the large gap between the two uterine horns (h), which join at the lower uterine segment (lus), and the single cervix (c). Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 31.11 from 1st ed)

difference between the two [66, 67]. Overall, the sensitivity and specificity of MRI are estimated at 88–93 % and 66–91 %, respectively, while those of transvaginal ultrasound are estimated at 53–89 % and 50–98 %, respectively [68]. One historical study by Ascher et al. in 1994 compared transvaginal ultrasound and MRI with regard to accuracy in diagnosis of adenomyosis and found a significant benefit with the usage of MRI; sonographic technological ability, however, has greatly improved in the intervening time period, and the authors did not report sensitivity or specificity for either modality [69]. Some instances in which the MRI procedure would likely be superior include subject obesity, which can severely limit ultrasound capability, and when concomitant leiomyomata and adenomyosis are present [66].

Fig. 6.14 Fibroids, T2-weighted sagittal (a) and axial (b) images of multiple uterine fibroids of low signal relative to the myometrium throughout the uterus in multiple locations, including submucosal (sm), myometrial (m), and exophytic (exo). Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 31.18 from 1st ed)

In our practice, characteristics of uterine fibroids are visualized with an MRI procedure prior to a planned laparoscopic myomectomy so as to plan for a minimum number of uterine incisions, surgical efficiency, and to minimize the chance of neglecting to remove an occult leiomyoma. Some have proposed the usage of MRI to assess ovarian volume and antral follicle counts in obese women with polycystic ovarian syndrome, but the issues of cost and patient inconvenience are definite drawbacks to widespread use of MRI [70].

Endometriomas are best seen on T1-weighted fat-suppression MRI images, with a sensitivity of 90 % and specificity of 98 % [71]. T1 images show a hyperintense ovarian cyst, and endometriomas appear hypo-intense on T2-weighted images. Although laparoscopy remains the gold standard for detection of endometriosis, Ha et al. proposed

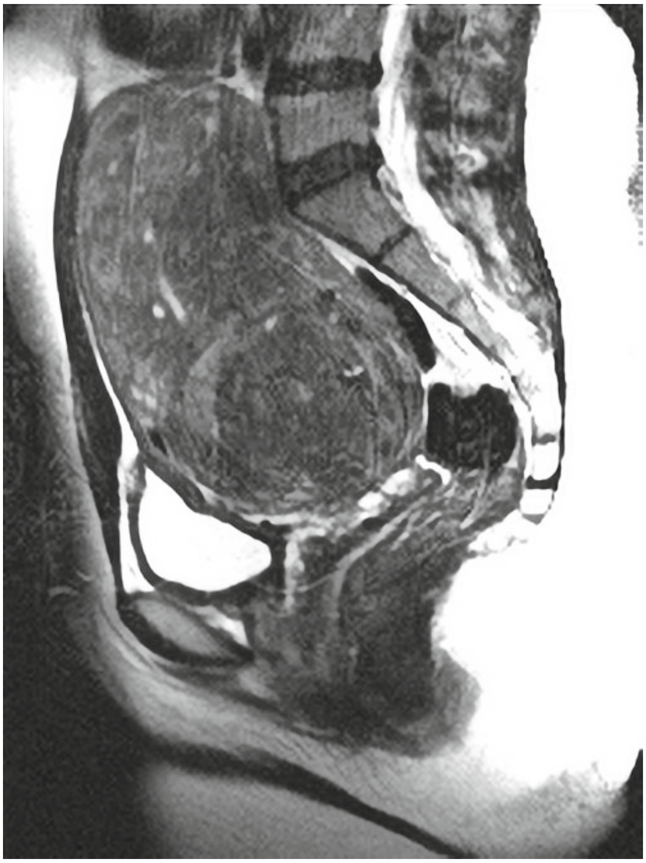

Fig. 6.15 Diffuse adenomyosis. Sagittal T2-weighted image. Reproduced with permission from Magen A, Veniero JC. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007. (Fig. 31.17 from 1st ed, part A only)

that fat-suppression T1-weighted MRI images could be used to noninvasively detect peritoneal implants with an accuracy of 77 % and sensitivity of 61 % [72].

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7 Amenorrhea

Melissa F. Wellons and Robert W. Rebar

Introduction

Amenorrhea refers to the absence of menstrual periods. It is classified as primary, if menstrual bleeding has never occurred by age 15 in the absence of hormonal treatment, or secondary, if menstrual periods are absent for >3 months in a woman with previous normal menstrual periods. Primary amenorrhea can also be diagnosed if a patient presents with no menses by age 13 along with a lack of development of secondary sex characteristics.

The diagnosis of secondary amenorrhea is generally applied after menses have been absent for a length of time equivalent to at least three of the previous menstrual cycle intervals or for 6 months.

Primary ovarian insufficiency (POI), which is also called premature ovarian failure (POF), refers to a loss of normal function of the ovaries before the age of 40. This condition is often but not always associated with depletion of ovarian follicles, as is seen in menopause. POI should be suspected in patients younger than 40 who present with either amenorrhea or signs of hypoestrogenemia and menstrual irregularity. Women with this condition may experience normal puberty and cyclic menses followed by oligomenorrhea and amenorrhea. POI is found in 10–28 % of women presenting with primary amenorrhea and 4–18 % of women presenting with secondary amenorrhea [1]. POI can result from several different disorders. It is important to note that in POI, residual oocytes may still exist [1]. This is why some researchers prefer the term "ovarian insufficiency" over "ovarian failure." When present, residual follicles usually exhibit episodic function, as opposed to the virtually inert oocyte-granulosa units seen in age-appropriate menopause [2]. As many as 20 % of women with POI will exhibit sporadic ovulatory cycles [3, 4]. Pregnancies have been reported in up to 8 % of the patients [3].

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Etiology

Primary Amenorrhea

There are multiple causes of primary amenorrhea (Table 7.1). While it is true that virtually any disorder that leads to secondary amenorrhea may also cause primary amenorrhea, certain disorders more commonly present as primary amenorrhea. The four most common etiologies of primary amenorrhea have been reported to be gonadal dysgenesis, Müllerian agenesis, hypothalamic disorders, and constitutional delay of puberty [5]. Less common causes include androgen insensitivity syndrome, inborn defects in gonadotropin secretion or response, and outflow obstructions of the genital tract, such as imperforate hymen and transverse vaginal septum.

Gonadal Dysgenesis

The term "gonadal dysgenesis" refers to all forms of abnormal gonads. This condition can occur in individuals with normal karyotypes as well as in a variety of abnormal or mosaic states. Gonadal dysgenesis accounts for almost half of all cases of primary amenorrhea. Gonadal dysgenesis with the stigmata of Turner syndrome is the most common, but Swyer syndrome is also associated with gonadal dysgenesis.

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Table 7.1 Classification of amenorrhea, both primary and secondary, and primary ovarian insufficiency[a]

I. Anatomic defects (outflow tract)
  A. Müllerian agenesis (Mayer-Rokitansky-Kuster-Hauser syndrome)
  B. Complete androgen resistance (testicular feminization)
  C. Intrauterine synechiae (Asherman syndrome)
  D. Imperforate hymen
  E. Transverse vaginal septum
  F. Cervical agenesis - isolated
  G. Cervical stenosis - iatrogenic
  H. Vaginal agenesis - isolated
  I. Endometrial hypoplasia or aplasia–congenital

II. Primary hypogonadism
  A. Gonadal dysgenesis
    1. Abnormal karyotype
      a. Turner syndrome 45,X
      b. Mosaicism
    2. Normal karyotype
      a. Pure gonadal dysgenesis
        i. 46,XX
        ii. 46,XY (Swyer syndrome)
  B. Gonadal agenesis
  C. Enzymatic deficiency
    1. 17α (alpha)-hydroxylase deficiency
    2. 17,20-lyase deficiency
    3. Aromatase deficiency
  D. Primary ovarian insufficiency (see also Table 7.2)
    1. X Chromosomal causes
    2. Mutations associated with a 46,XY karyotype
    3. Autosomal causes
    4. Environmental insults
    5. Immune disturbances
    6. Idiopathic causes

III. Hypothalamic causes
  A. Dysfunctional
    1. Stress
    2. Exercise
    3. Nutrition-related
      a. Weight loss, diet, malnutrition
      b. Eating disorders (anorexia nervosa, bulimia)
    4. Pseudocyesis
  B. Other disorders
    1. Isolated gonadotropin deficiency
      a. Kallmann syndrome
      b. Idiopathic hypogonadotropic hypogonadism
    2. Infection
      a. Tuberculosis
      b. Syphilis
      c. Encephalitis/meningitis
      d. Sarcoidosis

(continued)

Table 7.1 (continued)

    3. Chronic debilitating disease
    4. Tumors
      a. Craniopharyngioma
      b. Germinoma
      c. Hamartoma
      d. Langerhans cell histiocytosis
      e. Teratoma
      f. Endodermal sinus tumor
      g. Metastatic carcinoma

IV. Pituitary causes
  A. Prolactinomas
  B. Other hormone-secreting pituitary tumors (corticotropin, thyrotropin-stimulating hormone, growth hormone, gonadotropin)
    1. Mutations of FSH receptor
    2. Mutations of LH receptor
    3. Fragile X syndrome
  C. Autoimmune disease
  D. Galactosemia

V. Other endocrine gland disorders
  A. Adrenal disease
    1. Adult-onset adrenal hyperplasia
    2. Cushing syndrome
  B. Thyroid disease
    1. Hypothyroidism
    2. Hyperthyroidism
  C. Ovarian tumors
    1. Granulosa-theca cell tumors
    2. Brenner tumors
    3. Cystic teratomas
    4. Mucinous/serous cystadenomas
    5. Krukenberg tumors
    6. Nonfunctional tumors
    7. Metastatic carcinoma
  D. Space-occupying lesions
    1. Empty sella
    2. Arterial aneurysm
  E. Necrosis
    1. Sheehan syndrome
    2. Panhypopituitarism
  F. Inflammatory/infiltrative
    1. Sarcoidosis
    2. Hemochromatosis
    3. Lymphocytic hypophysitis
  G. Gonadotropin mutations (FSH)

VI. Multifactorial causes
  A. Polycystic ovary syndrome

[a]Adapted with permission from Loret de Mola RJ. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; and [26]

Disorders of the Genital Tract

Disorders of the genital tract encompass abnormalities of the Müllerian system as well as abnormalities of the external genitalia. Genital tract disorders will be found in 15 % of adolescents who present with normal adolescent development and primary amenorrhea. Common disorders of the genital tract include Müllerian agenesis, imperforate hymen, and transverse vaginal septum.

Müllerian Agenesis

Müllerian agenesis (Mayer-Rokitansky-Kuster-Hauser syndrome) is a condition in which all or part of the uterus and vagina are absent in the presence of otherwise normal female sexual characteristics. This condition accounts for approximately 10 % of cases associated with primary amenorrhea [6]. Renal abnormalities, skeletal abnormalities of the spine, and auditory deafness may be associated with Müllerian agenesis.

Outflow Obstructions

Imperforate hymen is the most frequent obstructive female genital tract anomaly, with an estimated frequency of approximately 0.1 %.

Transverse vaginal septum is less common, occurring in fewer than 1 in 20,000 females.

Androgen Insensitivity Syndrome

Although an abnormality of sexual differentiation, androgen insensitivity syndrome is identified in as many as 5 % of all patients presenting with primary amenorrhea [7]. Patients with androgen insensitivity commonly present with distinctive physical characteristics, including a blind vagina, eunuchoid habitus, breasts that have matured only to Tanner stage 3, and small nipples with pale areolae. Pubic and axillary hair is generally scant or absent. There may be fullness in the inguinal area from the presence of testicles there. The diagnosis can be confirmed by determining that serum testosterone levels are within or above the range normally found in males and by the presence of a 46,XY karyotype. The gonads should be removed after the age of sexual maturity to eliminate the possibility of gonadal tumors later in life, and estrogen should be provided exogenously. The disorder is due to the inability of biologically active testosterone to act normally in cells, generally because of the absence of the androgen receptor.

Rare Causes of Primary Amenorrhea

Isolated gonadotropin deficiency is characterized by a decrease or absence of endogenous gonadotropin-releasing hormone (GnRH) secretion, resulting in very low to undetectable levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Individuals with this disorder may have incomplete development of secondary sexual characteristics as well as primary amenorrhea. These characteristics may be accompanied by eunuchoid features and anosmia and, more rarely, color blindness.

LH Receptor Abnormalities

Abnormalities of the LH receptor in 46,XX females result in normal female sexual development and primary amenorrhea [8]. Serum LH may be normal or increased, and FSH will be normal, as will follicular phase estradiol levels. Progesterone will be low. The uterus in patients with LH receptor abnormalities is small, and the ovaries are consistent with anovulation.

Gonadotropin-Releasing Hormone Receptor Abnormalities

Abnormalities of GnRH receptors have also been found. If a patient presents with amenorrhea, cyclic pain, or a blind vaginal pouch, this possibility should be investigated.

Diagnosing Primary Amenorrhea

When diagnosing amenorrhea, a careful patient history is key. Physical and emotional stress, nutritional status, and genetic history should be explored. The clinician should gather information about the patient's family and disorders such as diabetes mellitus, as well as information about any previous medical disorders that may have been treated with gonadotoxic agents. The functional enquiry should examine secondary sexual development and the presence of hyperandrogenic symptoms; one should also look for evidence of galactorrhea. When galactorrhea is present, the examiner should note whether it is unilateral or bilateral, constant or intermittent. Galactorrhea of hormonal origin comes from multiple duct openings on the breast, in contrast to secretions occurring from a single duct, which are usually related to a local problem. That any breast secretion is in fact galactorrhea can be confirmed by examining the discharge under the microscope: galactorrhea presents as perfectly round, thick-walled fat globules of all different sizes. Galactorrhea may be white, yellow, or clear in color.

A detailed physical exam should also include a gynecologic examination as well as measurement of height, arm span, body mass index, pubertal status, signs of hyperandrogenism, skin manifestations of endocrine disorders, and a genital tract evaluation. Sexual development and growth milestones should be documented. The most important single feature in the history and physical exam is the presence or absence of breast development, which is a clear indication of the initiation of puberty. Breast development indicates the presence of biologically active estrogen, and pubic and axillary hair indicates the presence of biologically active androgen.

Gynecologic examination will usually reveal the presence of genital abnormities, including obstructive processes.

Imaging

Physical examination will often detect genital tract anomalies in patients with primary amenorrhea. Abdominal ultrasonography can be used to determine the presence or absence of a uterus. Magnetic resonance imaging (MRI) of the pelvis is probably the most effective imaging method for characterizing congenital anomalies. Patients without secondary sexual development should undergo radiographic determination of bone age. In patients with elevated prolactin, an MRI of the pituitary is indicated.

Laboratory Evaluation

Patients with primary amenorrhea should have an initial set of laboratory tests to determine the next series of diagnostic or therapeutic management steps. Pregnancy should be ruled out in patients with normal secondary sexual development. Levels of serum FSH, TSH, and prolactin should be measured in all patients regardless of sexual development. The estimation of TSH is still indicated to evaluate for subclinical hypothyroidism. Androgens should be evaluated if hirsutism is present or if androgen insensitivity syndrome is suspected. A karyotype is warranted in any young woman with elevated levels of FSH.

Diagnosing Secondary Amenorrhea

In secondary amenorrhea, menstruation begins at the appropriate age, but later stops for reasons other than pregnancy, lactation, or menopause. To arrive at this diagnosis, the length of amenorrhea should be equal to at least three of the previous cycle intervals, or 6 months, although patients with oligomenorrhea (menstruation <9 times per year or bleeding intervals >40 days) often have similar underlying pathology. Secondary amenorrhea affects at least 4 % of women and is more common in those whose weight is below or above the normal range. The four most common causes of secondary amenorrhea are hypothalamic amenorrhea, hyperandrogenic states, pituitary disorders, and POI.

Hypothalamic Forms of Amenorrhea

Hypothalamic forms of amenorrhea result from diminished GnRH input to the pituitary gland and commonly occur in women stressed mentally, emotionally, or physically as well as in those who are nutritionally deficient. Often a combination of these stressors is present and results in the anovulation. Menstrual cycle disturbances are common among competitive athletes, especially in those sports that encourage a low body weight. Menstrual irregularities appear to be greatest in ballet dancers (6–43 %) and middle- and long-distance runners (24–26 %). Hypothalamic amenorrhea is also common in women who have experienced a profound stress, such as rape, incest, or loss of someone particularly close. Severe eating disorders such as bulimia and anorexia nervosa can disrupt menstrual function in a similar manner.

Evaluation of Hypothalamic Amenorrhea

A detailed interview may identify stressors or emotional crises preceding the amenorrhea. Environmental factors such as job-related stress may also be present. The clinician should perform a careful review of the patient's lifestyle, including diet, exercise, and drug use [9].

Patients with hypothalamic amenorrhea typically have a history of normal menarche and regular menstruation, but several cases of primary amenorrhea have been reported. The physical examination should focus on identifying galactorrhea, thyroid dysfunction, and evidence of hyperandrogenemia such as acne and hirsutism. Oral examination may reveal the distinctive eroded "moth-eaten" dentition and enlarged salivary glands of a bulimic patient. The results of the pelvic examination should be normal except for a thinned vaginal mucosa or absent cervical mucus, which are characteristics of hypoestrogenism.

Laboratory evaluation should include measurement of serum FSH, prolactin, and TSH. FSH can be either low or normal. Estradiol levels will be low or within the lower limits of normal if measured. Most other pituitary hormones will be in the normal range. It should be noted that in many patients, the progestin challenge test (medroxyprogesterone acetate, 5–10 mg for 7 days) will not result in uterine bleeding, but there is very little reason to use this test in these hypoestrogenic women. When FSH levels are very low, it is important to rule out a central lesion.

The simplest treatment often includes counseling as well as estrogen replacement. Oral contraceptives can be used, but affected women should be told that they may be amenorrheic when the estrogen is discontinued, and infertility may be an issue. Estrogen is warranted in these women because they are at increased risk of accelerated bone loss. Because ovulation may precede the first menses, these women sometimes become pregnant unexpectedly, if contraception is not utilized in those who are sexually active.

Post-contraception Amenorrhea

Modern low-dose oral contraceptives do not affect fertility long term. However, as noted, women with amenorrhea prior to the administration of oral contraceptives may still be amenorrheic when the contraceptives are discontinued. A more common problem is amenorrhea after the intramuscular use of medroxyprogesterone acetate (e.g., Depo-Provera). Return to baseline fertility takes an average of 10 months after cessation of the drug [10].

Hyperandrogenic States

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is one of the most common causes of ovulation dysfunction. As originally described, large, pale polycystic ovaries with thickened capsules were found in women with amenorrhea (usually secondary), hirsutism, and "sterility" [11]. Over time it was recognized that PCOS was heterogeneous, with a wide clinical spectrum that included hirsutism, infertility, amenorrhea, obesity, and irregular menses. Defined early as "LH-dependent ovarian hyperandrogenism" commonly beginning around puberty, the definition was broadened by the Rotterdam Consensus Conference in 2004 to include the presence of two of the three following features after exclusion of other etiologies: (1) hyperandrogenism (clinical and/or biochemical), (2) oligo- and/or anovulation, and (3) polycystic ovaries. Utilizing this definition it is safe to say that PCOS is the most common endocrine disorder of women, affecting as many as 20–22 % of women of reproductive age. This last (and now most frequently used) definition has resulted in inclusion of many more women into the "PCOS spectrum" and may well contribute to confusion about the pathophysiology.

Common laboratory features include elevated levels of LH (compared to those found in the normal follicular phase) in many women, elevated levels of testosterone and virtually any other ovarian androgen measured, and decreased levels of sex hormone-binding globulin [12]. More recently, it has become recognized that insulin resistance and lipid and lipoprotein abnormalities are common in PCOS as well [13]. These laboratory abnormalities may put women with PCOS at higher risk of cardiovascular disease and metabolic abnormalities that shorten lifespan, but this remains to be established [14].

Management of PCOS is directed at treating the primary complaint, whether it is hirsutism, infertility, or glucose intolerance. Obese individuals should be encouraged to lose weight because signs and symptoms are not as severe in normal-weight women as they are in those who are overweight. It is known that adverse pregnancy outcomes, including pregnancy-induced hypertension and preeclampsia, gestational diabetes, preterm birth, and perinatal mortality, are increased in women with PCOS [15].

Other Hyperandrogenic States

There are other hyperandrogenic states, such as ovarian and adrenal tumors, that mimic PCOS and that can also result in amenorrhea. Similarly, Cushing syndrome can cause amenorrhea secondary to increased androgens. Another cause is adult-onset congenital adrenal hyperplasia. The ovary apparently can respond to increased androgens in only limited ways, and polycystic ovaries are invariably present in all of these disorders. To rule out these conditions, the patient's serum androgens should be measured, including total testosterone, dihydroepiandosterone sulfate (DHEAS), and 17-hydroxyprogesterone. Imaging of the ovaries and of the adrenal glands may be indicated in some cases.

Disorders of the Anterior Pituitary

Pituitary tumors often interfere with normal reproduction. Small pituitary tumors often present as irregular or absent menses or galactorrhea. Large pituitary tumors may manifest as headaches and compression of the optic chiasm and bitemporal hemianopsia related to their growth in a confined anatomical space.

Prolactin-Secreting Adenomas

Prolactin-secreting adenomas are the most common pituitary tumors. Hyperprolactinemia is associated with decreased estradiol concentrations as well as amenorrhea or oligomenorrhea. In patients presenting with hyperprolactinemia, the prevalence of a pituitary tumor is 50–60 % and the likelihood of a pituitary tumor is unrelated to the level of prolactin [16]. Whenever serum prolactin levels are consistently elevated, MRI or CT scanning of the pituitary should be performed [17].

The so-called "non-functioning" pituitary tumors commonly secrete the alpha subunit common to LH, FSH, and TSH and may present only with amenorrhea.

Postpartum Pituitary Necrosis (Sheehan's Syndrome)

Postpartum pituitary necrosis is a life-threatening condition usually preceded by a history of severe obstetrical hemorrhage with hypotension, circulatory collapse, and shock [18].

Pituitary Apoplexy

Pituitary apoplexy is a serious condition characterized by an acute infarction of the pituitary gland. Patients will experience a sudden onset of a severe retro-orbital headache and visual disturbances that may be accompanied by lethargy or a loss of consciousness.

Evaluation of Pituitary Adenomas

Pituitary adenomas can disrupt menses in women. Prolactin-secreting adenomas can also cause galactorrhea, headaches, and infertility. About one-third of women with amenorrhea will have elevated prolactin levels; one-third of women with galactorrhea and elevated prolactin levels will have normal menstrual cycles, and one-third of women will have high prolactin levels without galactorrhea [19]. As many as one-third of patients with secondary amenorrhea will have a prolactinoma, and when associated with galactorrhea, half of the patients will have normal findings on imaging of the sella turcica [19, 20].

Cushing Disease

Amenorrhea and galactorrhea are usually encountered with pituitary prolactinomas, but these symptoms may also precede adrenocorticotropic hormone (ACTH) or growth hormone-secreting tumors. If the patient presents with clinical symptoms of excessive glucocorticoid, suggesting Cushing disease, a corticotropin serum level test and a 24 h urine collection for free cortisol may be indicated.

Gonadal Dysgenesis

Very rarely, patients with gonadal dysgenesis may experience normal puberty and present with secondary amenorrhea, almost always before the age of 30.

Postirradiation Hypopituitarism

Exposure to therapeutic radiation sources for treatment of midline central nervous system tumors can place patients at increased risk for delayed development of hypopituitarism.

Diagnosing Primary Ovarian Insufficiency

Although the absence of ovarian function before the age of 40 can be the result of a physiologic process at an unusually young age, it is often due to a broad range of underlying etiologies (Table 7.2). Of all women, 10 % may become menopausal by age 45, and these women may have experienced an accelerated decline of fertility before age 32. These patients may be considered to have "early ovarian aging" in spite of having menstrual function. Regardless of etiology, the clinical results are decreased fertility and hypoestrogenemia. In most cases, the etiology of POI will not be clear. However, genetic errors such as single gene defects, abnormalities in the sex chromosomes (such as fragile X syndrome), and other poorly defined genetic diseases have been associated with POI. POI is a common cause of secondary amenorrhea, accounting for 4–18 % of cases. It is likely that many cases of POI result from genetic mutations [21], as new genetic causes of POI are being identified each year.

At present the etiology for the majority of patients with POI is unknown. However, for patients <30 years of age presenting with amenorrhea, a karyotype should be obtained to rule out chromosome abnormalities, since these conditions may be associated with an increased risk of ovarian malignancies.

Screening for Primary Ovarian Insufficiency

As in all cases of amenorrhea, the initial screening is with a serum thyroid-stimulating hormone (TSH), prolactin, and FSH. Diagnosis of POI is made in the presence of elevated serum FSH, normal serum prolactin, and normal serum TSH. Estradiol will also be decreased; thus, any progesterone challenge will not result in withdrawal bleeding. Because about half of women with POI do withdraw to a progestin, there is no point in utilizing this test [3].

Association with Autoimmune Disorders

POI is often associated with autoimmune disease. Up to 40 % of women with POI may have autoimmune abnormalities, the most common of which is autoimmune thyroiditis resulting in hypothyroidism [22, 23]. POI is also more common in women with insulin-dependent diabetes, myasthenia gravis, or parathyroid disease [24]. In 10–60 % of cases, Addison disease patients also may have autoimmune ovarian insufficiency. These higher-risk patients should be evaluated every other year for these abnormalities. In order to exclude other autoimmune disorders, patients with unexplained POI should undergo a complete evaluation such as calcium, phosphorus, fasting glucose, adrenal antibodies

Table 7.2 Causes of primary ovarian insufficiency[a]

I.	X Chromosomal causes
	A. Structural alterations or mutations in or absence of an X chromosome
	1. With the stigmata of Turner syndrome (45,X or mosaic)
	2. Without the stigmata of Turner syndrome
	a. Mutations in premature ovarian failure 1 (Xq26-q28)
	b. Mutations in premature ovarian failure 1 in association with Fragile X premutation (Xq27.3)
	c. Mutations in premature ovarian failure 2A (Xq22)
	d. Mutations in premature ovarian failure 2B (Xq21)
	e. Mutations in premature ovarian failure 4 in association with mutations in bone morphogenetic protein 15 (Xp11.2)
	B. Trisomy X with or without mosaicism
II.	Mutations associated with a 46,XY karyotype
	A. Mutations in Xp22.11-p21.2 (Swyer syndrome)
	B. Mutations in 5 cen
III.	Autosomal causes
	A. Mutations involving enzymes important for reproduction
	1. Galactosemia (galactose-1-phosphate uridyltransferase deficiency) (9p13)
	2. 17 α (alpha)–Hydroxylase deficiency (CPY17A1) (10q24.3)
	B. Mutations involving reproductive hormones, their receptors, and action
	1. Mutations of luteinizing hormone or follicle-stimulating hormone or both rendering them biologically inactive (theoretical)
	2. Mutations of inhibin (theoretical)
	3. Receptor mutations
	a. Follicle-stimulating hormone receptor (2p21-p16)
	b. Luteinizing hormone/human chorionic gonadotropin receptor (2p21)
	4. Mutations in the hormone action pathways
	C. Other mutations
	1. Blepharophimosis, ptosis, and epicanthus inversus, type 1 (BPES) (premature ovarian failure 3) (3q23)
	2. Premature ovarian failure 5 (newborn ovary homeobox) (7q35)
	3. Autoimmune polyendocrine syndrome, type 1(APS1) (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, APECED) (autoimmune regulator gene, AIRE) (21q22.3)
	4. Vanishing white matter leukodystrophy with ovarian failure (genes encoding the translation initiation factor E1F2B) (14q24, Chr 12, 1p34.1, 3q27, 2p23.3)
	5. Congenital disorders of glycosylation, type 1a (CDG1a) (genes encoding phosphomannomutase-2, PMM2) (16p13.3-p13.2)
IV.	Environmental insults
	A. Chemotherapeutic (especially alkylating) agents
	B. Ionizing radiation+
	C. Viral infection (documented for mumps)
	D. Surgical injury or extirpation
V.	Immune disturbances
	A. In association with other autoimmune diseases
	B. Isolated
	C. In association with congenital thymic aplasia
VI.	Idiopathic causes

[a]Reprinted with permission from Rebar RW. Premature ovarian failure. Obstet Gynecol. 2009 Jun;113(6):1355–63

to 21-hydroxylase enzyme, free T4, TSH, and thyroid antibodies [25]. Patients should also be screened for adrenal disease with an evaluation for anti-adrenal antibodies. If these are positive, then more sophisticated testing, such as a corticotrophin stimulation test, is in order (a fasting morning serum cortisol is not sufficiently sensitive).

Other Causes of Primary Ovarian Insufficiency

Although elevated serum FSH levels are virtually synonymous with ovarian disorders, there are less common conditions that can raise FSH but that are associated not with a primary ovarian problem but a central problem. These are pituitary adenomas that secrete FSH, specific enzyme defects such as 17-hydroxylase deficiency (P450c 17), or galactose-1-phosphate uridyl transferase deficiency (galactosemia).

Disorders of the Genital Tract

Intrauterine adhesions (i.e., Asherman syndrome) account for 7 % of cases of secondary amenorrhea. Another infrequent cause is an outflow obstruction secondary to cervical stenosis. This usually results from treatment of cervical dysplasia with modalities such as cryosurgery, electrocautery, or cold knife cone biopsy.

Diagnostic Approach to Secondary Amenorrhea

A careful history can help identify one of a wide variety of conditions that can stop menses. A physical examination will sometimes give clues as to the most likely etiologies. The initial laboratory evaluation is an important step as well, not only to rule out physiologic causes of amenorrhea (such as pregnancy) but also to detect subtle hormonal conditions that often have no other symptoms or physical signs.

History

Menstrual history, sexual activity, and means of contraception are basic to every history for patients with amenorrhea. Irregular menses over a long period of time suggest PCOS, but pregnancy should be ruled out. Several modern means of contraception are prone to iatrogenic amenorrhea.

Young women are at greater risk for POI related to abnormal karyotype. They are also at greater risk due to diet and exercise habits.

The history should note subtle symptoms of endocrinologic or systemic disorders, such as vaginal dryness and increased hair growth. Systemic diseases often have associated symptoms, such as the weight gain associated with both hypothyroidism and Cushing syndrome. Acne and increased midline hair are often signs of hyperandrogenic conditions.

Physical Examination

Physical examinations are often critical for discovering the underlying cause of secondary amenorrhea. For example, the clinician should be alert to signs of PCOS. PCOS patients will often be overweight with increased hair on the upper lip, chin, chest, and inner thighs. In particularly severe cases acanthosis nigricans may be present. Ovarian and adrenal tumors can produce sudden and dramatic hirsuitism. Short stature and Turner syndrome can suggest POI with a genetic basis.

Galactorrhea suggests hyperprolactinemia, although only one-third of women with elevated prolactin will have this finding. Cushing syndrome is often associated with central obesity, "moon facies," abdominal striae, and "buffalo hump," as well as hypertension and insulin resistance.

Gynecologic examination is occasionally illuminative in women with secondary amenorrhea. Speculum examination can sometimes reveal significant stenosis of the external cervix or vaginal atrophy associated with hypoestrogenemia. The bilaterally enlarged ovaries seen in PCOS can be detected through bimanual examination.

Laboratory Evaluation

All patients presenting with secondary amenorrhea should have an initial set of laboratory studies, including a pregnancy test, TSH, prolactin, and FSH. Elevated levels of TSH or prolactin are indications that one should evaluate the patient further for hypothyrodism or pituitary adenoma, respectively.

Progestin Challenge Test

The progestin challenge test is a traditional step in the evaluation of secondary amenorrhea. It is rarely, if ever, necessary in the contemporary evaluation of the patient with amenorrhea.

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8 Polycystic Ovary Syndrome

Erika B. Johnston-MacAnanny, John Kyun Park, and Sarah L. Berga

Introduction

Polycystic ovary syndrome (PCOS) refers to a complex, multidimensional disorder resulting in defects in reproduction and metabolism. Although one of the primary characteristic of PCOS is the presence of multiple cysts on the ovaries, the complexity of the syndrome is reflected in the wide range of its clinical manifestations, most notably insulin resistance, obesity, irregular menses, and signs of hyperandrogenism, such as hirsutism and acne.

Diagnostic Criteria

In 1990, a conference on PCOS sponsored by the National Institutes of Health led to diagnostic criteria based on a majority opinion of conference participants [1]. The criteria included hyperandrogenism and/or hyperandrogenemia, chronic anovulation, and exclusion of other known disorders. In 2003, a PCOS consensus workshop in Rotterdam sponsored by the European Society of Human Reproduction and Embryology (ESHRE)/American Society of Reproductive Medicine (ASRM) revised the diagnostic criteria for PCOS [2]. The revised criteria state that PCOS remains a diagnosis of exclusion, but that two out of the following three criteria must be present: (1) oligo- or anovulation, (2) hyperandrogenism and/or hyperandrogenemia, and (3) polycystic ovaries. A polycystic ovary is defined as having 12 or more follicles in one ovary measuring 2–9 mm in diameter, and/or increased ovarian volume of greater than 10 mL, which is the maximum size of a normal ovary [3] (Fig. 8.1). Administration of oral contraceptive pills and the presence of follicles >10 mm modify ovarian morphology; thus, the definition of a polycystic ovary does not apply to these clinical scenarios.

The differential diagnosis should include other causes of hyperandrogenism and menstrual dysfunction such as nonclassical congenital adrenal hyperplasia (CAH), hypothalamic amenorrhea, Cushing's syndrome and disease, hyperprolactinemia, thyroid disease, acromegaly, and androgen-secreting neoplasms of the ovary or adrenal gland.

Prevalence

The prevalence of PCOS is estimated to be 4–12 % of reproductive-age women. The largest US study on PCOS prevalence was published in 1998 [4]. Out of 277 women included in the study, 4.0 % had PCOS as defined by the 1990 NIH criteria. The prevalence was 4.7 % for white women and 3.4 % for black women. The inclusion of polycystic ovaries in the 2003 Rotterdam criteria calls for reevaluation of the prevalence of PCOS, as 21–23 % of normal women have polycystic-appearing ovaries on ultrasound [3].

Clinical Presentation

Hyperandrogenism

Clinical manifestations of hyperandrogenemia include hirsutism, acne, and male pattern alopecia. Hirsutism is defined as the growth of coarse, pigmented hairs in androgen-dependent areas such as the face, chest, back, and lower abdomen. Approximately 80 % of hirsute patients will have PCOS [5]. The modified Ferriman-Gallwey scoring system can be used

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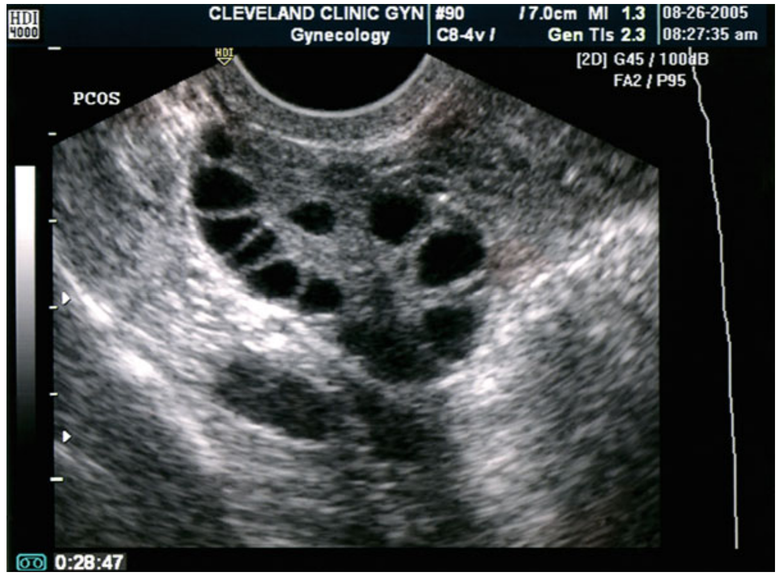

Fig. 8.1 This is the classical image of PCOS with an enlarged ovary containing an increased number of small follicles around the periphery of the cortex, resembling a string of pearls, along with a bright echogenic stroma

for clinical assessment of hirsutism. This system, which was originally used in the United Kingdom for a population of presumably Caucasian women, scores hair growth in nine body areas from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth) [6]. Other hyperandrogenic manifestations commonly found in PCOS patients include acne and alopecia [7, 8]. Acne is a result of androgen stimulation of the pilosebaceous unit with increased skin oiliness [7].

Obesity

Obesity is very common in PCOS, with the android pattern present in approximately 44 % of women with PCOS [9]. This central obesity is more characteristic of PCOS, as these patients have an increased waist-to-hip ratio compared to obese women without PCOS [10]. Hyperinsulinemia may stimulate central adiposity, which, in turn, exacerbates underlying or latent insulin resistance [11].

Insulin Resistance, Diabetes, and Acanthosis Nigricans

Insulin resistance and diabetes are important health concerns commonly seen in association with polycystic ovarian syndrome and will be discussed at length later in this chapter. Acanthosis nigricans is a dermatological condition of hyperkeratosis and increased skin pigmentation with raised, symmetrical, darkened, velvety plaques that commonly appear on the nape of the neck. It can also be found in the axilla, groin, and other intertriginous areas of the body. Elevated insulin has a mitogenic effect on basal cells of the epidermis, making acanthosis nigricans a relatively specific clinical marker of insulin resistance [12].

Irregular Menses and Infertility

Some of the menstrual abnormalities seen with chronic anovulation include secondary amenorrhea, oligomenorrhea, and dysfunctional uterine bleeding. Menarche typically begins at a normal or early age, but the menstrual irregularities often seen in adolescents may never resolve for the PCOS patient. The irregular menses may be masked in PCOS patients if they are on oral contraceptives. PCOS is the most common cause of anovulatory infertility, which often serves as the impetus for the patient to seek medical attention. Patients may report false-positive urinary ovulation predictor tests due to chronic elevation in luteinizing hormone (LH).

Miscarriage

The risk of a first-trimester spontaneous abortion is reported to be significantly higher for patients with PCOS. The spontaneous abortion rate in PCOS is reported to be 30 % [13]. In comparison, retrospective studies find the risk of spontaneous abortion to be 5–14 % for normal women [14, 15]. Of patients with recurrent miscarriage, 36–82 % have polycystic ovaries [13, 16, 17].

Several explanations have been offered. For example, Homburg et al. demonstrated that high concentrations of LH during the follicular phase in women with polycystic ovaries have a deleterious effect on rates of conception and are associated with early pregnancy loss [18].

Pathogenesis

Altered Gonadotropin Secretion

One of the well-described features of PCOS is an increase in LH and relative decrease in follicle-stimulating hormone (FSH) [19]. The relative decrease in FSH is the chief cause of anovulation. The pulsatile secretion of LH from the pituitary is increased in amplitude and frequency [20]. In addition, the pituitary has a greater LH response to gonadotropin-releasing hormone (GnRH) compared with normal women [20, 21].

The pulsatile secretion of GnRH cannot be studied in humans, so it must be inferred by detecting peripheral LH patterns. A study of PCOS women by Berga et al. found increased pulse frequency and amplitude for LH and α (alpha)-subunit, providing evidence for aberrant increases in GnRH pulse frequency (Fig. 8.2) [20]. Elevated LH is not caused by altered pituitary sensitivity to GnRH, as GnRH receptor blockade resulted in similar LH decreases in PCOS and normal women [22]. These findings suggest a derangement of the hypothalamic-pituitary axis, which appears to play a major role, because many of the cardinal features of PCOS can be traced to alterations in gonadotropins.

Neuroanatomical Considerations

The GnRH pulse generator refers to the synchronized pulsatile secretion of GnRH from neurons that are widely distributed in the medial basal hypothalamus. Knobil and associates conducted experiments with the Rhesus monkey to establish that the GnRH system exhibits rhythmic electrical behavior in the arcuate nucleus of the medial basal hypothalamus [23]. There was remarkable synchrony between pulses of GnRH in the portal blood and LH pulses in peripheral blood. This phenomenon was later studied in isolated human medial basal hypothalamus, where GnRH pulses were found to occur at a frequency of 60–100 min [24].

The secretion of GnRH into the portal vasculature also appears to be regulated by dynamic remodeling of GnRH neurovascular junctions. Morphological plasticity of the median eminence during the menstrual cycle has been demonstrated, where the maximal number of GnRH neuro-vasculature junctions are found during the LH surge [25].

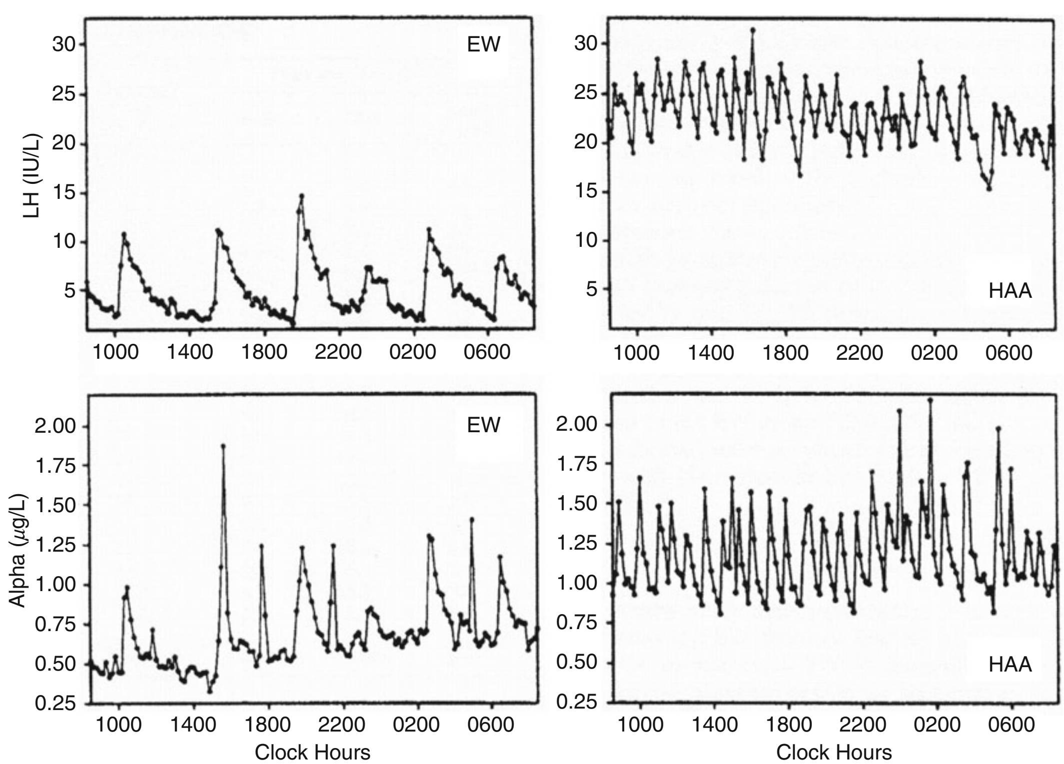

Fig. 8.2 Twenty-four hour concentration profiles of LH (*top*) and α(alpha)-subunit (*bottom*) in an eumenorrheic woman (EW) (*left*), studied in the follicular phase (day 2) and in a woman with hyperandrogenic anovulation (HAA)/PCOS (*right*). Reprinted with permission from Berga S, Guzick D, Winters S. Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. J Clin Endocrinol Metab 1993; 77(4):895–901. Copyright 1993, The Endocrine Society

GnRH Neuroregulation in PCOS

The GnRH pulse generator in PCOS patients is intrinsically faster, and the frequency is less likely to be suppressed with continuous estrogen and progesterone treatment [26].

Increased central adrenergic tone has been implicated as a cause of the aberrations of GnRH and gonadotropin secretion in PCOS. One possible mechanism is the increase in local blood flow and permeability of the portal vascular system, permitting the entry of increased amounts of GnRH [27]. Dopamine injection into the third ventricle led to a rapid increase in GnRH and prolactin inhibitory factor in portal blood, suggesting dopamine-mediated regulation of GnRH and prolactin inhibitory factor [28]. The identification of β(beta)-1-adrenergic and D1-dopaminergic receptors on GT-1 GnRH neurons provides a mechanism by which norepinephrine and dopamine could regulate gonadotropin release via direct synapses on GnRH neurons [29].

The role of insulin-like growth factor 1 (IGF-1) in modulation of GnRH cells has also been investigated. IGF-1 regulates growth, differentiation, survival, and reproductive function. The IGF receptor is a tyrosine kinase receptor located in the periphery and CNS, including the median eminence [30]. In PCOS women, an increased ratio of IGF-1 to their binding proteins correlated significantly with increased concentrations of circulating LH [21]. These findings suggest that IGF-1 can modulate GnRH neurons by inducing gene expression, resulting in more circulating LH.

Hyperandrogenemia

Circulating androgens are elevated in PCOS, with contributions from the ovary and adrenal glands. The elevated androgens can only be partially suppressed with combined oral contraceptive (COC) therapy. Daniels and Berga treated PCOS women with 3 weeks of COCs and found that androstenedione levels remained significantly higher compared to treated controls [26]. Pulse frequency of LH was suppressed in both PCOS women and controls, but the frequency remained significantly higher in PCOS patients (Fig. 8.3). This suggests there is reduced sensitivity of the GnRH pulse generator to suppression by sex steroids. The authors also suggest that GnRH drive in PCOS women may be intrinsically and irreversibly faster than in eumenorrheic women.

Theca Cell Function

Ovarian hyperandrogenism is driven by LH acting on theca cells, and the effect is amplified by the increased sensitivity of PCOS theca cells to LH [31]. Hyperandrogenism may also result from dysregulation of the androgen-producing enzyme P450c17, which has 17 α (alpha)-hydroxylase and 17,20-lyase activities. In contrast, in vivo studies do not find significant increases in androgen secretion in women with PCOS or normal women, despite considerable increases in insulin levels. A role for insulin is strongly suggested by the observation that reduction of hyperinsulinemia is associated with decreases of serum androgens. Treatment of PCOS patients with metformin, which reduces hepatic glucose production and secondarily lowers insulin, has been shown to decrease levels of testosterone, DHEAS, and androstenedione [32].

Adrenal Function

Excess adrenal androgen production is seen in PCOS women, with a 48–64 % increase in DHEAS and 11β (beta)-hydroxyandrostenedione. The underlying cause of elevated adrenal androgens is yet to be elucidated, but PCOS women do not have increased adrenocorticotropic hormone (ACTH) levels [33]. Increased adrenal androgen production in PCOS is likely caused by either altered adrenal responsiveness to ACTH or abnormal adrenal stimulation by factors other than ACTH.

Anovulation

The cause of anovulation in PCOS patients has yet to be clarified. However, several observations in granulosa function have been described that may give insight into this process.

Granulosa Cell Function

FSH levels are characteristically low in PCOS women, resulting in arrested follicular development. Insufficient granulosa cell aromatase activity was the basis of earlier studies that tried to explain poor follicular development, as follicular fluid estradiol concentrations were thought to be low. To the contrary, more recent studies found that PCOS granulosa cells are hyperresponsive to FSH in vitro, and estradiol concentrations from PCO follicles and normal follicles are no different [34]. A dose response study in PCOS women demonstrated a significantly greater capacity for estradiol production in response to recombinant human FSH compared with normal women [35]. The incremental response of serum estradiol was almost 2 times greater and considerably accelerated compared with that found in normal women.

Insulin Resistance

Although 50–70 % of PCOS patients have insulin resistance [36], it is not one of the diagnostic criteria of PCOS. The topic deservedly receives much attention, as many of the clinical signs and symptoms of PCOS may be attributed to excess insulin exposure. The precise molecular basis for insulin resistance is unknown, but it appears to be a post-receptor defect [37]. There is tissue specificity of insulin

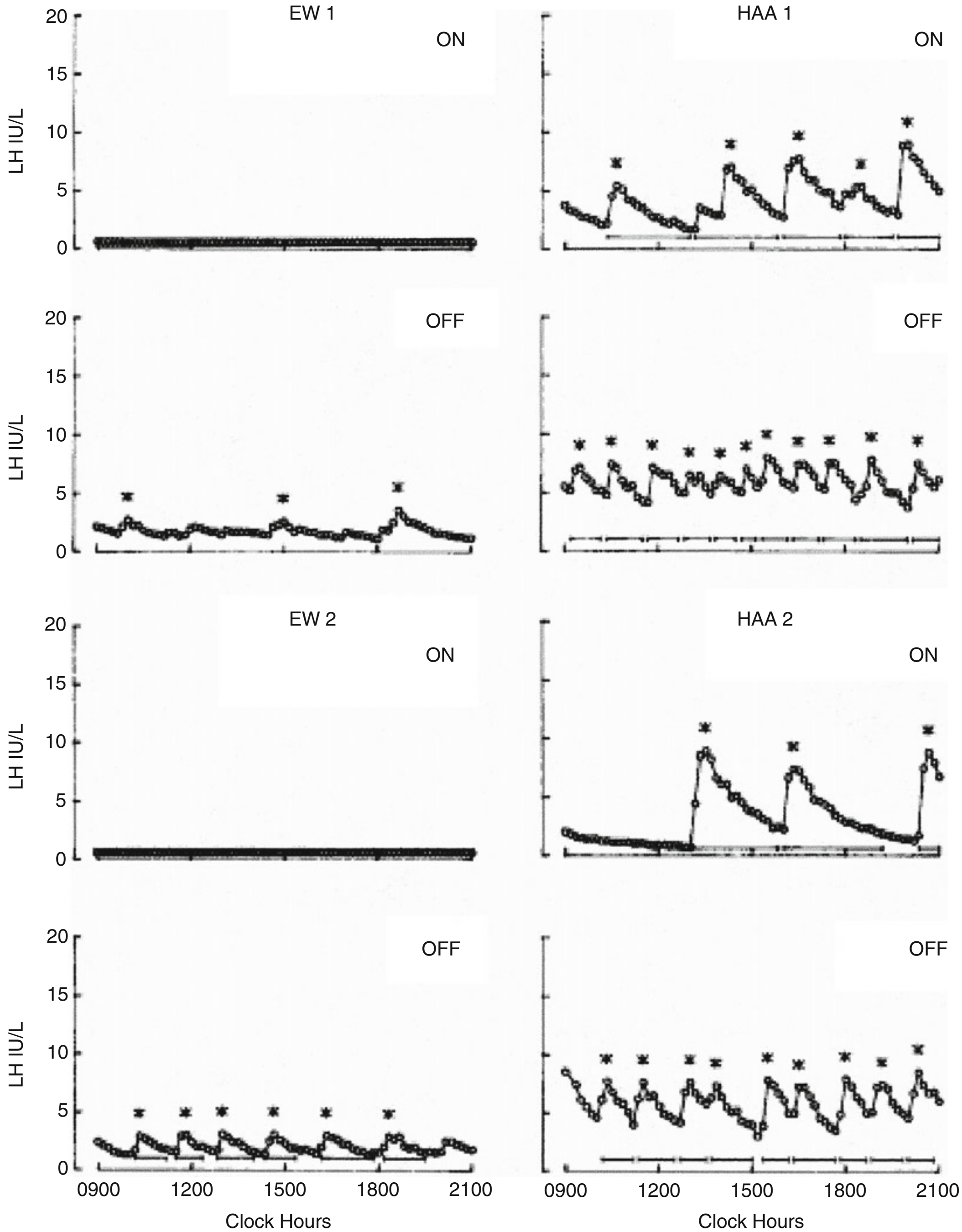

Fig. 8.3 Representative 12-h pulse patterns in two women with polycystic ovary syndrome/HAA are shown on the right side and those from eumenorrheic women on the left. "ON" means subjects were studied on day 21 of a combined oral contraceptive containing 35 μg of ethinyl estradiol and 1 mg of norethindrone. "OFF" refers to day 7 following cessation of the combined oral contraceptive. Reprinted with permission from Daniels T, Berga S. Resistance of gonadotropin releasing hormone drive to sex steroid-induced suppression in hyperandrogenic anovulation. J Clin Endocrinol Metab 1997; 82(12):4179–4183. Copyright 1997, The Endocrine Society

resistance in PCOS: muscle and adipose tissue are resistant, while the ovaries, adrenals, liver, skin, and hair remain sensitive. The resistance to insulin in skeletal muscle and adipose tissue leads to a metabolic compromise of insulin function and glucose homeostasis, but there is preservation of the mitogenic and steroidogenic function in other tissues. The effect of hyperinsulinemia on the sensitive organs results in downstream effects seen in PCOS, such as hirsutism [5], acanthosis nigricans [12], obesity [11], stimulation of androgen synthesis, increase in bioavailable androgens via decreased sex hormone-binding globulin (SHBG) [38], and, potentially, modulation of LH secretion.

In 1992, Hales and Barker proposed the concept that the environmental influence of undernutrition in early life increased the risk of type 2 diabetes in adulthood [39]. They discovered a relationship between low birth weight and type 2 diabetes in men from England. In the "thrifty phenotype hypothesis," malnutrition serves as a fetal and infant insult that results in a state of nutritional thrift. The adaptations result in postnatal metabolic changes that prepare the individual for survival under poor nutritional conditions. The adaptations become detrimental when the postnatal environment changes to one of an overabundance of nutrients, resulting in obesity and diabetes.

Insulin resistance is a component of the World Health Organization (WHO) definition of the metabolic syndrome, which is a cluster of risk factors for cardiovascular disease [40]. The WHO defines the metabolic syndrome as the presence of glucose intolerance or insulin resistance, with at least two of the following: hypertension, dyslipidemia, obesity, and microalbuminuria. Women with PCOS are 4.4 times more likely to have the metabolic syndrome, so it becomes prudent to screen these patients, especially in those with insulin resistance [41].

Lipid abnormalities are also more prevalent in PCOS patients. There can be a significant increase in total cholesterol, LDL cholesterol, and triglycerides, and a decrease in HDL cholesterol compared to weight-matched controls [42]. The dyslipidemia, impaired glucose intolerance, central obesity, hyperandrogenism, and hypertension seen in PCOS patients greatly increase the risk for cardiovascular disease. Based on this risk profile, women with PCOS have a sevenfold increased risk of myocardial infarction [43].

Laboratory Evaluation

In addition to confirming elevations of androgens, the laboratory evaluation of PCOS should have the objective of excluding other causes of hyperandrogenic anovulation. Androgen-producing tumors of the ovary and adrenals must be excluded. The adrenal glands contribute 98 % of circulating DHEAS, while both the ovaries and adrenals contribute equal amounts of circulating testosterone and androstenedione. If total testosterone is greater than 200 ng/dL or DHEAS is greater than 7,000 ng/dL, MRI is warranted to identify the hormone-secreting lesion. Measuring 17 α (alpha)-hydroxyprogesterone will screen for 21-hydroxylase deficiency, the most common enzyme deficiency in nonclassical CAH. A 17-hydroxyprogesterone level of greater than 3 ng/mL is defined as elevated and should be followed by an ACTH stimulation test, using 250 μg of synthetic ACTH given intravenously following an overnight fast. A 1-h increase of 17 α (alpha)-hydroxyprogesterone of more than 10 ng/mL is indicative of an enzyme defect in 21-hydroxylase.

Cushing's syndrome may masquerade as PCOS. Those who have additional signs of Cushing's syndrome, such as a moon facies, buffalo hump, abdominal striae, easy bruising, and proximal myopathy, should undergo screening with a 24-h urinary-free cortisol. In the work-up for anovulation, exclusion of prolactinoma should be performed. It is not uncommon to detect mild elevations in prolactin levels in PCOS patients. Thyroid-stimulating hormone (TSH) should be evaluated. LH, FSH, and estradiol levels should be obtained to exclude hypothalamic amenorrhea or premature ovarian failure.

Diabetes Screen/Evaluation of Insulin Resistance

The 2003 Rotterdam PCOS consensus group recommends a 2-h oral glucose tolerance test (OGTT) for obese PCOS patients and nonobese PCOS patients with risk factors for insulin resistance, such as family history of diabetes [2]. Defining insulin resistance is difficult, because the concept is nebulous with no universally accepted diagnostic strategy. The WHO defines insulin resistance as the lowest quartile of measures of insulin sensitivity. Women with PCOS are at significantly increased risk for impaired glucose tolerance and type 2 diabetes compared to age-, weight-, and ethnicity-matched controls [44]. If either the fasting glucose is 126 mg/dL or more, or the 2-h level is 200 mg/dL or more, diabetes is detected and should be confirmed with a repeat test. Impaired fasting glucose is defined as a glucose level between 100 and 126 mg/dL. Impaired glucose tolerance is defined as a 2-h glucose level between 140 and 200 mg/dL. It is also reasonable to obtain a fasting lipid profile in women suspected of having risk factors for cardiovascular disease. HgbA1C has been recently advocated as an accurate screening tool in evaluation of insulin resistance and diabetes in women with PCOS [45].

Treatment

There are many considerations when deciding on therapy for PCOS (Fig. 8.4). Identification of patient concerns is necessary when prioritizing goals and formulating a treatment plan. A combination of therapies may be warranted, and the practitioner should appropriately counsel the patient on the treatment expectations. Amelioration of long-term health risks should be emphasized, regardless of the primary complaints of the patient.

Weight Loss

Weight reduction should be a major component of any treatment plan for the overweight patient (BMI >25).

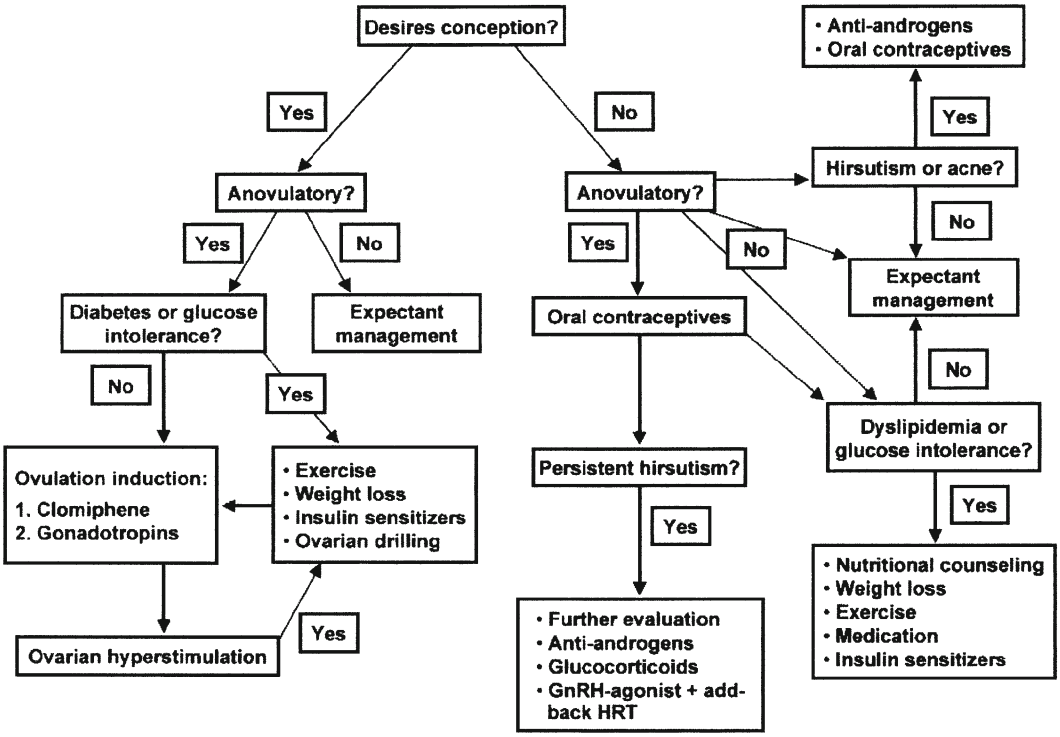

Fig. 8.4 Polycystic ovary syndrome treatment algorithm. Reprinted from Am J Obstet Gynecol, 179/6S, Berga S, The obstetrician-gynecologist's role in the practical management of polycystic ovary syndrome, 109S–113S, Copyright 1998, with permission from Elsevier

Any sustained improvement in weight should involve diet and exercise, and consultation with a nutritionist may be helpful for those with difficulty achieving weight reduction. Obese PCOS patients who achieve weight loss will have an increase in SHBG, decrease in free testosterone, and improvement in fasting insulin levels [46].

Oral Contraceptives

Combination oral contraceptives have been the mainstay of PCOS management for the patient not interested in conception. Contraceptives suppress pituitary LH and consequently reduce ovarian androgen secretion, increase SHBG, and reduce free testosterone, while regulating menses and reducing the risk of endometrial hyperplasia or malignancy. However, there may be mild attenuation of insulin sensitivity.

Korytkowski et al. have shown that short-term use of COCs in PCOS women results in a small decrease in insulin sensitivity and no change in the baseline elevation in triglyceride levels [47]. However, in normal women, COCs were shown to have an even more pronounced decline in insulin sensitivity, along with a significant elevation in triglyceride levels. The long-term effects of COCs upon insulin sensitivity and lipoprotein profiles have not been well documented. PCOS women are at greater risk for the development of diabetes and cardiovascular disease; thus, further investigation into the safety of long-term hormonal therapy is needed.

Hair Removal

In women with significant hirsutism, removal of unwanted hair, especially on the face, chest, and abdomen, is often an important concern. Shaving, plucking, waxing, and depilatories are the most common approaches used for temporary removal. These approaches do not induce coarser or faster hair growth, but must be repeated at frequent intervals.

Electrolysis is probably the most commonly used technique for permanent hair removal, wherein a fine needle is inserted into each hair follicle and an electrical current is applied. Hair follicles must be treated individually and several treatments may be needed to destroy the follicle. Usually, repeated treatments are required over a 12- to 18-month period. Possible side effects include pain, infection, hyper- or hypopigmentation, and keloid formation in susceptible women. Laser hair removal techniques, and the related intense pulsed light devices, are other options for hair removal. These techniques work by emitting light at various wavelengths, energy output, and pulse widths that are selectively absorbed by darker structures. For this reason, laser hair removal works best for light-skinned people with dark hair. As with electrolysis, laser treatments for hair removal must be repeated.

Topical Eflornithine Hydrochloride

Eflornithine hydrochloride (Vaniqa, Bristol-Myers Squibb, New York, NY) is a prescription-only topical cream approved by the FDA for reducing and inhibiting the growth of unwanted facial hair. This drug works by irreversibly inhibiting ornithine decarboxylase, an enzyme that facilitates cell division in hair follicles. The cream is applied twice a day to areas of unwanted facial hair, and <1 % is absorbed systemically. It is designated by the FDA as a Pregnancy Category C drug.

Noticeable results are usually observed after 4–8 weeks of therapy. Application must be continued for as long as inhibition of hair growth is desired, although facial hair growth is reduced for up to 8 weeks after discontinuation.

Antiandrogens

Antiandrogens are commonly used as an adjunct to oral contraceptive therapy for treatment of hirsutism, but they have also been found to improve ovulation and restore regular menses. It is important to remember that all antiandrogens are teratogenic and pose a risk of feminizing a male fetus, and thus should be used along with an effective form of contraception.

Spironolactone is an aldosterone antagonist, and it is the most commonly used adjunctive agent in the treatment of hirsutism. It competes for testosterone binding sites on the pilosebaceous unit, inhibits 5α (alpha)-reductase, and inhibits androgen production by interfering with cytochrome P450 [48]. The potassium-sparing effect warrants judicious use in the patient on potassium supplementation or preexisting hypertension.

Flutamide is a nonsteroidal antiandrogen that competes for the androgen receptor. Anovulatory PCOS patients treated with flutamide experienced resumption of ovulation with restoration of normal ovarian appearance with one dominant follicle [49]. This study also reported a reduction in plasma levels of LH, androstenedione, and testosterone. Liver toxicity is a rare but potentially serious side effect of flutamide.

Finasteride is a potent inhibitor of 5α (alpha)-reductase used for the treatment of prostatic hyperplasia with promising results as a treatment for hirsutism. All antiandrogens should be used along with a form of contraception, because they are teratogenic and pose a risk of feminizing a male fetus.

Insulin-Sensitizing Agents

Insulin-sensitizing agents have been shown to improve endocrine and reproductive abnormalities in PCOS. Metformin is the most thoroughly investigated insulin-lowering agent used in PCOS. It is a biguanide that primarily works by suppressing hepatic gluconeogenesis and, to a lesser degree, increases peripheral insulin sensitivity [50]. Thiazolidinediones (TZDs) are peroxisome proliferator activating receptor (PPAR-γ(gamma)) agonists that improve peripheral insulin sensitivity but do not appear to have an effect on hepatic glucose production [50]. This class of medications includes troglitazone, pioglitazone, and rosiglitazone.

Troglitazone is the oldest but was removed from the market in 2000 owing to hepatotoxicity. Rosiglitazone and pioglitazone are still available and appear to be safer. The role of insulin-sensitizing agents is still an area of active investigation.

Many studies have demonstrated the positive effects of metformin on the reproductive axis of PCOS patients, with one of the most comprehensive studies recently demonstrating a dramatic improvement after 6 months of treatment. Metformin administration to nonobese hyperandrogenic PCOS patients resulted in a reduction of (1) LH pulse amplitude; (2) androstenedione levels; (3) testosterone levels; (4) ovarian volume; and (5) Ferriman-Gallwey scores. Menstrual cyclicity was also improved in most patients [51]. The investigators did not determine if metformin increased the likelihood of ovulation or if FSH levels rose. Similarly, troglitazone-treated PCOS patients demonstrated improved ovulation, decreased hirsutism, decreased free testosterone, and increased SHBG [52].

Insulin-sensitizing agents have a favorable effect on hyperandrogenism by reducing LH secretion, thereby removing the main stimulus for pathologic ovarian and adrenal androgen production. The reduction in insulin levels elevates hepatic SHBG production, decreasing free androgen levels. The concurrent improvement in hyperinsulinemia and hyperandrogenemia conferred by the use of insulin-sensitizing agents may ameliorate hirsutism.

The improvement in ovulation and menstrual cyclicity in patients treated with insulin-sensitizing agents suggests improved fertility. Indeed, spontaneous and clomid-induced ovulation rates in metformin-treated women with PCOS are increased [53]. Spontaneous ovulation occurred in 34 % of those treated with 500 mg of metformin 3 times daily compared to only 4 % in the placebo group. Clomid-induced ovulation occurred in 90 % of women who received metformin compared to 8 % who received placebo. For those who are clomiphene-resistant, significant improvements in ovulation and pregnancy rates were reported in a randomized, double-blind, placebo-controlled trial for women pretreated with metformin [54]. Troglitazone alone and the combination of troglitazone plus clomiphene is also associated with increased rates (gamma) of ovulation and pregnancy in insulin-resistant women with PCOS [55].

Though metformin is a category B medication, its use throughout pregnancy is becoming more attractive. In one retrospective study, Jakubowicz et al. found a significant reduction in the rate of early pregnancy loss for PCOS women who conceived while taking metformin and continued the agent throughout pregnancy. The rate of early pregnancy loss in the metformin group was 8.8 % compared to 41.9 % in controls. In the women with a prior history of miscarriage, the early loss rate was 11.1 % for the metformin group compared with 58.3 % in the control group [56]. The efficacy of metformin for pregnancy loss is not yet clear, and safety data for this indication are lacking. In 2007, Legro et al. published a well-designed trial that concluded that live birth rate in PCOS patients prescribed clomiphene (22.5 %) was superior to metformin alone (7.2 %) and not dissimilar from combination clomiphene/metformin therapy (26.8 %) [57].

Metformin should not be given to those with conditions associated with elevated lactate levels, such as renal or hepatic disease, as there is a risk of lactic acidosis with an associated mortality of 50 % [58]. Although most studies of metformin in PCOS used a dose of 500 mg 3 times daily, no studies have been performed to determine the optimal dosing regimen for improvement in insulin sensitivity, reduction of androgens, and resumption of ovulation. A dose–response study of type II diabetic patients demonstrated that the 2,000-mg daily dose was optimal for improvement of glucose homeostasis [59], but the relevance of this dose to the PCOS population remains to be investigated.

Metformin should be initiated in a stepwise approach, titrating the dose slowly over several weeks in order to minimize side effects. Most patients will experience gastrointestinal symptoms such as nausea, diarrhea, indigestion, and abdominal discomfort. Side effects will resolve in several days for most patients, which allows incremental dosing increases on a weekly basis up to a maximum dose of 1,000 mg bid. Baseline serum creatinine should be obtained with yearly monitoring to avoid lactic acidosis.

There are no guidelines currently on the long-term use of metformin to prevent or improve health outcomes in patients with PCOS. One of the serious reactions of TZD is hepatotoxicity. Initiation of treatment requires baseline liver function studies along with periodic monitoring.

Ovarian Surgery

Ovarian Wedge Resection

Ovarian wedge resection is a surgical procedure used for PCOS patients that has been found to restore both regular menses and ovulation in the majority of cases. This procedure, originally performed by laparotomy, consisted of removing a wedge of ovarian tissue and reconstructing the ovary. Laparoscopic techniques for ovarian wedge resection have described.

The primary disadvantage of this approach is the formation of significant pelvic adhesions, which occurs in at least one-third of the patients. The concern is that these adhesions might actually decrease fertility and increase the risk of pelvic pain. Another concern is that the restoration of ovulation is unlikely to be permanent, since the ovaries are not the causal agents in this complex systemic disorder. However, the actual long-term effectiveness of wedge resection has never been reported.

Ovarian Drilling

A laparoscopic variant with similar results to ovarian wedge resection is called ovarian drilling. This procedure involves making multiple punctures in the ovarian cortex and destroying ovarian tissue using unipolar electrosurgery or laser. The results and complications for this approach appear to be similar or slightly less than those for ovarian wedge resection, although a prospective randomized study has never been done. There are additional concerns about long-term effects of ovarian drilling on ovarian function.

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9 Abnormal Uterine Bleeding

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Background and Definitions

Abnormal uterine bleeding (AUB) is the primary reason for at least one-third of all gynecology visits [1]. Despite its frequency, AUB is often a difficult diagnostic and therapeutic challenge and accounts for more than half of all hysterectomies performed in the United States. Approximately 20 % of hysterectomy specimens removed for AUB have no visible pathology [2]. This suggests that many cases of AUB are potentially treatable using hormonal therapy or other systemic or local treatment modalities.

It is important for gynecologists to develop a safe and cost-effective approach to the diagnosis and management of AUB. The most expedient methods for evaluation and treatment are dependent on understanding the various causes of AUB and the corresponding presenting symptoms.

Definition

AUB is broadly defined as any uterine bleeding that occurs outside the parameters of normal menstruation during the reproductive years. It includes bleeding originating from either the uterine fundus or cervix and does not include bleeding that originates in the lower genital tract (i.e., the vagina or vulva). However, these causes can be difficult to distinguish clinically. Therefore, both of these origins should be considered in all patients presenting with bleeding from the uterus.

The terms "menorrhagia" and "metrorrhagia" have now been replaced by more descriptive terms, including "heavy menstrual bleeding" and "intermenstrual bleeding." A new classification system is also being used to classify AUB according to the etiology [3]. The acronym PALM COEIN classifies the causes of AUB into structural abnormalities and nonstructural abnormalities (Table 9.1).

Abnormal bleeding can occur during childhood, the reproductive years, and after menopause. Since the differential diagnoses and corresponding diagnostic approaches are markedly different during these time periods, AUB in women before and after the reproductive years is considered separately in Chaps. 4 and 10.

Normal Menstruation

The concept of normal menstruation is somewhat subjective and often varies between individual women and certainly between cultures. However, normal menstruation (eumenorrhea) can be defined as bleeding that occurs after ovulatory cycles every 21–35 days, lasts 3–7 days, and is not excessive. Normal menstruation should not cause severe pain or include passage of identifiable clots.

The total amount of blood lost during a normal menstrual period has been estimated to average 30 mL and should be <80 mL. However, menstrual blood loss is difficult to estimate clinically, because much of the menstrual effluent is dissolved endometrium [4]. If the patient is changing pads or tampons more than once per hour, this is considered to be outside the parameters of normal menstruation.

The different bleeding patterns of AUB often give hints to the etiology and can be used to guide the appropriate diagnostic work-up. Due to the marked variation in presentation and the possible existence of multiple causes of bleeding, presentation alone cannot be used to clinically exclude common conditions.

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Table 9.1 PALM COEIN classification for abnormal uterine bleeding (AUB)

PALM (structural)
*P*olyp
*A*denomyosis
*L*eiomyoma
*M*alignancy and hyperplasia
COEIN (non-structural)
*C*oagulopathy
*O*vulatory dysfunction
*E*ndometrial
*I*atrogenic
*N*ot yet classified

Table 9.2 Common uterine conditions associated with AUB

Pregnancy
Early normal pregnancy
Spontaneous abortion
Ectopic pregnancy
Gestational trophoblastic disease
Infection
Pelvic inflammatory disease
Endometritis
Cervicitis
Neoplasms
Benign
Leiomyoma
Endometrial polyps
Endocervical polyps
Malignant
Endometrial carcinoma
Cervical carcinoma
Adenomyosis

Dysfunctional Uterine Bleeding

Dysfunctional uterine bleeding (DUB) is a term that refers to excessive uterine bleeding in cases in which no uterine pathology can be identified and is therefore a diagnosis of exclusion [5]. Due to the development of a greater understanding of AUB and the availability of more sophisticated diagnostic techniques, this term is less frequently used today.

In many cases that would have been referred to as DUB in the past, modern diagnostic techniques identify either uterine or systemic pathologies that (1) result in anovulation (e.g., hypothyroidism), (2) result from anovulation (e.g., endometrial hyperplasia or carcinoma), or (3) coexists with anovulatory bleeding but may or may not be causal (e.g., leiomyomata). Bleeding unrelated to uterine pathology can usually be determined to be a result of chronic anovulation (polycystic ovary syndrome [PCOS] and related conditions), exogenous steroid hormones (contraceptives or hormone replacement therapy), or hemostatic disorders (e.g., von Willebrand disease).

Treatment is most likely to be effective when specific causes of AUB can be identified. Since the term "DUB" is used to refer to widely divergent causes of AUB, a national consensus group recently concluded that this term is unlikely to improve diagnosis or treatment and thus no longer has any usefulness in clinical medicine [6].

Abnormal Uterine Bleeding Caused by Uterine Conditions

AUB can be grouped according to the basic pathophysiology of the various etiologies. The clinician must keep in mind that any individual patient can simultaneously have more than one cause of uterine bleeding (Table 9.2). Therefore, the work-up should include an appropriate evaluation encompassing both likely and serious anatomic and systemic etiologies.

Pregnancy

It is important to exclude pregnancy in every case of AUB in a reproductive-aged woman, no matter how obvious any alternative diagnosis may be. Pregnancies are the most common cause of AUB in the reproductive age group, including normal pregnancies, spontaneous abortions, ectopic pregnancies, and gestational trophoblastic disease.

First-trimester bleeding occurs in up to 25 % of all pregnancies and is associated with an increased risk of many common complications [7]. In approximately half of these cases, bleeding will be an early symptom of impending spontaneous abortion, whereas the remaining half will ultimately prove to have a viable pregnancy. Ectopic pregnancies currently make up 2 % of all pregnancies and commonly present with AUB as one of the presenting symptoms [8]. Gestational trophoblastic disease is another pregnancy-related problem that presents as AUB in >80 % of cases [9].

Uterine Pathology

It is a priority for gynecologists to precisely identify uterine pathology that might contribute to uterine bleeding. Most of these diagnoses can be determined to be related to infection and neoplasm. Additionally, a common uterine pathology related to AUB is adenomyosis.

Infection

Infection, in the form of endometritis, is an under-recognized cause of AUB and is often the basis of what appears to be AUB. In obvious cases of pelvic inflammatory disease, approximately 40 % of patients will present with vaginal bleeding [10]. However, subclinical endometritis can also result in AUB.

Chronic endometritis was diagnosed in the past only when plasma cells were found on endometrial biopsy. More recent studies have shown an association between AUB and endometritis that manifests only as reactive changes in the surface endometrium and not in association with the presence of a particular type of inflammatory cell [11]. Other studies have verified that subclinical endometritis is a common finding in patients diagnosed with AUB and can be related to any of a number of different pathogens [12].

Cervicitis is another commonly recognized cause of AUB and is characterized by postcoital spotting. Postcoital bleeding is the most common presenting symptom in women found to have *Chlamydia* infections [13]. In addition to common sexually transmitted diseases (i.e., chlamydia and gonorrhea), other vaginal flora and pathogens can also cause cervicitis [14].

Neoplasms

AUB can be a presenting symptom for gynecologic neoplasms involving the cervix, uterine fundus, or ovaries. These neoplasms can be benign (e.g., endometrial or endocervical polyps, leiomyoma) or malignant (e.g., endometrial or cervical carcinoma). Focal intracavitary lesions account for up to 40 % of cases of AUB [15]. Neoplasms of the ovary can indirectly cause irregular bleeding by interfering with ovulation, as discussed below. Some of the most common neoplasms known to cause AUB are reviewed below.

Leiomyomas

These benign tumors of the myometrium are remarkably common and by age 50 can be found in almost 70 % of white women and >80 % of black women upon ultrasonographic examination [16]. Many of these leiomyomas are subclinical, and estimates of symptomatic leiomyomas range from 20 to 40 %.

Submucosal and intracavitary leiomyomas that distort the uterine cavity are most likely to result in menorrhagia, presumably because of a direct effect on the adjacent endometrium. Large intramural leiomyomas can sometimes result in menorrhagia. However, the majority of leiomyomas that are intramural, subserosal, or pedunculated on the external uterine surface are not associated with AUB.

Adenomyosis

This benign condition involves the invasion of endometrium into the myometrium. Microscopic examination of the uterus reveals endometrial glands and stroma deep within the endometrium surrounded by hypertrophic and hyperplastic myometrium. This histopathologic diagnosis is found in over 60 % of hysterectomy specimens [17]. Clinically, two-thirds of patients with adenomyosis will complain of menorrhagia and dysmenorrhea, and pelvic examination usually reveals a diffusely enlarged and tender uterus.

Diagnostic tests that help suggest the diagnosis of adenomyosis include both transvaginal ultrasonography and magnetic resonance imaging. The sensitivity for ultrasonography approaches 50 %, and the sensitivity of MRI ranges from 80 to 100 % [17]. Perhaps in the future, there will be more effective diagnostic testing for adenomyosis and treatments other than hysterectomy.

Endometrial Polyps

Endometrial polyps are localized overgrowths of the endometrium that project into the uterine cavity. These polyps may be broad-based (sessile) or pedunculated. Endometrial polyps are common in both pre- and postmenopausal women and are found in at least 20 % of women undergoing hysteroscopy or hysterectomy [18]. The incidence of these polyps rises steadily with increasing age, peaks in the fifth decade of life and gradually declines after menopause.

In premenopausal women complaining of AUB, studies have shown that from 5 to 33 % will be found to have endometrial polyps [19]. Endometrial polyps are commonly found in patients with a long history of anovulatory bleeding, suggesting that polyps may be the result of chronic anovulation in some women. Polyps can also be found in women complaining of post-menstrual spotting or bleeding in ovulatory cycles or during cyclic hormonal therapy. Endometrial polyps in premenopausal women are almost always benign [18]. However, the risk of endometrial malignancy increases with age, and one study reported the risk of malignancy in polyps in women >65 years old was >50 %.

Endometrial Hyperplasia

It is unlikely that endometrial hyperplasia causes AUB. However, this condition is most often found in premenopausal women with AUB with prolonged anovulation [20]. Although endometrial hyperplasia is not in itself a health risk, it is both a precursor to and marker for concurrent endometrial cancer, particularly in the presence of atypia.

Endometrial Cancer

The single most important disease to identify early in the evaluation of a peri- or postmenopausal woman with AUB is endometrial cancer. Approximately 20 % of endometrial cancer is diagnosed in women before menopause and 5 % before the age of 40 years [21]. After the menopause, approximately 10 % of women with AUB will be found to have endometrial cancer, and the incidence rises with each decade of life thereafter.

Endocervical Polyps

These soft, fleshy growths originate from the mucosal surface of the endocervical canal. They usually arise from a stalk and protrude through the cervical os, although some may be broad-based. They usually range in size from 3 to 20 mm, but occasionally can be larger.

Endocervical polyps are known to be more frequent in women on oral contraceptives and with chronic cervicitis; however, the etiology remains unclear. Microscopically, endocervical polyps consist of a vascular core surrounded by a glandular mucous membrane and may be covered completely or partially with stratified squamous epithelium. In some polyps, the connective tissue core may be relatively fibrous. Endocervical polyps removed from women taking oral contraceptives often show a pattern of microglandular hyperplasia [22].

Endocervical polyps are relatively common in sexually active women, but are rare before menarche. Many endocervical polyps are asymptomatic and are discovered incidentally on visual examination of the cervix. In other instances, these polyps can manifest as intermenstrual and/or postcoital spotting.

Cervical Cancer

Cervical dysplasia can be found in up to 17 % of women presenting with postcoital spotting, and 4 % will have invasive cancer [23]. In the absence of a visible lesion, Papanicolaou smears and colposcopy (if indicated) are important diagnostic tools. In the presence of a visible cervical lesion, it is critical to biopsy the lesion to confirm the clinical diagnosis.

Table 9.3 Causes of AUB unrelated to pregnancy or uterine pathology

Exogenous hormones
Hormone contraceptives
Hormone replacement therapy
Ovulation defects
Physiologic oligo-ovulation
Perimenarchal
Perimenopasual
Polycystic ovary syndrome
Hyperandrogenic states
Congenital adrenal hyperplasia, adult-onset
Cushing's syndrome
Ovarian and adrenal tumors
Systemic diseases that interfere with ovulation
Hypothyroidism
Hyperprolactimemia
Renal failure
Liver disease
Endometrial atrophy
Menopause
Premature ovarian failure
Hypogonadotropic hypogonadism
Exogenous progestins
Hyperandrogenemia
Coagulopathy
Hereditary bleeding disorders
Von Willebrand disease
Disorders of platelet function and fibrinolysis
Acquired bleeding abnormalities
Idiopathic thrombocytopenia purpura
Leukemia
Aplastic anemia
Anticoagulation therapy

Abnormal Uterine Bleeding Unrelated to Uterine Pathology

Many women experience heavy or irregular menstrual bleeding that is not caused by an underlying condition of the uterus. Anovulatory bleeding is one of the most common underlying causes; however, a number of other unrelated causes, such as exogenous hormones and bleeding disorders, must also be considered (Table 9.3).

Exogenous Hormones

Hormonal therapy is one of the most common causes of AUB. Specifically, irregular bleeding is the most common symptom of women receiving contraceptive therapy and hormone replacement therapy (see Chap. 10) and the most common reason for discontinuation of these therapies.

Hormone Contraceptives

Approximately ten million women in the United States use some type of hormone contraception, including combination oral contraceptives, progestin-only pills, depot medroxyprogesterone acetate injections, progestin-containing intrauterine devices, subdermal levonorgestrel implants, transdermal combination hormone patches, and intravaginal rings. In addition to being a common reason to visit primary care physicians and gynecologists, AUB is the major reason for contraception discontinuation and subsequent unplanned pregnancy.

During the first 3 months of combination oral contraceptive use, as many as one-third of women will experience AUB. For the vast majority of women, the most effective treatment approach is patient reassurance and watchful waiting. As the uterus adapts to the new regimen of hormonal exposure, the monthly withdrawal bleeding becomes regular, lighter, and less painful than natural menstruation in most women.

If abnormal bleeding persists beyond 3 months while a patient is on hormonal contraceptives, other common causes should be excluded. In young, sexually active women, sexually transmitted diseases should be excluded, because in one study, almost one-third of women on oral contraceptives who experienced abnormal bleeding were found to have otherwise asymptomatic *Chlamydia trachomatis* infections [24]. If no cause for AUB other than hormonal therapy is found, treatment options include the use of supplemental estrogen or changing to an oral contraceptive with a different formulation that has a different progestin or higher estrogen content.

Women using progestin-only contraceptives have a greater risk of continued AUB than those using combined oral contraceptives. Prolonged exposure to progestins results in a microscopic condition sometimes called "pseudoatrophy" (see the section on Atrophic Endometrium). When reassurance is not sufficient, administration of supplemental estrogen during these bleeding episodes is sometimes useful.

Ovulation Defects

Abnormal or absent ovulation is a common cause of AUB during the reproductive years. A brief description of normal menstrual physiology (which is covered in depth in Chap. 1) is useful in understanding anovulation as an underlying cause of AUB.

Normal Menstruation

Each month, the endometrium of normally ovulating women is exposed to physiologic levels of estradiol (50–250 pg/mL), accompanied in the last 14 days of each cycle by progesterone (mid-luteal phase >12 nmol/L). The result is a structurally stable endometrium 5–20-mm thick as measured by transvaginal ultrasound.

Withdrawal of progesterone and estrogen results in menstruation, which involves the breakdown and uniform shedding of much of the functional layer of the endometrium, which is enzymatically dissolved by matrix metalloproteinases [25]. Normal menses occur every 28 ± 7 days, with duration of flow of 4 ± 2 days, and a blood loss of 40 ± 40 mL [26]. Hemostasis is achieved by a combination of vasoconstriction of the spiral arterioles and normal coagulation mechanisms.

Oligo- and Anovulation

Irregularity or absence of ovulation is common among reproductive-aged women not using hormonal contraception. In the perimenarchal years, adolescents often have anovulatory cycles as part of the maturation process, but only occasionally do they complain of clinically significant AUB. In the perimenopausal years, anovulatory cycles again become more common for many women. These episodes of endometrial exposure to unopposed estrogen increase the risk of not only AUB but also endometrial hyperplasia and endometrial cancer. During the intervening years, both chronic and intermittent intervals of anovulation can occur, usually as a result of a treatable underlying condition.

Mechanism of Anovulatory Bleeding

Anovulation results in AUB as a result of chronic exposure of the endometrium to estrogen, without the effect of cyclic exposure to postovulatory progesterone. Endometrium that is exposed to unopposed estrogen becomes abnormally thickened and structurally incompetent. The result is asynchronous shedding of portions of the endometrium unaccompanied by vasoconstriction.

The bleeding associated with unopposed estrogen exposure is usually heavy. Since the blood has not been lysed by endometrial enzymes, blood clots are often passed, resulting in increased menstrual cramping in many women. Prolonged periods of bleeding also appear to predispose to subclinical endometritis, which can further exacerbate bleeding, and is often unresponsive to hormonal therapy.

Polycystic Ovary Syndrome

The most common cause of chronic anovulation is a disorder that can present with a constellation of symptoms and is referred to as PCOS (see Chap. 8). PCOS is a heterogeneous endocrine and metabolic disorder that affects 6–10 % of reproductive age women [27]. This syndrome is diagnosed when a woman without an underlying condition is found to have two out of the following three criteria: (1) oligo- or anovulation, (2) clinical and/or biochemical evidence of hyperandrogenemia, and (3) polycystic ovaries [28]. These women have circulating estrogen levels in the normal range, but anovulatory progesterone levels.

PCOS is believed to result from insulin resistance in many women [27]. In today's culture, insulin resistance is often the result of obesity. However, only 70 % of women with PCOS are obese [28]. Insulin resistance will be found in approximately 75 % of women with PCOS who are obese, but <40 % of those who are not obese [29].

The mechanism whereby insulin resistance results in PCOS is intriguing [30]. Insulin increases production of androgens by both the ovaries (primarily androstenedione and testosterone) and adrenal gland (primarily dihydroepiandrosterone). In the ovary, insulin increases androgen secretion by both theca cells, which are LH-dependent, and ovarian stroma cells. These increased androgens contribute to hirsutism and may contribute to the increased body mass often seen in PCOS patients. These androgens can be aromatized peripherally in both fat and muscle to estrogen (primarily estrone), which acts on the pituitary to increase secretion of LH, which in turn stimulates the ovaries to secrete more androgens in concert with insulin. The positive-feedback loop that results is believed to be the cause of many cases of

PCOS. The accuracy of this interpretation is supported by the observation that in many overweight patients, either weight loss or the use of an insulin-sensitizing agent (e.g., metformin) will simultaneously improve insulin resistance and restore regular ovulatory cycles [30].

Systemic Diseases That Can Mimic PCOS

Some patients that are oligo- or anovulatory can have an underlying systemic disease, which makes these patients clinically indistinguishable from PCOS. Although some diseases can be detected with appropriate testing, not all of these systemic conditions can be treated such that the symptoms completely resolve.

Conditions that result in signs and symptoms identical to PCOS can be divided into two groups. The first group includes conditions that cause hyperandrogenemia, which in turn can interfere with ovulation and result in a clinical picture identical to PCOS [31]. These include adult-onset congenital adrenal hyperplasia, Cushing's syndrome and disease, and androgen-secreting neoplasms of the ovary or adrenal gland. Adult-onset congenital adrenal hyperplasia should be suspected whenever PCOS symptoms occur simultaneously with menarche. Cushing's and androgen-secreting tumors should be suspected when hyperandrogenism and ovulation dysfunction present rapidly in a woman with previously normal menstrual cycles.

The second group consists of any systemic condition that can interrupt ovulation. Both hypothyroidism and hyperprolactinemia are relatively common conditions that can have no other symptoms other than interfering with ovulation. Simple blood tests can screen for these conditions in the initial evaluation of PCOS. In addition, any serious systemic disease can interfere with ovulation—most notably, renal failure and chronic liver disease. These systemic disorders can also affect hemostasis. Patients with serious systemic diseases, however, usually manifest significant symptoms in addition to ovulatory dysfunction and AUB [32].

Endometrial Atrophy

Endometrial atrophy from any cause can result in AUB and is usually described as spotting. The significance of this type of AUB is that it is indistinguishable from the earliest symptoms of endometrial cancer and thus must be carefully evaluated in the peri- and postmenopausal woman.

Hypoestrogenemia is most commonly the result of surgical or natural menopause. Although natural menopause occurs at an average age of approximately 51 years, 2 % of women undergo premature menopause before the age of 40 years. Hypoestrogenemia also occurs in women with normal ovaries who lack gonadal hormonal stimulation due to pituitary or hypothalamic pathology, descriptively grouped together as having hypogonadotropic hypogonadism. Causes of this condition include hypothalamic amenorrhea, usually secondary to conditions such as anorexia nervosa, repetitive or prolonged strenuous exercise, or starvation, and the relatively uncommon pituitary failure. Hypoestrogenemia can also occur secondary to hyperprolactinemia.

Histologically, hypoestrogenemia leads to atrophy of both endometrial glands and stroma. Scanty, small glands can be seen in dense stroma. The result is thinning of the endometrium, which can be <5-mm thick by transvaginal ultrasonography.

Prolonged exposure to exogenous progestins, with or without estrogen, can also result in endometrial atrophy. Long-term use of combined oral contraceptives results in poorly developed glands lined by a single layer of low columnar to cuboidal cells. Secretory changes are minimal, but stromal decidualization is present, resulting in discordance between small inactive glands and decidualized stroma. Numerous granular lymphocytes are often present. Progestin-only contraception results in endometrial atrophy with sparse, narrow glands lined by flattened epithelium in a spindle-cell stroma without decidual reaction. Women with hyperadrogenemia can develop a similar clinical and histological picture.

Coagulopathy

A surprisingly common cause of menorrhagia is one of the several inborn or acquired conditions that can interfere with the body's normal hemostatic mechanisms.

Hereditary Bleeding Disorders

Von Willebrand disease and less common disorders of platelet function and fibrinolysis are characterized by excessive menstrual bleeding that begins at menarche and is usually regular. As many as 20 % of adolescents who present with menorrhagia significant enough to cause anemia or hospitalization have a bleeding disorder, and should therefore undergo an evaluation for coagulopathy. However, it is important to remember that most AUB in this age group is probably due to anovulation [33].

The most common bleeding disorder is von Willebrand disease, which affects 1–2 % of the population [34]. This hereditary deficiency (or abnormality) of the von Willebrand factor results in decreased platelet adherence, with von Willebrand factor interacting with platelets to form a platelet plug. A fibrin clot will then form on this plug. There are three main types of von Willebrand disease. The mild form (type 1) is responsible for over 70 % of cases and is characterized by an absolute decrease in the protein. The mechanism by which an abnormal factor leads to bleeding at the level of the endometrium is unclear. The vast majority of women with this disease report AUB, specifically menorrhagia. The prevalence of this disorder in adults can range from 7 to 20 %. Other inherited conditions include thrombocytopenias and rare clotting factor deficiencies (e.g., factor I, II, V, VII, X, XI, XIII).

Acquired Bleeding Disorders

New onset of extremely heavy menses not amenable to hormonal therapy can sometimes be related to acquired bleeding abnormalities. Such abnormalities include idiopathic thrombocytopenia purpura (ITP) or hematologic diseases affecting platelet production, such as leukemia. Other systemic disorders, such as sepsis and liver disorders, can also cause an acquired hemostatic disorder resulting in bleeding.

Anticoagulant Therapy

Excessive bleeding can rarely be a significant problem for women taking anticoagulant therapy, such as warfarin or heparin. Fortunately, most women taking anticoagulants do not have problems with AUB, which is considered to be an adverse reaction to anticoagulant therapy. Life-threatening genital bleeding in women taking anticoagulants is rare, but may lead to emergency hysterectomy [35].

Clinical Evaluation of AUB

The work-up for AUB should be tailored to the clinical presentation of the patient and, importantly, the age should be taken into consideration. At the same time, the clinician must be aware of common causes of AUB that might not be clinically obvious but still must be excluded.

An important point to keep in mind is that AUB can often have more than one cause. Sometimes subtle comorbid conditions, such as endometritis, can make single-factor therapy surprisingly ineffective [12]. In other women, obvious causes of chronic anovulation can be associated with endometrial hyperplasia and/or cancer. Careful evaluation of the patient for multiple simultaneous causes of AUB is important.

History

A careful history is the most important factor in determining the appropriate diagnostic approach. This should include the patient's menstrual patterns and history, the extent of recent bleeding, sexual activity, and contraception. Important questions include symptoms of pregnancy, infection, changes in body hair, excessive bleeding, and systemic disease. Current medication and information about prior Papanicolaou smears are also important. The review of systems should include symptoms of systemic disease, such as weight gain or loss, abdominal swelling, somnolence, and nipple discharge.

Pregnancy

In reproductive-aged women, the presence of signs and symptoms of pregnancy are important to ascertain. Current contraceptive methods and past pregnancy history are also important.

Characterization of Bleeding

Once pregnancy is excluded, the amount and character of the bleeding is important to ascertain. Careful, stepwise retrospective questioning will usually give a clear picture of the bleeding pattern over the previous days, months, and even years. In nonemergency cases of bleeding, the use of a prospective menstrual calendar is an excellent way to document the problem as well as the response to therapy. It is important to determine when the bleeding problems were first noticed, since menorrhagia starting at menarche should alert the clinician to the possibility of an underlying bleeding disorder.

The amount of bleeding is probably the most difficult to elicit on history, since normal or heavy menstrual bleeding can be very subjective. For research purposes, menorrhagia can be defined as a monthly blood loss of >80 mL on three consecutive menses as measured by the alkali hematin method [36]. Unfortunately, this type of accurate evaluation is neither cost-effective nor readily available.

In adolescents with menorrhagia, it is important to determine any past history of excess bleeding during surgical, dental, or obstetric procedures since this has been found to be predictive of von Willebrand disease [37]. Interestingly, in this same study, epistaxis and easy bruising were not clear discriminatory symptoms.

Physical Examination

The physical examination is intended to detect both gynecologic and systemic diseases. Special care should be taken to document the presence of hirsutism, acne, or other signs of excess androgens, as well as galactorrhea.

The pelvic examination begins with a speculum examination to inspect the cervix for polyps, signs of infection, or inflammation. A bimanual examination is important to determine uterine size, adnexal masses, and the presence and character of any tenderness.

Laboratory Testing

Laboratory evaluation is an important part of the initial evaluation of all patients with AUB (Table 9.4). However, rather than ordering every possible test at the first visit, laboratory tests should be obtained in a stepwise fashion based on presentation (Fig. 9.1).

Table 9.4 Laboratory testing for AUB

All patients	
	Pregnancy test
	Complete blood count (including platelets)
	Papanicolaou smear
	Cervical tests for gonorrhea and chlamydia
Anovulation	
	Thyroid-stimulating hormone
	Prolactin
Obesity	
	Type II diabetes screen: HgA1c
Hirsutism	
	Testosterone
	DHEA-S
>40 years of age	
	Endometrial biopsy
New-onset menorrhagia	
	Prothrombin time
	Activated partial thromboplastin time
	Bleeding time
Menorrhagia since menarchy	
	Above plus
	Iron profile, serum creatinine
	Factor VII level
	VWf antigen
	Ristocetin cofactor
	Platelet aggregation studies
	If the above are negative, consider
	Factor XI level
	Euglobulin clot lysis time

The most important test for all reproductive-aged women complaining of AUB is a beta-HCG test for pregnancy. For all cases, except the most insignificant bleeding, a CBC (including platelets) is important to detect significant anemia and disorders of platelet production or survival. Unless precluded by extremely heavy bleeding, a Papanicolaou smear should be performed on any woman who has not had recent screening as per the current screening guidelines. For patients with apparent oligo- or anovulation, thyroid-stimulating hormone (TSH) and prolactin testing will detect subtle pituitary function disorders that might present with AUB as the earliest symptom. Since cervical and uterine infections are common, cervical tests for gonorrhea and chlamydia are helpful in women with intermenstrual spotting, as well as any woman at risk for these infections.

Several patient groups may require additional ancillary tests. Obese patients with apparent AUB are at increased risk for type II diabetes. Several authors recommend measurement of hemoglobin A1c (HbA1c) as a good diabetes screen that does not require fasting or a return visit for provocative testing. Patients with hirsutism or other evidence of androgen excess should be screened for ovarian and adrenal malignancies with total testosterone and DHEAS. All women >40 years old should have an endometrial biopsy after pregnancy is excluded to detect endometrial hyperplasia or cancer.

PCOS and adult-onset congenital adrenal hyperplasia may sometimes be indistinguishable by clinical presentation, since both disorders are often characterized by hirsutism, acne, menstrual abnormalities, and infertility [38]. Unfortunately, no discriminatory screening test exists for this heterologous condition, which is most commonly caused by 21-hydroxylase or 11 beta-hydroxylase deficiency. If ovulation dysfunction and signs of androgen excess begin at the time of puberty, such women should be investigated appropriately (see Chap. 4).

Hemostatic Disorders

Patients with new onset of significant menorrhagia should be evaluated for bleeding disorders with prothrombin time, activated partial thromboplastin time, and bleeding time [39]. Any patient with a history of menorrhagia since menarche, especially with a history of surgical or dental-related bleeding or postpartum hemorrhage, should be evaluated for hereditable bleeding disorders. These tests include specific tests for von Willebrand disease, such as von Willebrand factor antigen, von Willebrand factor functional activity (ristocetin cofactor activity), and factor VIII level. These levels can fluctuate; therefore, these tests should be repeated if clinical suspicion is high. Normal ranges should be adjusted for the observation that von Willebrand factor levels are 25 % lower in women with blood type O compared with other blood groups. Further studies, such as platelet aggregation studies, may also be required [39]. If these studies are negative, factor XI level and euglobulin clot lysis time can be evaluated.

Malignancies and Premalignancies

Endometrial Biopsy

AUB in women 40 years of age to menopause can often be attributed to anovulatory bleeding, which is a normal physiological response to declining ovarian function. However, the risk of endometrial hyperplasia and carcinoma increases with age. For this reason, once pregnancy has been excluded, an endometrial biopsy should be obtained in all women older than 45 years of age who present with AUB. Endometrial biopsy should also be performed in all women who are younger than 45 years of age who have a history of persistent AUB, unopposed estrogen exposure, or failed medical management [40].

Imaging and Hysteroscopy

Over the last 2 decades, our ability to visualize the uterine cavity and adnexa has dramatically increased. In addition to the bimanual pelvic examination, the only other available methods were hysterosalpingogram (HSG) and dilation and curettage. Although the radiation exposure and discomfort

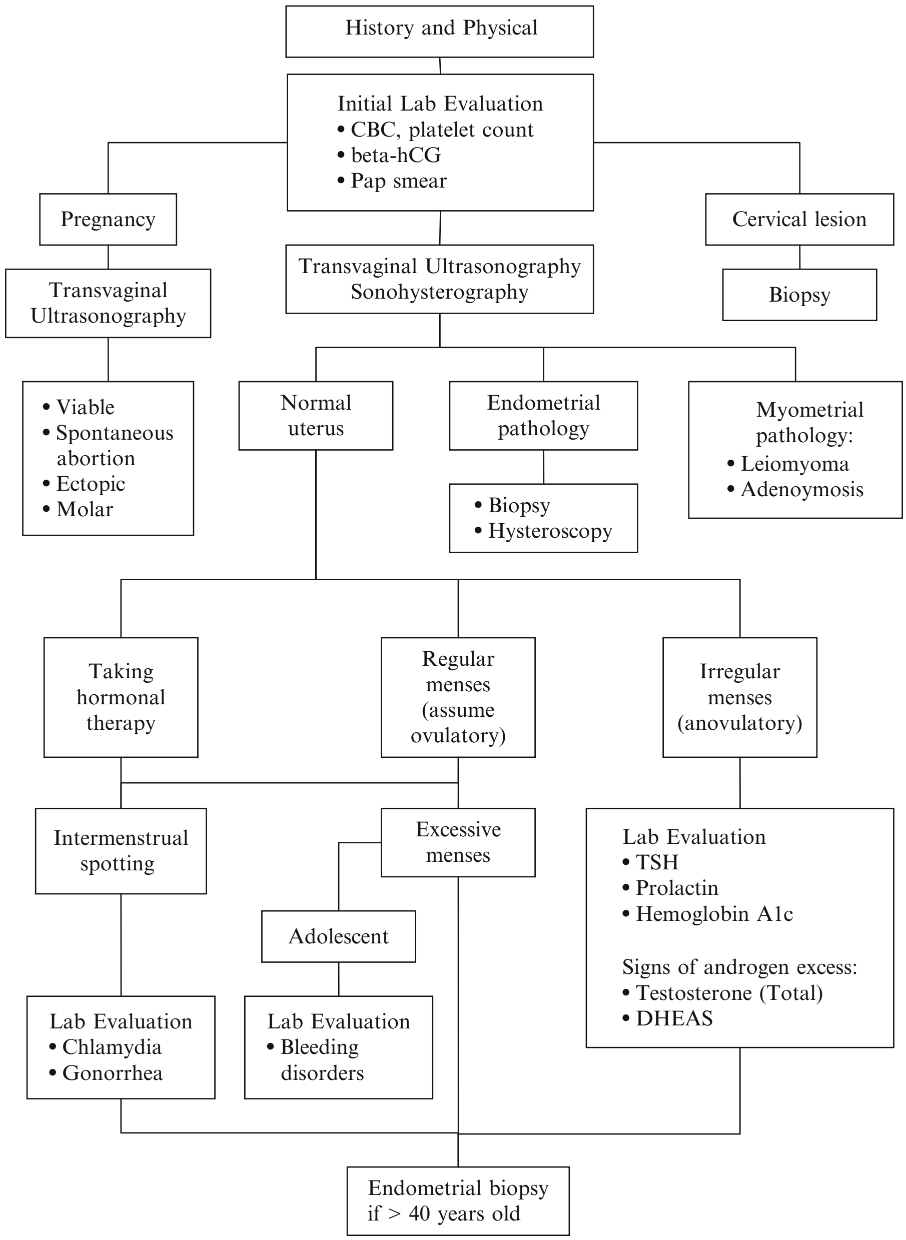

Fig. 9.1 Algorithm for evaluating women with AUB

associated with HSG are both considered acceptable, this technique effectively identifies only marked abnormalities of the uterine cavity. Lesions <1 cm in size are often missed. Likewise, the previously blind procedure of dilation and curettage gave the operator only the roughest idea of the depth and contour of the uterine cavity. Intrauterine findings at the time of hysterectomy were often a surprise. In obese patients in whom bimanual examinations are difficult, unexpected ovarian masses at laparotomy were commonplace.

Transvaginal Ultrasonography

Today, transvaginal ultrasonography and sonohysterography have made unexpected findings at surgery a rare occurrence (see Chap. 6). Ultrasonography and sonohysterography have become important steps in the evaluation of AUB. Transvaginal ultrasonography can accurately determine uterine size and configuration, and reveal the nature of both palpable and nonpalpable adnexal masses. Knowledge about the size and location of leiomyoma and the potential that an ovarian mass might be malignant is invaluable prior to surgery. Sonohysterography can be used to accurately visualize most intrauterine abnormities once pregnancy has been excluded. Accurate evaluation of the uterine cavity is of the utmost importance for the evaluation and treatment of AUB. This procedure involves injection of sterile saline into the uterus while a transvaginal sonogram is performed. It may cause a

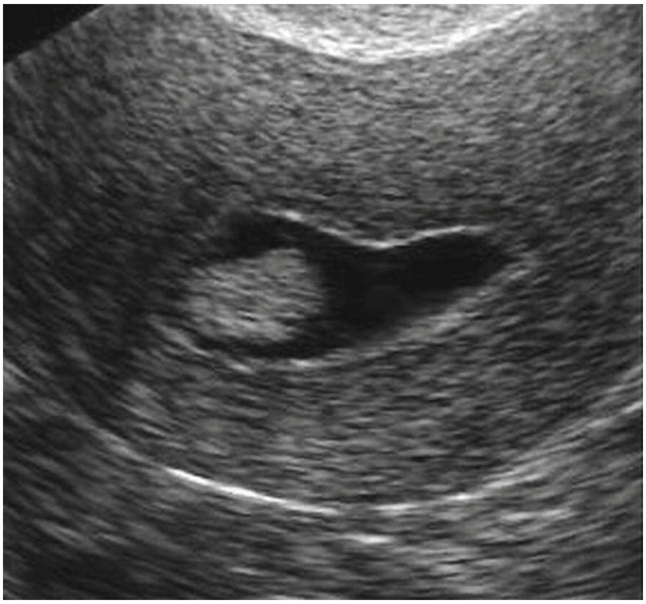

Fig. 9.2 Endometrial polyps diagnosed by sonohysterography

small amount of discomfort to the patient. When the uterine cavity is distended with saline, intracavitary lesions (e.g., polyps, fibroids, cancer) as small as 3 mm can be clearly seen (Fig. 9.2).

Office Hysteroscopy

Office hysteroscopy (see Chap. 16) is another excellent outpatient method for visualizing the uterine cavity. The discomfort and risk is somewhat more than sonohysterography, and the procedure can be difficult in the presence of cervical stenosis or when the cervix is difficult to visualize. However, the color photographs depicting the lesion can be very informative for patients.

Management of AUB Unrelated to Pregnancy or Uterine Pathology

In more than half of patients presenting with AUB, the etiology will be determined to be unrelated to pregnancy or underlying uterine pathology. Treatment of these cases consists of managing underlying systemic medical conditions or comorbidities and normalizing the endometrium with exogenous hormone therapy, when necessary. In patients with hypothyroidism, for example, restoration of a euthyroid state with thyroid hormone replacement will restore normal ovulatory function in most cases. Likewise, treatment of underlying conditions, such as hyperprolactinemia, PCOS, or other endocrine dysfunction, such as an adrenal enzyme deficiency, may restore normal menstrual function.

Anovulatory Bleeding

For heavy, acute anovulatory bleeding, the primary focus of initial management is the expedient bleeding cessation by achieving rapid structural stability of the endometrium. After stabilization of the endometrium, long-term therapeutic goals for women who do not desire pregnancy include promoting synchronous endometrial shedding at regular intervals or achieving amenorrhea with exogenous hormone administration. This approach should prevent subsequent episodes of heavy bleeding that require emergent evaluation and management.

Hemodynamic Stabilization and Initial Evaluation

While hemorrhagic shock secondary to AUB is rare, many patients will present with critically low hemoglobin levels and symptomatic anemia. Healthy women of reproductive age can often compensate physiologically for severe anemia and thus have minimal symptoms. In contrast, older women, particularly those with underlying cardiovascular disease, may present with significant symptoms from heavy uterine bleeding. Initial management of hemodynamically unstable patients includes intravenous fluid resuscitation and blood product replacement, as indicated.

With stabilization, an expedient evaluation should be performed to detect pregnancy or anatomic pathology. Pertinent laboratory tests should be obtained, including CBC and quantitative beta-HCG. Transvaginal ultrasonography is an important initial diagnostic tool to evaluate for the presence of underlying anatomic pathology. However, differentiating intrauterine lesions such as leiomyomata or polyps from clots may be difficult in the setting of acute bleeding. Hormonal therapy, as discussed below, should be initiated to decrease uterine bleeding. Endometrial evaluation is an important part of the evaluation for many women, depending on age and risk factors for endometrial hyperplasia or malignancy, but can safely be deferred until the bleeding decreases with medical management. Therefore, hormonal therapy does not have to be delayed until histologic sampling can be performed.

Dilation and Curettage

In cases in which massive, life-threatening, anovulatory uterine bleeding is present, dilation and curettage provides a rapid means to decrease bleeding and evaluate for endometrial pathology. As a surgical approach to the management of AUB, disadvantages include the risks of anesthesia, which depend on an individual patient's underlying medical conditions, and the small surgical risks of the procedure.

Table 9.5 "Taper" oral contraceptive regimen for treatment of AUB using low-dose (30 μg ethinyl estradiol), monophasic oral contraceptive pills

Day	Frequency
1–2	One tablet 4 times daily
3–4	One tablet 3 times daily
5–19	One tablet daily
20–25	Expect menses
26	Start oral contraceptives at standard dosage

Dilation and curettage has no long-term therapeutic effect; therefore, long-term treatment must be initiated postoperatively. In most cases of AUB, medical management can be safely used as the first-line treatment.

Intravenous Estrogen Therapy

In the absence of life-threatening bleeding requiring surgical intervention, AUB should be initially managed with hormonal therapy. Historically, first-line therapy consists of the administration of intravenous conjugated estrogens, 25 mg every 4 h, until the cessation of bleeding or for 24 h [41]. Given that this therapy is often associated with nausea, concomitant intravenous or oral antiemetics should be administered. Patients who do not respond to estrogen therapy may require dilation and curettage, as described above.

Given the pathophysiology of anovulatory bleeding, namely, prolonged exposure to unopposed estrogen, treatment with estrogen may seem paradoxical. By simulating clotting at the capillary level and promoting vasoconstriction, estrogen acutely decreases uterine bleeding related to asynchronous shedding [42].

Oral High-Dose Combined Hormonal Therapy

With heavy, non-emergent bleeding or when bleeding has decreased to a level consistent with heavy menses or less, an oral contraceptive therapy taper can be initiated (Table 9.5). This approach may be utilized for women with heavy bleeding in the outpatient setting who do not require hospitalization. As with intravenous estrogen, nausea is a common side effect, and oral antiemetics should be provided to optimize compliance.

Women at Increased Risk for Cardiovascular Disease or Venous Thrombosis

In women at increased risk for cardiovascular disease, venous or arterial thromboembolism, estrogen-containing oral contraceptives may be relatively or absolutely contraindicated. This includes women with a history of thromboembolic disease and woman over 35 years of age who have additional risk factors for thromboembolism (e.g., cigarette smoking, hypertension, diabetes). Although no studies have been published using short-term, high-dose intravenous or oral estrogen in these patients, at least one case of fatal pulmonary thromboembolism has been reported with intravenous therapy [43]. Certainly, high-dose estrogen therapy should be used in these patients in the acute setting only if the benefits outweigh the risks. The Medical Eligibility Criteria for Contraception provides evidence-based guidelines for use of hormonal agents in the setting of various medical comorbidities and can be an important reference in the management of high-risk patients, particularly when considering options for long-term therapy [44].

Women with a Coagulopathy

Women with menorrhagia secondary to von Willebrand's disease may be successfully treated with long-term oral contraceptive therapy, as described below. Other medical treatments used by hematologists for acute episodes include desmopressin acetate, antifibrinolytic agents, and plasma-derived concentrates of von Willebrand factor.

Long-Term Treatment of AUB

The most appropriate long-term management of AUB depends on the underlying etiology of AUB as well as a patient's reproductive desires. Women who do not desire pregnancy may initiate combined oral contraception or other hormonal therapies, such as cyclic progestins or a progestin-containing intrauterine device. Additionally, non-hormonal therapy with tranexamic acid, an anti-fibrinolytic agent, may be a therapeutic option. For anovulatory women wishing to become pregnant, ovulation induction is usually the most appropriate treatment. Two important considerations, endometrial synchronization and subclinical endometritis, deserve mention, because they may serve to optimize medical management of AUB.

Endometrial Synchronization

For women with chronic irregular bleeding, synchronizing the endometrium prior to the initiation of cyclic hormones may be a helpful first step, as synchronization can reduce breakthrough bleeding encountered with subsequent therapy. Two approaches to synchronization include the use of a potent progestin or an oral contraceptive taper. Medroxyprogesterone acetate, in the dose of 10 mg per day for 14 days, usually improves the presenting bleeding episode within 2–3 days and serves to thin the endometrial lining prior to withdrawal bleeding. Patients should be counseled that they may experience moderately heavy bleeding within 1–2 days of stopping the medroxyprogesterone. Oral contraceptives should be started on the Sunday following this withdrawal bleeding. Alternately, taper therapy with oral contraceptives (Table 9.4) can be used for women presenting with heavy prolonged bleeding.

Antibiotics for Subclinical Endometritis

While few studies have evaluated the impact of subclinical endometritis on the clinical presentation of AUB, an emerging body of literature supports a relationship between these clinical diagnoses. A recent study demonstrated that chronic endometritis is the most frequent histologic finding in endometrial biopsies performed on women with AUB [45]. In another study, 81 % of patients with irregular bleeding or vaginal discharge had positive endometrial cultures for *Mobiluncus*, and treatment with metronidazole resolved their irregular bleeding [46]. In a study of 100 hysterectomies performed for irregular bleeding or fibroids, 25 % of the endometrial cavities were found to harbor organisms, including *Gardnerella vaginalis*, *Enterobacter* and *Streptococcus agalactiae* [47]. Finally, a study of college-age women presenting with abnormal bleeding while on oral contraceptives found that 29 % were infected with *Chlamydia* [24].

These studies suggest that subclinical endometritis may impact the clinical presentation of AUB. Therefore, when apparently anovulatory AUB does not respond to standard medical management with hormonal therapy, subclinical endometritis may be a coexisting disorder to address. Cervical evaluation for common pathogens (chlamydia and gonorrhea) followed by appropriate antibiotic therapy is important. In women with negative cultures who do not respond to cyclic hormonal therapy, empiric therapy with a broad-spectrum antibiotic (e.g., metronidazole or a cephalosporin) may be considered. However, to date, prospective trials assessing the impact of treatment of clinical endometritis on AUB outcomes have not been conducted.

Ovulation Induction

Restoration of ovulation for women desiring fertility is of paramount importance. Accomplishing regular ovulatory cycles may occur with the treatment of any underlying condition responsible for anovulation. For example, in patients with hyperprolactinemia, using a dopamine agonist to normalize prolactin levels will often result in ovulation and pregnancy. In the case of PCOS, recent studies have demonstrated that insulin sensitizing agents, such as metformin, can promote ovulation (see Chap. 8). However, a recent randomized clinical trial did not demonstrate an improvement with live birth rates when adding metformin to clomiphene citrate [48].

While waiting for systemic therapies to result in resumption of ovulation and normal menses, monthly induction of withdrawal bleeding with an oral progestin should be considered to avoid ongoing AUB. In women not using combined oral contraception, the use of micronized progesterone (200–300 mg daily for 14 days) will result in reasonable withdrawal bleeding and will be safe should pregnancy occur. For patients who do not resume ovulation with systemic therapy, induction of ovulation using clomiphene citrate or injectable medications should be considered (see Chap. 8).

Oral Contraceptives

For decades, combined oral contraceptive pills have been the first-line therapy for managing AUB, and studies have demonstrated that combined oral contraceptives decrease the duration and amount of menstrual flow as well as dysmenorrhea [49]. In addition, extending the number of consecutive days of active pills and decreasing the annual number of menses may further minimize menstrual-related symptoms [50]. Extended-cycle regimens increase the risk of spotting and breakthrough bleeding when compared with standard monthly cycle regimens, but the risk generally decreases over time [51].

Progestins

Progestins represent another option for long-term management of AUB. The administration of progestins, such as 10 mg medroxyprogesterone or 300 mg micronized progesterone, daily, from day 15 to 26 of each cycle, will regulate menses in anovulatory patients. Cyclic progestin therapy represents a safe and effective approach to managing AUB and does not have the side effects or risks associated with oral estrogen. Additionally, cyclic progestin therapy provides endometrial protection against hyperplasia and cancer. Side effects of progestin therapy include mood changes or depression, nausea, breast tenderness, and bloating. Luteal phase progestin therapy has been demonstrated to be less efficacious in ovulatory AUB (menorrhagia) when compared with nonsteroidal antiinflammatory drugs (NSAIDs), tranexamic acid, or a progesterone-releasing intrauterine system [52].

Levonorgestrel-Containing Intrauterine Device

While originally developed for contraception, the levonorgestrel intrauterine device (IUD) represents a highly effective treatment of both menorrhagia and dysmenorrhea. The local release of levonorgestrel into the uterine cavity suppresses endometrial growth and has been shown to decrease menstrual blood loss by as much as 97 % [53]. While many women will experience irregular or intermenstrual bleeding in the first 6 months of use, approximately 50 % will have amenorrhea by 24 months [54]. While most of the progestin acts locally within the uterus, levonorgestrel can be detected in the systemic circulation among IUD users [55]. Therefore, other side effects, such as hirsutism, acne, weight change, nausea, headache, mood changes, and breast tenderness, may occur. Although the initial costs of the levonorgestrel IUD may be higher than other medical treatment options, they provide very cost-effective long-term therapy of AUB.

GnRH Analogues

Administration of a GnRH agonist results in pituitary downregulation, hypoestrogenemia, and complete cessation of menses. GnRH agonists initially increase ovarian stimulation, called a "flair," prior to suppression of the hypothalamic-pituitary-ovarian axis. GnRH antagonists avoid this flair, but have only recently become available, and their clinical utility remains to be determined. Symptoms of estrogen deprivation, most notably hot flashes, mood alterations, and bone loss, result from the use of all GnRH analogues. These side effects may be averted with the use of "add-back" therapy with daily administration of norethindrone 5 mg orally. While GnRH analogues play an important role in the initial management of AUB, they are rarely used for long-term therapy given the expense and side effect profile.

Tranexamic Acid

Tranexamic acid, an anti-fibrinolytic agent, is an emerging therapy for the treatment of AUB. Recent Cochrane analysis has confirmed efficacy and patient tolerance of tranexamic acid in the treatment of menorrhagia, and in Europe this medication has become the preferred treatment for women with heavy menstrual bleeding [56]. Recently, the FDA approved tranexamic acid for use in the treatment of menorrhagia. This therapy is administered orally at a dose of 1,300 mg three times daily for 5 days, initiated with onset of menses. To date, studies have not demonstrated an increased risk of venous or arterial thromboembolism [57]. However, tranexamic acid should not be concomitantly administered with combined oral contraception or in women with an increased risk of thromboembolism.

Nonsteroidal Antiinflammatory Drugs

Prostaglandins significantly impact endometrial hemostasis, and by inhibiting prostaglandin synthesis, NSAIDs serve to decrease menstrual blood loss. NSAIDs may reduce menstrual blood loss by 20–40 % [58]. While naproxen has been the most extensively studied NSAID, no member of the drug class offers distinct advantages for AUB [59]. Additionally, NSAIDs provide an effective treatment for dysmenorrhea, which is often present in those with AUB.

Surgical Treatment

For women with AUB refractory to medical management who do not desire childbearing, surgical treatment modalities, including endometrial ablation or hysterectomy, should be considered. Endometrial ablation is a minimally invasive surgical procedure that has been demonstrated to have less morbidity, shorter recovery, and greater cost-effectiveness than hysterectomy in the short term. Importantly, endometrial ablation does not provide reliable contraception, and patients who become pregnant after ablation have markedly increased risks of adverse pregnancy outcomes, such as PPROM or abnormal placentation. Therefore, reliable contraception is necessary, and permanent sterilization should be considered [60].

Hysterectomy remains a reasonable option for some women with AUB who fail medical management. As many as 20 % of women who initially undergo endometrial ablation will require hysterectomy within 5 years, and some studies have demonstrated a higher satisfaction rate in women who initially underwent hysterectomy rather than endometrial ablation [61].

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10 Menopause

James H. Liu and Francisco Arredondo

Introduction

Natural menopause occurs between the ages of 45 and 55, with a median age of 51 for women in industrialized countries [1]. Available evidence suggests that in lesser developed countries with lower socioeconomic and nutritional levels, this event takes place earlier [2, 3]. These differences in the onset of menopause support the hypothesis that menopause might not only be genetic, but rather, a biological marker echoing society's longevity.

The significant medical and technological gains of the past century in medicine, and, to some extent, the constant betterment of living standards, have yielded an improvement in life expectancy. In some countries, increases of more than 40 years of life expectancy have been observed during the last 100 years alone [4]. The life expectancy for women at birth in the United States is 80.4 years of age and is 5 years longer than men [5].

As a consequence of this demographic evolution, a large proportion of the female population will spend over one-third of their lives after the menopause. In 1950 there were 220 million women in the world over the age of 50. More than half of these women live in the so-called developed world (112 million). In 1990, there were 467 million women age 50 around the globe, and by 2030 this number is expected to approach 1.2 billion. Roughly three out of four of these menopausal women will be in the developing world (total of 912 million) [6]. At a national level, the United States Census Bureau calculated a total of 35.5 million women older than 50 during 1990, and by 1997 the menopausal population in the United States represented almost 30 % of the total female population. An estimated 6,000 US women reach menopause daily. By 2020, the menopausal population in the United States older than age 55 is expected to reach 46 million.

These demographic changes will reflect an "inverted pyramid" phenomenon in certain countries. In this scenario, the elderly to non-elderly population ratio will significantly change, with obvious challenging consequences in the economic balance between productivity and expenditure. For these reasons, understanding the physiological nature of the menopausal state, associated diseases, and its full impact to society are fundamental for health providers and health policy makers. The present chapter will aim at providing an overview of this challenge.

Menopause Physiology and Pathophysiology

The supply of primordial follicles in the female gonad is predetermined before birth and diminishes with age until it is unable to provide enough mature follicles to sustain menstrual cyclicity [7]. The peak number of germ cell count is found at 20 weeks of gestation, with decreased numbers at birth and puberty. Based on the numbers of follicles at three successive stages of development which were obtained by counting follicles in histological sections of ovaries from 52 normal women, a mathematical model was developed to describe the rates of growth and death of ovarian follicles (atresia) in human ovaries between ages 19 and 50 [8]. While the number of oocytes dwindles throughout a woman's life [9], there seems to be a transition at age 38 when the rate of follicle disappearance is augmented considerably with age. As a consequence, an estimated total of 1,500 follicles remained at 50 years of age from the 300,000 present at age 19 years. This rate of decline in small follicles appears to be responsible for ovarian failure, and therefore menopause, and the transition to midlife in our species.

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Table 10.1 Factors associated with earlier onset of menopause

Smoking
Genetic factors
Family history of early menopause
Pelvic surgery
Total abdominal hysterectomy
Unilateral oophorectomy
Metabolic factors
Type 1 diabetes mellitus
Galactose consumption
Galactose-1-phosphate uridyl transferase deficiency
Ovulation patterns
Nulliparity
Shorter menstrual cycles
Non-use of birth control pills

Recently, these concepts have been modified by Tilly and coworkers, who observed in the mouse model that there is a pool of germ cells that can proliferate and sustain oocyte and primordial follicle development in the *postnatal* mammalian ovary [10]. Moreover, in a chemotherapy-induced ovarian failure mouse model, his group was able to demonstrate that bone marrow transplantation is associated with a new population of immature oocytes [11]. Taken together, these new observations suggest that the control mechanism(s) of oocyte atresia can be modified.

Age of Menopause

The mean age of menopause in normal women in the United States ranges between 50 and 52 years [1]. This number was based on a cross-sectional study, which is associated with recall bias. However, these findings have been confirmed in a large prospective cohort study of mid-aged women: the Massachusetts Women's Health. The cohort of 2,570 women that were followed up confirmed that the median age of natural menopause was 51.3 years, with a mean difference of 1.8 years between current smokers and nonsmokers. This study also showed that smokers not only show an earlier menopause but also a shorter perimenopause [12].

In other parts of the world, population-based surveys have shown an earlier onset of menopause. For example, the median age of onset of menopause was 48 in a survey of 742 United Arab Emirates women [13]. In certain regions of India, such as the state of Himachal Pradesh, the mean age onset of menopause can be as low as 43.5 years, according to data from 500 postmenopausal women [14].

Factors Affecting the Onset of Menopause

Table 10.1 includes several factors that have been linked with an earlier onset of menopause. Among these factors, smoking has been the most often linked environmental agent to early age onset of menopause. However, these studies have been inconclusive with regard to duration and intensity of smoking. Midgette et al. concluded after a review of 14 studies that the risk of being menopausal was approximately doubled for current smokers compared with nonsmokers among women 44–55 years of age [15]. However, in 2004 van Asselt et al., using data from a Dutch population-based cohort of 5,544 women, assessed the effect of smoking duration and intensity on age at menopause correcting for the chronologic age-dependency of the variables concerned. After their modeling, they concluded, like previous researchers, that smoking lowers the menopausal age. However, the reduction in the menopausal age appears not to be dependent on smoking duration and that smoking cigarettes could have an effect only around the time of menopause itself [16]. In other words, the number of cigarettes smoked during perimenopause is apparently more significant than smoking history as the culprit of earlier age of onset of menopause among smoking women.

Women treated with total abdominal hysterectomy appear to be at risk of early menopause. Concurrent unilateral oophorectomy was associated with an even earlier onset. However, previous tubal ligation does not influence the age of menopause.

Time Course of Oocyte Pool Depletion

It is estimated that the entire process from initiation of the primordial follicle growth until complete maturation and finally ovulation is over 150 days [17]. The majority of follicles will experience atresia by apoptosis at some point in their developmental process [18]. Fifty percent of apoptosis occurs at the small antral follicle stage of 2.1–5 mm. On the other hand, the atresia of the resting follicles in the human fetus seems to be set off by a process of necrosis rather than apoptosis [19]. It seems clear now that the age-related decline in ovarian function in women is the result of the decline in both quantity and quality of the resting ovarian follicle pool. Recently, a total of 182 resting follicles from a young cadre of women (25–32 years) was compared with 81 resting follicles from the less young group (38–45 years) for signs of age-related changes by transmission-electron microscopy. De Bruin et al. concluded that, in resting follicles, the morphological changes with age are different from the changes seen in quality decline by atresia. The morphological changes with age specifically comprise the mitochondria, the dilated smooth endoplasmic reticulum, and the Golgi complex.

Genetic Contribution

The age of natural menopause is determined by the interplay of genetic and environmental factors [20]. There are some cross-sectional [21] and case–control [22] population studies suggesting the existence of genetic variability in the age of menopause to be as high as 70 %. However, a study conducted in the Netherlands in 164 mother–daughter pairs with a natural menopausal age estimated a hereditability of 44 % (95 % CI 36 %, 50 %). The authors conclude that these estimates are more accurate because previous studies that were done in twins and siblings are overestimates because siblings shared many environmental factors [23].

Stages of Reproductive Aging

Until recently, there was an absence of a relevant organized nomenclature system for the different stages of female reproductive aging. With this in mind, the Stages of Reproductive Aging Workshop (STRAW) was held in Park City, Utah, on July 23 and 24, 2001, and updated 10 years later (Fig. 10.1) [24].

Conceptually, the adult life of a woman can be divided into three major periods: reproductive, menopausal transition, and postmenopause. As is noted in Fig. 10.1 the anchor for the staging system is the final menstrual period (FMP). For clinical research purposes, five stages occur before and two after this anchor point. Stages −5 to −3 cover the reproductive period; stages −2 and −1 are the menopausal transition; and stages 1 and 2 are the postmenopause.

Among the most concrete achievements of the STRAW workshops was the development of clear and specific nomenclature that was previously vague and confusing in the literature. The authors of the STRAW workshops recognize that this is an evolving field and these concepts will change as the knowledge advances

Menopausal Transition

Stage −2 (early) and −1 (late) encompass the menopausal transition and are defined by menstrual cycle and endocrine changes. The menopausal transition begins with variation in menstrual cycle length in a woman who has a monotropic FSH rise and ends with the FMP (not able to be recognized until after 12 months of amenorrhea). For most women at these stages, day 3 follicular phase FSH levels are variable and anti-Müllerian hormone levels and antral follicle counts are low.

Postmenopause

Stage +1 (early) and +2 (late) encompass the postmenopause. The early postmenopause is defined as 5–6 years since the FMP. The participants agreed this interval is relevant because it encompasses a further dampening of ovarian hormone function to a permanent level as well as accelerated bone loss. Stage +1 was further subdivided into segment "a," the first 12 months after the FMP, and segments "b and c," the next 1–5 years. Stage +2 has a definite beginning, but its

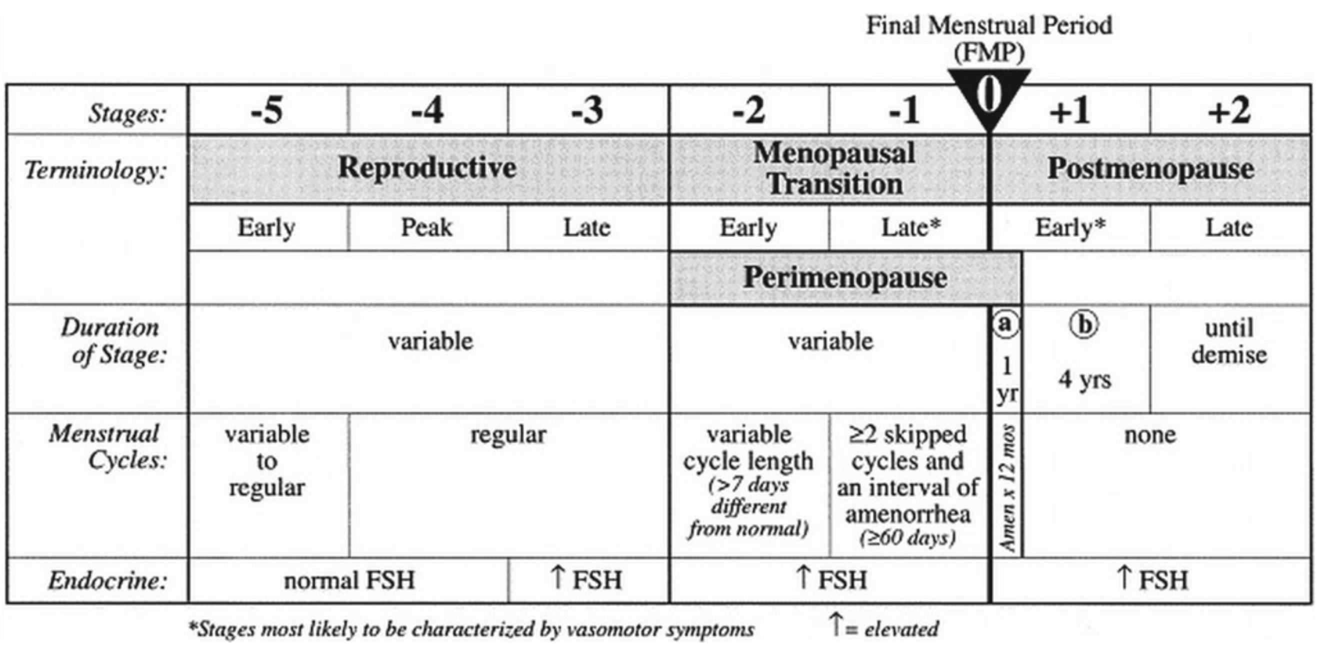

Fig. 10.1 The STRAW + 10 staging system. Reproduced with permission from Harlow SD, Gass M, Hall J, Lobo R, Maki P, Rebar RW, et al. Executive summary of stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging. Menopause 2012;19(4):387–95

duration varies, since it ends with death. Further divisions may be warranted as women live longer and more information is accumulated.

Perimenopause

Perimenopause literally means "about or around the menopause." It begins with stage −2 and ends 12 months after the FMP. "The climacteric" is a popular but vague term that we recommend be used synonymously with perimenopause. Generally speaking, the terms *perimenopause* and *climacteric* should be used only with patients and in the lay press and not in scientific papers.

Clinical Signs and Symptoms of Menopause

The diagnosis of menopause can be established when the absolute level of serum FSH is elevated. The threshold for the diagnosis of menopause will vary according to the assay employed. In any event, the level will be two standard deviations above from the normal value of a reproductive-age woman on cycle day 3. Anti-Müllerian hormone levels will typically be very low and may be undetectable. LH is of little value in the evaluation or diagnosis of menopause.

The clinical diagnosis of menopause is established retrospectively once a patient has had more than 12 months of amenorrhea in conjunction with vasomotor symptoms such as hot flashes and headaches. At this point, the patient has made the transition in the STRAW classification from −1 to +1. The range of symptoms due to immediate estrogen deficiency in women during STRAW stages –1 to +1 includes hot flashes and urogenital changes [29–31].

Hot Flashes

Vasomotor symptoms (hot flashes) are the most characteristic trait of estrogen deficiency. It is experienced at least once in 75 % of women during menopause. It is also one of the most puzzling symptoms of menopause, because the etiology and physiology remain incompletely understood [25]. It is thought to be the result of a hypothalamic dysregulation from estrogen withdrawal that culminates in peripheral vasodilatation and increase in blood flow. This results in heat loss and a decrease in core body temperature. The hypothalamic dysfunction is also manifested by simultaneous pulse of LH and presumably GnRH that is coincident with the hot flash. "Hot flash," "hot flush," "night sweats," and vasomotors symptoms are words frequently used to describe the same experience. Hot flashes are defined subjectively as the recurrent transient sensation of heat, and could be accompanied by palpitations, perspiration, chills, shivering, and feeling of anxiety. It is then followed by a heat dissipation response that habitually begins in the face, neck, chest, and often becomes generalized [26].

While menopause is the most common cause of hot flashes, there are other causes that should be considered. Fever by far is the most common cause of hot flashes, especially when coupled with a night sweat; thus, if during an episode of hot flashes the oral temperature is elevated, then the cause of the fever should be sought.

In general we can divide the potential causes of hot flashes into seven categories: systemic diseases, neurological, alcohol–medication interaction, drugs, food additives, eating, and miscellaneous [27] (Fig. 10.2). However, it is important to emphasize that these other causes are much less common than those associated with low estrogen levels.

Systemic Diseases

The most common systemic disorders associated with hot flashes are carcinoid syndrome, mastocytosis, pheochromocytoma, medullary thyroid carcinoma, pancreatic carcinoma, and renal cell carcinoma.

Carcinoid Syndrome

These patients present with neuroendocrine tumors of the bowel. Carcinoid tumors can be localized to the bronchus, pancreatic islets, retroperitoneum, liver [28], and even in the ovary [29]. It is thought these tumors probably arise from gastrointestinal or bronchopulmonary pluripotential stem cells [30]. The carcinoid syndrome clinically has a classic triad of diarrhea, flushing, and valvular heart lesions. Skin flushing is the most common sign and is present in over 90 % of patients. The mechanism of flushing is at least partially due to serotonin release, but other substances such as kinins, substance P, neurotensin, and prostaglandin may play a role [37].

Mastocytosis

Mast cell proliferations can be limited to the skin (cutaneous) or can spread to extracutaneous tissues (systematic). Vasomotor-like symptoms may be present in these patients because the mast cell granules contain a number of acid hydrolases, leukotrienes, histamine, heparin, and slow-reacting substance [31].

Pheochromocytoma

These tumors often arise from the adrenal medulla. Most of their clinical characteristics are due to the production, storage, and secretion of catecholamines. A key finding in these patients is hypertension, which is present in over 60 % of patients. A significant number of them suffer of hot flashes. Documenting increased urinary catecholamines makes the diagnosis [32].

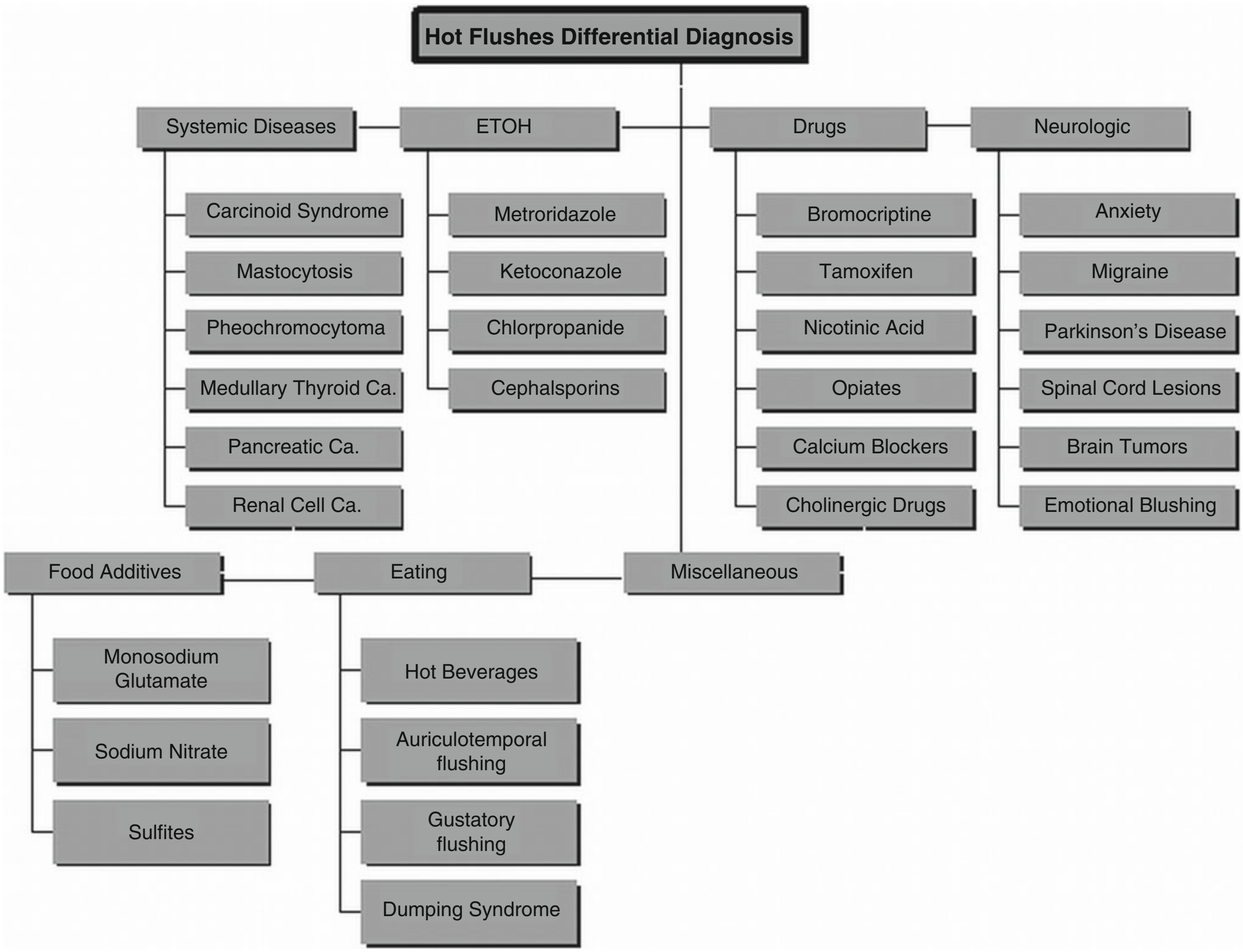

Fig. 10.2 Differential diagnosis of flushing. Adapted from [27]

Medullary Carcinoma of the Thyroid

This is a malignant tumor that originates from the parafollicular or thyroid C cells. These tumor cells typically produce an early biochemical signal (hypersecretion of calcitonin) [33]. This cancer can occur sporadically, but many times is inherited in an autosomal-dominant pattern as a part of a syndrome: multiple endocrine neoplasia type 2 [34]. Other bioactive substances that can be secreted by medullary carcinomas and may be responsible for the vasomotor symptoms include ACTH, corticotropin-releasing hormone, and prostaglandins [34].

Neurological Flushing

Anxiety or emotional blushing, migraines, Parkinson's disease, spinal cord lesions (autonomic hyporeflexia), and brain tumors can also be associated with hot flashes.

Alcohol–Medication Interaction

Alcohol and numerous drugs are associated with vasomotor symptoms. In some cases, the drug is not the real vasoactive agent, but rather, a metabolite or another mediator triggered by the drug ingested (i.e., histamine release). Some drugs will only create vasomotor symptoms when combined with alcohol [35]. Others, such as calcium channel blockers, have a direct impact in the vessels [36]. On the other hand, tamoxifen or bromocriptine produce hot flashes by triggering different mediators.

Vasodilators (nitroglycerin, prostaglandins), calcium channel blockers, nicotinic acid, opiates (i.e., morphine), amyl nitrite, cholinergic drugs, bromocriptine, thyrotropin-releasing hormone, tamoxifen, clomiphene, triamcinolone, and cyclosporine are the most commonly cited medications.

Food Additives and Eating Habits

Monosodium glutamate, sodium nitrite, and sulfites are the most common food additives associated with hot flashes. Hot beverages, auriculotemporal flushing (cheese, chocolate, lemon, highly spicy foods), gustatory flushing (chewing chili pepper), dumping syndrome (seen in patients after gastric surgery and triggered by a meal, hot fluid, or hypertonic glucose) are examples of symptoms associated with ingesting food or beverage.

The main limitation in our ability to assess the value of a treatment for hot flashes is the lack of an objective measure. This complex task is due to the current inability to reliably identify when a hot flash has taken place. The main objective method used today is sternal skin conductance monitoring, which has some limitations, but the main weakness is the failure of sternal skin conductance to provide any information on duration, intensity, and interference with patient activities. Therefore, all data are derived from imperfect methods [32].

Morbidity Associated with Hot Flashes

Sleep Disturbances

The relationship between hot flashes and interference with sleep is controversial. Multiple epidemiological studies have shown an association between awakening and arousal from sleep and hot flashes in menopausal women [37–39]. This has led to the commonly held conception that hot flashes and night sweats cause awakening, which subsequently create fatigue, and possibly decrease performance and quality of life [40]. The flaw in these studies is that these hypotheses have not been properly tested in controlled laboratory investigations. Also, neither of these investigations screened out patients with apnea and other sleep disturbances that are also prevalent in menopause and may represent a confounder factor.

Recently, Freedman et al. studied 31 patients between the ages of 46 and 51 who were classified into three groups: premenopausal asymptomatic (cycling), postmenopausal asymptomatic (asymptomatic), and postmenopausal symptomatic (symptomatic). They then assessed several outcome measures: sleep electroencephalogram recordings, sternal skin conductance to record hot flashes, multiple sleep latency test to assess sleepiness, simple and divided attention performance tests, sleep and fatigue questionnaires. There were no significant differences among the three groups on any sleep variable. Of the awakenings taking place within 2 min of a hot flash, 55.2 % happened before the hot flash, 40.0 % after the hot flash, and 5 % simultaneously. Of arousals taking place within 2 min of a hot flash, 46.7 % occurred before, 46.7 % after, and 5.6 % simultaneously. There were no significant group differences on any self-report measure or on any performance measure. They concluded that there is no evidence that hot flashes produce sleep disturbance in symptomatic postmenopausal women [41]. Although the use of estrogen will effectively relieve postmenopausal nocturnal vasomotor symptoms, sleep quality was not changed [42].

Migraines

There is sufficient observational data to suggest a link between hormones and migraines [43, 44].

However the relationships between menopause and migraine are still debated. Observational studies suggest that migraine worsens just before menopause and improves after cessation of menses in approximately two-thirds of cases.

Neri studied 556 postmenopausal women for the prevalence and characteristics of headaches and found that many had migraine with aura. Interestingly, women with prior migraine generally improved with the onset of spontaneous menopause. In contrast, women with bilateral oophorectomy usually experienced worsening of their migraines [45]. More recently, a cross-sectional community-based study of 1,436 women using the 1988 International Headache Society Criteria showed the highest prevalence of migraines in the perimenopausal group (31 %) and the lowest (7 %) in the postmenopausal group [46].

Urogenital Changes

The lack of estrogen has been associated with the onset of vulvo-vaginal atrophy with thinning of the cells lining the vulva, urethra, and vagina. Epithelial secretions decline and with time lead to dryness of the vaginal tissues. The vaginal pH will increase and the bacterial flora will change. The percentage of superficial vaginal cells will decline and basal-appearing cells will predominate. The persistent dryness of the vaginal mucosal surfaces may lead to symptoms of vaginitis, pruritus, dyspareunia, and even stenosis. Other symptoms that may be related to estrogen deprivation in the urogenital tissues are dysuria, urgency incontinence, and urinary frequency. It is unclear whether all these symptoms are related to the lack of estrogen or whether they are part of a degenerative process of aging. It is postulated that changes in estrogen levels changes the composition of the collagen content and the connective tissues in the urogenital area [47].

Data suggest that lack of estrogen increases the likelihood of menopausal women to experience recurrent urinary tract infections (UTIs). A randomized, placebo-controlled trial of vaginal estrogen in 93 postmenopausal women demonstrated that patients being treated could reduce their number of UTIs per year. This study observed 0.5 episodes of UTI in the treatment groups vs. 5.9 episodes in the placebo group [48]. On the other hand, data from the Heart and Estrogen/Progestin Replacement Study (HERS) showed that UTI frequency was higher in the group randomized to hormone treatment, although the difference was not statistically significant (odds ratio 1.16, 95 % confidence interval 0.99, 1.37) [49].

However conflicting these results are, from the clinician's point of view it seems prudent to attempt a trial of vaginal estrogen therapy to address those postmenopausal with urogenital symptoms. It should be considered in the presence of a vaginal pH greater than 4.5. Like FSH, elevated vaginal pH appears to be a good prediction of estrogen status [50, 51].

Long-Term Morbidity Associated with Postmenopausal Status

The two major long-term risks associated with menopause are osteoporosis and cardiovascular disease.

Osteoporosis

In this section, we will discuss its relationship as a long-term risk factor in menopausal women. The demographic changes and longevity increase described at the beginning of this chapter, coupled with the fact that osteoporosis rises dramatically with age, make [52] osteoporosis a serious economical burden for health care systems in our society. The estimated total direct expenditure (hospitals and nursing homes) for osteoporotic and associated fractures was $17 billion in 2001 ($47 million each day) [53]. The National Osteoporosis Foundation estimates that 50 % of white women will suffer at least one osteoporosis-related fracture in their remaining lifetime. At least 90 % of hip and spine fractures among elderly women can be attributed to osteoporosis [54]. These statistics are the result of an accelerated decline in bone-mass after menopause. However, this decline begins approximately at age 35, when bone resorption is greater than bone formation. After menopause there are two periods of net bone loss: An accelerated stage that begins with the onset of menopause (1–3 years) and continues for 5–8 years [55] (STRAW 0, 1, and 2) and a prolonged, slower stage of bone loss that remains throughout STRAW 2. The initial accelerated phase may account for bone loss of up to 30 % [56].

The three most common osteoporotic-related fractures are hip, vertebral, and wrist. The most common are vertebral fractures, accounting for 700,000 cases a year in the United States [60]. These should be suspected in postmenopausal women with back pain, loss of height, and kyphosis. In one observational study of 7,223 postmenopausal women over 65 years of age, patients with radiographically detected vertebral fractures were found to have significantly more limited-activity days, whether they were symptomatic or not [57]. These data should raise awareness for the clinician of the decreased quality of life that menopausal patients may experience even with asymptomatic fractures.

The second most common fracture is hip fracture, accounting for 300,000 cases a year in the United States [60]. These are without doubt the more serious consequence of osteoporosis in postmenopausal women. One in five women will die within 1 year postfracture and one in two will have permanent loss of function [58]. Lastly, distal forearm fractures occur in 250,000 patients a year in the United States [60]. Only half of the patients that suffer these fractures recover full function of the arm in 6 months [59].

Cardiovascular Disease

The American Heart Association has designated cardiovascular disease a "silent epidemic." Despite the overall decline in the mortality rate due to cardiovascular disease in the United States, the absolute number of deaths due to cardiovascular disease is actually increasing [60], in part due to the demographic changes in our society described in the introduction of this chapter. Cardiovascular disease that encompasses heart attacks and strokes combined are responsible for more deaths than all other causes combined in postmenopausal women [61, 67]. The burden and threat of this disease during menopause is in part due to the lack of perception of its magnitude by both physicians and patients. Nothing exemplifies this better than a 1995 Gallup survey, which revealed that four out of five women aged 45–75 were unaware that cardiovascular disease was the first cause of death for their age group. Instead, most of the women quoted cancer, specifically breast cancer, as their most probable cause of death. In reality this represents only 4 % of the causes of death in this age group. The primary care physicians questioned did not do much better. Thirty-two percent were unaware that heart disease was the main cause of death in this age group of women [62].

The incidence of cardiovascular disease and particularly myocardial infarction dramatically increases after menopause and approximates the mortality of this entity in men [63, 64]. Furthermore, bilateral oophorectomy or premature ovarian failure increases the risk of cardiovascular disease beyond that of natural menopause [65]. Despite this seemingly logical association between estrogen cardioprotection and other coherent data from observational studies, the Women's Health Initiative group of trials and the HERS have found no role for estrogen as a primary or secondary prevention for cardiovascular disease in postmenopausal women.

Substantive evidence from epidemiological studies and clinical research indicate that the best tools are preventive measures and lifestyle habit modifications: smoking cessation, blood pressure control, lowering cholesterol, and promoting exercise.

Medical Treatment of Menopause

The results of the Women's Health Initiative study (WHI) have altered the principles of medical practice in menopausal women. We have changed from the concept of prevention of chronic diseases encompassed in the term "hormone replacement therapy" to the concept of "hormone therapy." Thus, the US Food and Drug Administration (FDA) and professional organizations such as the American College of Obstetricians and Gynecologists recommend that the use of estrogenic-containing medications be restricted to the treatment of vasomotor and vaginal symptoms. They also affirm that the lowest effective dose be prescribed for the shortest duration of time [66–68].

More detailed analysis of the WHI results has suggested a more individualized approach to hormone therapy. For this reason, the North American Menopause Society and the Endocrine Society have modified their guidelines for hormone therapy in 2012 [69]. Current evidence suggests that the absolute risks of estrogen therapy between the ages of 50 and 59 are low and the individual benefit may favor a longer duration of therapy. In contrast, the duration of use of estrogen with progestin should be limited because of the increased risk of breast cancer associated with 3–5 years of use.

Principles of Hormone Therapy

During the last 3 decades, the clinical opinion on the use of estrogen during menopause has changed drastically. Initially, estrogen was recommended as a short-term treatment for menopausal symptoms. Later, on the basis of observational studies, estrogen was given for long-term prevention of heart disease and an improved quality of life. The Women's Health Initiative Hormone Therapy trial, however, demonstrated that estrogen was not effective for the prevention of cardiovascular disease.

Key Findings from the Women's Health Initiative

The WHI was a group of clinical trials designed to examine the impact of hormone therapy on cardiovascular disease and breast cancer, the effect of low-fat diet on breast and colon cancer, and the impact of vitamin D in calcium supplementation on fractures and colon cancer [70].

These trials included:

- A randomized controlled trial of 16,608 asymptomatic postmenopausal women ages 50–79 years with a uterus comparing conjugated estrogens (0.625 g) and a progestin, medroxyprogesterone (MPA) (2.5), daily vs. placebo. The primary outcome measure of this trial was coronary heart disease (CHD) and breast cancer. The secondary outcome measures were stroke, congestive heart failure, angina, peripheral vascular disease, coronary revascularization, pulmonary embolism, deep venous thrombosis, ovarian cancer, endometrial cancer, hip fractures, diabetes mellitus requiring therapy, death from any cause, and quality-of-life measures
- A randomized controlled trial on 10,739 asymptomatic postmenopausal women 50–79 years without a uterus (hysterectomized) comparing conjugated estrogens (0.625 mg/day) vs. placebo
- A dietary modification randomized controlled trial of 48,837 postmenopausal women 50–79 years to either sustained low fat (20 %) or self-determined diet. The primary outcome measures were breast and colorectal cancer. The secondary outcome measures included stroke, congestive heart failure, angina, peripheral vascular disease, coronary revascularization, ovarian cancer, endometrial cancer, hip fractures, diabetes mellitus requiring therapy, and death from any cause
- A calcium/vitamin D supplementation diet trial of 38,282 postmenopausal women in which the primary outcome measure was hip fractures and the secondary outcome measures were death from any cause, breast and colon cancer
- A cohort observation group of 93,676 postmenopausal patients

In May of 2002 the clinical trial that aimed to assess the cardiovascular effects of estrogen and progestin therapy in postmenopausal women with intact uterus was halted. The Data and Safety Monitoring Board reported that the estrogen/progestin treatment group had an increased risk in cardiovascular disease, thromboembolism, and breast cancer after 5.2 years of follow-up [71]. In 2004, after 6.8 years of follow-up, the estrogen-alone trial was halted [72]. In this clinical trial, estrogen-only treatment demonstrated an increase risk in strokes similar to the one found in the estrogen–progestin clinical trial previously halted. There was also reported a lack of benefit on cardiovascular disease incidents and a probable increase in dementia. Surprisingly, the breast cancer risks in the estrogen treatment group were lower than the placebo. The risks and benefits findings of the WHI are summarized in Table 10.2 [73].

It should be emphasized that the WHI trial did not intend to evaluate the effects of estrogen or estrogen–progesterone on vasomotor symptoms because hot flashes were not the major complaint among the majority of subjects. Therefore, these results must be translated into the specific needs of our patients when they request relief for hot flashes or other postmenopausal symptoms. It is also critical to point out that the serious adverse events in patients treated with estrogen therapy is low and calculated to be 2 out of 1,000 women treated per year [74]. Health care providers and patients must balance the benefits of estrogen treatment vs. the absolute risk for adverse events. Today, more than ever, the concept of

Table 10.2 Women's Health Initiative Findings: outcomes associated with use of combined estrogen and progestin and estrogen alone in healthy postmenopausal women, aged 50–79 years

	Estrogen and progestin		Estrogen[a]	
Outcome	RR (95 % CI)	Average Absolute Risk difference[b]	RR (95 % CI)	Average Absolute Risk difference[b]
Cardiovascular				
Deep venous thrombosis	2.07 (1.49–2.87)	13	1.47 (1.04–2.08)	6
Pulmonary embolism	2.13 (1.39–3.25)	8	1.34 (0.87–2.06)	11
Coronary heart disease	1.24 (1.00–1.54)	7	0.91 (0.75–1.12)	–5
Ischemic stroke	1.44 (1.09–1.90)	8	1.39 (1.10–1.77)	12
Cancer				
Breast	1.24 (1.02–1.50)	8	0.77 (0.59–1.01)	–7
Colorectal	0.63 (0.43–0.92)	–6	1.08 (0.75–1.55)	1
Ovarian	1.58 (0.77–3.24)	8	NYR	NYR
Endometrial	0.81 (0.48–1.36)	–4	N/A	N/A
Other				
Probable dementia[c]	2.05 (1.21–3.48)	23	NYR	12
All fractures	0.76 (0.69–0.83)	–44	0.70 (0.63–0.79)	–56
Hip fractures	0.67 (0.47–0.96)	–5	0.61 (0.41–0.91)	–6
Mortality	0.98 (0.82–1.18)	–1	1.04 (0.88–1.22)	+3

RR relative risk compared to placebo; *CI* confidence interval; *N/A* not applicable (hysterectomized women); *NYR* not yet reported
[a]Hysterectomized
[b]Annual per 10,000 women
[c]Women aged 65–79 years

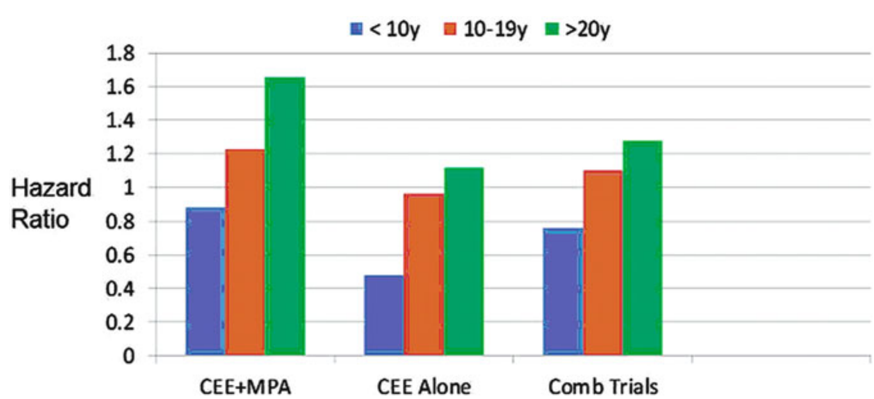

Fig. 10.3 Hazard ratio of cardiovascular risk for estrogen alone and estrogen with progestin relative to placebo controls in the WHI. Created with data from [75]

individualized menopausal care should be applied in the clinical setting.

The WHI investigators have performed a secondary, stratified analysis of the cardiovascular risk by age. These data suggest that estrogen or estrogen with progestin have potential cardiovascular benefits if started early in the menopause (aged 50–55 or less than 10 years from menopause), whereas those started on hormone therapy after 60 or more than 10 years from menopause are at increased risk (Fig. 10.3). These newer findings are in keeping with observational studies and other cardiovascular preventive trials. Taken together, these data suggest that there is a "window or time" of opportunity for use of estrogen therapy and it has been termed the "timing hypothesis" [75, 76]. Studies to test this hypothesis are ongoing.

The WHI hormone trial is the first randomized controlled trial that demonstrates that estrogen actually decreases the risk of fractures in a low-risk population. However, when all the risks and benefits are weighted, it can be concluded in this study that estrogens are not indicated as an overall preventive measure in postmenopausal women and the potential harm outweighs the potential long-term benefit. Thus, at the present time, use of estrogen should be limited to the treatment of symptomatic menopausal women for the shortest possible time at the lowest dose. Table 10.3 shows a comparison between the WHI and HERS trials.

Table 10.3 Impact of estrogen–progestin treatment on cardiac events in a healthy menopausal population (WHI) vs. a population with a prior history of cardiac events (HERS)

WHI-CHD by year of follow-up				HERS-risk of cardiac events		
Year	Estrogen–progestin $n = 8{,}000+$	Placebo	Hazard ratio and confidence interval	Estrogen–progestin $n = 1{,}383$	Placebo $n = 1{,}380$	Relative hazard (risk) and Confidence Interval
1	42 cases	23 cases	1.81 (1.09–3.01)	57 cases	38 cases	1.52 (1.01–2.29)
2	38	28	1.34 (0.82–2.18)	47	48	1.00 (0.67–1.49)
3	19	15	1.27 (0.64–2.50)	35	41	0.87 (0.55–1.37)
4	32	25	1.25 (0.74–2.12)	33	49	0.67 (0.43–1.04)
5	29	19	1.45 (0.81–2.59)			1.06 (0.69–1.62)
>6	28	37	0.70 (0.42–1.14)			0.98 (0.72–1.34)

Key Findings of the Heart and Estrogen/Progestin Replacement Study

While the WHI aimed to test the hypothesis that hormone therapy prevented cardiovascular disease in healthy postmenopausal women (primary prevention), the HERS intended to evaluate whether hormone therapy decreased the risk of CHD in postmenopausal women with established coronary disease. In this randomized trial, all the 2,763 postmenopausal women had uteruses, and were allocated to either placebo ($n = 1{,}383$) or 0.625 mg of conjugated equine estrogens plus 2.5 mg of MPA daily ($n = 1{,}380$) [77, 78]. The primary outcome measures were (1) nonfatal myocardial infarction and (2) CHD death.

The results of HERS trial have been reported in two publications, HERS and HERS II. The HERS report is the result of randomized, blinded, placebo-controlled trial for 4.1 years and the HERS II reflects the unblinded follow up for 2.7 more years [84, 85]. Both of the studies demonstrated that in patients with established heart disease, the use of estrogen and progestin does not prevent additional cardiovascular events. There were no differences in the primary or secondary outcomes of patients in the placebo or the treatment group.

The conclusion of HERS and HERS II studies is that postmenopausal hormone therapy should not be recommended for the purpose of reducing the risk of cardiovascular events.

Key Findings the Million Women Study

The Million Women Study is a prospective observational study that included 1,084,110 British women aged 50–64 recruited between 1996 and 2001. This study was undertaken by the UK National Health Service Breast Screening Programme targeting women ages 50–64 undergoing routine screening once every 3 years. Because approximately only half of these patients had ever taken estrogen after menopause, the aim of the study was to investigate the relation between various combinations of hormone therapy and two main outcomes: breast cancer and mortality [79]. All patients filled out a questionnaire and were monitored in this manner. This questionnaire is available at http://www.millionwomenstudy.org. The major strength of this study was the unparalleled database size, which was sufficiently powered to quantify absolute and relative risks and enabled researchers to discern the effects among different preparations of hormones in use among postmenopausal women. One weakness of the study was that hormone use vs. nonuse was determined on admission to the study and was not modified during follow-up, even though there were potential multiple crossover treatments in some of the subjects. The authors reached these conclusions:

- Current use of hormone therapy is associated with an increased risk of incident and lethal breast cancer
- The risk is substantially greater for estrogen–progestin combinations of postmenopausal hormone therapy
- The mortality rate due to breast cancer was 27 % less in hormone users than in non-hormone users. This is could be explained by in part by more frequent medical care and early detection

The relative risks for invasive breast cancer in relation to current of use of hormone therapy and type of hormone preparations are illustrated in Figs. 10.4 and 10.5.

Candidates for Hormone Therapy During Menopause

It is clear that the treatment paradigm has shifted when it comes to whom should we recommend the use of estrogens during menopause. After reviewing the evidence in the randomized controlled trials, it seems prudent to state that estrogen should not be used to prevent long-term chronic diseases such as cardiovascular disease and dementia.

Nevertheless, patients with moderate to severe vasomotor symptoms should weigh the risk and benefits and might consider using estrogen. It seems that the group of patients with vasomotor symptoms and osteoporosis or at risk for osteoporosis are good candidates to consider prescribing estrogen. Estrogen is the most effective treatment to alleviate vasomotor symptoms and has been shown to reduce the fracture risk in postmenopausal women.

HRT use at baseline	Cases/population	Relative risk (95% FCI)*
Never users	2894/392 757	1.00 (0.97–1.04)
Current users	3202/285 987	1.66 (1.60–1.72)
Last use <5 years previously	579/81 875	1.04 (0.95–1.12)
Last use 5-9 years previously	207/28 395	1.01 (0.88–1.16)
Last use >10 years previously	79/12 568	0.90 (0.72–1.12)

x^2 for heterogeneity between ever users=161.5, p<0.0001

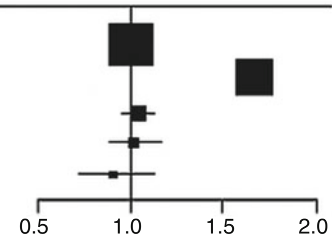

Fig. 10.4 Relative risk of incident invasive breast cancer in relation to recency of use of HRT. *FCI* floated CI. *Relative to never users, stratified by age at first birth, family history, of breast cancer, body-mass index, region, and deprivation index. Reprinted from The Lancet, 362, Breast cancer and hormone-replacement therapy in the Million Women Study, 419–27, Copyright 2003, with permission from Elsevier

HRT use at baseline	Cases/population	Relative risk (95% FCI)*
All never users	2894/392 757	1.00 (0.96–1.04)
All past users	1044/150 179	1.01 (0.95–1.08)
Current users of:		
Oestrogen only	991/115 383	1.30 (1.22–1.38)
Oestrogen-progestagen	1934/142 870	2.00 (1.91–2.09)
Tibolone	184/18186	1.45 (1.25–1.67)
Other/unknown types	93/9548	1.44 (1.17–1.76)

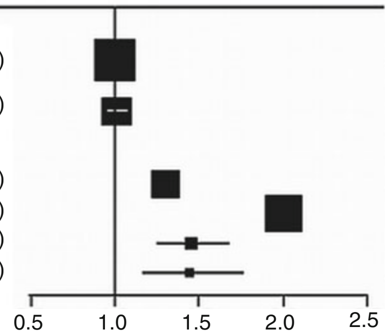

Fig. 10.5 Relative risk of incident invasive breast cancer in relation to recency and type of HRT used. *FCI* floated CI. *Relative to never users, stratified by age at first birth, family history, of breast cancer, body-mass index, region, and deprivation index. Reprinted from The Lancet, 362, Breast cancer and hormone-replacement therapy in the Million Women Study, 419–27, Copyright 2003, with permission from Elsevier

For these reasons, medical management of menopausal symptoms has evolved to a personalized approach. Personal choice is important and some will never take hormone therapy under any circumstances. The physician must consider many variables before offering hormone therapy in light of individual cardiovascular, osteoporosis, and breast cancer risk and the severity of estrogen-deficient symptoms.

General Principles of Drug Therapy

For patients on hormone replacement therapy, there are two important concepts that must be discussed. First, is the projected duration of therapy. Professional societies such as the North American Menopause Society and the Endocrine Society suggest that the lowest effective dose be utilized consistent with the treatment goals. If it is for the treatment of vasomotor symptoms, it could be for a limited period of time. The duration of therapy may vary depending on the individual. Those begun on hormone therapy earlier in the menopause (prior to age 55) appear to have a cardiovascular benefit. Secondly, the effectiveness of therapy should be quantified. Typically, this consists of assessing symptoms and signs of hypoestrogenemia and monitoring bone mineral density.

The choice of drug regimen should be based on the specific needs of the patient. For example, a breast cancer patient with severe genitourinary atrophy who has failed nonhormonal regimens may benefit from a locally applied estrogen medication that has minimal systemic absorption, provided this is with the acquiescence of her oncologists.

Hot flashes can be controlled with continuous estrogens rather than cyclic. If the hot flashes are not controlled, a progestin can be added if this has not already been used. Increased metabolism of estrogen by concomitant use of anti-seizure drugs should be considered and substitution with a transdermal delivery with reduced hepatic metabolism should be considered. Patients that do not or cannot use estrogens can be offered a selective serotonin reuptake inhibitor.

All patients with a uterus should be given a progestin. Table 10.4 shows the most common estrogen–progestin regimens. The progestin can be given cyclically, such as the first 12–14 days of each month or continuously (daily). The majority of patients on cyclic therapy will have a monthly withdrawal bleed. The continuous regimen will induce irregular bleeding initially but will eventually result in

Table 10.4 Common estrogen and progestin treatment regimens

Regimen	Bleeding pattern	Possible side effects
E days 1–30, cyclic P days 1–12	Bleeding ~ day 14 or 15	Breast tenderness, mood disturbances, headaches
E days 1–25, cyclic P days 14–25	Bleeding ~ day 27 or 28	Same as above, may have hot flashes days 27–30
Long cycle (low-dose E daily, P every 3 months for 14 days	Occasional spotting, with quarterly withdrawal bleeding	Same as above, but on a quarterly basis

amenorrhea. Patients with a uterus require endometrial monitoring to detect possible hyperplasia or cancer. With cyclic regimens, bleeding occurs after10–12 days of progestin therapy. Unscheduled bleeding or a change in the pattern of bleeding warrants investigation. A transvaginal ultrasound should be performed. If the endometrial thickness is less than 5 mm, endometrial cancer is ruled out. If the endometrial thickness is 5 mm or greater, an endometrial sampling should be performed. If the thickness is less than 5 mm but bleeding persists or recurs, then endometrial sampling should also be performed.

Absolute contraindications to estrogens include acute venous thrombosis, pulmonary embolism, cardiovascular or liver disease. Untreated endocrine-sensitive tumors and undiagnosed vaginal bleeding are also contraindications. Relative contraindications include the chronic forms of the previous mentioned conditions as well as uncontrolled hypertension and hypertriglyceridemia. Some traditional relative contraindications, such as seizure disorders, migraines without scotomata, gallbladder disease, or myomas, are a matter of debate. Patients with a personal or family history of venous thromboembolism should be screened for a possible thrombophilia before starting hormone replacement therapy.

Hormone Preparations for Menopausal Therapy

The use of hormone therapy has some documented increased risks including stroke, breast cancer, CHD, and venous thromboembolism. Nevertheless, this does not mean that postmenopausal hormone therapy must never be used. Postmenopausal women with severe vasomotor symptoms, vaginal dryness, and/or other symptoms that decrease the quality of life continue to be appropriate indications in the absence of contraindications such as history of coronary artery disease, thrombophilia, and history of thromboembolism. Here we will discuss the different estrogen preparations to address menopausal symptoms.

The concept of "bioidentical" has been promoted by the public and some practitioners. This refers to the use of steroids that are naturally found in humans and sometimes to the concept of individualizing the doses by specifically compounding a product. There are no data to support the superiority or the perceived safety of this approach.

Systemic Estrogen Therapy

Systemic absorption can occur with oral, transdermal, or vaginal preparations. All estrogens are metabolized in the liver but the oral forms will have a dominant "first pass effect" that is manifested by elevated binding globulins, triglycerides, and clotting factors. Estrogen therapy may increase thyroxine requirements due to the induced hepatic production of thyroid-binding globulin.

Oral Estrogen

There are several preparations in the oral form of estrogen. The most commonly used preparation is (Premarin) (Table 10.5). This compound of conjugated equine estrogens derived from pregnant mare's urine consists mostly of estrone sulfate, equilin sulfate, dihydroequilin sulfate, and many other minor estrogenic compounds. There are also synthetic conjugated synthetics such as Cenestin that are derived from plant sources. Esterified estrogens, such as Estratab or Menest, are derived from plant sources as well. Ethinyl estradiol is found in oral contraceptives but also in one product for hormone therapy called Femhrt. Estrace is a micronized form of estradiol. As described in Table 10.5, there are many options available with different dosages and potencies. However, there is no major difference in their efficacy provided that equivalent doses are used [80].

Lower-dose regimens have been approved by the FDA for the treatment of vasomotor symptoms and osteoporosis and include Prempro 0.45 mg conjugated estrogen/1.5 mg MPA, and Prempro 0.3 mg/1.5 mg. Trials with these combination estrogen–progestin regimens show no evidence of endometrial hyperplasia in women treated regardless of the dose [81]. These clinical trials have also demonstrated that low doses of estrogen are adequate for the prevention of bone loss. It is important to emphasize a basic tenet of hormone therapy that the lowest dose should be used to achieve the desired clinical response.

Transdermal Estrogen

All transdermal systems currently available contain the same estrogen: 17ß-estradiol at different dosages. A dose of 50 µg/day in a transdermal delivery system is bioequivalent to an oral dose of 0.625 of conjugated equine estrogens [82]. The transdermal system delivery with the lower dose that has been approved for prevention of osteoporosis only has 0.014 mg/day of estradiol (Menostar) [83].

Table 10.5 Estrogen products, routes of administration, and available doses

Product	Available doses
Oral	
Synthetic conjugated estrogens (Cenestin)	0.3 mg, 0.625 mg, 1.25 mg
Equine conjugated estrogens (Premarin)	0.3 mg, 0.45 mg, 0.625 mg,
	0.9 mg, 1.25 mg, 2.5 mg
Micronized estradiol (Estrace, Gyndodiol)	0.5 mg, 1.0 mg, 2 mg
Esterified estrogens (Menest)	0.3 mg, 0.625 mg, 1.25 mg
	2.5 mg
Estropipate (Ogen, Ortho-Est)	0.625 mg, 1.25 mg, 2.5 mg
Transdermal systems	
17-beta estradiol (Estraderm, Vivelle, Alora Climara, Esclim, Menostar)	0.025 mg, 0.0375 mg, 0.05 mg
	0.075 mg–0.1 mg/day
	14 μg/day
17-beta estradiol plus	0.045–0.05 mg/day plus
norethindrone acetate or	0.14, 0.15, 0.25 mg/day
Levonorgestrel (Combipatch, Climara Pro)	
Topical	
17-beta estradiol (Estrasorb, EstroGel)	3.48 g/day delivers 0.5 mg/ estradiol/day
	1.25 g/day delivers 0.75 mg/ estradiol/day
Injectable	
Estradiol valerate in oil (Delestrogen)	10–40 mg/mL
Estradiol cypionate in oil (Depo-Estradiol)	5 mg/mL
Vaginal	
Tablets (Vagifem)	10, 25 μg estradiol/tablet
Creams	
Estrace	0.1 mg ethinyl estradiol/g
Premarin	0.625 conjugated equine estrogen mg/g
Ogen	1.5 mg estropipate/g
Rings	
Estring	Estradiol 2 mg/3 months/ring (7.5 μg/day)
Femring	Estradiol 0.05 mg to 1 mg/day for 90 days (systemic absorption)

Topical Estrogen

There are multiple products: Estrogel, Divigel, Elestrin, Evamist, and Estrosorb. A randomized controlled trial of 225 menopausal women demonstrated that a topical gel containing 1.25 or 2.5 g of gel containing 17ß (beta)-estradiol was much more effective than placebo in alleviating the frequency and intensity of moderate and severe hot flashes in symptomatic women [84].

Vaginal Estrogen

When the target organ of the hormonal preparation is the urogenital tissue, vaginal route of administration seems to be the most appropriate way to provide relief to symptomatic postmenopausal women. The safety and efficacy of the vaginal delivery have been widely studied, and there are cream, tablets, and ring preparations available in the market (see Table 10.4). The Estring vaginal ring delivers local estradiol without significant estrogen absorption. It is effective for 3 months. Other intravaginal products such as Femring release estradiol at a rate of 50 μg/day, which will have systemic absorption that necessitates consideration of an endometrial effect.

There are some general rules of equivalence between these products. Ethinyl estradiol 5 μg, 0.625 mg of conjugated or esterified estrogens, 2 mg of micronized estradiol, and 0.05 mg of a transdermal estradiol appear to be equivalent.

Selective Estrogen Receptor Modulators

New pharmaceutical agents to treat or prevent chronic diseases such as osteoporosis are being developed. The mixed estrogen agonist–antagonist action such as selective estrogen receptor modulators (SERMs) have been developed to specifically target tissues such as bone, vaginal tissues, and their selective binding with estrogen receptors makes them a good option for some patients [85]. There are many reasons why SERMs produce the tissue-selective effects such as differences in receptor binding affinities and interaction with different coactivators and corepressors that are present in target tissues [86].

Tamoxifen

Tamoxifen is a SERM that may act as an estrogen receptor agonist (i.e., uterus) or as an antagonist (i.e., breast) [87]. As a result, tamoxifen can have beneficial effects for osteoporosis, reduction in breast cell proliferation, and cardiovascular disease. Unfortunately, tamoxifen also stimulates the endometrial proliferation and increases vasomotor symptoms.

In the largest prevention-oriented randomized clinical trial aimed to test the hypothesis that tamoxifen reduces the incidence of breast cancer in patients at high risk for the disease, fracture risk was determined as a secondary endpoint. In The National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 Trial, 13,338 women were randomized to placebo or 20 mg of tamoxifen a day for 5 years. The trial demonstrated a significant decrease in the incidence of invasive and noninvasive breast cancer, and a decrease in the incidence of vertebral, wrist (Colle's), and hip fractures in the treatment group when compared with the placebo. In this same study, patients in the tamoxifen group did not experience a higher rate in ischemic heart disease. However, the risk for endometrial cancer, especially in women over 50 years of age, was increased (risk ratio = 2.53; 95 % confidence interval = 1.35–4.97) with all of the endometrial cancers detected in early stage (I) [88].

Raloxifene

Raloxifene was discovered over 20 years ago in an effort to develop an antiestrogenic drug to treat and/or prevent breast cancer and was previously known as keoxifene [89, 90]. Raloxifene reduced the risk of osteoporosis in postmenopausal women without increasing their risk for uterine cancer or endometrial hyperplasia [91, 92]. Raloxifene at 60 mg/day is currently approved for the prevention and treatment of osteoporosis.

Some clinicians prefer to use bisphosphonates as a first option because of their more potent antiresorptive activity than raloxifene [93]. A comparative trial compared the two classes of drugs. In the "EFFECT" trial (*EF*ficacy of *F*OSAMAX vs. *E*VISTA *C*omparison *T*rial), patients in the alendronate group were found to have substantially greater increases in bone mineral density (BMD) than raloxifene at both lumbar spine and hip sites at 12 months. Lumbar spine BMD increased 4.8 % with alendronate vs. 2.2 % with raloxifene ($P<0.001$). The increase in total hip BMD was 2.3 % with alendronate vs. 0.8 % with raloxifene ($P<0.001$). Decreased bone turnover was more significant with alendronate than raloxifene. Overall tolerability was comparable. However, the number of patients reporting vasomotor symptoms was considerably higher with raloxifene (9.5 %) than with alendronate (3.7 %, $P=0.010$), while an equal number of patients reported adverse gastrointestinal side effects [92].

Besides the positive impact in intermediate outcome measures such as BMD and bone markers, raloxifene has been proven to reduce the risk of osteoporotic vertebral fractures. In the MORE trial (Multiple Outcomes of Raloxifene Evaluation), a multicenter, international, double-blind placebo controlled trial, 7,705 women aged 31–80 years in 25 countries who had been postmenopausal for at least 2 years were randomized to either of three groups: 60 mg/day, 120 mg/day of raloxifene, or to placebo. After 36 months of follow-up, the risk of vertebral fracture was reduced in both study groups receiving raloxifene (for 60 mg/day group: relative risk [RR], 0.7; 95 % confidence interval [CI], 0.5–0.8; for 120-mg/day group: RR, 0.5; 95 % CI, 0.4–0.7) [94]. This study also demonstrated that the rate of breast cancer was less common in the treatment arms that in the placebo. Thirteen cases of breast cancer were confirmed among the women assigned to raloxifene vs. 27 among the women assigned to placebo (relative risk [RR], 0.24; 95 % confidence interval [CI], 0.13–0.44; $P<0.001$) [95]. Like the tamoxifen P-1 Trial, the Study of Tamoxifen and Raloxifene (STAR) P-2 Trial demonstrated that raloxifene was as effective as tamoxifen with a reduction in breast cancer risk of 50 %. Women on raloxifene had 36 % fewer uterine cancers and 29 % fewer blood clots [96].

Raloxifene studies have also shown improvement in some intermediate cardiovascular biomarkers such as serum LDL, lipoprotein (a), homocysteine, and plasma fibrinogen [97, 98]. To test the hypothesis that raloxifene reduces risk of coronary events (coronary death, nonfatal myocardial infarction [MI], or hospitalized acute coronary syndromes other than MI) the Raloxifene Use for the Heart (RUTH) trial was begun in 1998. A total of 10,101 menopausal women were randomized to raloxifene 60 mg/day or placebo and followed for a median for 5.6 years. Raloxifene did not alter the risk of CHD [99].

Tibolone

Tibolone is a synthetic steroid that has shown to be effective in alleviating menopausal symptoms and preventing bone loss. It has been widely used in Europe and the rest of the world since 1988 but is not available in the United States. Tibolone has estrogenic actions in the brain, vagina, and bone tissues, but lacks estrogenic activity in the endometrium and in the breast tissue. Its multifaceted hormone properties appear to be due to its rapid conversion into three metabolites: 3α(alpha)- and 3β(beta)-hydroxy-tibolone, each with estrogenic effects, and the Δ(delta)^4-isomer, with progestogenic and androgenic effects. The tissue-selective actions of tibolone are the product of metabolism, enzyme control, and receptor activation that vary in responsive target tissues. This biotransformation takes place principally at the liver and intestine. These pharmacological characteristics make tibolone unique and different from the typical SERMs [100].

More recently, an even lower dose of tibolone was evaluated in an RCT. This study included 90 postmenopausal women who were followed for 2 years and were allocated to tibolone 2.5 mg ($n=30$), tibolone 1.25 mg ($n=30$), and a control group ($n=30$). All subjects received 1,000 mg of calcium per day. Gambacciani et al. demonstrated tibolone to be effective at lower doses not only at preventing bone loss as measured by BMD but also was successful at alleviating vasomotor symptoms [101].

Another interesting potential benefit of tibolone on postmenopausal women is the positive effect shown on libido in some clinical trials. In a small randomized trial, patients with postmenopausal changes in sexual desire were allocated to 2.5 mg/day of tibolone ($n=14$) or to 500 mg/day of calcium [102]. This trial showed that the patients treated with tibolone experience an improvement in sexual desire after the third month of treatment, which was maintained until the end of treatment (12 months) [101]. Similar beneficial findings in sexual desire were shown in a small study of 50 postmenopausal patients that were treated with either tibolone or conjugated estrogens and medroxyprogesterone [103].

In the large cohort Million Women Study, there was increased relative risk of breast cancer in current users of tibolone (RR 1.45, 95 % confidence intervals 1.25–1.68) [104]. These results were not anticipated, since tibolone does not increase breast density and has been shown to have much less estrogenic activity in breast tissues than other agents in postmenopausal clinical trials [105, 106].

In summary, clinical data support the use of tibolone as a viable option for hormone therapy during menopause. Its versatile actions in different target tissues have demonstrated to have beneficial effects for the relief of vasomotor symptoms and treatment of osteoporosis. There is suggestive evidence that tibolone may positively impact libido in postmenopausal women. Unfortunately, there are few randomized trials, and there is lack of the long-term clinical evidence of tibolone benefits on cardiovascular and neurological systems.

Androgens

The use of androgens for postmenopausal women is the subject of much debate. Reduced androgen levels in the menopausal women are due in part to a gradual decline of adrenal precursors such as DHEA and DHEAS with a further decline in ovarian production of testosterone. Combinations of esterified estrogens and methyltestosterone have been used for decades for the treatment of severe hot flashes especially in younger, surgical menopause women [107], whereas the use of methyltestosterone alone at low doses did not relieve hot flashes [108]. Currently, no combination estrogen–androgen products are available in the United States.

Testosterone is the key sex steroid implicated in the mediation of sexual desire and coital frequency in both men and women. In contrast to women after surgical menopause, women undergoing natural menopause do not experience an abrupt decline in testosterone levels [109]. The production of testosterone from the menopausal ovary will gradually decline with aging. The concept of sexuality in women is complex and not directly linked to levels of serum androgens. Nonetheless, there have been many clinical trials that have assessed the use of testosterone. In a recent cross-sectional cohort study of 2,311 perimenopausal women, the Study of Women Across the Nation (SWAN), longitudinal data indicate that total testosterone levels declined from baseline by −12.8, −21.4, −22.4, and −26.4 % during the initial 4 years of follow-up. Other adrenal-derived, less potent androgens such as DHEA sulfate did not appreciably change [110]. Taken together, these observations suggest that an androgen deficiency state may not be unique to women with surgical menopause but rather the decline in androgens may be clinically significant in women who are undergoing the natural transition into the menopause. What remains unclear is whether these inevitable physiological changes in circulating testosterone concentrations are associated with an increased incidence of hypoactive sexual desire disorder (HSDD) as defined by a loss of libido that is associated with psychological distress.

Validated instruments to evaluate and quantify sexual desire in surgically menopausal women [111] have been established. In randomized, double-blinded, placebo-controlled clinical trials, transdermal testosterone administration at a dose of 300 μg/day to surgically menopausal women significantly enhanced sexual functioning and increased the frequency of total satisfying sexual activity [112–114]. These testosterone patches are currently not available in the United States.

Progestins

Oral progestins that are typically used are MPA, micronized progesterone (Prometrium), and norethindrone. These hormones can be used cyclically or continuously. MPA 5–10 mg or micronized 200 mg progesterone can be given for the first 12 days of each month. Lower doses are used for continuous use. Typical doses for this are 2.5 mg MPA (Prempro), 1 mg norethindrone (Femhrt), or 100 mg micronized progesterone.

Some progestins are formulated with an estrogen such as Prempro, which contains MPA, or Femhrt, which contains norethindrone 0.5–1 mg. A low-dose levonorgestrel intrauterine device is under investigation as a method of endometrial protection.

Nonhormonal Therapies

It is clear that estrogen is the most effective and studied medication to treat vasomotor symptoms. However, given the results of the WHI, more patients and physicians have considered non-estrogen treatment methods for the treatment of vasomotor symptoms.

Clonidine

Clonidine is an antihypertensive medication that acts centrally as an α2-adrenergic agonist and can be administered orally or via a transdermal delivery system. Clonidine has been used to treat hot flashes not only in postmenopausal women but also in post-orchiectomized men [115]. The evidence to support its use is limited. One randomized controlled trial 15 patients to placebo and 14 to transdermal clonidine. Subjects were followed for 8 weeks, and 86 % of the patients in the treatment group had a significant decrease in the frequency of vasomotor episodes, 73 % decrease in the severity of flushes, and 67 % decrease in the duration. In the placebo groups, these benefits were present in 36 %, 29 %, and 21 %, respectively [116]. One characteristic consistent throughout the retrospective and prospective clinical trials of clonidine was the high percentage of patients with side effect: dry mouth in up to 40 % of patients, drowsiness and

Table 10.6 Randomized, controlled trials of selective serotonin reuptake inhibitors (SSRIs) for the treatment of vasomotor symptoms in postmenopausal women

SSRI/groups	Subjects	Endpoint	Length of the study	Comments
Venlafaxine (2000)	191	Reduction average daily hot flashes	4 weeks	Side effects: nausea, dry mouth, constipation, decrease appetite
Placebo	50	27 %		
37.5 mg/day	49	37 %		Other benefits: decrease depression scores, improved quality-of-life scores
75 mg/day	43	61 %		
150 mg/day	49	61 %		
Venlafaxine (2005)	61	Reduction patient perceived hot flash score	12 weeks	Side effects: dry mouth, sleeplessness, decreased appetite
Placebo	32	15 %		
37.5 mg/day × 1 week then				
75 mg/day × 11 weeks	29	51 %		
Paroxetine (2003)	165	Mean change daily hot flash composite score	6 weeks	Clinical global impression also improved significantly in treatment groups
Placebo	56	37.8 %		
12.5 mg/day	51	62.2 %		
25 mg/day	58	64.6 %		
Fluoxetine (2002)	81	Hot flash frequency and hot flash score	1-week documentation	Randomized cross-over trial
Placebo/fluoxetine 20 mg	41	36 %	4 weeks each period	Well tolerability of fluoxetine
Fluoxetine 20 mg/placebo	40	50 %	Total of 9 weeks	

dizziness in up to 35 %, skin rash and irritation in 15 % of oral and up to 50 % in the transdermal system.

If no other treatment fits the need of the symptomatic postmenopausal patient, clonidine can be tried in a dose of 2.5 mg of a transdermal system and changed every week. Clonidine also may be prescribed and divided orally in doses of 0.1–0.4 mg/day.

Selective Serotonin and Norepinephrine Reuptake Inhibitors

This group of drugs (Table 10.6) seems to effectively decrease vasomotor instability in menopausal women by increasing the availability of serotonin and/or norepinephrine in the central nervous system. These compounds should be considered a possible option to control hot flashes in women not willing to take estrogen or they are contraindicated. There are several open label trials and enough randomized controlled trials to currently recommend them as a reasonable option. There are studies that evaluate citaprolam, paroxetine, and sertraline. Perhaps the most widely studied and used is venlafaxine at doses of 37.5–75 mg/day. Venlafaxine and its active metabolite desvenlafaxine 100 mg/day (Pristiq) have both serotonin and norepinephrine reuptake inhibition. In general, the efficacy of these compounds is approximately a 50–60 % reduction in severe hot flash frequency relative to baseline. The placebo response rates in these same study populations range from 30 to 40 % [117].

Gabapentin

Gabapentin is an (gamma)-aminobutyric acid analogue approved in 1994 for the treatment of seizures and has been used by neurologists to treat neuropatic pain, essential tremor, and migraines [118]. In a randomized, double-blind controlled trial that included 59 menopausal women with seven or more severe hot flashes a day; a dose of 900 mg of gabapentin a day was given for 12 weeks. Gabapentin was associated with a 45 % reduction in hot flash frequency and a 54 % reduction in hot flash composite score (frequency and severity combined into one score) from baseline, compared with 29 % ($P = 0.02$) and 31 % ($P = 0.01$) reductions, respectively, for placebo [119]. One out of five patients taking gabapentin will experience dizziness and somnolence. To reduce these side effects and improve efficacy, an extended-release formulation of gabapentin has undergone phase three trials with promising results using divided doses of 1,800 mg daily. There were significant reductions in hot flash frequency and severity relative to placebo [120].

Paced Respirations

This behavioral modification technique uses a slow, deep breathing exercise to control hot flashes. Freedman and Woodward first described this technique and validated that the deep breathing component alone was effective in reducing hot flashes, whereas progressive muscle relaxation techniques were ineffective [121]. The protocol utilized by Freedman consists of slow, deliberate, deep breathing at a target rate of six to eight breaths per minute to be done for 15 min twice a day. Additionally, deep breathing is applied during a hot flash. This regimen is risk-free and could be considered a first-line treatment for vasomotor symptoms.

Dietary and Lifestyle Recommendations During Menopause

There is little doubt that prevention is the best strategy to live a healthy life. As health care providers, we should regard the onset of menopause as a signal for future living rather than as the decline of a woman's life. This phase is a great window of opportunity for health care providers to help patients to establish dietary and lifestyle habits to maximize her physical, social, mental, and sexual opportunities.

Calcium Recommendations

One of the key elements to prevent osteoporosis is to maintain a positive calcium balance. This task becomes challenging during menopause because calcium absorption declines with age. Furthermore, the lower levels of estrogen during menopause decrease the levels of 1,25-dihydroxyvitamin D, with the subsequent consequence of even less calcium absorption [122]. Nordin et al. measured radiocalcium absorption in 262 postmenopausal women ages 40–87 and demonstrated a late age-related decrease (only after >75 years of age) in calcium absorption in postmenopausal women in addition to the decline that occurs at menopause. He concluded that this decrease could be due to a decline in either the active calcium transport or diffusion component of the calcium absorption system [123].

The following are the recommendations for calcium intake in postmenopausal women [124]:

- 1,000 mg/day for women between 25 and 50 years
- 1,000 mg/day for postmenopausal women on estrogen replacement therapy
- 1,200 mg/day for postmenopausal women not on estrogen therapy
- For all women over 50 years, daily intake is recommended to be 1,200 mg/day, although further research is needed in this age group
- Adequate vitamin D is essential for optimal calcium absorption. Dietary supplements, hormones, drugs, age, sun exposure, and genetic factors influence the amount of calcium required for optimal skeletal health
- Calcium intake, up to a total intake of 2,000 mg/day, appears to be safe in most individuals
- The preferred source of calcium is through calcium-rich foods such as dairy products. Calcium-fortified foods and calcium supplements are other means by which optimal calcium intake can be reached in those who cannot meet this need by ingesting conventional foods

These guidelines are based upon calcium from the diet plus any calcium taken in supplemental form and all are based on elemental calcium.

The preferred method of reaching optimal calcium intake is through dietary sources. Among the foods with higher content of calcium are dairy products, green vegetables (e.g., broccoli, kale, turnip greens, Chinese cabbage), calcium-set tofu, some legumes, canned fish, seeds, nuts, and certain fortified food products, like bread and cereal. A good rule of thumb to calculate the daily intake of calcium is to multiply the number of milk servings (8 oz = 240 mL = 1 cup) by 300 mg [125].

Due to the challenges of achieving adequate calcium intake via food sources, many physicians advocate the use of calcium supplements. There are two main types of calcium compounds available in the market: calcium carbonate and calcium citrate. Some authors advocate the use of calcium citrate (Citracal) over calcium carbonate (Os-Cal), arguing that calcium citrate is better absorbed than calcium carbonate, especially with an empty stomach but even when taken with a meal [126, 127]. On the other hand, the calcium carbonate is usually less expensive, and according to at least one study in absorbability, bioavailability, and cost-effectiveness, the carbonate formulation is a better supplement choice in a population at risk for both low BMD and hip fracture [128].

Vitamin D

Vitamin D is not usually found in food. It is produced by the skin in response to light exposure. There is controversy regarding the right amount of oral intake of vitamin D that is required. A serum 25-OH vitamin D level of >than 20 ng/mL is considered adequate for bone and overall health. The dose recommended by the Recommended Dietary Allowance (RDA) in women 50–70 years old is 600 IU/day. For those >70 years, a dose of 800 IU/day is recommended. Those living in Northern climates should consider a higher daily dose not to exceed 4,000 IU/day [129].

Exercise

Many exercise recommendations to maintain a health-related fitness is based on studies done in men. However, a recent systematic review of all the randomized controlled exercise trials in postmenopausal women delineates some benefits in intermediate outcome measures. In this systematic review, Asikainen et al. assessed 28 randomized controlled trials with 2,646 subjects. Based on this review of the studies on postmenopausal women, they describe specific guidelines for health professionals to counsel their patient to adequately develop an exercise program [130]:

- Early postmenopausal women could benefit from 30 min of daily moderate walking in one to three episodes combined with a resistance-training program twice a week
- For a sedentary person, walking is a feasible way to start exercise by incorporating it into everyday life
- A feasible way to start resistance training is to perform 8–10 repetitions of 8–10 exercises for major muscle groups starting with 40 % of 1 repetition maximum
- Resistance training initially requires professional instruction, but can thereafter be performed at home with little or no equipment as an alternative for a gym with weight machines
- Warm-up and cool-down with stretching should be a part of every exercise session

The training described above probably will preserve normal bodyweight. When combined with a weight-reducing diet, exercise will likely reduce bone loss and increase muscle strength. Based on limited evidence, such exercise might also improve flexibility, balance, and coordination, and decrease hypertension and improve dyslipidemia [130].

Tobacco and Alcohol

Cigarette smoking and excessive alcohol intakes are related to increase risk of bone loss and cardiovascular disease. In women, more than two drinks per day are associated with an elevated risk of developing hypertension. On the other hand, mild consumption of alcohol (≤ 7 units/week) in women may be associated with a cardioprotective effect. In a 12-year prospective study that included 85,709 healthy women aged 35–59, Fuchs et al. concluded that mild to moderate consumption of alcohol could be associated with a decreased mortality rate for women, but mainly for those at high risk for CHD [131]. Nevertheless, we ought to be cautious when counseling for mild alcohol intake, since it is also associated with increased incidence of breast cancer [132]. These confounders make it difficult to provide a solid recommendation for alcohol consumption for women.

Alternative Medicine During Menopause

Botanical Dietary Supplements

In recent years more natural products have gained popularity as alternatives for treatments of vasomotor symptoms. Among these products phytoestrogens are perhaps the ones with more widespread use. Phytoestrogens are plant-derived substances structurally related to estrogens that have weak affinity to estrogen receptors [133]. Phytoestrogen-containing dietary supplements commonly employed to relieve vasomotor symptoms include flaxseed, red clover extract, evening primrose oil, and soy compounds among others. The difficulty of consuming these botanical dietary supplements is that the purity, potency, and effectiveness are not well established; however, they are popularly believed to be safe and effective for the treatment of menopausal symptoms [134]. These products are used by as many as 46–79 % of menopausal patients in some surveys [135, 136].

A review of randomized controlled trials of complementary and alternative medicine for the treatment of menopausal symptoms by Esenberg et al. evaluated a total of 29 randomized controlled trials of complementary and alternative therapies for hot flashes and other menopausal symptoms; of these, 12 dealt with soy or soy extracts, 10 with herbs, and 7 with other therapies. They concluded that while soy products seem to have modest benefit for hot flashes, studies are not definitive. Isoflavone preparations seem to be even less effective than soy products. Black cohosh may be effective for menopausal symptoms, especially hot flashes, but the lack of adequate long-term safety data (mainly on estrogenic stimulation of the breast or endometrium) precludes recommending long-term use [137].

Also, a more recent systematic review of 25 randomized controlled trials from the Cochrane Library and MEDLINE from 1966 to March 2004 involving a total of 2,348 patients concluded that the available evidence suggests that phytoestrogens available as soy foods, soy extracts, and red clover extracts do not improve hot flushes or other menopausal symptoms [138].

In summary, the current clinical data on the effectiveness of botanical dietary supplement is mainly from open label trials, which are burdened with methodological flaws because of the large placebo effect. For this reason, it is useful to refer to the North American Menopause Society position statement on the treatment of vasomotor symptoms:

> For mild hot flashes, lifestyle-related strategies such as keeping the core body temperature cool, participating in regular exercise, and using paced respiration have shown some efficacy without adverse effects. Among nonprescription remedies, clinical trial results are insufficient to either support or refute efficacy for soy foods and isoflavone supplements (from either soy or red clover), black cohosh, or vitamin E; however, no serious side effects have been associated with short-term use of these therapies [139].

Progesterone Cream from Yam Root

Progesterone can be synthesized commercially from the wild yam. Its use for alleviating menopausal symptoms is growing in the United States. The scientific evidence is controversial at best. A 12-month randomized controlled trial of 102 healthy postmenopausal women given either a quarter teaspoon of cream (containing 20 mg progesterone) or placebo to the skin daily showed that while there was a significant improvement in the relief of vasomotor symptoms (83 % treatment group vs. 19 % in the placebo group), there was no difference in the BMD [140].

In contrast, a randomized, double-blind, placebo-controlled, crossover trial performed in 23 postmenopausal women with vasomotor symptoms, using a topical cream containing wild yam extract (*Dioscorea villosa*), vitamin E, and other oils, found no detrimental side effects, nor any improvement in menopausal symptoms as documented in weekly diaries for 3 months [141]. Similar results were found in a parallel, double-blind, randomized, placebo-controlled trial in 80 symptomatic postmenopausal women comparing the effect of a transdermal cream containing a progesterone (32 mg/daily) with a placebo cream. This study showed no changes in mood characteristics or sexual feelings nor was there any change in blood lipid levels or in bone metabolic markers, despite a slight elevation of blood progesterone levels [142].

Acupuncture

A limited number of studies have evaluated Chinese acupuncture in alleviating mainly vasomotor symptoms. The lack of systematic controls in some of these studies makes it difficult to interpret their effectiveness.

In a small prospective open trial, 11 menopausal symptomatic patients underwent acupuncture for 5 weeks and experienced a significant improvement in menopausal vasomotor symptoms without any changes in reproductive hormones or psychosocial or sexual symptoms as measured by the Menopause Specific Quality of Life Questionnaire [143].

A pilot study evaluated the effectiveness of acupuncture for the treatment of menopausal symptoms in 15 patients with breast cancer treated with tamoxifen 20 mg/day. With the exception of libido, all the other dimensions of the Green Menopause Index showed significant improvement ($P<0.001$) with acupuncture [144].

Wyon et al. randomized 45 postmenopausal women with complaints of vasomotor symptoms to three study groups: electro-acupuncture, superficial needle insertion, or oral estradiol treatments for 12 weeks with a 6-month follow-up. The patients in the electro-acupuncture group had a decrease in the mean number of hot flashes from 7.3 to 3.5, while superficial needle insertion (i.e., placebo) patients decreased the mean number of hot flashes from 8.1 to 3.8. In the estrogen group, the number of flushes decreased from 8.4 to 0.8. The Kupperman index and the general climacteric symptoms score decreased, and remained unchanged for 24 weeks after treatment in all groups. Superficial needle insertion and electro-needle stimulation were similar in efficacy for acupuncture treatment of vasomotor symptoms, although not as effective as estrogen [145].

In Sweden, a randomized single-blind controlled design was used to evaluate the effects of electro-acupuncture on general psychological distress and relate to experience of climacteric symptoms in 30 postmenopausal women. This study had a group of patients with electro-acupuncture and another group with extremely superficial needle insertion, this one functioning as a near-placebo control. Patients were treated for 12 weeks, and general psychological wellbeing, mood, and experience of climacteric symptoms were used as outcome measures. This study showed enhancement in the mood scale in the acupuncture group, but climacteric symptoms and psychological wellbeing improved in both placebo and treatment groups, suggesting that electro-acupuncture is not any better than superficial needle insertion for amelioration of climacteric symptoms or improvement of wellbeing [146].

Acupuncture may create tissue damage occasionally, but usually will not cause serious complications (i.e., pneumothorax, cardiac tamponade). The most common serious risk is the transmission of hepatitis viruses or other infection agents through inadequately sterilized needles. Disposable needles, the standard of care in the United States, have eliminated this threat. In summary, alternative treatment for vasomotor symptoms lack carefully conducted placebo-controlled trials. Until these are evaluated, all recommendations should be guarded.

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Male Infertility

11

Mark Gibson and Ahmad O. Hammoud

Introduction

The male accounts for nearly half of known instances of infertility. Determining the prevalence of male infertility is hampered by lack of thresholds for normal and infertile measures in the semen analysis and other tests of sperm quality and function. Thorough evaluation of the male factor should be a part of the infertility and is essential for defining the course and content of a couple's care. This chapter identifies diagnoses as found in the history, by examination, and in the laboratory; each contributes valuable information. Pathophysiology and management for principal diagnoses are also presented. For many infertile men, attribution of cause for semen abnormalities is not possible. For them, and for men whose conditions are not amenable to specific therapy, intrauterine insemination (IUI) and assisted reproductive technology (ART) with intracytoplasmic spermatozoa injection (ICSI) offer pathways to fertility.

History

Coital Function

Adequate frequency of intercourse and erectile function with ejaculation are essential. Semen quality may decline with daily ejaculation, leading to prescription for alternate day intercourse. However, many studies show better semen quality with daily or more frequent ejaculation [1–5]. Prescribed timing for intercourse can create dysfunction and marital stress related to on-demand performance [6]. Because of this, and because ovulation prediction may have up to a day of error, advice to have intercourse "every day or two" during the fertile portion of the cycle without reference to ovulation prediction may be helpful. It allows some spontaneity without compromise of the chance for optimal timing. Sexual dysfunction frequently accompanies infertility [7]. Men unable to achieve appropriate coital frequency and function should be evaluated for hypogonadism (see section "Endocrine Evaluation") [8]. Findings will usually be normal and provision for marital/sexual counseling is then appropriate [9].

Ejaculatory dysfunction may be psychogenic, occurs after retroperitoneal node dissection, results from use of some medications, is common in men with diabetes, and is not possible for most men with spinal injury [10, 11]. Induction of ejaculation is often successful using high-amplitude vibratory stimulation, which is less stressful than electroejaculation, and allows for home use and home insemination for some men [12–16]. Induced ejaculation can be complicated by autonomic dysreflexia, so initial attempts should include monitoring for this complication, which can be blunted with the use of nifedipine [17]. Semen quality is often poor in men with spinal cord injury, so the principal benefit of induced ejaculation may be avoidance of testicular spermatozoa extraction (TESE) for ICSI. When azoospermia is found on an initial induced ejaculation, second attempts, or use of other methods may yield semen with sperm sufficient for ART with ICSI [18–20].

Surgery, Injury, and Infection

Childhood surgery involving the reproductive tract and/or inguinal region can imply abnormalities resulting from defective androgen synthesis or action that may explain later

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impaired spermatogenesis. Alternatively, surgery may injure the ductal system. The assemblage of abnormal androgen-dependent development, genital malignancy, and impaired spermatogenesis is suggested to comprise "testicular dysgenesis syndrome" [21–24]. A history of injury, torsion, or vasectomy reversal may explain subsequent infertility. Genital tract infection may compromise semen quality, but does not cause infertility except in cases of post-infective obstruction [11, 25–27]. Retroperitoneal node dissection compromises ejaculatory function, adding to the damage to germinal epithelium from chemotherapy [12].

Cytotoxic Medications

Chemotherapy for malignant or rheumatologic disease frequently causes azoospermia. This effect depends on the agents used, their doses, whether there was also radiation used [28–31]. Effects may be transient [29]. There is no evidence that these exposures affect health of offspring [30]. Resources for fertility preservation should be provided for men anticipating cytotoxic therapy [32, 33].

Lifestyle

Evidence for adverse effects of tobacco and alcohol on fertility is mixed [34–40]. Studies have not linked recreational drugs to infertility [39]. Obesity is associated with poor semen and reduced fertility in some, but not all studies [39, 41–45]. Genetic variation in hormone metabolism may explain a portion of the variability in obesity's effect on semen quality [46]. Effects of obesity may be mediated by coexisting disturbances in insulin resistance, leptin, systemic inflammation, sleep apnea, and testicular thermoregulation [43, 47]. Reversibility of obesity's effect has not been shown, and rapid weight loss may harm semen quality [48]. High intensity endurance training alters hormone levels and some semen measures but has not been shown to cause infertility [49–51]. Most medications have not been investigated for effects on human male fertility. Agents of concern for semen quality or ejaculation include serotonin reuptake inhibitors, anti alpha-adrenergic antihypertensives, and sulfasalazine [11, 44, 52]. Anabolic steroid abuse and testosterone replacement reversibly cause fertility impairment [53, 54]. Nutrition is important for spermatogenesis; dietary elements of interest include selenium, dietary antioxidants, zinc, folate, and folate indirectly via its metabolism [55–61]. Clear linkage between adequacy of these in the diet and infertility has not been shown and limited studies of supplementation have not shown benefit [60, 62].

Environmental Exposures

Air pollutants and heavy metal exposure may impair semen quality but evidence for causation of infertility is weak, and for thresholds for effect are unclear [63–65]. Recent interest has focused on organochlorines, dioxins, phthalates, phytoestrogens, and chemical mixtures as found in pesticides and tobacco smoke because of mechanistic hypotheses for endocrine disruption and some demonstrated effects on semen quality [66, 67]. Effects are found for PCBs inconsistently, and DDT exposure appears to have minimal effect on semen parameters [68–70]. Pesticide exposure may affect semen quality and fecundability [71]. Overall, however, the literature is not consistent regarding effects of man-made xenobiotics [72–74].

Physical Examination

Clinical Signs of Hypogonadism

Clinical hypogonadism may be suspected with findings of decreased muscle mass, decreased sexual hair, and increased subcutaneous tissue but there is wide normal variation in expression of sexually dimorphic characteristics.

Examination of Scrotal Contents

Testis size reflects aggregate seminiferous apparatus, not endocrine tissue, and is normal at 30 cc, or a length of 4 cm. Testes smaller than this may reflect decreased sperm production, especially when they exhibit less-than-normal turgor. The vas deferens should be palpable. Absence is usually bilateral, with normal testis findings and azoospermia, and is usually a result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) complex. Before undertaking ART, partners should be screened to exclude abnormal CFTR genes that would place offspring at risk for cystic fibrosis. Unilateral absence of the vas deferens may indicate CFTR mutations or renal abnormalities and calls for genetic evaluation and urinary tract imaging [75].

Varicocele

Varicocele is posturally expressed and more often left sided. Clinically inevident varicocele is of questionable significance, though can be identified with ultrasound, which may be useful when findings are equivocal. Implications of varicocele for fertility (and response to corrective treatment) are proportional to size of the lesion, but a standard classification is lacking [76]. It is common—the prevalence generally given is 15 %—and though more prevalent among infertile men is also commonly innocent. Men with varicocele may have normal semen and fertility or may have infertility, reduced testicular volume, and severely abnormal semen [77, 78]. Proposed mechanisms for infertility include altered testicular thermoregulation, increased seminal plasma reactive oxygen species, and compromised testosterone production [79]. Varicocele effects on semen may be progressive, which argues for surgical treatment for men with normal semen except that this effect is neither consistent nor predictable [80, 81]. Treatment of varicocele is discussed below, under treatment of oligoasthenoteratospermia (OAT).

The Laboratory

Semen Analysis

Pioneering work by Macleod established norms for semen measures based on time required to achieve pregnancy in subjects with currently pregnant partners [82–84]. Standards for semen analysis were promulgated by the World Health Organization (WHO) in 1987, 1992, 1999, and most recently in 2010 [85]. Yet, excepting the most severely abnormal specimens, semen analysis does not clearly distinguish men with normal fertility from men with infertility [86, 87]. Moreover, because infertility often is multifactorial, and semen exhibits large intra-individual variation, assigning a precise contribution of semen findings to a couple's infertility is usually not feasible [88, 89]. The WHO guidelines published in 2010 are aggregated from findings among fertile men in five studies in eight countries (Table 11.1) [85, 86]. Cutoffs stipulated that 95 % of the values were normal, with a one-sided distribution that assumed upper end values do not represent disease. Values below the fifth percentile for these fertile subjects were designated abnormal. The new guidelines simplify quantification of motility from designation of grades to "progressive" or "non-progressive." Morphology is described by "strict" (or "Tygerberg") criteria and poses some difficulties, as this important measure is subjectively determined and difficult to standardize across laboratories [82, 90–93]. The new, often lower, cutoffs for normal do not solve the problem of finding values denoting infertility, and have raised concerns regarding clinical application [94–97]. Nonetheless, semen analysis can be used effectively when its limitations are understood [98]. Fertility is a continuously varying characteristic such that reference values cannot segregate absolute fertility from absolute infertility and instead lie within a zone of ill-defined subfertility [87, 93]. Very importantly, decisions to treat with the assumption of infertility on the part of the male (e.g., varicocelectomy, decision for ART/ICSI) should not be made on the basis of results of a single semen analysis, owing to large variations in an individual's semen measures over time [88, 89].

Table 11.1 Lower reference limits (fifth percentile) and their 95 % confidence intervals for semen parameters and 50th percentile values from fertile men whose partners had a time-to-pregnancy of 12 months or less

	N	5th percentile (95 % CI)	50th percentile
Semen volume (mL)	1,941	1.5 (1.4–1.7)	3.7
Sperm concentration (106/mL)	1,859	15 (12–16)	73
Total number (106/ejaculate)	1,859	39 (33–46)	255
Total motility (PR, NP, %)[a]	1,781	40 (38–42)	61
Progressive motility (PR, %)[a]	1,780	32 (31–34)	55
Normal forms (%)	1,851	4 (3.0–4.0)	15
Vitality (%)	428	58 (55–63)	79

Adapted from [86]

[a]PR, progressive motility (WHO, 1999 grades a, b); NP, nonprogressive motility (WHO, 1999 grade c)

Morphology: A Key Measure of Fertility

Efforts to correlate semen findings with fertility consistently highlight the importance of sperm morphology [83, 87]. Refined standards for morphology, in part based on mucus penetrating capability, led to elaboration of the strict criteria [99]. Morphology by strict criteria varies independently of sperm density and motility and independently predicts success with IUI and ART [91, 100–104]. Strict morphology assessment correlates only roughly with tests of function (e.g., hamster egg penetration test) and has replaced these as predictors of fertilization success in vitro or requirement for ICSI in most ART programs.

Other Measures for Sperm

The advent of ART and ICSI gave impetus to the quest for measures predicting ability to fertilize oocytes and to produce successful embryos. Efforts to this end centered on two principal arenas: first, functional tests of the binding of sperm to the oocyte with execution of the acrosome reaction and second, the integrity of sperm DNA. Examples of functional tests include the hamster egg penetration test, hemizona binding assay, and the zona-induced acrosome reaction (ZIAR) [91, 102, 105–107]. Results of such tests show correlation with morphology, particularly of the acrosome, but are not inconsistently aligned with measures of strict morphology [99, 108, 109]. Sperm function tests remain poorly validated and standardized, their clinical relevance is uncertain, and they are not part of the routine fertility assessment [98, 110].

Fragmentation of DNA is present to varying degrees in sperm, and its extent can be assessed by techniques such as the flow cytometry-based sperm chromatin structure assays (SCSA), terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick-end labeling (TUNEL), the single-cell gel electrophoresis assay (also known as COMET), among several [111–113]. The correlation of findings from these tests with semen measures is often poor, and their ability to predict natural fertility, IUI success, and ART outcomes is variable [111, 113–116]. DNA damage is increased in the presence of varicocele and improves after repair [117]. Measures for DNA damage may be markers for toxicant exposure and oxidative injury to sperm, inflammation, or exposure to certain medications [67, 118–121]. Because of differing results among different tests, lack of standardization, and conflicted data as to utility routine use of DNA fragmentation testing is not justified by current evidence [95, 113, 122, 123]. Emerging work suggests that epigenetic alterations and defects in DNA packaging (protamines, histones) may reflect abnormal spermatogenesis or constitute primary disorders of fertility [124–127].

Special Findings in the Evaluation of Semen

Agglutination of sperm is detected and graded on wet-mount examination. If extensive and associated with a history testicular trauma or vasectomy reversal, it suggests the presence of antisperm antibodies. Evidence suggesting antibodies should be substantiated with specific testing, most commonly with the immunobead test [128–130]. Antibodies may be directed at a variety of antigens on different regions of spermatozoa, with differing consequences for fertility [131, 132]. Pregnancy can occur spontaneously in the presence of antisperm antibodies, but IUI has been used successfully when it does not [133]. Rapid dilution of semen upon collection for IUI may be beneficial [134]. ART has also been used as treatment for antisperm antibodies [133]. The addition of ICSI addresses concern for interference of antibodies with fertilization, but is currently without evidence for clinical benefit [135, 136].

Absent motility occurring with normal measures of vitality indicates one of several ultrastructural defects affecting ciliary function in primary ciliary dyskinesia syndrome. With chronic respiratory infection and situs inversus, the diagnosis of Kartagener's syndrome can be made [137–139]. These disorders are autosomal recessive gene defects affecting the several proteins critical for normal ciliary ultrastructure and movement. Chronic/recurrent respiratory function in these men is due to impaired mucociliary function. Evaluation of sperm tail ultrastructure by electron microscopy can confirm diagnosis, but the classic findings on semen analysis with a typical history of respiratory disease and clinical findings for situs inversus are sufficient for clinical diagnosis. Pregnancy is achieved with ART and ICSI [140, 141].

Absent or minimal ejaculate after orgasmic masturbation suggests retrograde ejaculation or ejaculatory duct obstruction. Distinction between the two depends upon post-ejaculation urine analysis, which will show abnormally elevated sperm numbers after retrograde ejaculation. Causes include anatomic disruptions from prostate surgery, and neurologic dysfunction related to diabetes, demyelinating disorders, or sequelae of retroperitoneal node dissection. Pharmacologic disruption of the ejaculatory signaling pathway may occur with alpha-adrenergic blockers used for urine flow with prostatic hyperplasia. Medical treatments using alpha sympathomimetic agents (ephedrine, phenylephrine) or tricyclic antidepressants may help in some instances [142, 143]. More often, harvesting of sperm from post-ejaculatory urine that has been alkalinized by bicarbonate ingestion is done so that IUI or ART may be undertaken [144–146].

Clinical Categories of Severe Semen Abnormalities

Oligoasthenoteratospermia

Abnormalities of semen among infertile males are rarely limited to a single parameter and commonly present as subnormal values for sperm density, motility, and normal morphology. This constellation is often termed OAT, or OAT syndrome. OAT, when severe, should be evaluated for genetic, chromosomal, and endocrine origins as described below for the evaluation of azoospermia. OAT is often idiopathic [147]. Two groups of OAT have been described, one affected primarily in density and motility and the other in sperm morphology, with the latter showing higher correlation with the presence of sperm aneuploidy [148, 149]. OAT is associated with sperm aneuploidy in many studies, with the rate of aneuploidy found in normal and abnormally formed sperm from men with OAT being similar to that in abnormally formed sperm from normal semen specimens [150–153]. Elevated frequencies of aneuploidy in sperm from men with OAT likely explain the increased aneuploidy in embryos created from their sperm using ICSI [154]. Other sperm abnormalities in OAT include increased DNA fragmentation, mitochondrial abnormalities, epigenetic alterations, and disordered chromatin organization [155–157]. Management of infertility due to OAT includes consideration of donor insemination, ART with ICSI, and attempts at medical therapy (discussed below). Surgical or embolic treatment for varicocele for men with OAT may be appropriate.

Treatment of Varicocele

Treatment of varicocele with surgery or embolization is performed for discomfort and for infertility. Additionally, when low serum testosterone levels are present, they may be corrected with surgical treatment [78, 158]. Improvement in semen varies widely after varicocelectomy, but even azoospermic individuals may show return of sperm to the ejaculate after surgery [159]. Surgical techniques have advanced in recent decades to reduce unintended vascular or lymphatic injury [160, 161].

Despite beneficial effects of varicocelectomy on semen quality, endocrine function, and pain, its role as a fertility treatment is controversial; analyses of literature using live birth as the outcome of interest yields conflicting conclusions [160, 162–164]. The advent of ART and ICSI for male factor infertility adds complexity to knowing varicocelectomy's role [164, 165]. Pregnancy as a result of varicocelectomy may occur over an extended window of time (as is true for pregnancy among normally fertile couples) and, therefore, youth of a couple, and lack of urgency would favor an attempt at correcting infertility with varicocelectomy, prior to advancement of care to ART. The presence of pain or hypoandrogenism would favor surgery, as would religious or other barriers to ART. Alternatively, progression to ART, without varicocele correction, may be preferred for couples not comfortable accepting its uncertain benefit and for couples for whom time to pregnancy is a concern, especially where female age is a factor.

Azoospermia

Azoospermia requires examination of centrifuged semen for confirmation. It may be due to obstruction, inadequate gonadotropins, or defective germinal epithelium. The latter two comprise the two causes for nonobstructive azoospermia (NOA), and the laboratory plays an important role in differentiating between them. The nature of the semen is helpful in distinguishing the causes of azoospermia [166]. Smaller semen volumes and greater acidity without fructose suggest absent seminal vesicles (CBAVD), which can be confirmed on scrotal examination. Smaller semen volumes will also be seen in severe hypogonadism. Normal semen volumes with normal pH suggest primary testicular (germinal) defects or ductal obstruction at the level of the vas or epididymis. Surgery for cryptorchidism in childhood is a risk factor for NOA and these occurring together may implicate the putative "testicular dysgenesis syndrome," warranting careful testicular examination for mass [21, 24, 167]. Surgery for cryptorchidism or for torsion that harms the contralateral ducts or history of prior epididymitis may account for proximal ductal obstruction. Exogenous androgen use may profoundly suppress spermatogenesis [53, 54]. Examination of the scrotal contents helps differentiation of ductal disorders (the presence of normal testicular volume and turgor, palpable ductal abnormalities) from primary testicular and endocrine control disorders (small testes of reduced turgor). Congenital bilateral absence of the vas is evident from palpation and has implications for CFTR mutation screening in the event of ART.

Endocrine Evaluation

The principal value of endocrine testing in evaluation of azoospermia lies in distinguishing testicular from central causes of NOA. It is rarely helpful in the evaluation of OAT or sexual dysfunction. Some degree of elevation of follicle-stimulating hormone (FSH) levels is expected with primary testicular disorders, and though cutoff values for this are elusive, such findings usefully contrast with the very low FSH, luteinizing hormone (LH) and testosterone levels seen in hypogonadotropic disorders [168].

Principal hypogonadotropic disorders are either congenital (frequently as classic Kallman's syndrome, which includes ansomia), in which impaired pubertal development may have led to androgen replacement, or acquired disorders, in which the principal concern is pituitary or juxtapituitary neoplasm [169–171]. Therefore, when adult-onset hypogonadotropic hypogonadism is diagnosed, the serum prolaction levels should be determined; CNS and pituitary imaging should be performed if it is elevated, or if there is evidence for global pituitary insufficiency (central hypothyroidism, hypoadrenalism, or diabetes insipidus) or symptoms of intracranial mass. Treatment of endocrine disorders for fertility restoration is discussed below.

Chromosomal and Genetic Evaluation

NOA of testicular origin and severe OAT warrant genetic and chromosomal evaluation, especially prior to ART. Identifiable chromosomal and genetic abnormalities are common among men requiring ICSI [172–175]. Five percent of ICSI patients exhibit chromosome errors, and these involve the sex chromosomes in approximately two-thirds of instances. Frequency of chromosomal errors increases with the severity of semen impairment, reaching 10 % or greater among men with the most profound deficits in sperm density [172, 176]. Y-chromosomal microdeletion is roughly as common as chromosomal error in this population and also most prevalent among men with the greatest depression of spermatogenesis [172, 173].

Sex chromosome aberrations are the most frequent of the few chromosome abnormalities compatible with adult life in men. Klinefelter syndrome (XXY and mosaics) and XYY syndrome both include infertility and each occurs in one to two per 1,000 births [174, 175, 177]. Non-mosaic Klinefelter's syndrome is common among azoospermic individuals, and men mosaic for the disorder frequently present with abnormal semen [176]. Klinefelter's patients have harvestable sperm with TESE, more often than not. Testicular volume and hormone levels are of limited utility in predicting TESE success [168, 178, 179]. Because spermatogenesis may be focal, microdissection may provide the best TESE success rate [178, 180]. Fluorescence in situ hybridization (FISH) to exclude embryonic aneuploidy should be considered if ART/ICSI is undertaken for Klinefelter's syndrome [181]. Autosomal-balanced translocations (and the Robertsonian translocation form of these) are associated with infertility, recurrent abortion, and rarely, offspring with deficits owed to unbalanced chromosomes [174–176]. Effects of autosomal translocations and inversions arise through disruption of normal meiotic bivalents such that azoospermia, due to meiotic arrest, or oligospermia occur. Interchromosomal effects, whereby other, normal chromosomes are collaterally damaged during meiotic errors, add to the reproductive morbidity of autosomal chromosomal rearrangements [182]. Frequencies of sperm aneuploidy, likelihood of embryonic aneuploidy, and successful reproduction vary widely according to the defect present [174, 183].

High percentages of embryos from men with structural rearrangements have aneuploidy, and preimplantation genetic diagnosis (PGD) can increase the likelihood of transferring normal embryos [184]. It is most clearly of use in cases of recurrent abortion, in which ART/PGD may shorten the time to implantation of a normal embryo, or in the uncommon cases in which abnormal offspring with unbalanced chromosomal complement have been born [185]. PGD technologies will be transformed by the advent of emerging array technologies [174].

It is likely that genetic lesions often explain severe male infertility, but only a few are described [186, 187]. Microdeletions in the AZF region of the Y-chromosome long arm have been extensively studied. A prevalence of 7.4 % among infertile men is estimated, and their likelihood is proportional to the severity of spermatogenic abnormality. Deletions in the various AZF subregions ("a," "b," or "c") or combinations of them occur with differing frequencies and with differing implications for the degree of impairment of spermatogenesis, and the likelihood of retrievable sperm if there is NOA. Large areas of deletion and deletions involving the a and b subregions are associated with failure to retrieve sperm on TESE [181, 186, 188]. Because of this, testing for AZF deletion is advisable before attempting TESE for NOA. Mutations with severe functional consequence for the androgen receptor lead to infertility and intersex conditions and lesser lesions to infertility alone [189–191]. Such mutations were found in 1 % of a large series of men with severe oligospermia undergoing ICSI [173].

Identification of chromosomal or genetic causes for male infertility thus has implications for health of embryos and offspring, and can predict TESE success for NOA. Using results of genetic and chromosomal evaluation of the male to provide counseling about pregnancy likelihood and outcomes is an important element of care for couples treated for severe male factor infertility [172–174, 188, 192].

Treatment of Azoospermia and OAT

Treatments available for azoospermic disorders include donor insemination in all cases, surgical repair for some ductal obstructions, and ART with TESE and ICSI in selected instances [193–195]. Genetic and chromosome evaluation should be encouraged prior to ART for non-ductal azoospermia (see above). CBAVD signals a high likelihood of CFTR mutation, and ART care should always include screening for these in the female partner prior to ART. When azoospermia is associated with varicocele, treatment may restore sperm to the ejaculate of some patients [159, 196, 197]. Administration of gonadotropins can be effective as sole therapy for hypogonadotropic disorders or to provide ejaculated sperm for ICSI. Fertility can be restored with dopamine agonists for most men with pituitary tumors; when treated surgically, gonadotropins are usually required (see below).

Medical Regimens

Medical therapy for male infertility falls into three categories: replacement of deficient gonadotropins for men with hypogonadism of central origin, empiric direct or indirect augmentation of gonadotropins for men with unexplained infertility, and use of nutritionals and supplements.

Hypogonadal Males with Central Deficiencies of Gonadotropins

Induction of spermatogenesis in constitutional hypogonadotropic hypogonadism, including patients with anosmia (Kallman's syndrome), can be accomplished with administration of pulsatile gonadotropin-releasing hormone (GnRH), which precisely targets the pathophysiology, but is cumbersome [198]. Fertility in Kallman's syndrome and idiopathic or postsurgical hypogonadotropic states is often achievable with administration of human chorionic gonadotropin (hCG) alone, typically in doses of 1,500–2,000 IU twice weekly, but many patients will require co-administration of FSH [199, 200]. When required, FSH doses as low as 150–225 IU weekly may be sufficient [201]. Pregnancies often occur once there are sperm densities that are usually considered oligospermic [200].

Surgical management of prolactinomas in men is complicated by a high rate of persistent hypogonadotropic hypogonadism and recurrent hyperprolactinemia such that replacement gonadotropins are still necessary for fertility [202]. Medical therapy has emerged as a preferable course for most cases. Treatment with the dopamine agonist Cabergoline allows for regression of lesion size, normalization of the hypothalamic–pituitary–testicular axis, normalization of androgens, and restoration of spermatogenesis in a majority of instances, including cases of large prolactinomas [170, 171, 203].

Empiric Therapies for Idiopathic OAT

There is a limited literature supporting a variety of empiric therapies for unexplained male infertility [204]. These treatments presume etiologies such as minimally defective gonadotropin secretion, oxidative insult, or nutritional deficiency. Administration of gonadotropins for men with apparently intact hypothalamic–pituitary–gonadal axes and poor semen quality is supported by limited trial data [205, 206]. Indirect enhancement of gonadotropin secretion with antiestrogens (tamoxifen citrate and clomiphene citrate) is less cumbersome and costlier than gonadotropin administration and is also supported by limited evidence [205, 207–210]. Use of aromatase inhibitors has also shown some promise, especially for men with low ratios of circulating testosterone to estradiol [211, 212]. Among supplements, zinc and folate may improve semen parameters [213]. Studies of carnitines and of antioxidants suggest possible benefits [210, 214–216]. Evidence for these several putative therapies for unexplained male infertility is hampered high intra-individual variation in semen values, limited expression of results in terms of pregnancies, and the likely heterogeneous nature of underlying causes. None of these is adequately supported by high-quality evidence [217]. Large and carefully conducted clinical trials for the treatment of idiopathic OAT that utilize pregnancy as the outcome are needed [147].

Intrauterine Insemination

IUI is widely employed for infertility due to mild or moderate male factor or unknown cause. Often the latter, may have undiagnosed mild male factor. The rationale for IUI is based on several conjectures, including the bypass a hostile vaginal and or cervical environment, reducing the distance for sperm transport, selection of the most fertile sperm, concentrating fertile sperm at the site competition for fertilization, reducing the concentration of spermatotoxic molecules in the seminal fluid (capacitation inhibitors, free radical, etc.), and improved timing of the ovum–sperm exposure. IUI require that the female has spontaneous or inducible ovulation, and has normal anatomy, including normal uterine cavity and patent fallopian tubes. Timing IUI is determined by home kits for detection of the LH surge, or by a triggering injection of hCG given when follicle diameters reach at least 18–20 mm. If hCG is used, artificial insemination is timed 32–36 h after the injection. Success rates are not affected by whether the endogenous LH surge or hCG administration is used for timing [218]. Frequent intercourse through midcycle appears preferable prescribed abstinence prior to collection of the specimen for IUI [1, 2, 4]. Semen is prepared for insemination using one of several aimed at selecting the most fertile pool of sperm and, importantly, to separate sperm from seminal prostaglandins which can cause painful contractions. Density gradient preparation is commonly used, although there is no evidence of the superiority of any of the sperm preparation techniques [219].

The prepared sperm is typically concentrated to a volume of ½ to 1 cc and injected into the uterine cavity gently, using a sterile catheter passed through the cervical canal after wiping the cervix free of secretions or excess mucus. Triggering of upper reproductive tract infection with IUI is a rare complication. IUI shows a higher pregnancy rate when compared to intracervical insemination in couples with unexplained infertility but may not be superior to timed intercourse when done without superovulation [220, 221]. Although superovulation adds efficacy to IUI, and pregnancy rates show some proportionality to numbers of maturing follicles, this must be balanced against significant risks for high-order multiple pregnancy [220, 222–224]. Double inseminations have been proposed to increase success. However, most studies show little evidence of the benefit of this maneuver, which increases the cost and complexity of IUI considerably [225–228]. IUI success depends on female age and quality of semen. Older women fare poorly, and pregnancies are uncommon if they are older than 40 [229]. Pregnancy success is a function of total motile sperm in the insemination; a preparation that contains five million motile sperm appears to be the threshold for benefit of IUI, although preparations with fewer sperm may rarely yield pregnancy [224, 230, 231]. Artificial insemination is typically attempted for three to four cycles; series do not show a significant increase in cumulative pregnancies beyond that [224]. A recent critical review found a limited number of adequately conducted trials, few subjects overall for evaluation, and thus limited evidence for a benefit for IUI vs. timed intercourse [221]. It is likely that couples vary in the degree to which IUI might benefit them, and that substantial benefit for some couples, and little benefit for others, underlies the generally unencouraging statistics for IUI outcomes. A trial of IUI is often selected in hopes that success will obviate the need to progress to the more invasive and costly undertaking of ART.

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Female Infertility and Fertility Preservation

12

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Introduction

The inability to conceive, no matter what the etiology, can lead to a great deal of frustration for patients and their partners. With advances in assisted reproductive technologies (ART), there are many options for patients to build a family. In addition, more women are seeking care to discuss the possibility of preserving their fertility. When women present with their concerns, they should be informed of basic infertility testing, possible treatment options, as well as general reproductive health education.

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Definitions

Infertility is associated with a broad spectrum of definitions and classifications (Table 12.1). The medical definition of infertility is 1 year of unprotected intercourse without successful conception. Utilizing this strict interpretation, infertility is a common problem, affecting at least 10–15 % of all couples. Based upon observational data, the remaining 85–90 % of couples attempting conception will achieve a pregnancy within that 1-year time frame [1, 2]. When viewed across the entirety of the reproductive lifespan, the problem becomes even more common, and up to 25 % of women can have an episode of undesired infertility for which they actively seek medical assistance [3]. It is important to note that fecundity rates are dependent on female age. Data have suggested that fertility in women generally peaks between the ages of 20 and 24 [4, 5]. It remains fairly stable until approximately ages 30–32, at which time it begins to decline progressively [6, 7]. This decline accelerates markedly after the age of 40. Women at the age of 20 have a fecundity rate of approximately 20 % per cycle. In general, this is the peak fecundity rate and it can be considered the "gold standard" when comparing success rates. Subsequently, fertility rates decrease by 4–8 % in women ages 25–29; 15–19 % by ages 30–34; 26–46 % by ages 35–39; and 95 % at 40–45 years of age [8, 9]. Although these are considered the general fecundity rates per age group, as the length of time a couple is infertile increases, the overall chance of a successful pregnancy declines [10, 11].

The fecundity rates of individual therapies will reveal the same diminution across time as the natural model. With each failed cycle of therapy, the chances of relative success with the next cycle are decreased. Not all therapies will lead to success in all patients, nor will all patients become pregnant with any therapy. It is imperative that patients fully understand this concept from the very inception of their treatment. It will greatly increase their understanding of the implications of their therapies and make it far easier to understand the possible failure of those therapies when it occurs.

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Table 12.1 Basic definitions[a]

Infertility	One-year period of unprotected intercourse without successful conception
Subfertility	An ability to conceive a pregnancy that is decreased from aged matched and population matched controls
Fecundability	The probability that actions taken in a single menstrual cycle will result in a pregnancy
Fecundity	The probability that actions taken in a single menstrual cycle will result in a live birth
Primary infertility	A patient who has never been pregnant
Secondary infertility	A patient with a previous history of a pregnancy regardless of outcome, i.e., spontaneous abortion, ectopic pregnancy, still birth or live birth
Chemical pregnancy	A pregnancy diagnosed by a positive beta-hCG titer that spontaneously resolves before clinical verification by other means such as transvaginal ultrasonography
Clinical pregnancy	A pregnancy diagnosed by a positive beta-hCG titer and clinically verified, usually with transvaginal ultrasound (i.e., intrauterine sac or fetal cardiac activity) or in cases of miscarriage, by pathological examination

[a]Modified with permission from Horowitz GM. Female infertility. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Causes of Infertility and Subfertility

The actual percentages that individual factors are found to be the primary cause of infertility vary widely between studies. The 2009 assisted reproductive technology success rates, published by the Centers for Disease Control and Prevention, reported the following primary diagnoses: male factor (18.8 %), unexplained infertility (13.5 %), diminished ovarian reserve (11.5 %), tubal factor (7.7 %), ovulatory dysfunction (6.8 %), endometriosis (4.2 %), uterine (1.4 %), multiple factors (28.4 %), and other (7.7 %) [12].

The initial evaluation of the infertile couple is to determine what can be improved or corrected in order to establish a successful pregnancy. Each question asked at the initial interview, each laboratory test requested, and every diagnostic procedure performed must always reflect the need to categorize the problem as simply as possible in order to suggest the appropriate remedy.

Initial Evaluation of the Infertile Couple

The initial visit will help identify the specific causes of infertility and suggest the appropriate treatment. The first visit helps lay down the sense of understanding and trust necessary between physician and patients, especially in such an emotionally charged situation as fertility. Patients should understand from the initial counseling session that although they have presented asking for medical advice concerning their infertility, the eventual therapeutic decisions are solely theirs to make. It is at this initial evaluation that the physician should discuss possible treatment with the patients. Not all therapies will work in all patients, and not all patients will become pregnant regardless of the therapy. The couple should be given a concise outline of the possibilities of care and all of the information necessary to make an intelligent decision concerning their options.

Primary Elements of the Initial Infertility Evaluation

History

The data collected as part of a careful medical history will often identify signs and symptoms of a specific disease or possible cause of infertility and allow a specific focus for the evaluation. Sending a questionnaire to the patients prior to their initial evaluation can be very helpful. By filling out these data sheets before their initial visit, the patients will be able to (1) find out the answers to the questions that they do not immediately know the answers to, especially those about family history; (2) have collated the data from other physicians concerning testing already performed and other therapies attempted; and (3) be able to better understand the nature of the first evaluation prior to their arrival.

Demographics

It is important to determine where the patient has lived. Extra-genital *Mycobacterium tuberculosis* infections remain one of the most common causes of pelvic inflammatory disease in the third world [13–15]. In areas where tuberculosis is an endemic disease, such as Vietnam and the Philippines, tuberculus epididymitis and salpingitis are common. If recent diagnostic testing has not been done, placement of a PPD should be performed and the results drive further investigation. Even in the United States, up to 2–5 % of tubal disease can be tubercular in nature [16]. With any suspicion of tubercular disease, further evaluation of the female genital tract, such as endometrial biopsy and evaluation of the fallopian tubes, should be performed.

Menstrual History

Detailed information should be obtained about the following:

- Onset of menses
- Development of secondary sexual characteristics such as the development of breasts (thelarche), pubic hair (pubarche) and axillary hair, and the prepubertal growth spurt (adrenarche). If any of these events have been delayed or aberrant in nature or timing, different diagnostic questions must be raised
- Cycle length and characteristics

- Onset and severity of dysmenorrhea
- If dysmenorrhea is present, when does the pain begin? As an example, the pain of endometriosis frequently decreases with the actual onset of menstrual bleeding
- Mitigating factors or agents that decrease the dysmenorrhea
- Factors that make the dysmenorrhea worse
- Onset and severity of dysuria, especially with the onset of menses. Endometriotic lesions of the bladder can cause significant dysuria at the time of the menses
- Onset and severity of dyschezia. Endometriotic lesions of the recto-sigmoid can cause significant pain at the time of the menses. The nature of the stool may also change markedly in this case, with an increase in the frequency of bowel movements and the nature of the stool [17, 18]
- Presence or absence of mid-cycle spotting
- Presence or absence of premenstrual symptoms

Gynecological History

Gynecologic questioning should include questions about the following:

- Previous sexually transmitted diseases
- Previous use of an intrauterine device (IUD)
- Previous abnormal Pap smear
- Previous instrumentations of the cervical os, such as a dilatation for curettage. Any instrumentation of the os can tear the collagenous fibers of the cervix and, hence, lead to cervical factor infertility
- Previous use of hormonal contraception; duration and time of discontinuation
- History of endometriosis, fibroids, ovarian cysts

Obstetrical History

Although this portion is short in women with primary infertility, couples with secondary infertility should be carefully questioned about the following:

- Gravity, parity, pregnancy outcomes, and any associated complications
- Length of time necessary to conceive each pregnancy event, noting whether it was with the current partner
- Mode of delivery with each successful pregnancy event. One of the indications for cesarean delivery is malpresentation, and a common cause of malpresentation can be a Müllerian anomaly [19]
- Mode of treatment of each unsuccessful pregnancy event. Was there instrumentation of the uterus in these previous losses? Instrumentation can lead to the formation of adhesions and Asherman's syndrome

Medical History

Past or current medical diseases for which the patient has been under the care of a physician can be very important and should include the following:

- Any prescription medications she is currently taking
- Past medical admissions to the hospital
- History of common communicable diseases such as rubella, rubeola, varicella, and/or mumps
- Allergies

Surgical History

Past surgery, its indications and outcomes, especially abdominal surgery, can be important. Surgeries should be listed and considered regardless of the organ system involved or the seeming lack of anatomic association with reproduction. Developmental abnormalities of the reproductive tract can frequently be associated with those of other anatomic sites.

Family History

There are many common genetically determined diseases such as congenital adrenal hyperplasia that can have a significant effect on ovulation and fecundity. Such family information would be helpful in determining the need for further provocative testing.

- Number and age of siblings and fertility histories
- Her mothers' current age, the age of her menopause and cause (i.e., was it a natural menopause or one surgically induced)? If surgically induced, what was the indication for this surgery? Did she have difficulty in conceiving? If so, what therapies were used to achieve success?

Questions should be raised about a familial history of any of the following:

- Thyroid disease
- Atherosclerotic cardiovascular disease
- Cancer
- Diabetes mellitus
- Birth defects
- Heritable diseases. Populations at specific risk for genetic disease should be appropriately screened at the time of the initial evaluation per recommendations made by the American College of Obstetricians and Gynecologists and the American Society of Reproductive Medicine (Table 12.2).

Social History

Several environmental exposures are known to have adverse effects on fertility:

- Occupation and the possibility of toxic exposures in the workplace
- Use of tobacco. Smoking can have a marked negative effect upon fecundity and ovarian reserve [20–22]. Smoking cessation should be discussed within the context of any medical evaluation
- Use of alcohol
- Use of recreational or illicit drugs
- Use of herbal preparations, vitamin supplements, megavitamins. Herbal preparations are commonly used in the United States. As many as 32 % of the population use some type of herbal preparation purchased over the counter [23]. Many of these products have ingredients that

Table 12.2 Elements of the infertility evaluation[a]

	Tests
Preconception Screening	Current Pap smear
	ABO, Rh factor typing
	Verify immunity
	Rubella titer
	Varicella titer
	Appropriate genetic screening (see Table 12.3)
	Sexually transmitted diseases
	Recommended screening
	Syphilis screen (VDRL/RPR)
	Hepatitis B surface antigen (HBsAg)
	HIV 1 and 2
	Hepatitis C antibody (HCA)
	High-risk patients
	Gonorrhea
	Chlamydia (RNA/DNA-based testing)
	Donor gametes or ART patients
	All tests above, plus:
	Cytomegalovirus (CMV)
	Human T-cell lymphocyte virus (HTLV) Types I and II
Infertility Testing	Semen analysis
	Ovulatory function
	TSH
	Prolactin
	Basal body temperature chart
	Mid-luteal serum progesterone
	Urinary LH surge detection
	Ovarian reserve testing
	Day 3 FSH
	Clomiphene Citrate Challenge Test (CCCT)
	Anti-Müllerian hormone
	Antral follicle count
	Imaging studies
	Vaginal ultrasonography
	Sonohysterography
	Hysterosalpingogram
	Diagnostic laparoscopy

[a]Modified with permission from Horowitz GM. Female infertility. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

contain active hormones, estrogen disrupters, vasoactive amines, and/or anti-inflammatory ingredients, all of which may have a marked effect on the menstrual cycle, fecundity, and pregnancy

- The nutritional status of the patient should also be reviewed. It should be determined if the patient is taking adequate amounts of folic acid, calcium, and vitamin D

Sexual History

Coital Frequency and Timing

It is important to be aware of the association of coital timing and the probability of successful conception. Activated sperm can last for up to 80 h in the female reproductive tract [24, 25], and the general recommendation has been that the "fertile window" is 6 days leading up to ovulation. Intercourse is most likely to result in pregnancy when it occurs in the 3 days leading up to ovulation.

Sexual Dysfunction

Whether it is caused by a specific organic disease, the treatment of that disease, or the use of self-prescribed substances that interfere with sexual performance, this data should be gleaned at the outset of the evaluation. Many substances such as many blood pressure medications, alcohol consumption, and/or the use of many of the recreational drugs can lead to both erectile dysfunction in the male and desire phase sexual dysfunction in the female.

Dyspareunia

Questions concerning painful intercourse should be specific in order to typify the type of dysfunction that this pain represents:

- Is the pain insertional in nature? A lack of lubrication at the initiation of coitus and the pain that it can engender does not necessarily represent the presence of an organic problem specific to the reproductive tract
- Do the patients utilize an artificial lubricant on a regular basis? Although most commercially available vaginal lubricants are not spermicidal in their basic nature, use can form an amalgam with the semen placed into the vagina during ejaculation. This may lead to a decrease in sperm motility and the number of sperm that enter the cervix. It should be suggested to the patients that alternative methods of increasing vaginal lubrication be used during times of high relative fecundity
- Is there deep thrust dyspareunia? Deep thrust dyspareunia can be a very common gynecological problem, but it is usually an episodic or intermittent complaint [26]. The etiology of this symptom stems from the relative immobility of the pelvic organs and arises from rapid stretching of the uterosacral and cardinal ligaments due to the sudden movement of the cervical/uterine unit during coitus. Deep thrust dyspareunia should raise the suspicion of an organic disease, such as endometriosis or adenomyosis [27–30]
- Is there increased pain with orgasm? Localized production of prostaglandins and endoperoxidases in both endometriosis and adenomyosis can intensify the contractions of an orgasm and cause sensitization of c-afferent nerve fibers in the pelvis, thereby eliciting greater pain with each of these individual contractions [31]

Sexual Orientation

In the United States alone, it is estimated that 1.1–3.5 % of women identify themselves as lesbians [32]. Many of these women will present for medical therapy given this absolute male factor infertility, either alone or with a partner. Traditionally, many physicians have altered their history taking and diagnostic regimen in lesbian populations due to the seeming absence of significant risk factors for pelvic inflammatory disease and other sexually transmitted diseases. However, this is not always the case.

Numerous studies have reported that 53–99 % women who identify themselves as lesbians have at some time had sex with men, and 25–30 % of these women continue to have sex with men [33]. Up to 25 % of this population has been pregnant at one time, and over 60 % of those who had been pregnant report having one or more induced abortions [34]. Erroneous assumptions about the reproductive health and history of lesbians may place them at increased risk for delayed detection of risk factors and adverse outcomes. This in turn may decrease the success rates of any suggested therapy. Sexual orientation should be established at the initial evaluation, but the work-up should remain standardized.

Review of Systems

There are several portions of the general review of systems that must be stressed in the evaluation of the infertile female. Each is indicative of a specific hormonal or physiologic abnormality that can be closely associated with anovulation and hence with infertility or subfertility.

Headaches

Patients should be questioned to find out both the frequency of headaches, as well as the need for self-medication with nonsteroidal anti-inflammatory medications (NSAIDs) and the dosage taken. Headaches can be associated with pituitary lesions such as craniopharyngiomas [35, 36] and prolactinomas [37, 38]. Prolactinomas are a relatively common cause of anovulation. Each can cause hormonal derangements that lead to anovulation and infertility.

Additionally, frequent headaches of any etiology may lead a patient to self-medicate with large doses of over-the-counter NSAIDs. It has been suggested that at high doses, these medications can interfere with the inflammatory processes of ovulation and implantation [39, 40]. Patients should also be advised to avoid taking these medications during their therapeutic protocols in order to prevent these abnormalities.

Visual Changes

The most common presenting feature in space-occupying lesions of the pituitary, such as a craniopharyngioma or macroadenomas, is visual impairment [41]. As the size of the pituitary increases, the relatively small area of the sella turcica forces the gland superiorly towards the optic chiasm. Compression of the optic nerve at the optic chiasm most commonly may cause bitemporal hemianopsia, or bilateral loss of the peripheral visual fields, although total blindness due to optic atrophy can occur rarely.

Changes in Weight

As in all of medicine, radical changes in weight in either direction may be indicative of an underlying organic problem and should be appropriately investigated.

Heat and Cold Intolerance

Temperature intolerance is a common clinical feature of both hypothyroidism [42] and hyperthyroidism [43]. Both extremes of altered thyroxine secretion have been shown to cause menstrual irregularities and anovulation as outlined elsewhere in this book.

Physical Examination

In the female partner, the physical examination may reveal pertinent medical facts that may directly impact reaching the appropriate diagnosis. Several portions of the general physical examination should be specifically stressed in the evaluation of the infertile female.

Weight and Body Mass Index

The connection between increased body mass indices and anovulatory infertility is important to discuss with the patient. Anovulation, oligo-ovulation, subfertility, and infertility have all been associated with patients who are significantly above or below their ideal body weight [44]. Documentation of patients' vital statistics is generally more comparable across large populations when determined as a Body Mass Index (BMI) rather than a simple listing of absolute body weight and height.

Thyroid Abnormalities

As outlined elsewhere in this text, abnormalities of the thyroid gland can have marked effect on the menstrual cycle and, hence, on fecundity. The thyroid gland is one of the largest of the endocrine glands and lies in the anterior neck, immediately below the prominence of the thyroid cartilage.

Breast Examination

As the primary care physicians for the majority of American women, especially those of reproductive age, the gynecologist has long been well versed in the proper screening and the evaluation for breast disease. In the fertility evaluation, the physician should play close attention to the symmetry of the breasts or any evidence of galactorrhea.

It is quite common to have some dissymmetry in the breasts [45]. This should, however, be a developmental finding and not an ongoing and progressive finding. Most patients are aware of any relative inequality in the size and shape of their breasts and any dissymmetry should be directly questioned at the time of the examination.

Galactorrhea is the active secretion of breast milk at a physiologically inappropriate time (i.e., a time other than during pregnancy or when the patient is actively nursing). Usually white in color, breast milk can be differentiated from a pathologic discharge in several ways. Secretions that have been hormonally induced usually arise from multiple ductal openings and are commonly found bilaterally. Pathological discharges, on the other hand, are elicited from single ducts and are primarily unilateral in nature. The specimen generally does not need to be sent for cytologic evaluation unless there is a suspicion of breast disease.

Abdomen

The abdomen should be evaluated for evidence of an organic disease that can have a negative effect on fecundity. As an example, the violaceous striae associated with Cushing's syndrome can be noted on the skin of the abdomen and over the hips [46]. Finding these purplish streaks or marked central obesity would thereby suggest an evaluation for hypercortisolemia. Obesity itself should also trigger concern for the effects of body mass on fertility as mentioned above.

Skin

There are two specific dermatologic findings that should alert a reproductive physician of possible underlying pathology: hirsutism and acanthosis nigricans. Hirsutism, the overgrowth of terminal hair, can trigger recognition of possible hyperandrogenism to help guide the direction of your laboratory examination of the patient.

Acanthosis nigricans is characterized by hyperpigmented, velvety plaques in body folds such as the neck and axilla, although other areas can be involved as well. The formation of these characteristic plaques is stimulated by hyperinsulinemia, often the consequence of obesity-associated insulin resistance. Since insulin resistance is associated with polycystic ovarian syndrome, the finding of acanthosis nigricans is an indication for further investigation.

Gynecological Examination

The primary purpose of the gynecological examination is to identify abnormal reproductive anatomy. Abnormalities in the exam suggest specific organic disease states or structural anomalies that can have marked effects on fecundity. These, in turn, focus the evaluation and direct further investigation into the causes of the patient's complaint. Important abnormalities readily identifiable by gynecological exam include clitoromegaly and abnormalities of the cervix, uterus, and fallopian tubes.

In the non-erect state, the clitoris is generally 0.5–1.5 cm in length and partially covered by a prepuce or hood of skin. Although no consistent standard exists for clitoromegaly, it can be liberally defined as length >20 mm or clitoral width >10 mm. Developmental endogenous or exogenous abnormalities of the clitoris are rare. In most cases, enlargement is due to inappropriate androgen exposure. Identification of abnormal breadth and length of the clitoris will require questions about the possible ingestion of exogenous androgens, possible exposure in utero to androgenic substances taken by the patient's mother, and will certainly tailor part of your endocrinologic screening.

The most common cervical abnormality associated with decreased fertility is cervical stenosis. Cervical stenosis decreases fertility apparently by diminishing the mucus bridge from the vagina to the endocervix necessary for sperm transport. Intrauterine insemination (IUI) using washed sperm has proven to be an excellent treatment for this condition.

Malformations of the cervix can include transverse ridges, cervical collars, hoods, coxcombs, pseudopolyps, and cervical hypoplasia and agenesis [47]. These uncommon malformations can be idiopathic developmental imperfections, or the results of obstetrical trauma and surgery. In the not too distant past, cervical malformations were commonly linked to in utero exposure to diethylstilbesterol (DES), which was frequently prescribed to pregnant women from the 1940s through the early 1970s to prevent miscarriages. Although in utero DES exposure is unlikely in younger women, a woman in her mid-forties or older found to have a cervical malformation should be questioned about possible in utero exposures.

Cervical motion tenderness is sometimes elicited by gentle lateral movement of the cervix. Cervical motion tenderness can be associated with either active or antecedent pelvic infection. Its physiological basis is that movement of the cervix causes movement of the adnexae as well. If the fallopian tubes or ovaries are actively infected, this will cause sliding of inflamed parietal peritoneum upon another surface, thereby causing the internal equivalent of rebound tenderness. Consequently, if cervical motion during the bimanual examination elicits a painful response, an active pelvic infection should be suspected. However, other causes of cervical motion tenderness should also be considered.

An important cause of cervical motion tenderness is pelvic adhesions. The adnexa can be adhered to surrounding pelvic structures as a result of previous infection or endometriosis. Even in the absence of adhesions, endometriosis can cause cervical motion tenderness when it involves pelvic structures attached to the cervix, including the vaginal apex, the uterosacral ligaments and cardinal ligaments, and the inferior portion of the broad ligaments.

The bimanual examination can often identify uterine abnormalities associated with decreased fecundity, including leiomyomata, adenomyosis, or Müllerian anomalies. Findings such as uterine enlargement, irregularity, or tenderness are often indications further evaluation. Any adnexal mass palpable during the pelvic examination should be evaluated further, as the fallopian tubes are not normally palpable on bimanual examination.

Diagnostic Testing

After the completion of a thorough medical history and physical examination, further testing is required and can be subdivided into two categories: (1) preconception screening that should be performed on every woman considering pregnancy and (2) the basic infertility evaluation that will further direct evaluation and treatment. Based on these tests or specific findings in the medical history or the physical examination, it may be necessary to perform more directed and invasive diagnostic procedures as well. An outline of such testing is listed in Table 12.2.

Preconception Screening

The American College of Obstetricians and Gynecologists and the American Society for Reproductive Medicine recommends women preparing for pregnancy have an up-to-date Papanicolaou smear, blood type and antibody screen, and verification of immunity to Rubella. If immunity cannot be proven, it is recommended that patients receive the measles, mumps, and rubella (MMR) vaccine; additionally more physicians are also testing for varicella immunity in the preconception period, and if the woman is not immune, she should receive the varicella vaccination 1 month prior to conception. Women should also be up-to-date on their attempting tetanus-diptheria-pertussis vaccine [48]. As mentioned previously, preconception genetic screening should be offered to specific ethnic groups (Table 12.3).

Table 12.3 Genetic screening for various ethnic groups[a]

Ethnic group	Disorder
Ashkenazi Jews	Cystic fibrosis Tay-Sachs disease Canavan disease Familial dysautonomia
Non-Hispanic Whites	Cystic fibrosis
African Americans	Sickle cell anemia
Mediterranean populations	Beta thalassemia
Southeast Asians, Chinese	Alpha-thalassemia

[a]Modified with permission from Horowitz GM. Female infertility. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Screening of women and partners for sexually transmitted infections (STIs) is an important part of the infertility evaluation to detect current infections and determine women at increased risk of having pelvic adhesions related to previous infections. The Food and Drug Administration (FDA) requires that all anonymous and directed sperm donors are tested for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell lymphotropic virus (HTLV) I and II, chlamydia, gonorrhea, and cytomegalovirus (CMV) [49]. Although the FDA does not hold the same requirements for intimate partners, such screening can minimize risk to the partner and offspring. There are different state determined mandates and laboratory requirements, so it is recommended that all patients be screened for at least HIV1 and 2, HBV, HCV, and syphilis.

Infertility Evaluation

Male Factor

The semen analysis is the foundation of screening for male infertility problems. This test provides important information about both sperm quality and quantity, but does not assess sperm function. The semen sample is analyzed for volume, viscosity, pH, and color of the ejaculate, sperm concentration, motility, morphology, forward progression of the sperm, and the presence of white or red blood cells suggestive of infection.

Men with persistently abnormal semen analyses should be seen by a reproductive urologist for further evaluation.

Uterine, Tubal, and Peritoneal Factors

Ultrasonography

An excellent adjunct to the physical examination is transvaginal ultrasonography, which allows for examination of the pelvic anatomy, particularly the uterus and ovaries [50, 51].

Conditions diagnosed by ultrasound include uterine anomalies, hydrosalpinges, and endometriomas. Since these conditions might not be detectable on physical examination and are often treatable, transvaginal ultrasound is strongly recommended prior to proceeding with specific treatment.

Sonohysterography

Sonohysterography (SHG) is an ultrasound-based test in which a fluid medium is instilled through the cervix into the uterine cavity in order to evaluate the uterine anatomy. This technique has long been used for the delineation of subtle endometrial and intracavitary lesions such as polyps, fibroids, and endometrial cancers.

Hysterosalpingography

Hysterosalpingography (HSG) is a radiographic evaluation that allows visualization of the inside of the uterus and can determine tubal patency [52]. Radiographic contrast dye, either water- or oil-based, is injected into the uterine cavity through the cervix. The dye fills the uterine cavity and spills into the abdominal cavity if the fallopian tubes are open.

An HSG can identify endometrial polyps, submucosal fibroids, intrauterine adhesions (synechia), uterine and vaginal septa, and uterine cavity abnormalities. More importantly, the HSG can determine whether the fallopian tubes are open or blocked and whether the blockage is located proximally, at the junction of the tube and the uterus, or distally, which usually manifests as a hydrosalpinx. If tubal ligation reversal is planned, HSG will demonstrate the point at which the tubes are blocked. Although the primary purpose of the HSG is not therapeutic, both oil- and water-soluble media have been shown to increase subsequent pregnancy rates for several months after the procedure [53].

Diagnostic Laparoscopy

Laparoscopy is an important diagnostic tool for many infertile women. It is the only way to accurately diagnose the extent of endometriosis and intraperitoneal adhesions. It is also a way to directly identify any abnormalities of the uterus, fallopian tubes, and ovaries. Laparoscopy can have a therapeutic benefit whenever endometriosis or pelvic adhesions are diagnosed. Laparoscopy has traditionally been a part of the basic female infertility evaluation. However, because of the cost and small but real risks of surgery, laparoscopy is not always performed prior to a trial of treatment. Most reproductive specialists advocate doing laparoscopy when there is a high index of suspicion for endometriosis or pelvic adhesions based on history or physical examination.

Cervical Factor

Cervical factor is rarely the primary cause for infertility. Traditionally, reproductive physicians used to perform a post-coital test (PCT) to evaluate for cervical factor infertility. This test was typically performed by examining a specimen of cervical mucus microscopically immediately prior to ovulation, 2–12 h after coitus. The presence of motile sperm was considered a normal result. The PCT is no longer considered to be an important part of the infertility evaluation [54, 55]. The test has poor predictive value, and results rarely alter treatment decisions. If the PCT is repeatedly abnormal, the patient is treated with IUI, the most effective treatment for cervical factor infertility. If the PCT is normal, most patients are still treated with IUI, since it is also an effective treatment for unexplained infertility, and improves pregnancy rates over other forms of insemination, regardless of the cause of infertility [56].

Ovulatory Factors

The only absolute proof of ovulation is conception. For practical purposes all other diagnostic tests are indirect evidence. Women who have regular menstrual cycles (occurring at intervals of 25–35 days) and significant moliminal symptoms, such as breast tenderness, bloating, and dysmenorrhea are most likely ovulatory. However, more accurate determination of ovulatory function is an important part of an infertility investigation.

Basal Body Temperature Charts

A basal body temperature (BBT) chart is the most traditional method for documenting ovulation, and is based upon the general effects of progesterone on core BBT. It is inexpensive and simple, yet can be tedious and does not prospectively inform patients of ovulation. In most ovulatory women, a sustained rise in BBT is indicative of ovulation. This can occur anywhere between 1 and 5 days after the mid-cycle LH surge and up to 4 full days after ovulation has already occurred [57].

For this test, the woman takes her temperature every morning and plots the results on graph paper. For greatest accuracy, the BBT needs to be a measured at rest, prior to rising from bed in the morning [58]. A digital thermometer is most commonly used although an oral thermometer with a scale able to differentiate temperature to tenths of a degree will suffice. At rest, the BBT generally fluctuates between 97.0 and 98.0 °F during the follicular phase of the menstrual cycle. Progesterone levels of >5 ng/mL raise the hypothalamic set-point for basal temperature by approximately 0.6 °F.

Classic studies on ovulation prediction and the use of the BBT revealed that 95 % of biphasic cycles are ovulatory and only 80 % of monophasic cycles are actually anovulatory [59, 60]. This indicates a 5 % false-positive rate and a 20 % false-negative rate.

Serum Progesterone

Another method for documenting that ovulation has occurred is the measurement of serum progesterone levels. With the resolution of the corpus luteum from the previous menstrual cycle, serum progesterone levels remain below 1 ng/mL during most of the follicular phase. They rise during the late follicular phase to 1–2 ng/mL, an increase partially responsible for the change in pituitary sensitivity to gonadotropin-releasing hormone (GnRH) that creates the mid-cycle LH surge [61].

After ovulation, progesterone levels rise steadily until they peak 7–8 days after ovulation. Any level of serum progesterone >3 ng/mL provides reliable evidence that luteinization of the follicle, and hence ovulation has occurred [62]. A mid-luteal level of >12 ng/mL was originally considered a measure of adequate luteal function; however, because progesterone secretion is pulsatile, no level can reliably predict quality [63, 64].

There are several ways to determine the appropriate time to measure mid-luteal progesterone levels. In the past, the serum progesterone level was measured on day 21 of the menstrual cycle, based on the classic 28-day menstrual cycle [65]. Since normal menstrual cycles fluctuate in length, the average cycle length of an individual patient can be used to determine the day likely to be 7 days prior to her next menses. If patients are using urinary ovulation predictor kits, they can test their progesterone level a week after their surge. Although measurement of serum progesterone levels can be used as a documentation ovulation and an adequate luteal phase, similar to the BBT, this test cannot be used prospectively to predict when ovulation will occur.

Urinary LH Measurements

The mid-cycle LH surge is the harbinger of future ovulation. For this reason, detection of a mid-cycle LH rise in the urine can predict ovulation before it happens. There are over-the-counter products designed specifically to detect the LH surge in urine. They are simple colorimetric tests designed to change color when the urinary LH concentration exceeds a threshold associated with the mid-cycle LH surge.

To consistently detect the timing of the LH surge, testing must be performed daily, generally starting 2–3 days before the expected day of ovulation. As the LH surge is a succinct event, generally lasting only between 48 and 50 h, the majority of cycles will test positive on only 1 day. Because it is a colorimetric test based on an absolute concentration, test results will vary both by the time of the day they are taken and by the patient's general volume of fluid intake. Patients should be advised not necessarily to limit their fluid intake, just to limit it in the time immediately preceding the time they intend to perform the test. The first urine of the day should not be used for this test.

Women in the northern hemisphere generally start their LH surge early in the morning. Since it takes several hours for LH to subsequently appear in the urine, the best results correlate with testing done in the late afternoon or early evening [66, 67].

Ovulation will generally follow an afternoon or early evening urinary detection of LH within 14–26 h [66]. Consequently, if these tests are being used to time coitus or an intrauterine insemination (IUI), the day after the first positive test will have the highest success rate [68]. Although the accuracy of many of these ovulation predictor kits can vary, the most accurate kits predict ovulation within the next 24–48 h with 90 % accuracy [66, 69, 70].

Endometrial Biopsy

The endometrial biopsy is no longer recommended as part of the standard infertility evaluation. Although painful and costly, this office test was once considered the gold standard for the diagnosis of luteal phase deficiency [65]. However, a large multicenter study showed convincingly that out-of-phase biopsies do not discriminate between fertile and infertile women [71].

Hormonal Causes for Ovulatory Factors

Thyroid-Stimulating Hormone

Hypothyroidism is a relatively common problem in women. It can present as ovulation dysfunction and have minimal other symptoms. The simplest screening test for hypothyroidism is the measurement of thyroid-stimulating hormone (TSH) and should be drawn at the initial evaluation. When TSH is elevated, this is suggestive of hypothyroidism and should be repeated with a measurement of free T_4 [72]. When TSH is abnormally low, it can indicate hyperthyroidism. Further testing is required to verify this diagnosis.

Prolactin

Hyperprolactinemia can cause menstrual disruption, oligomenorrhea, amenorrhea, and consequently infertility [73, 74]. Hyperprolactinemia is another relatively common clinical entity and can be caused by a myriad of pathological processes. Prolactin secreting adenomas are the most common pituitary tumor in women [75].

There has been some concern in the past that prolactin evaluation after a breast examination might lead to spurious elevation, since breast or nipple stimulation can markedly increase serum prolactin levels during pregnancy [76]. However, in the nonpregnant patient, routine breast examination does not acutely alter serum prolactin levels [77].

It should also be remembered that thyrotropin-releasing hormone (TRH) is a potent prolactin stimulating substance [78]. Since TRH, as well as TSH, is elevated in hypothyroid states, prolactin secretion will also be elevated in such circumstances. To avoid confusion, TSH and prolactin should be drawn together at the initial evaluation.

Table 12.4 Ovarian reserve testing[a]

These tests may provide prognostic information to the following patients:

- Any patient >35 years of age
- Any patient with a significant history of ovarian trauma due to:
  - Surgery
  - Infection
  - Endometriosis
- Unexplained infertility of any age
- Smokers
- History of chemotherapy or radiation therapy
- Significant history of autoimmune disease
- Family history of early menopause
- Previous tubal ligation
- Previous poor response to gonadotropin stimulation
- Planning treatment with assisted reproductive technology

[a]Modified from American Journal of Obstetrics and Gynecology, 179/3, Sharara FL, Scott RT, Seifer DB, The detection of diminished ovarian reserve in infertile women, 804-812, Copyright 1998, with permission from Elsevier

Evaluation of the Ovarian Reserve

The age-related decline in fertility is primarily due to the relentless and progressive diminution of oocyte numbers and quality. Consequently, there comes a time in every female patient's reproductive life when it becomes exceedingly difficult to achieve a pregnancy with her own oocytes. Accurate assessment of ovarian function is a core part of an infertility evaluation. Certain indications for ovarian reserve have been identified (Table 12.4); however, it is increasingly popular to check these tests on all patients that present for reproductive evaluation.

Day 3 FSH and Estradiol

As the remaining oocyte pool decreases, the FSH secreted by the pituitary will rise in order to force the failing ovary to work harder. An early follicular (basal) FSH level drawn on cycle days 2–5 can have predictive value on fertility potential [79–81]. Estradiol levels should be drawn in conjunction with basal FSH levels. It can confirm a low FSH level is not falsely suppressed because of a prematurely elevated early follicular phase estradiol level (>60–80 pg/mL) [82].

The specificity of basal FSH levels for predicting who will respond poorly to stimulation reaches 83–100 %; however, the sensitivity varies widely, from 10 to 80 % [83]. When basal FSH levels are elevated (10–20 IU/L or higher), success with even the most aggressive therapies, including in vitro fertilization, are greatly diminished [84].

Clomiphene Citrate Challenge Test

The clomiphene citrate challenge test (CCCT) is a provocative examination of endocrine dynamics [85]. In this test a basal FSH level is measured on day 3 of the cycle. The patient is then given clomiphene citrate 100 mg daily on cycle days 5 through 9, and the FSH level is measured again on cycle day 10. This test is considered abnormal if either the day 3 or day 10 FSH levels are elevated.

Antral Follicle Count

The antral follicle count (AFC) utilizes transvaginal ultrasound in the early follicular phase to determine the number of follicles between 2 and 10 mm in diameter. A low AFC, fewer than 10 antral follicles, has been shown to correlate with poor ovarian response to stimulation [86].

Anti-Müllerian Hormone

Anti-Müllerian hormone (AMH) is produced by granulosa cells and is a reflection of the primordial follicle pool. It is a convenient test, because it can be measured at any time in the menstrual cycle [87, 88]. Although exact cut-offs for good versus poor ovarian reserve have not been established, lower levels (<1.0 ng/mL) have been correlated to poor ovarian response [89, 90].

Treatment

If pregnancy is not achieved after normalization of any problems that have been diagnosed, the standard approach is to enhance fertility in a stepwise fashion, beginning with the least expensive, lowest technology and progressing as needed to the most expensive, highest technology treatment that the couple desires until pregnancy is reached or the couple decides to no longer pursue the goal of conceiving a child. The most common alternatives are to adopt a child or remain childless.

The physician's role throughout this process is to assist the couple in making the best decisions they can about treatment, considering their medical conditions, emotional states, financial situation, and ultimate goals.

Fertility Preservation

In the modern era of successful treatment of many malignancies, the majority of cancer patients will survive their diagnosis. By the age of 39, the prevalence of a female being diagnosed with cancer is about 2 %. An overall 5-year survival rate of greater than 80 % is expected in this patient population [91]. Consequently, as more women survive cancer, fertility preservation has become an increasingly important issue. Fertility preservation strategies have been designed to protect or regain reproductive function in patients exposed to chemotherapy and/or radiation therapy, whether treatment is for malignancy or other systemic disease, such as lupus. Advances in ART, including ovarian tissue cryopreservation and transplantation, oocyte cryopreservation, and novel ovulation induction approaches, offer new hope to women at risk of being rendered sterile by their treatments.

The treatment options initially offered to cancer survivors have more recently been applied to female patients that are interested in prolonging childbearing. Frequently referred to as "elective" oocyte cryopreservation, the treatment strategies are similar and may give females more reproductive options in the future.

The following section will review the pathophysiology of chemotherapy- and radiotherapy-induced gonadal toxicity, as well as the indications and the outcomes of techniques used for fertility preservation.

Patient Population

The patient population that will seek fertility preservation for a diagnosis of cancer in the reproductive years is small, yet the impact of lost reproductive capacity is large. The overall incidence of cancer in women less than 40 years of age is 754 per 100,000 [91]. The most common malignancies in reproductive-age women are breast, thyroid, melanoma, and cervical or uterine cancer [91]. The mainstay of treatment of all of these malignancies remains surgery, chemotherapy, and/or radiation.

Premature gonadal failure is a well-known consequence of ovarian exposure to chemotherapeutic drugs and radiation therapy. A wide variety of malignant and nonmalignant conditions during the reproductive years are treated with gonadotoxic chemotherapy. In general, radiotherapy is used cautiously in children and adolescents because of its late sequelae on immature and developing tissues [92]. Pelvic radiotherapy is most commonly used to provide local disease control for solid tumors, including bladder, rectum, uterus, cervix, or vagina, all of which are more common in adult women.

Chemotherapy and Ovarian Damage

The extent of chemotherapy-induced gonadotoxicity is variable. Histologic sections of the ovary following treatment with cytotoxic drugs known to cause ovarian failure show a spectrum of changes, ranging from decreased numbers of follicles to absent follicles to fibrosis. The exact incidence of premature ovarian failure following chemotherapy is difficult to establish, because many factors contribute to ovarian toxicity.

Cytotoxic Drug Targets

Cytotoxic drugs can destroy maturing follicles, impair follicular maturation, deplete primordial follicles, or some combination of these effects. Destruction of maturing follicles results in temporary amenorrhea, whereas destruction of primordial follicles results in permanent amenorrhea secondary to ovarian failure.

The menstrual dysfunction that occurs during chemotherapy is not always due to the direct toxic effects on the ovary. Severe illness, malnutrition, and general mental and physical stress can also interfere with normal function of the hypothalamic-pituitary-ovarian axis. Short-term disruption of a menstrual cycle can also be the result of destruction of growing follicles rather than primordial follicles. Destruction of all growing follicles will delay menses for at least 3 months, since it takes a primordial follicle approximately 85 days to reach the stage of ovulation. It is important to note that a normal menstrual cycle after treatment with chemotherapy is not a reliable predictor of a patients' fertility status.

Risk Factors for Gonadal Damage

The most important risk factors for gonadal damage are the age of the patient, the drug class, and cumulative dose of the drug. The risk of gonadal damage increases with the age of the woman. This is most likely due to the presence of fewer remaining oocytes compared to younger patients. In one study of women who had received mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) for Hodgkin's disease, the subsequent amenorrhea rate was 20 % for women <25 years old, compared to 45 % for those ≥25 years old [93].

Additionally, cytotoxic chemotherapeutic agents are not equally gonadotoxic. Cell cycle nonspecific chemotherapeutic agents are considered to be more gonadotoxic than cell cycle specific ones (Table 12.5). Alkylating agents are among the most gonadotoxic of these cell cycle nonspecific drugs, and women who have received high-dose alkylating agent therapy are at highest risk for premature ovarian failure. Cyclophosphamide is considered to be the most gonadotoxic member of this category.

Predicting Ovarian Failure

Premature ovarian failure does not consistently occur in patients receiving multi-agent chemotherapy, regardless of age or type of chemotherapeutic agent. Most young patients with Hodgkin's disease treated with multi-agent chemotherapy and radiation to a field that does not include the ovaries will be fertile, although their fertility will begin to decrease at a younger age than matched controls [94]. A spontaneous conception was reported in a young woman with premature ovarian failure after fourteen courses of an alkylating agent combined with pelvic irradiation for treatment of Ewing's

Table 12.5 Gonadotoxic chemotherapeutic agents

Toxicity	Medication class	Medication
High	*Alkylating agents*:	Cyclophosphamide
	Chloroethylamine	Chlorambucil
	Mechlorethamine	Nitrogen mustard
	Nitrosurea	L-phenylalanine mustard
	Alkylalkane sulfonate	Carmustine
	Methylhydrazine derivative	Lomustine
		Busulfan
		Procarbazine
Intermediate	Platinum complexes	Cisplatinum
	Anthracyclines	Carboplatinum
		Doxorubicin
Low	Antimetabolites	Methotrexte
	Vinca alkaloids	5-Fluorouracil
	Antibiotics	6-Mercaptopurine
		Vincristine
		Vinblastin
		Bleomycin
		Dactinomycin

sarcoma of the pelvis [95]. This exemplifies the difficulties in predicting the probability of ovarian failure after chemotherapy, which also makes it difficult to evaluate the efficacy of treatment aimed at preserving ovarian function.

Markers for Gonadal Damage

Ovarian reserve testing, which was discussed earlier in the chapter, should be offered both pre- and posttreatment. Basal FSH levels (if the patient has menstrual cycles), AMH, inhibin-B, and transvaginal ultrasound measured AFC can be useful markers for ovarian function after chemotherapy [96].

Radiation Therapy and Damage to Pelvic Organs

Pelvic radiotherapy damages both the ovaries and the uterus. Ovarian damage from radiotherapy results in impaired fertility and premature ovarian failure [97–105]. Irradiation-induced uterine damage manifests as impaired growth and blood flow [106]. The effects on subsequent pregnancies can be substantial.

Ovarian Damage

The ovarian follicles are remarkably vulnerable to DNA damage from ionizing radiation. Irradiation results in ovarian atrophy and reduced follicle stores [104]. As a result, serum FSH and LH levels progressively rise and estradiol levels decline within 4–8 weeks following radiation exposure. On the cellular level, irradiation of oocytes results in rapid onset of pyknosis, chromosome condensation, disruption of the nuclear envelope, and cytoplasmic vacuolization. The irreversibility of this damage has been attributed to the lack of germLine stem cells in the ovary.

Risk Factors for Ovarian Damage

Cancer patients are at high risk for premature ovarian failure after treatment with pelvic or total body irradiation. The degree of ovarian damage is related to the patient's age and the total dose of radiation to the ovaries. It is generally estimated that a single dose of 5.0 Gy (Gray) will cause permanent ovarian failure in over 90 % of postpubertal women [107]. When looking at specific age groups, a prepubertal girl may have permanent ovarian failure if exposed to 12 Gy, but only 2 Gy may cause the same result in women over the age of 45 [98].

The dose response of the ovaries to irradiation has been demonstrated in several studies [101–103]. It is estimated that as little as 3 Gy is enough to destroy 50 % of the oocyte population in young, reproductive-age women [100]. When the mean radiation dose to the ovary was 1.2 Gy, 90 % of patients retained their ovarian function. When mean dose was 5.2 Gy, only 60 % retained ovarian function.

Ovarian failure will occur in virtually all patients exposed to pelvic radiation at the doses necessary to treat cervical cancer (85 Gy), rectal cancer (45 Gy), or total body radiation for bone marrow transplantation (8–12 Gy exposed to the ovaries). The addition of chemotherapy to radiotherapy further decreases the dose required to induce premature ovarian failure.

Even if the ovaries are not directly in the radiation field, radiation scatter can reduce ovarian function. Given this risk, it is very important to discuss with the radiation oncologist the expected dose that will be delivered to the ovary either directly or through scatter.

Uterine Damage

Young women exposed to radiotherapy below the diaphragm are at risk of impaired development of the uterus, in addition to ovarian failure. Long-term cancer survivors treated with total body irradiation and marrow transplantation are at risk of impaired uterine growth and blood flow. Despite standard estrogen replacement, the uterine size of young girls is often reduced to 40 % of the normal adult size. The younger the girl is when irradiated, the more the uterus appears to be affected.

It has been demonstrated in women treated with total body irradiation that sex steroid replacement in physiological doses significantly increases uterine volume and endometrial thickness, as well as reestablishes uterine blood flow. However, it is not known whether standard regimens of estrogen replacement therapy are sufficient to facilitate uterine growth in adolescent women treated with total body irradiation in childhood.

Pregnancy after Radiation Therapy

Pregnancies achieved by survivors of childhood cancer who have received pelvic irradiation must be considered high risk [106, 108]. It is likely that radiation damage to the uterine musculature and vasculature adversely affects prospects for pregnancy in these women. Even if the uterus is able to respond to exogenous sex steroid stimulation, and appropriate ARTs are available, pregnancy prognosis remains guarded. Common obstetric problems reported in these patients include early pregnancy loss, premature labor, and low-birth-weight infants [109, 110].

Fertility Preservation Strategies

A wide variety of strategies have been reported in an effort to preserve ovarian function and fertility in women undergoing chemotherapy and/or radiotherapy. Fertility-sparing procedures, pharmacologic options, and ARTs will all be discussed here (Table 12.6).

Surgical Techniques

In general, the conventional therapy for a gynecologic malignancy consists of removal of the uterus, tubes and ovaries. However, there are specific circumstances that may allow a more conservative surgical approach.

Cervical Cancer

Cervical cancer is typically treated with surgery or radiation therapy, dependent on the stage at presentation. Those with early stage disease, Stage IA1, may be treated with cervical conization and close follow-up. Women who desire future fertility and are diagnosed with Stage IA2 and Stage IB disease may opt for a radical trachelectomy, which involves removal of the cervix, surrounding tissues, and lymph node dissection [111]. Patients treated with trachelectomy may require ART to achieve a future pregnancy, and should be aware that they are at increased risk of second-trimester loss and preterm birth after this procedure [112].

Ovarian Cancer

Ovarian tumors classified as low malignant potential, germ cell, sex cord-stromal, or early epithelial malignancies have the potential to be treated with conservative surgery. Most ovarian cancers diagnosed in the reproductive years tend to be unilateral and are less likely to have metastasized. The surgical option most likely to succeed in removing the

Table 12.6 Fertility preservation strategies

	Technique	Pros	Cons	Experimental vs. Established
Surgical	Fertility-preserving surgery	May be able to conceive and carry future pregnancy Less aggressive Inexpensive	May leave residual disease Requires close follow-up May still require ART	Established
	Ovarian transposition	Drastically reduces ovarian exposure to radiation	Requires surgery May fall back into the field Can cause pain	Established
Pharmacologic	GnRH agonists	Minimal delay in treatment	Not proven Side effects similar to menopause	Experimental
Assisted reproductive technology	Embryo cryopreservation	Highest success rates	Elevated hormone levels Time-consuming Requires a male partner Expensive	Established
	Oocyte cryopreservation	Moderate success rates Does not require a male partner	Elevated hormone levels Time-consuming Expensive	Established
	Ovarian tissue cryopreservation	No delay in treatment Does not require a male partner Prepubertal females can use	Requires surgery Ovarian tissue ischemia Possible reexposure to cancer cells	Experimental
	In vitro maturation	Minimal delay in treatment Does not require a male partner	Limited experience with this procedure	Experimental

cancerous tissue, as well as preserve fertility potential, is unilateral oophorectomy with conservation of the remaining normal ovary and the uterus. These women should still undergo complete staging and be monitored closely by a gynecologic oncologist for possible recurrence.

Endometrial Cancer

Both complex endometrial hyperplasia with atypia and early stage endometrial adenocarcinoma (Stage IA1) can be treated conservatively. A hysteroscopy with dilatation and curettage, followed by high-dose progesterone therapy, is the standard treatment for those women who desire fertility preservation. Unfortunately, recurrence is common and close periodic evaluation is required to avoid progression. It is also important to stress to these patients that they should pursue childrearing sooner rather than later, and then have a complete hysterectomy and bilateral salpino-oophrectomy to ensure a disease-free survival.

Ovarian Transposition

Transposition of the ovaries out of the field of radiation appears to help maintain ovarian function in patients scheduled to undergo gonadotoxic radiation therapy. This technique can be utilized for gynecologic, colon, rectal, and anal cancers. Transposition of the ovaries has been reported to reduce the radiation dose to each ovary by approximately 90–95 % compared to ovaries left in their original location [110].

There are two techniques available, lateral and medial transposition. Lateral transposition appears to be more effective than medial transposition. A compilation of 10 case reports and a small series showed an ovarian failure rate of 14 % after lateral transposition compared to 50 % after medial transposition [113].

Ovarian transposition can be performed by either laparotomy or laparoscopy. When surgery is required for the treatment of cervical cancer or during staging and treatment of ovarian cancer, lateral ovarian transposition can be performed simultaneously. However, if a surgical procedure is not required for treatment, the transposition can easily be performed in as an outpatient procedure. The ovaries have a tendency to migrate back to their original position, so it is recommended to complete the procedure immediately prior to the initiation of radiation therapy [103, 114, 115].

Most ovaries will maintain function if they are transposed at least 3 cm from the upper edge of the field [116]. It has been shown that approximately 80 % of women undergoing laparoscopic ovarian transposition will maintain ovarian function after radiation therapy for various indications [117].

Ovarian failure following transposition can be on the basis of several different mechanisms. Ovarian failure may result if the ovaries are not moved far enough out of the radiation field. Another reason for failure would be ovarian migration back to their original position. Ovarian failure following transposition may also be due to compromised ovarian blood flow from surgical technique or radiation injury to the vascular pedicle [118].

Pregnancies have been reported after ovarian transposition. Some have occurred spontaneously, but others required reversal of the procedure.

Pharmacologic Protection

Gonadotropin-Releasing Hormone Agonists

One of the first medical strategies attempted was to mimic the premenarchal state by administering GnRH agonists [119, 120]. This was based on the observation that ovaries of premenarchal girls seemed to be less sensitive to cytotoxic drugs than adult ovaries. The hypothesis is that suppression of FSH and LH will inhibit the normal physiologic loss of primordial follicles by recruitment and subsequent atresia. Other possibilities include a protective effect of decreased ovarian perfusion secondary to the resultant hypo-estrogenic milieu, or a direct gonadal effect mediated through sphingosin-1-phosphate or germline stem cell preservation [121].

The most compelling study of this approach to date was performed in primates. A prospective randomized controlled trial using rhesus monkeys demonstrated that administration of GnRH agonists protected the ovary against cyclophosphamide-induced damage [122]. To date, there are a few studies that have confirmed these results in humans [123–126]. A prospective clinical trial published in 2008 followed 111 patients diagnosed with Hodgkin lymphoma. They were treated with chemotherapy and a monthly injection of GnRH agonist versus chemotherapy alone. In those that received the GnRH agonist, 96.9 % eventually resumed her menses or conceived a child, compared to 63 % of the controls [127].

There have been a few randomized trials with varying results. The largest was a trial of triptorelin in breast cancer patients reported by Del Maestro et al. [128]. Twelve months after their last dose, the rate of early menopause was 25.9 % in the women receiving chemotherapy alone and 9.8 % in those who received triptorelin with their chemotherapy. On the other hand, Isamil-Kahn et al. stopped their randomized trial early because of a lack of benefit after 49 women were enrolled [129].

The data are still not clear on the effectiveness of GnRH agonists as a potential strategy for fertility preservation.

Assisted Reproductive Technology

The application of ART for patients interested in fertility preservation depends on multiple factors, such as the type of cancer, treatment planned, time until treatment will start, and presence of a partner. There are multiple options available to the patient; some are considered established practices and others are experimental techniques. The overall goal is to preserve embryos, oocytes, or ovarian tissue for these women prior to treatment, so they may have options to reproduce in the future.

Embryo Cryopreservation

Embryo cryopreservation is the only established technique approved by the American Society of Reproductive Medicine for fertility preservation [130]. The challenge with this option is that it is only available to those women with a partner or those willing to use donor sperm to create embryos for cryopreservation. The chance of successful outcome is the highest with embryo cryopreservation. The likelihood of a live birth from a cryopreserved embryo in a woman under the age of 40 years old is 28.5–38.7 % [131]. In general, the post-thaw survival rate of embryos ranges between 76 and 93 %, and the clinical pregnancy rate is 37.5–62.5 % [132].

A typical IVF cycle for fertility preservation can be done in a few weeks from start to finish; the time constraint is sometimes dependent on where the patient is in her current menstrual cycle. Some centers have offered natural cycle-IVF for breast cancer patients. During this process, a single oocyte is aspirated during a woman's spontaneous menstrual cycle. Unfortunately, cancellation rates are high and the pregnancy rates are very low for this protocol (7.2 % per cycle and 15.8 % per embryo transfer) [133, 134].

Most centers will use mild ovarian stimulation with a GnRH antagonist to prevent ovulation [135]. In those patients who present in the luteal phase of the menstrual cycle, a GnRH antagonist can be started immediately to help down-regulate LH and initiate luteolysis. Ovarian stimulation is started at the same time, thereby reducing the time to retrieval to less than 3 weeks. Reports in the literature have identified similar dosage requirements, numbers of oocytes retrieved, and fertilization rates in women who started in the luteal phase compared to those who started at in the follicular phase of their menstrual cycle [135, 136].

Tamoxifen and Letrozole

There is some concern that the high estrogen levels obtained during an IVF cycle may decrease long-term survival for breast cancer patients. In those women with hormone-sensitive tumors, stimulation with tamoxifen, a nonsteroidal antiestrogen, or letrozole, an aromatase inhibitor, may be beneficial.

In a manner similar to clomiphene citrate, tamoxifen (40–60 mg) is started on day 2 or 3 of the cycle and given daily for 5–12 days. Letrozole has more recently been utilized as an ovulation induction agent as well. Adding letrozole to a standard gonadotropin stimulation protocol has been shown to lower total serum estradiol levels [137].

To date, there is no correlation between the ovarian stimulation immediately prior to chemotherapy and an increase in breast cancer recurrence rates [137, 138].

Oocyte Cryopreservation

The option of oocyte cryopreservation with a plan for future fertilization is no longer considered an experimental technique by the ASRM [130]. The greatest concern about utilizing oocyte cryopreservation is that the success rate in the past was significantly lower than with embryo cryopreservation. Early studies reported a low survival, fertilization, and pregnancy rate with thawed oocytes [139]. However, in women without partners, freezing mature or immature oocytes may be the only practical option.

The structural complexity of the oocyte is most likely responsible for the reduced success rate in oocyte cryopreservation. Unlike fertilized embryos, the subcellular organelles in oocytes are far more complex and more sensitive to thermal injury [140, 141]. Improvements in the cryopreservation technique have led to significant improvements in the overall outcome of oocyte cryopreservation. The advent of vitrification for cryopreservation, rather than the slow-freeze protocol, has reduced the damage caused from ice-crystal formation and subsequent cellular damage [142]. Recent reports have seen survival rates after a thaw of 75–81 %, fertilization rates of 77 %, and clinical pregnancy rates of 38 % [143]. In those pregnancies that have resulted from oocyte cryopreservation, there appears to be no increase in chromosomal abnormalities, birth defects, or developmental deficits [144].

Cryopreservation of Ovarian Tissue

Ovarian tissue cryopreservation is an experimental procedure introduced to preserve fertility in women who cannot delay treatment and undergo the stimulation necessary to create embryos or oocytes for cryopreservation [145]. It is the only option currently available to prepubertal females. It involves removal of small strips of ovarian cortical tissue and freezing it as an avascular graft, in an effort to save thousands of primordial follicles for future use. When the patient is in remission, the ovarian tissue can then be transplanted back to the ovary or to the patient's subcutaneous tissue. It is also possible that one day the primordial follicles can be matured in vitro.

One of the concerns about returning tissue back to the patient is the theoretical risk of reintroducing cancer cells into the circulation. This concern may limit its use in malignancies that have a high chance of involving the ovaries, including leukemias and potentially breast cancers.

An additional limitation to the procedure is the loss of a large fraction of follicles during the initial ischemia that occurs after transplantation [146–148]. Previous work indicated that while loss due to freezing is relatively small, up to two-thirds of follicles are lost subsequent to transplantation.

Both the return of ovarian function and spontaneous pregnancies have been reported in patients after orthotopic transplantation [149–152]. The live births reported have all been after the tissue was transferred back to the pelvis. In some patients, this does not exclude the possibility that ovarian function resumed from areas in the ovary that had not been removed and reimplanted.

In Vitro Maturation

In vitro maturation is another potential modality for obtaining oocytes with little or no ovarian stimulation. This technique is considered an experimental procedure and is only performed in a few centers around the world. It appears the most effective in patients with polycystic ovarian syndrome where numerous immature oocytes can be retrieved. The entire process takes less than a week, which can be attractive to physicians and patients who are eager to start cancer treatment. An ultrasound is performed on day 6–8 of the cycle and human chorionic gonadotropin (hCG) is given to help increase oocyte maturity at the time of retrieval. Oocyte retrieval is scheduled 36 h later and immature oocytes are obtained and incubated in special culture media. If the oocytes mature, as determined by extrusion of the first polar body, fertilization is attempted by intracytoplasmic sperm injection. The embryos formed are then cryopreserved. If there is no partner, the mature oocytes can be cryopreserved.

This procedure is significantly less successful than those mentioned earlier. To date, over 300 live births have resulted from this procedure; however, significantly fewer embryos will be obtained per cycle and the chance of implantation, pregnancy, and live birth is low [153].

In vitro maturation of primordial follicles obtained from frozen thawed ovarian cortical strips is not yet possible. Contrary to in vitro maturation of oocytes from antral follicles, which requires days to mature, in vitro maturation of oocytes derived from primordial follicles would require months.

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13 Recurrent Early Pregnancy Loss

Paul Robert Brezina and William Hanna Kutteh

Introduction

Recurrent early pregnancy loss (RPL) is a profound personal tragedy to couples seeking parenthood and a formidable clinical challenge to their physician. When to evaluate a couple and what constitutes a complete evaluation is, at the time of the writing of this chapter, in a state of flux. The American College of Obstetrics and Gynecology has withdrawn their 2001 Practice Bulletin on early RPL and have not issued a replacement for over 1 year. The American Society for Reproductive Medicine (ASRM) has recently released a committee opinion after extensively evaluating available evidence over a 7-year period [1].

While spontaneous abortion occurs in approximately 15 % of clinically diagnosed pregnancies of reproductive-aged women, recurrent pregnancy loss occurs in about 1–2 % of this same population [2]. Great strides have been made in characterizing the incidence and diversity of this heterogeneous disorder, and a definite cause of pregnancy loss can be established in over half of couples after a thorough evaluation [3, 4]. A complete evaluation will include investigations into genetic, endocrinologic, anatomic, immunologic, and iatrogenic causes. The occurrence of recurrent pregnancy losses may induce significant emotional distress, and in some cases, intensive supportive care may be necessary. Successful outcomes will occur in over two-thirds of all couples [4, 5].

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Definition of Pregnancy Loss

The traditional definition of RPL included those couples with three or more spontaneous, consecutive pregnancy losses. Ectopic and molar pregnancies are not included. The ASRM has defined RPL as "a distinct disorder defined by 2 or more failed clinical pregnancies" [1]. For purposes of determining if an evaluation for RPL is appropriate, pregnancy "is defined as a clinical pregnancy documented by ultrasonography or histopathological examination" [1]. Several studies have recently indicated that the risk of recurrent miscarriage after two successive losses is similar to the risk of miscarriage in women after three successive losses; thus, it is reasonable to start an evaluation after two or more consecutive spontaneous miscarriages to determine the etiology of their pregnancy loss, especially when the woman is older than 35 years of age, or when the couple has had difficulty conceiving [6].

Those couples with primary recurrent pregnancy loss have never had a previous viable infant, while those with secondary recurrent loss have previously delivered a pregnancy beyond 20 weeks and then suffered subsequent losses. Tertiary recurrent loss refers to those women who have multiple miscarriages interspersed with normal pregnancies.

Recurrence Risk

The main concerns of couples with recurrent miscarriage when they present to our recurrent pregnancy loss center are to find the cause and to establish the risk of recurrence. In a first pregnancy, the overall risk of loss of a clinically recognized pregnancy loss is 15 % [7, 8]. The true risk of early pregnancy loss, however, is estimated to be around 50 % because of the high rate of losses that occur before the first missed menstrual period. Furthermore, as women age, this rate likely rises, owing to chromosomal errors introduced through meiotic nondisjunction errors during oocyte maturation.

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Studies that evaluated the frequency of pregnancy loss, based on highly sensitive tests for quantitative hCG, indicated that the total clinical and preclinical losses in women aged 20–30 is approximately 25 %, while the loss rate in women aged 40 or more is at least double that figure [7, 8]. The ability to predict the risk of recurrence is influenced by several factors, including maternal age, parental and fetal karyotypes, the gestational age at which prior losses occurred, and the presence of various maternal laboratory findings [8–14].

Etiologies, Diagnosis, and Treatment of Recurrent Pregnancy Loss

Traditionally, the chief causes of RPL have been thought to be due to embryonic chromosomal abnormalities, maternal anatomic abnormalities such as a uterine septum, luteal phase defects, and antiphospholipid antibodies. Other factors, such as infection and hypercoaguable state, have also been considered but to a somewhat lesser degree.

When to initiate an RPL workup has been a source of recent debate. Classically, conducting a workup for RPL was recommended after three miscarriages. Recent data do not necessarily support this traditional evaluation protocol [15, 16]. The evaluation of healthy women after a single loss is usually not recommended, as this is a relatively common, sporadic event. However, the risk of another pregnancy loss after two miscarriages is only slightly lower (24–29 %) than that of women with three or more spontaneous abortions (31–33 %) [7]. Therefore, evaluation and treatment can reasonably be started after two consecutive miscarriages [1, 4]. Furthermore, additional testing such as chromosomal testing of the products of conception from a second miscarriage may confer a cost-saving measure [15, 16]. Based on available data, we outline a new strategy for the workup of RPL (Fig. 13.1).

An evaluation of an RPL patient should always include a complete history, including documentation of prior pregnancies, any pathologic tests that were performed on prior miscarriages, any evidence of chronic or acute infections or diseases, any recent physical or emotional trauma, history of cramping or bleeding with a previous miscarriage, any family history of pregnancy loss, and any previous gynecologic surgery or complicating factor. A summary of the diagnosis and management of recurrent pregnancy loss includes an investigation of genetic, endocrinologic, anatomic, immunologic, and iatrogenic causes (Table 13.1).

We outline a proposed algorithm for the evaluation and treatment of RPL (see Fig. 13.1). Under this new schema, no diagnostic/therapeutic action is recommended following one miscarriage. Fetal karyotype is recommended to be obtained after the second miscarriage. Products of conception (POC) to be sent for karyotype may be obtained from early nonviable pregnancies either via traditional D+C or with in-office aspiration. POC may be sent for traditional karyotype or, as we recommend, be sent for 23 chromosome pair microarray evaluation.

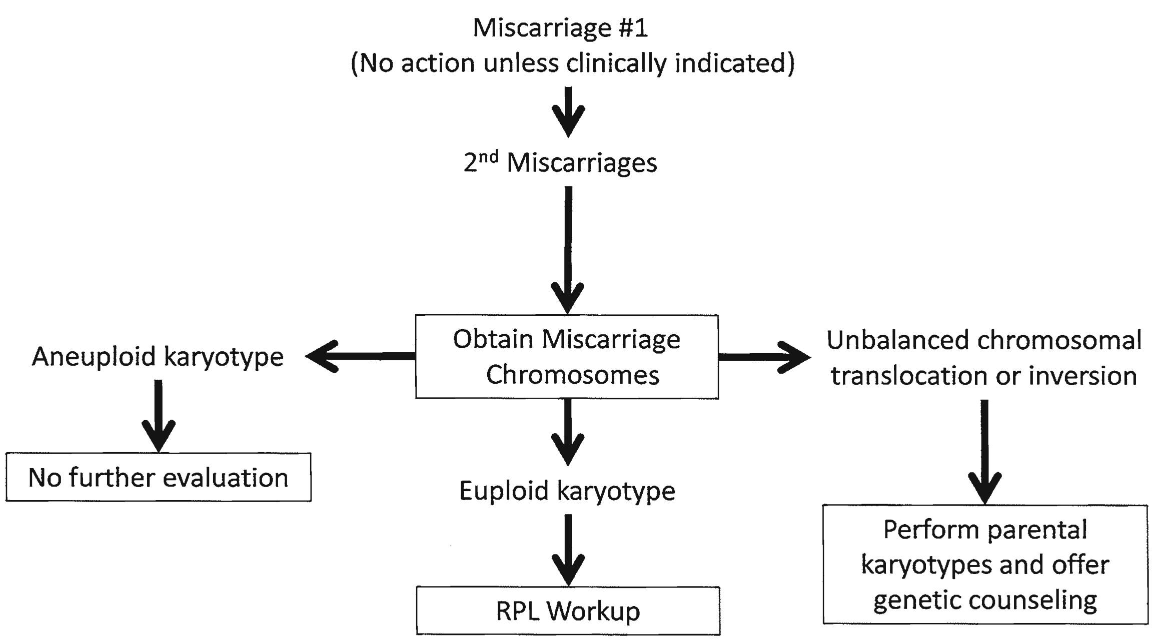

Fig. 13.1 Initial evaluation for early RPL. This figure outlines an algorithm for the initial evaluation of early RPL. *Arrows* are provided that guide the reader through various outcomes possible during the RPL evaluation and appropriate "next steps" in diagnostic management

Table 13.1 Diagnosis and management of recurrent pregnancy loss

Etiology	Diagnostic evaluation	Therapy
Genetic	Karyotype partners	Genetic counseling
	Karyotype POC[a]	Donor gametes, PGD[b]
Anatomic	Hysterosalpingogram	Septum transection
	Hysteroscopy	Myomectomy
	Sonohysterography	Lysis of adhesions
	Transvaginal 3D US[c]	
Endocrinologic	Midluteal progesterone	Progesterone
	TSH	Levothyroxine
	Prolactin	Bromocriptine, Dostinex
	HgbA1c	Metformin
Immunologic	Lupus anticoagulant	Heparin + aspirin
	Antiphospholipid antibodies	Heparin + aspirin
	Anti-β(beta)2 glycoprotein	Heparin + aspirin
Psychologic	Interview	Support groups
Iatrogenic	Tobacco, alcohol use, obesity	Eliminate consumption
	Exposure to toxins, chemicals	Eliminate exposure

[a] Products of conception
[b] Preimplantation genetic diagnosis
[c] Transvaginal three-dimensional ultrasound
[d] Indications for testing and treatment are not clearly established

The results of this POC karyotype guide further evaluation. If the POC are found to be aneuploid, no further evaluation or treatment is recommended at that juncture because the cause for the loss is known, although all future early miscarriages should also be subject to karyotypic evaluation. If an unbalanced chromosomal translocation or inversion is identified in the fetal POC, then the workup would focus on performing parental karyotypes and offering appropriate therapeutic options, such as preimplantation genetic testing. If the fetal POC are found to be chromosomally normal, then a full RPL workup is to be done. If the fetal POC karyotypes have not been performed, then we recommend a full RPL workup after at least two miscarriages. The full RPL workup is outlined in Fig. 13.2.

What constitutes a full RPL is a topic of debate. We recommend including an anatomic evaluation, endocrinologic evaluation, testing for autoimmune factors, evaluating lifestyle and environmental factors, and obtaining parental karyotypes if the karyotyic status of prior POC is unknown. Not included in this evaluation are more controversial types of testing and therapies, such as those dealing with microbiologic factors, thrombophilic factors, immunotherapy, and other evaluations, although these may be appropriate in certain clinical situations. In the following section, we describe the physiologic background, diagnostic approaches, and therapy of the various components of our proposed RPL workup. In addition, we address other more controversial proposed etiologies of RPL.

Anatomic Causes of RPL

Anatomic causes of RPL are typically diagnosed using hysterosalpingography (HSG) or sonohysterography. Hysteroscopy, laparoscopy, or magnetic resonance imaging (MRI) can supplement these tests as needed. Recently, transvaginal three-dimensional ultrasonography has been introduced and has allowed an accurate and noninvasive diagnosis of congenital uterine anomalies. The treatment of congenital and acquired uterine anomalies often involves corrective surgery.

Congenital Malformations

Congenital malformations of the reproductive tract result from failure to complete bilateral duct elongation, fusion, canalization, or septal resorption of the Müllerian ducts. Müllerian anomalies were found in 8–10 % of women who had had three or more consecutive spontaneous abortions who underwent HSG or hysteroscopic examination of their uteri [3, 4, 17]. Inadequate vascularity compromising the developing placenta and reduced intraluminal volume have been theorized as possible mechanisms leading to pregnancy loss.

The most common congenital abnormality associated with pregnancy loss is the septate uterus. The spontaneous abortion rate is high, averaging about 65 % of pregnancies in some studies [18]. A septum is primarily composed of fibromuscular tissue that is poorly vascularized. This lack of vascularization may compromise decidual and placental growth. Alternatively, a uterine septum may impair fetal growth as a result of reduced endometrial capacity or a distorted endometrial cavity [18]. Uncontrolled studies suggest that resection of the uterine septum results in higher delivery rates than in women without treatment. Other congenital abnormalities, such as uterine didelphys and bicornuate and unicornuate uterus, are more frequently associated with later trimester losses or preterm delivery.

Intrauterine Adhesions

Intrauterine trauma resulting from endometrial curettage or endometritis is associated with a risk for the development of adhesions. Intrauterine adhesions (synechiae) are an acquired uterine defect that has been associated with recurrent miscarriage. The severity of adhesions may range from minimal to complete ablation of the endometrial cavity. The term Asherman's syndrome is often used to describe intrauterine adhesions associated with oligomenorrhea or amenorrhea.

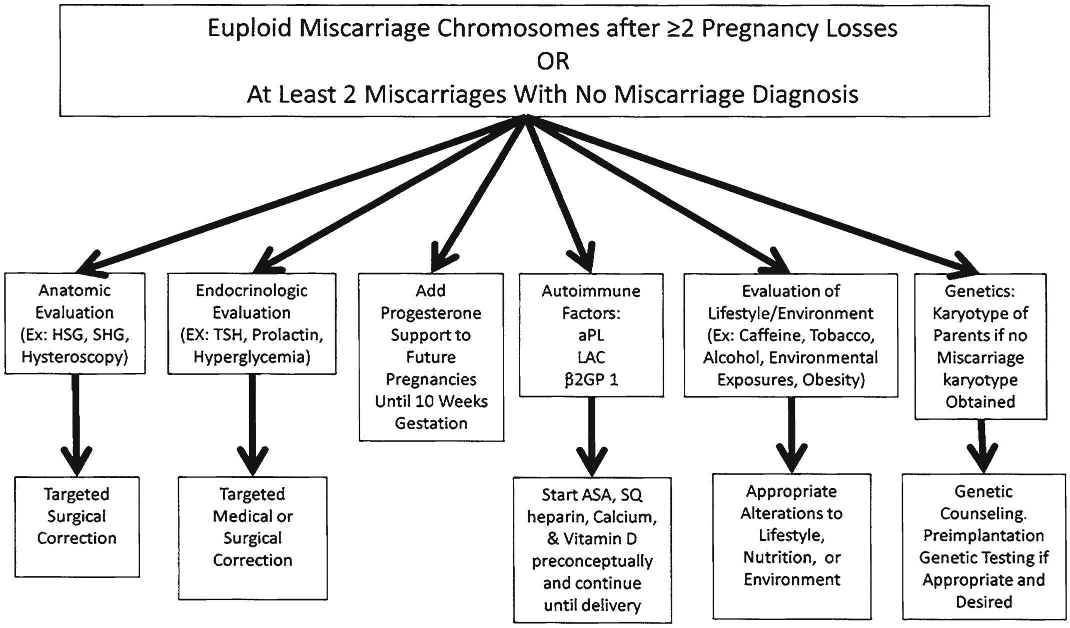

Fig. 13.2 Workup for early RPL. This figure outlines an algorithm for the full workup of early RPL. *Arrows* are provided that guide the reader through various outcomes possible during the RPL evaluation and appropriate "next steps" in diagnostic and therapeutic management. *HSG* hysterosalpingogram; *SHG* sonohysterogram; *TSH* thyroid stimulating hormone; *aPL* antiphospholipid antibodies; *LAC* lupus anticoagulant; *β(beta)2GP1*, *β(beta)2* glycoprotein 1; *ASA* aspirin

These adhesions are thought to interfere with the normal placentation and are treated with hysteroscopic resection. The insertion of an intrauterine balloon catheter for 1week is recommended after resection of synechiae by some physicians to help prevent reformation of adhesions. During this time, antibiotic prophylaxis with doxycycline (100 mg twice a day) is given to prevent endometritis. Patients may also be given estrogen and progestin for 1 month.

Intrauterine Masses

Intrauterine cavity abnormalities, such as submucosal leiomyomas and polyps, can contribute to pregnancy loss. Depending on the size and location of the leiomyoma, it may partially obliterate or alter the contour of the intrauterine cavity, providing a poorly vascularized endometrium for implantation or otherwise compromising placental development. Uterine leiomyomas and polyps may also act like an intrauterine device (IUD), causing subacute endometritis. Until recently, it was felt that only submucous leiomyomas should be surgically removed prior to subsequent attempts at pregnancy. However, several recent studies investigating the implantation rate in women undergoing in vitro fertilization have clearly demonstrated decreased implantation with intramural leiomyomas in the range of 30 mm [19]. When smaller leiomyomas are identified, it is unclear if myomectomy is beneficial [20].

Incompetent Cervix

Cervical incompetence can be considered as an acquired uterine anomaly that is associated with RPL. The diagnosis of cervical incompetence is based on the presence of painless cervical dilation resulting in the inability of the uterine cervix to retain a pregnancy. Cervical incompetence commonly causes pregnancy loss in the second, rather than first, trimester. It may be associated with congenital uterine abnormalities such as septate or bicornuate uterus. Rarely, it may be congenital following in utero exposure to diethylstilbestrol [21]. It is postulated that most cases occur as a result of surgical trauma to the cervix from conization, loop electrosurgical excision procedures, overdilaton of the cervix during pregnancy termination, or obstetric lacerations [22].

Endocrinologic Causes of RPL

Endocrine factors may contribute to 8–12 % of recurrent pregnancy loss. Therefore, an endocrinologic evaluation is a critical component of the RPL workup.

Luteal Phase Deficiency

Maintenance of early pregnancy depends on the production of progesterone by the corpus luteum. Between 7 and 9 weeks of gestation, the developing placenta takes over the progesterone production. Luteal phase deficiency (LPD) is defined as an inability of the corpus luteum to secrete progesterone in high enough amounts or for too short a duration. The preponderance of evidence suggests that LPD is a preovulatory event most likely linked to an alteration in the preovulatory estrogen stimulation that may indicate poor oocyte quality and a poorly functioning corpus luteum [23, 24]. Classically, the diagnosis is based upon results of endometrial biopsy, though this is currently not recommended as a diagnostic modality. Most authors advocate the measurement of serum progesterone levels in the luteal phase for the diagnosis of LPD, with levels below 10 ng/mL considered abnormal [25]. However, progesterone levels are subject to large fluctuations because of pulsatile release of the LH hormone. Moreover, there is a lack of correlation between serum levels of progesterone and endometrial histology [26]. While conflicting data exist, a recent Cochrane review evaluating 15 trials concluded that there was a benefit to the routine administration of progesterone to all women with a history of RPL [27, 28]. Progesterone is available either as intravaginal suppositories (50–100 mg twice daily starting the third day after LH surge and continuing for 8–10 weeks) or as intramuscular injections (50 mg IM daily).

Untreated Hypothyroidism

Untreated hypothyroidism may increase the risk of miscarriage. A study of over 700 patients with recurrent pregnancy loss identified 7.6 % with hypothyroidism [29]. Hypothyroidism is easily diagnosed with a sensitive TSH test, and patients should be treated to become euthyroid (defined for the purposes of RPL as between 1.0 and 2.5 μIU/mL) before attempting a next pregnancy [1, 30]. It has also been suggested that thyroid antibodies are elevated in women with recurrent pregnancy loss. A retrospective study of 700 patients with recurrent pregnancy loss demonstrated that 158 women had antithyroid antibodies, but only 23 of those women had clinical hypothyroidism on the basis of an abnormal TSH value [31]. The presence of antithyroid antibodies may imply abnormal T-cell function; therefore, an immune dysfunction rather than an endocrine disorder may be responsible for the pregnancy losses. The Endocrine Society recommend that patients with RPL be treated to keep a TSH level of between 1.0 and 2.5 μIU/mL in the first trimester [30]. For TSH levels found to be between 2.5 and 10 μIU/mL, a starting levothyroxine dose of at least 50 μg/day is recommended [30].

Abnormal Glucose Metabolism

Patients with poorly controlled diabetes are known to have an increased risk of spontaneous miscarriage, which is reduced to normal spontaneous loss rates when women are euglycemic preconceptually [32]. Testing for fasting insulin and glucose is simple, and treatment with insulin-sensitizing agents can reduce the risk of recurrent miscarriage [33]. More recently, determining the average load of blood glucose through testing of hemoglobin A1C has become an increasingly utilized modality to evaluate insulin resistance [1]. Because there is strong evidence that obesity and/or insulin resistance is associated with an increased risk of miscarriage, weight reduction in obese women is a first step in the treatment. Metformin seems to improve pregnancy outcome, but the evidence for this treatment is limited to a few cohort studies. Metformin is a category B medication in the first trimester of pregnancy and appears to be safe. Other endocrine abnormalities, such as thyroid disorders and diabetes, should be corrected prior to conception.

Hyperprolactinemia

Normal circulating levels of prolactin may play an important role in maintaining early pregnancy. Data from animal studies suggest that elevated prolactin levels may adversely affect corpus luteal function; however, this concept has not been proven in humans [34]. A recent study of 64 hyperprolactinemic women showed that bromocriptine therapy was associated with a higher rate of successful pregnancy and that prolactin levels were significantly higher in women who miscarried [35].

Diminished Ovarian Reserve

Follicle-stimulating hormone (FSH) is thought to be a marker of the number of follicles available for recruitment on any given menstrual cycle. Therefore, elevated levels of FSH in the early follicular phase of the menstrual cycle are representative of diminished ovarian reserve, a condition in which there is a low number of follicular units available for recruitment.

More recently, other markers, such as decreased anti-Müllerian hormone, have been introduced to identify diminished ovarian reserve. Although the frequency of elevated day-3 FSH levels in women with recurrent miscarriage is similar to the frequency in the infertile population, the prognosis of recurrent miscarriages is worsened with increased day-3 FSH levels [36]. Although no treatment is available, testing may be helpful in women over the age of 35 with recurrent pregnancy loss, and appropriate counseling should follow.

Autoimmune Factors as the Cause of RPL

Immunologic Disorders

Autoimmune Factors: Maternal Response to Self

In some instances, there is a failure in normal control mechanisms that prevents an immune reaction against self, resulting in an autoimmune response [37]. Autoantibodies to phospholipids, thyroid antigens, nuclear antigens, and others have been investigated as possible causes for pregnancy loss [29]. Antiphospholipid (aPL) antibodies include the lupus anticoagulant, anti-beta 2 glycoprotein I antibodies, and anticardiolipin antibodies. There is still controversy concerning testing for other phospholipids, but an increasing number of studies suggest that antibodies to phosphatidyl serine are also associated with pregnancy loss [38]. Women with systemic lupus erythematosus and aPL have increased risks for miscarriage compared to those with lupus and negative aPL [39].

Antiphospholipid Antibody Syndrome

Antiphospholipid antibody syndrome (APS) is an autoimmune condition characterized by the production of moderate to high levels of antiphospolipid antibodies and certain clinical features (Table 13.2) [40]. The presence of antiphospholipid antibodies (anticardiolipin and lupus anticoagulant) during pregnancy is a major risk factor for adverse pregnancy outcome [41]. In large meta-analysis of studies of couples with recurrent abortion, the incidence of APS was between 15 and 20 %, compared to about 5 % in nonpregnant women without a history of obstetrical complications [42, 43].

Table 13.2 Clinical and laboratory characteristics of antiphospholipid antibody syndrome[a]

Clinical	Laboratory
Pregnancy morbidity	Positive IgG anticardiolipin[b]
≥1 unexplained death at ≥10 weeks or	Positive IgM anticardiolipin[b]
Delivery at ≤34 weeks with severe PIH or	Positive lupus anticoagulant test
Three or more losses before 10 weeks	IgG anti-β(beta)2 glycoprotein 1[b]
Thrombosis	IgM anti-β(beta)2 glycoprotein 1[b]
Venous	
Arterial, including stroke	

PIH pregnancy-induced hypertension
[a]Modified from [40]. Patients should have at least one clinical and one laboratory feature at some time in the course of their disease. Laboratory tests should be positive on at least two occasions
[b]≥99th percentile

Several mechanisms have been proposed by which antiphospholipid antibodies (aPL) might mediate pregnancy loss. Classically, it was believed that aPL antibodies induced thromboses in vessels surrounding the placental-maternal unit, resulting in placental infarction and fetal death. However, recent data suggest that the primary mechanism by which aPL antibodies lead to miscarriage may be via a deleterious effect conferred directly on trophoblastic cells and/or endothelial cells [44, 45]. aPL antibodies can interact with cultured human vascular endothelial cells with resultant injury and/or activation [44]. Furthermore, aPL have been demonstrated to inhibit secretion of human placental chorionic gonadotropin and to inhibit the expression of trophoblast cell adhesion molecules (alpha-1 and alpha-5 integrins, E and VE cadherins) [45]. These mechanisms could explain RPL secondary to aPL antibodies early in the first trimester [42].

APS is treated with a combination of low-dose heparin (5,000–10,000 units subcutaneously every 12 h), and low-dose aspirin (81 mg PO daily) appears to be effective and may reduce pregnancy loss by 54 % in women with APS [46, 47]. Aspirin alone does not appear to reduce miscarriage rates [48]. Unfractionated heparin is preferred to low-molecular-weight heparin (LMWH), based on available data [49].

Treatment with steroids is not recommended based on current evidence [42, 46]. Aspirin should be started preconceptually, and heparin should be started after the first positive pregnancy test [47]. Treatment should be continued until the time of delivery because these women are at an increased risk for thrombosis. Postpartum thromboprophylaxis is reasonable for a short interval to prevent thrombosis when the risk is high [43]. The adverse reactions associated with heparin include bleeding, thrombocytopenia, and osteoporosis with fracture. Calcium (600 mg twice daily) with added vitamin D supplementation (1000 IU daily) and weight-bearing exercise are encouraged to decrease the risk of osteoporosis. In any pregnant woman starting on heparin, the platelet count should be monitored weekly for the first 2 weeks after initiation and after any dosage change. Women with APS should consider avoiding the use of estrogen-containing oral contraceptives in the future.

Immunotherapy

Immunotherapy for alloimmune disorders is based on the hypothesis that spontaneous abortion occurs due to a failure of maternal immunological adaptation to the developing conceptus, resulting in a form of transplantation rejection. Although some randomized, double-blinded studies have shown an increase with therapies such as paternal leukocyte immunization, trophoblast immune infusion, intravenous intralipid therapy, and immunoglobulin infusion in successful pregnancy outcomes, others have not confirmed these results [50–54]. A Cochrane review of 19 trials of various forms of immunotherapy did not show significant differences between treatment and control groups [55]. There is currently insufficient evidence to recommend the use of these therapies for RPL. Testing for Th-1and Th-2 profiles, parental HLA-profiles, alloantibodies, NK cells, antiparental cytotoxic antibodies, or embryotoxic factor assessment is currently not clinically justified.

Antinuclear Antibodies

Approximately 10–15 % of all women will have detectable antinuclear antibodies regardless of their history of pregnancy loss. Their chance of successful pregnancy outcome is not dependent on the presence or absence of antinuclear antibodies. Treatments such as steroids have been shown to increase the maternal and fetal complications without benefiting live births [56]. Thus, routine testing and treatment for antinuclear antibodies is not indicated.

Microbiologic

Certain infectious agents have been identified more frequently in cultures from women who have had spontaneous pregnancy losses [57]. These include *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Chlamydia*. Other less frequent pathogens include *Toxoplasma gondii*, rubella, HSV, measles, CMV, coxsackievirus, and *Listeria monocytogenes*. It is important to be aware that none of these pathogens have been causally linked to RPL. Because of the association with sporadic pregnancy losses and the ease of diagnosis, some clinicians will test women with RPL and treat for the appropriate pathogen in both parents.

Thrombotic Disorders

Thrombophilias are thought to be responsible for more than half of maternal venous thromboembolisms in pregnancy; however, ACOG recommends that only patients with a personal or family history of thromboembolic events should be tested [58].

The recommended evaluations are as follows:

- Factor V Leiden screening with activated protein C resistance using a second-generation coagulation assay is probably the most cost-effective approach. Patients with a low APC resistance ratio (<2.0) should then be genotyped for the factor V Leiden mutation
- Prothrombin G20210A gene mutation using PCR
- Antithrombin activity with normal levels between 75 and 130 %
- Protein S activity with normal levels between 60 and 145 %
- Protein C activity with normal levels between 75 and 150 %

Genetic Factors as the Cause of RPL

A variety of genetic factors may result in failure of a pregnancy to develop. These include aneuploidy (the gain or loss of a chromosome), chromosomal imbalances as a result of parentally harbored translocations or inversions, deletions or duplications of genetic information within chromosomes, and single-gene mutations. Broadly, genetic factors may be divided into embryonic errors derived from known parental chromosomal abnormalities and embryonic errors that arise de novo in apparently chromosomally normal parents.

Parental Chromosomal Disorders

Parental chromosome anomalies occur in 3–5 % of couples with RPL as opposed to 0.7 % in the general population. These include *translocations*, *inversions*, and the relatively rare *ring chromosomes*. Balanced translocations are the most common chromosomal abnormalities contributing to recurrent pregnancy loss [59]. In recurrent pregnancy loss, this abnormality is found more frequently in the female partner at a ratio of 2:1 up to 3:1 (female:male). In addition to genetic errors resulting from the parental balanced translocation, recent data from preimplantation genetic testing has shown that embryos resulting from parents harboring a balanced reciprocal translocation also have rates of unrelated chromosomal aneuploidy at rates exceeding 35 % [60].

Translocations are where chromosome segments are exchanged between nonhomologous chromosomes. There are two main types of translocation: reciprocal and Robertsonian. Robertsonian translocations involve two acrocentric chromosomes (numbers 13, 14, 15, 21, 22) that fuse near the centromere region with loss of the short arms. Studies indicate that when the Robertsonian translocation is maternal, there is a greater risk that the fetus will exhibit an unbalanced phenotype [61]. In reciprocal translocations, the type of rearrangement results from breakage of nonhomologous chromosomes, with reciprocal exchange of the broken-off segments [62, 63]. Balanced reciprocal translocations are thought to directly contribute to both infertility and recurrent pregnancy loss (RPL) [64, 65].

Parental balanced chromosomal errors such as translocations or inversions are diagnosed through karyotyping of couples. Chromosomal abnormality in one of the parents can be found in up to 3–5 % of couples who experience multiple spontaneous abortions. If no fetal POC is available and the couple has a history of at least two consecutive or three non-consecutive fetal losses, we recommend obtaining parental karyotypes. Treatment of parental balanced chromosomal translocations/inversions may be addressed through preimplantation genetic testing which will be addressed later in this chapter.

Recurrent Aneuploidy

The first chromosomally abnormal abortus was documented in 1961, and since then, a large body of data on the chromosomal status of spontaneous abortuses has accumulated. The overall frequency of chromosome abnormalities in spontaneous abortions is at least 50 % [14, 66–69]. Of these abnormalities, most are numerical: 52 % are trisomies, 29 % are monosomy 45,X, 16 % are triploidies, 6 % are tetraploidies, and 4 % are structural rearrangements [70]. Evidence suggests that some couples are at risk for conceptions complicated by recurrent aneuploidy. Empirically, the birth of a trisomic infant places a woman at an approximately 1 % increased risk for a subsequent trisomic conceptus [71]. Germline mosaicism has been reported in recurrent cases of Down syndrome and may also be responsible for recurrent aneuploidy in some couples [72]. The vast majority of embryonic aneuploidy is thought to be a result of maternal meiotic nondisjunction during oocyte development, although abnormalities arising from the sperm component are possible, especially in couples with male factor infertility.

There is significant debate in the professional community as to how prevalent aneuploidy is among RPL embryos. Emerging data suggest that RPL patients may have lower rates of embryonic aneuploidy in first-trimester miscarriages as compared to all women. For example, a study evaluating 4,873 embryos via single nucleotide polymorphism (SNP) microarray showed the rate of aneuploidy found using trophectoderm biopsy at the blastocyst stage in RPL was significantly lower (32 %) than the rate of aneuploidy found with cleavage-stage biopsy (61 %) [73]. Therefore, it is possible that couples with a diagnosis of RPL may produce embryos with aneuploidy at a high rate. It is less clear, however, what percentage of these aneuploid embryos progress to the blastocyst stage. Other data from small studies suggest that the rate of aneuploidy in embryos in RPL is higher than 65 % [74, 75]. Therefore, the true rate of embryonic aneuploidy in couples with a diagnosis of RPL is currently a topic of great debate among reproductive endocrinologists.

Chromosomal Deletions and Duplications

In contrast to aneuploidy, which is the gain or loss of an entire chromosome, chromosomal deletions and duplications of discrete regions of chromosomes in euploid embryos are also possible. The impact of these regions of deletions and duplications is still unclear. Small deletions and duplications are also known as copy number variations and have been shown to be extremely common and are present in most phenotypically normal adults. Recently, however, many of these copy number variations have been linked to a variety of medical disorders. The impact of larger deletions and duplications is ill defined as it relates to embryo development. However, certain deletions and duplications in which a critical area of a chromosome is significantly altered may certainly lead to significant medical disorders or arrest of a developing embryo.

Developmental Errors in Euploid Embryos

Another cause of first-trimester miscarriage appears to be failure of chromosomally normal embryos to develop properly. Much as a women with Müllerian agenesis are chromosomally normal but have a separate developmental abnormality, serious developmental abnormalities that involve vital structures may result in euploid embryos. Small studies have suggested that the rates of significant anatomic abnormalities in chromosomally normal embryos taken from first-trimester miscarriages in women with RPL may exceed 25 % [75].

Preimplantation Genetic Testing

Genetic causes of RPL may be subdivided into embryo abnormalities that are the result of known parental abnormalities (such as parental balanced translocations or inversions) and embryo aneuploidy in parents believed to be chromosomally normal. Preimplantation genetic testing is a technology that is designed to minimize the effects of these and other embryonic genetic abnormalities. Preimplantation genetic testing is accomplished by performing an in vitro fertilization (IVF) cycle, removing a cell(s) from the resultant embryos or oocytes, evaluating this cell for genetic abnormalities, and using the results to determine which embryos are ideal for uterine transfer. When a known parental genetic abnormality is identified, as in parental balanced translocations, this practice is referred to as preimplantation genetic diagnosis, or PGD. When this process is executed to determine which embryos are aneuploid in parents believed to be chromosomally normal, the procedure is referred to as preimplantation genetic screening, or PGS.

PGD for structural aberrations such as translocations and inversions is a generally accepted utilization of preimplantation genetic testing. In contrast, PGS is far more controversial. A 2007 publication in the *New England Journal of Medicine* by Mastenbroek et al. showed no benefit from PGS [76]. This was followed by major medical societies discouraging the routine use of PGS [77]. Since this time, newer technologies, such as microarrays, have been introduced that are capable of evaluating the ploidy status of all 23 pairs of chromosomes instead of the 9–14 pairs of chromosomes evaluated with older fluorescence in situ hybridization (FISH) technologies [78]. Additionally, performing embryo biopsy at the blastocyst, as opposed to the cleavage stage, seems to confer superior pregnancy rates [79, 80].

Recent data evaluating pregnancy rates in RPL patients using 23 chromosome microarrays are encouraging [73, 81]. PGS as a treatment modality is currently widely utilized. Of 27,630 PGT IVF cycles reported over the past 10 years to the ESHRE PGD Consortium, collecting data from around the globe, 61 % ($n=16{,}806$), were performed for PGS [78]. Despite this high rate of utilization, however, large and well-conducted randomized controlled trials are necessary to firmly establish the efficacy of PGS and define which patient populations may benefit from these technologies. Furthermore, PGS is far from a full-proof technology in determining the ploidy status of an embryo. Embryo mosaicism, the presence of more than one cell line within the same embryo, has been shown to be as high as 50 % in cleavage-stage embryos and as high as 10 % in blastocysts [82, 83]. Therefore, the cell taken at the time of embryo biopsy may not always be representative of the genetic composition of the embryo. Furthermore, technical limitations such as failure to successfully amplify genomic DNA, genomic contamination, and the possibility for human error may be another source of diagnostic error. It is vital that providers explain to patients contemplating utilizing preimplantation genetic testing the risks, benefits, and alternatives of the technology in detail. PGS may be a viable option for couples with recurrent pregnancy loss shown to be resultant from embryo aneuploidy to reduce the incidence of future miscarriage.

Lifestyle Issues and Environmental Toxins

Couples experiencing recurrent pregnancy losses are often concerned that toxins within the environment may have contributed to their reproductive difficulty. It is important that health-care providers, counseling patients about exposures to substances in the environment, have current and accurate information in order to respond to these concerns.

Cigarette Smoking

Cigarette smoking reduces fertility and increases the rate of spontaneous abortion. The data evaluating smoking and miscarriage are extensive and involve approximately 100,000 subjects. The studies suggest a clinically significant detrimental effect of cigarette smoking that is dose dependent, with a relative risk for miscarriage among moderate smokers (10–20 cigarettes a day) being 1.1–1.3 [84]. Patients should be aggressively counseled to stop cigarette smoking prior to attempting pregnancy.

Alcohol Consumption

Alcohol consumption is associated with a risk of spontaneous abortion [85]. The minimum threshold dose for significantly increasing the risk of first-trimester miscarriage appears to be two or more alcoholic drinks per week [86, 87]. When personal habits, cigarette smoking, and alcohol are utilized in the same individual, the risk of pregnancy loss may increase fourfold. Couples should be counseled concerning these habits and strongly encouraged to discontinue these prior to attempting subsequent conception [88].

Obesity

Obesity, defined as a body mass index over 30, has been associated with an increased risk of miscarriage. Obesity ($BMI > 30\ kg/m^2$) has been shown to be an independent risk factor for first-trimester miscarriage [24]. The association is strongest in women with $BMI > 40$. The etiology of this phenomenon is unclear. However, many studies have linked obesity to a generalized increase in systemic inflammatory responses [89].

Caffeine Intake

Several studies have shown that caffeine in excess of 300 mg/day (three cups of coffee per day) is associated with a modest increase in spontaneous abortion, but it is not clear if this relationship is causal [90].

Ionizing Radiation

The studies of atomic bomb survivors in Japan showed that in utero exposure to high-dose radiation increased the risk of spontaneous abortions, premature deliveries, and stillbirths [91]. Diagnostic X-rays in the first trimester that deliver less than 5 rads are not teratogenic [92]. Large doses (360–500 rads) used in therapeutic radiation, however, induce abortion in offspring exposed in utero in the majority of cases. Adverse effects of chronic low-dose radiation on reproduction have not been identified in humans [84].

Outcome

The treatment of RPL should be directed at the cause. Given the good outcome for most couples with unexplained recurrent abortion in the absence of treatment, it is difficult to recommend unproven therapies, especially if they are invasive and expensive. Explanation and appropriate emotional support are possibly the two most important aspects of therapy. In fact, in one study, antenatal counseling and psychological support for couples with recurrent abortion and no abnormal findings resulted in a pregnancy success rate of 86 %, compared with a success rate of 33 % for women who were given no specific antenatal care [17].

In approximately 60 % of all cases of recurrent pregnancy loss, a complete evaluation will reveal a possible etiology [3, 4]. Abnormal findings during the evaluation should be corrected prior to attempting any subsequent pregnancy. If no cause can be found, the majority of couples will eventually have a successful pregnancy outcome with supportive therapy alone [93]. Once a pregnancy occurs, the patient should be monitored closely, with evaluation of quantitative hCG levels at least twice and documentation of adequate progesterone levels. Early sonography should be scheduled, and any encouraging results should be communicated to the couple. In women with a history of RPL, the presence of a normal embryonic heart rate between 6 and 8 gestational weeks that is confirmed with repeat sonography in 1 week is associated with a live birth rate of 82 % [94].

Couples who have experienced RPL want to know what caused the miscarriage. Unexplained reproductive failure can lead to anger, guilt, and depression. Anger may be directed toward their physician for not being able to solve their reproductive problems. Feelings of grief and guilt following an early loss are often as intense as those following a stillbirth, and parents experience a grief reaction similar to those associated with the death of an adult. The couple should be assured that exercise, intercourse, and dietary indiscretions do not cause miscarriage. Any questions or concerns that the couple may have about personal habits should be discussed.

Thankfully, the prognosis for women with RPL to eventually deliver with medical therapy is quite good. A recent study evaluating 987 women with RPL found that the chances of achieving a live birth within 5 years of initial physician consultation was in excess of 80 % for women under the age of 30 and approximately 60–70 % for women ages 31–40 [5].

Women who suffer RPL have already begun to prepare for their baby, both emotionally and physically, as compared to couples with infertility who have never conceived. When a miscarriage occurs, a couple may have great difficulty informing friends or family about the loss. Feelings of hopelessness may continue long after the loss. Patients may continue to grieve and have episodes of depression on the expected due date or the date of the pregnancy loss. Participation in support groups or referral for grief counseling may be beneficial in many cases (SHARE Pregnancy and Infant Loss Support, Inc., http://www.nationalshareoffice.com).

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Induction of Ovulation

14

Alan M. Martinez and Steven R. Lindheim

Introduction

Induction of ovulation or stimulation of ovarian follicular development remains a key component of therapy in the infertility patient. Several applications exist, including restoration of ovulation in anovulatory women, ovarian superovulation (stimulating more than one mature follicle) in ovulatory women, and controlled ovarian hyperstimulation (stimulating the development of multiple dominant follicles) for assisted reproduction.

Ovulation induction can be achieved through several avenues, including disruption of the negative estrogen feedback loop, direct gonadotropin administration, inhibition of androgen conversion to estrogen, use of insulin-sensitizing agents, and laparoscopic surgical intervention. This chapter will review a brief history of these agents, the underlying scientific basis, and clinical guidelines regarding the use of different medications for the purpose of promoting follicular recruitment and ovulation. Included in this chapter is a brief overview of follicular development and ovulation, along with a description of the anatomical axes involved in this process.

History of Ovulation Induction

In the early1900s, observation that anterior pituitary lesions resulted in atrophy of the gonads provided the first evidence of an endocrine pituitary-gonadal axis. The terms "prolan A and prolan B" were initially coined to describe the two gonadotropins of the pituitary gland. In the late 1920s, the first description of urine from pregnant women stimulating gonadal function was observed. In 1931, Fevold and Hisaw successfully isolated and purified follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This was followed by the first successful induction of ovulation using human pituitary gonadotropins in 1958 and the first pregnancy in 1960 [1, 2].

In the late 1950s, the first nonsteroidal estrogen antagonist (MER-25) was tested in women and observed to resume menses in previously amenorrheic women. This was followed by Greenblatt's work in 1961; Greenblatt achieved the first application of clomiphene citrate to induce ovulation (MRL-41), which was subsequently FDA-approved in 1967 for ovulation induction [3].

During the 1970s, two additional modalities for anovulation were introduced, including gonadotropin-releasing hormone (GnRH) and prolactin-inhibiting agents. GnRH was isolated, and synthesized in the laboratory, and ultimately shown to be effective when given in a pulsatile fashion in 1980 by Knobil. From 1974 to 1977, research demonstrated that bromocriptine was effective in reducing prolactin levels, restoring LH pulsatility, and normalizing menstruation and ovulation.

In 2000, the first case series of women utilizing the aromatase inhibitor letrozole as an ovulation-induction agent was reported, with subsequent studies providing evidence of its effectiveness when compared to clomiphene citrate [4]. These historical events have paved the way for the current approaches to ovarian stimulation and continue to be the basis of treatment in the infertility patient.

Physiologic Basis of Follicular Development and Ovulation

An understanding of normal physiology is an important basis for understanding ovulation induction. Ovulation is comprehensively discussed in Chap. 3. The following section provides a brief overview of folliculogenesis.

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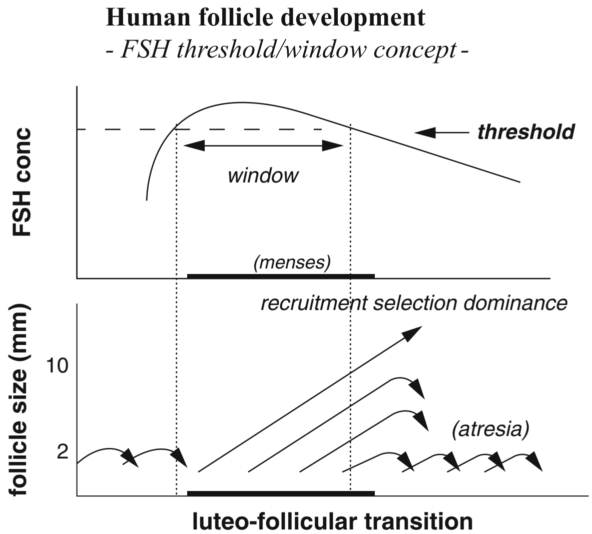

Fig. 14.1 The intercycle rise in serum FSH concentrations exceeds the threshold for recruitment of a cohort of follicles for further development. The number of follicles recruited is determined by the time ("window") for which the serum FSH is above the threshold at which recruitment occurs (reproduced with permission from Mitwally MI, Casper RI. Induction of ovulation. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [9])

Folliculogenesis

Ovarian folliculogenesis is regulated by both endocrine and intraovarian mechanisms. The main follicular component of the ovarian cortex is the primordial follicle, consisting of an oocyte arrested at the diplotene stage of meiosis I, and surrounding granulosa cells [5]. Although initial follicular growth appears to be independent of pituitary gonadotropins, FSH activates granulosa cell proliferation and differentiation [6].

During the early stage of follicle development, the granulosa cells proliferate to form a preantral follicle, and the theca cells differentiate, forming a fluid-filled space called an antrum. At this point, the antral follicle becomes dependent on gonadotropins for growth and development. However, <1 % of primordial follicles present at the time of birth will ever proceed to ovulation, with the majority of follicles degenerating by atresia [7].

During the later part of the luteal phase, gonadotropin levels increase, and once a "FSH threshold" is achieved, growth of a limited cohort of follicles proceeds [8] (Fig. 14.1). The time period above which the threshold is exceeded (the FSH "window") determines the number of recruited follicles for a given cycle. In a normal cycle, one follicle continues developing despite falling FSH levels secondary to an increased sensitivity to FSH, while the remaining follicles respond to falling FSH levels by undergoing atresia [9] (see Fig. 14.1).

When the follicle reaches a certain level of development, high estrogen levels initiate a mid-cycle LH surge, promoting luteinization and oocyte maturation, ultimately triggering ovulation [10]. The preovulatory gonadotropin surge induces ovulation and follicular cell differentiation, forming the corpus luteum that produces high levels of estrogen and progesterone. Adequate luteal progesterone is secreted to allow maintenance of pregnancy until a conceptus-derived source of progesterone, the placenta, can produce adequate amounts of this steroid to support pregnancy [11].

Ovulation Induction

Ovulatory Dysfunction Classification

Anovulation or oligo-ovulation has been traditionally classified into three groups: World Health Organization (WHO) type I, II and III anovulation [12]. It should be noted that anovulation secondary to hyperprolactinemia is categorized as a fourth entity.

Women in WHO Group I suffer from a defect at the level of the hypothalamus/pituitary, comprising 5–10 % of anovulatory women. They are estrogen-deficient with normal or low FSH or prolactin levels and have no space-occupying lesion in the hypothalamic-pituitary region. They typically have amenorrhea and do not bleed in response to progestin challenge. Causes may include emotional, physical, or nutritional stress, excessive weight loss/exercise, Kallmann syndrome, and other isolated gonadotropin deficiency states.

Women in WHO Group II comprise 75–85 % of anovulatory women, and have normal estradiol, FSH, and normal or elevated LH levels. They typically experience oligomenorrhea, but they may have anovulatory cycles or amenorrhea with bleeding in response to a progestin challenge. Included in this group are women with polycystic ovary syndrome (PCOS).

Women in WHO Group III comprise 10–20 % of anovulatory women and include those with elevated gonadotropins secondary to primary ovarian failure, mainly due to diminished ovarian reserve and the loss of ovarian follicles. These women are resistant to various methods of ovarian stimulation, and the best approach for their anovulation-associated infertility is oocyte donation.

Women with hyperprolactinemia have inhibited gonadotropin secretion and comprise 5–10 % of anovulatory women. These women have either oligomenorrhea or amenorrhea, should be screened to establish the existence of a mass lesion, and should be evaluated for clinical hypothyroidism and for use of medications that may alter prolactin levels.

Ovulation-Induction Indications

Normal follicular development involves a sequence of events orchestrated by local ovarian factors and remote endocrine factors from the pituitary, hypothalamus, thyroid, and adrenal glands, which are critical in coordinating the interaction between the hypothalamus, pituitary and ovaries resulting in ovulation. A derangement in any of these systems may result in dysfunctional ovarian follicular development or even the complete failure of ovulation.

Medications Used for Ovulation Induction

For more than 40 years, clomiphene citrate has been the most commonly used oral ovulation-induction agent. Originally utilized in the treatment of postmenopausal breast cancer, the aromatase inhibitor (AI) letrozole has now also become a commonly used agent for ovulation induction. Although not FDA-approved for ovulation induction (approved for type 2 diabetes), the insulin-sensitizing agent metformin has been introduced into clinical practice for ovulation induction for patients with PCOS and significant insulin resistance.

Finally, the use of injectable gonadotropins has served as a means to enhance follicular recruitment in women in whom a poor response to oral ovulation-induction agents is seen. Specifically, hypogonadotropic hypogonadism (WHO Group I) women, clomiphene-resistant women, and women with unexplained infertility are groups that may benefit from this approach. In some instances, sequential treatment with clomiphene citrate or letrozole and exogenous gonadotropins has been used to achieve desired follicular recruitment and pregnancy, with reduced costs and similar pregnancy rates when compared to injectable gonadotropins alone.

Patients from WHO Group IV with hyperprolactinemia may be treated with dopamine agonist therapy, specifically bromocriptine or cabergoline. These agents have the ability to normalize prolactin levels in approximately 60–85 % of women, with 50–75 % ultimately experiencing ovulatory cycles.

Clomiphene Citrate

Structure and Pharmacokinetics

Clomiphene citrate is a nonsteroidal triphenylethylene derivative chemically related to diethylstilbestrol and tamoxifen. It is considered a "selective estrogen receptor modulator" that exhibits both estrogen agonist and antagonist properties depending on the prevailing levels of endogenous estrogen. Estrogen agonist properties are manifest only when endogenous estrogen levels are extremely low; otherwise, clomiphene citrate acts mainly as an antiestrogen [13].

Clomiphene is metabolized by the liver and excreted primarily in the stool. About 85 % of an administered dose is eliminated after approximately 6 days, although traces may remain in the circulation for much longer [14]. Clomiphene citrate is a racemic mixture of two distinct stereoisomers, enclomiphene and zuclomiphene, that have different properties. Available evidence indicates that enclomiphene is the more potent anti-estrogenic isomer and the one primarily responsible for the ovulation-inducing actions of clomiphene citrate [13–15].

Pharmacodynamics and Mode of Action

Clomiphene citrate, due to its structural similarity to estrogen, binds to estrogen receptors (ER) throughout the body, but differs from natural estrogen in that the binding duration is over weeks and not days. This extended binding capacity in the hypothalamus ultimately depletes ER concentrations by interfering with normal receptor replenishment [3]. Depletion of ER results in a reduced estrogen negative feedback on gonadotropin-releasing hormone (GnRH) production by the hypothalamus and gonadotropins (FSH and LH) by the pituitary; thus, upregulation of these hormones stimulates ovarian follicular development.

Indications for Clomiphene Citrate Treatment

- Induction of ovulation in women with anovulatory infertility
- Stimulation of multifollicular ovulation or enhancing ovulation in ovulatory infertile women (e.g., unexplained infertility)
- Infertility patients without evidence of tubal factor (specifically hydro)
- Patients with unilateral tubal occlusion
- Used as first line for many subfertility patients with near-normal sperm

Clomiphene citrate is the initial treatment of choice for most anovulatory or oligo-ovulatory subfertile women who have normal reproductive hormone parameters (WHO type II anovulation, e.g., PCOS). Clomiphene citrate is effective only in the presence of normal circulating estrogen levels, with the potential for estrogen negative feedback on gonadotropin production. Women who do not respond to clomiphene citrate are those with very low circulating estrogen levels, such as WHO types I and III, or women with a defective hypothalamic-pituitary axis, such as those with Sheehan syndrome and Kallmann syndrome. In the normal ovulatory woman, such as in the case of unexplained infertility, unilateral tubal disease (excluding hydro), and in the case of subfertility with near-normal sperm, the goal is to increase mature follicular development to overcome the apparent subfertility.

Clomiphene Citrate Administration

Clomiphene citrate is administered orally for 5 days, after the onset of spontaneous or progestin-induced menses. In anovulatory women, ovulation rates, conception rates, and pregnancy outcome are similar regardless whether treatment is started on cycle day 2, 3, 4, or 5 [16]. Treatment typically begins with a single 50-mg tablet daily, increasing by 50-mg

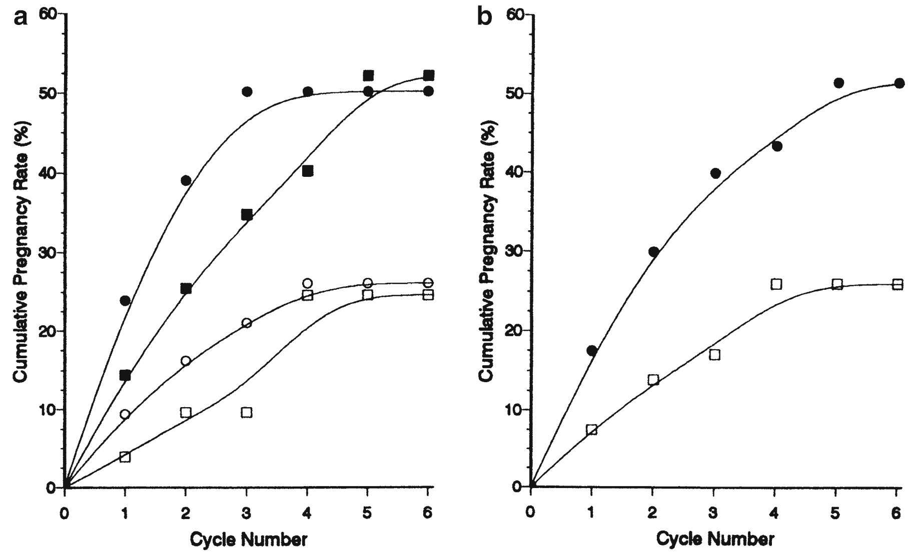

Fig. 14.2 Cumulative pregnancy rates with clomiphene citrate with intrauterine inseminations in patients with ovulatory and anovulatory infertility by Kaplan–Meier life table analysis. (**a**) *Filled circles* ≤30 years of age; *closed squares*, 31–35 years of age; *open circles* 36–40 years of age; *open squares* ≥41 years of age. (**b**) Groups collapsed into two: *closed circles* ≤35 years of age; *open squares* >35 years of age (reproduced from Agarwal SK, Buyalos RP. Clomiphene citrate with intrauterine insemination: Is it effective therapy in women above the age of 35 years. Fertil Steril. 1996;65(4):759-63, with permission from The American Society for Reproductive Medicine)

increments in subsequent cycles if resultant follicular maturation is not achieved. Although the dose required for achieving ovulation is correlated with body weight, there is no reliable way to predict accurately what dose will be required in an individual woman.

The effective dose of clomiphene citrate for most women ranges from 50 to 200 mg/day, although some women will be found to be very sensitive to clomiphene citrate and only require doses as low as 12.5–25 mg/day. Most women will ovulate at the lower doses, with 52 % ovulating after 50 mg/day and another 22 % after treatment with 100 mg/day. Although higher doses are sometimes required, the ovulation rates are usually low at these does, with only 12 % ovulating after 150 mg/day, 7 % after 200 mg/day, and 5 % after 250 mg/day. Most women who fail to respond to 150 mg/day of clomiphene citrate will ultimately require alternative or combination treatments [17, 18]. Traditionally, progestin withdrawal is utilized and incremental increases of clomiphene citrate are used in these anovulatory women. However, recent data from the Reproductive Medicine Network (RMN) suggest that pregnancy rates are not compromised and even significantly improved with redosing in the same cycle, even when follicular growth is not observed in the initial clomiphene citrate treatment regimen.

Once the effective dose of clomiphene citrate is established, there is no indication for further increments unless the ovulatory response is lost. Higher doses do not improve the probability of conception, but may increase the risk of hyperstimulation and a multiple-gestation pregnancy.

Pregnancy rates are highest during the first three cycles of clomiphene citrate treatment. The chances of achieving pregnancy are significantly lower beyond the third treatment cycle and uncommon beyond the sixth treatment cycle [17] (Fig. 14.2). Generally speaking, failure to conceive within six clomiphene citrate–induced ovulatory cycles is a clear indication to expand the diagnostic evaluation to exclude other factors, change the overall treatment strategy, or both [19].

Outcome of Clomiphene Citrate

Clomiphene citrate treatment has been reported to successfully induce ovulation in 60–80 % of properly selected women. More than 70 % of those who ovulate respond at the 50-mg or 100-mg dosage level. In young anovulatory women, cumulative conception rates between 60 % and 70 % are observed after up to three successfully induced ovulatory cycles, and rates of 70–85 %, after five. Overall, cycle fecundity is approximately 15 % in women who ovulate in response to treatment [17]. Important factors that adversely affect treatment outcome are increased age (particularly > 35 years), presence of other treated or untreated infertility factors, and increasing duration of infertility [20].

Adverse Effects and Risks of Clomiphene Citrate

Clomiphene citrate is generally well tolerated. Some side effects are relatively common but rarely threaten completion of treatment. Hot flashes are dose dependent and occur in approximately 10 % of clomiphene citrate–treated women.

Visual disturbances, including blurred or double vision, scotomata, and light sensitivity, are generally uncommon (<2 % prevalence). Less specific side effects include breast tenderness, pelvic discomfort, nausea, and premenstrual symptoms, all observed in 2–5 % of clomiphene citrate–treated women [21].

Congenital Anomalies

Clomiphene citrate is considered pregnancy risk category X. Although studies in rats and mice have shown a dose-related increase in some types of malformations and an increase in mortality, studies in humans have not found an association between clomiphene and congenital defects [22, 23]. A small study of pregnancy outcome in women inadvertently exposed to clomiphene citrate during the first trimester also found no increase in the prevalence of congenital anomalies [24].

Pregnancy Loss

More than one study has suggested that pregnancies resulting from clomiphene citrate are at increased risk of spontaneous abortion compared to spontaneous pregnancies. Studies analyzing the rate of miscarriage in 62,228 clinical ART pregnancies in the United States (1996–1998) support this increased rate [25, 26]. However, the results of these studies are not conclusive, with pregnancy loss possibly related to other comorbidities, such as insulin resistance, genetic factors related to PCOS or unexplained infertility, endometriosis, and advancing maternal age [27].

Antiestrogenic Effects and Fertility

Owing to the relatively long half-life of clomiphene citrate isomers, there are undesirable and unavoidable antiestrogenic effects on the endocervix, endometrium, ovary, ovum, and embryo. These are more apparent at higher doses or after longer duration of treatment. Although the quality and quantity of cervical mucus production is altered, these changes offer no prognostic value on overall pregnancy outcome [28–30].

There are reported conflicting effects of clomiphene citrate on the endometrium that may be secondary to different methodologies used for endometrial assessment [28–33]. A reduction in endometrial thickness below the level thought to be needed to sustain implantation (<7 mm) was found in up to 30 % of women receiving clomiphene citrate for ovulation induction or for unexplained infertility and has been confirmed by other studies [28–30]. The administration of estrogen concomitantly with clomiphene citrate treatment has yielded conflicting results, with reports of increased endometrial thickness and improved pregnancy rates [34, 35], whereas others have reported no benefit [36] or even a deleterious effect of estrogen administration [37].

Failure of Clomiphene Citrate Treatment

Treatment failure can be divided into two groups. The first, clomiphene resistance, includes patients who fail to ovulate with clomiphene citrate treatment. The second, clomiphene pregnancy failure, includes patients who ovulate with clomiphene citrate treatment but fail to achieve pregnancy.

Clomiphene pregnancy failure may be related to a wide variety of underlying factors, including endometriosis, undiagnosed tubal factor, male factor, or endometrial receptivity factors. However, the success in achieving pregnancy with alternative ovarian stimulation protocols (injectable gonadotropins or aromatase inhibitors) supports the hypothesis that persistent antiestrogenic effects associated with clomiphene citrate might play a role in the discrepancy between ovulatory rates and pregnancy rates [38, 39].

Aromatase Inhibitors

A number of aromatase inhibitors have been developed over the last 30 years, with the most recent, third-generation aromatase inhibitors licensed mainly for breast cancer treatment in postmenopausal women [40]. Recently, the success of using aromatase inhibitors for induction of ovulation has been reported, and the physiologic mechanism of compensatory gonadotropin stimulation from these agents is now commonly used to enhance follicular growth in the infertile patient [41–44].

Aromatase Description

Aromatase is a microsomal member of the cytochrome P450 hemoprotein-containing enzyme complex superfamily (P450arom, the product of the CYP19 gene). Aromatase catalyzes the rate-limiting step in the production of estrogens— that is, the conversion of androstenedione and testosterone via three hydroxylation steps to estrone and estradiol, respectively [45]. Aromatase activity is present in many tissues, such as the ovary, brain, muscle, liver, adipose, and breast tissue, and in malignant breast tumors. The main sources of circulating estrogens are the ovaries in premenopausal women and adipose tissue in postmenopausal women [46]. Aromatase is a good target for selective inhibition because estrogen production is a terminal step in the biosynthetic sequence.

Types of Aromatase Inhibitors

The first aromatase inhibitor to be used clinically was aminoglutethimide, which induces a medical adrenalectomy by inhibiting many other enzymes involved in steroid biosynthesis [47]. Although an effective agent in postmenopausal breast cancer, the need for concurrent corticosteroid replacement complicates usage, and side effects like lethargy, rashes, nausea, and fever result in 8–15 % of patients stopping treatment [48].

The third-generation aromatase inhibitors available include two nonsteroidal preparations, anastrozole and letrozole, and a steroidal agent, exemestane [49]. Anastrozole, ZN 1033 (Arimidex, AstraZeneca, London, UK), and letrozole, CGS 20267 (Femara, Novartis, Basel, Switzerland), are available for clinical use for treatment of postmenopausal breast cancer. These triazole (antifungal) derivatives are reversible, competitive agents with greater intrinsic potency than that of aminoglutethimide. They have a mean half-life of approximately 45 h (range, 30–60 h), with metabolism primarily by the liver. Main adverse events include hot flashes, nausea, vomiting, asthenia, headache, and leg/back pain, based on studies in postmenopausal women [49].

Dosing for letrozole includes 2.5-mg, 5-mg, or 7.5-mg regimens for the duration of 5 days, similar to clomiphene citrate starting cycle days 3–7. There is also evidence for a single-dose regimen (20 mg on day 3), which may be similarly efficacious to the traditional multidose protocol [42].

Mechanism of Action for Ovulation Induction

Several hypotheses have been postulated for the mechanisms underlying the effectiveness of aromatase inhibitors in ovulation induction [50]. In the central hypothesis, diminished estrogen production by inhibiting aromatization increases gonadotropin secretion, resulting in follicular growth. Another centrally mediated mechanism suggests that estrogen can modulate the activin/inhibin/follistatin system, leading to FSH production through a direct effect on the pituitary gonadotropes [51–54].

In addition, because aromatase inhibition does not antagonize ER in the hypothalamus, follicle growth is accompanied by increasing concentrations of both estradiol and inhibin. This results in a normal secondary-feedback loop, limiting FSH response (development of one to two mature follicles), significantly reducing the risk for ovarian hyperstimulation and multiple gestations [55]. Only with the addition of FSH to an aromatase inhibitor is there an apparent increase in higher-order multiple ovulation.

A second peripheral hypothesis involves an increased follicular sensitivity to FSH. This may result from temporary accumulation of intraovarian androgens, since conversion of androgen substrate to estrogen is blocked by aromatase inhibition. Recent data support a stimulatory role for androgens in early follicular growth in primates [56], with androgen accumulation in the follicle-stimulating insulin-like growth factor I (IGF-I), which may synergize with FSH to promote folliculogenesis [57–60].

Another part of the peripheral hypothesis involves ER in the endometrium. Aromatase inhibition, with suppression of estrogen concentrations in the circulation and peripheral target tissues, may result in possible upregulation of ER in the endometrium. This in turn may result in increased endometrial sensitivity to estrogen, causing rapid proliferation of endometrial epithelium and stroma, with improved blood flow to the uterus and endometrium [61]. As a result, normal endometrial development and thickness occur by the time of follicular maturation, even in the face of the observed lower-than-normal estradiol concentrations in aromatase inhibitor–treated cycles [41, 50, 62, 63].

Potential Advantages Compared to Clomiphene Citrate

The significantly shorter half-life (45 h) compared to clomiphene citrate allows for rapid elimination from the body [64, 65]. Since no ER down-regulation occurs, no adverse effects on estrogen target tissues are observed. Aromatase inhibitors are therefore an interesting alternative to clomiphene citrate, particularly in cases with recurrent clomiphene citrate failure [43, 44], an alternative to treatment in clomiphene-resistant anovulatory women, and present a cost-effective option prior to considering gonadotropin therapy [66]. In summary, letrozole appears to be as effective as clomiphene citrate for ovulation induction, has lower multiple pregnancy rates, and is well tolerated in the anovulatory woman.

Pregnancy Outcomes and Controversies Surrounding Letrozole

The aromatase inhibitor letrozole is considered a pregnancy category X agent, with an unknown safety profile during lactation. There is evidence of embryotoxicity, fetotoxicity, and teratogenicity in animal models that warrant this designation. Although originally designed and implemented as an FDA-approved treatment for postmenopausal breast cancer, the off-label use of letrozole for ovulation induction has become a common entity. The observed lower multiple-gestation rate and decreased antiestrogenic effect on the endometrium compared to clomiphene citrate have resulted in letrozole becoming an alternative treatment modality in ovulation-induction cycles. The short half-life of letrozole and use in the early follicular phase minimize the exposure of this agent to a developing fetus. Studies have shown that both miscarriage and ectopic pregnancy rates following letrozole treatment in ovulation-induction cycles were found to be similar to those in women who spontaneously conceived [55]. Also, the risk of congenital malformations has not been found to be increased in mothers who conceived using letrozole when compared to a group of age-matched controls [67].

Insulin-Sensitizing Agents

Insulin resistance and hyperinsulinemia are recognized features of PCOS, with hyperinsulinemia contributing significantly to hyperandrogenism and chronic anovulation. It is logical that agents that improve insulin action in the body (insulin sensitizers) will help infertile PCOS women with insulin resistance to achieve ovulation by lowering their hyperinsulinemia. This is particularly true in PCOS patients who fail to ovulate with clomiphene citrate treatment. De Leo et al. reviewed the benefits of insulin-lowering agents in the management of PCOS with extensive discussion of the effect of these agents on endocrine, metabolic, and reproductive functions [68].

Although it is believed that the endocrine and reproductive benefits observed during treatment with insulin sensitizers in PCOS women are due to improving insulin resistance, insulin sensitizers may work through other mechanisms to achieve ovulation in these patients. There is evidence that the insulin sensitizers, metformin and thiazolidinediones, can modulate steroid production through a direct suppression of steroidogenesis enzymes [69, 70].

Metformin

Metformin, a biguanide anti-diabetic drug, was first described in the scientific literature in 1957 [71]. It is an insulin-sensitizing agent (i.e., improves peripheral insulin sensitivity) that suppresses hepatic gluconeogenesis and decreases intestinal absorption of glucose. Studies demonstrate that treatment with metformin can restore menses and cyclic ovulation in many amenorrheic PCOS women [72, 73].

Effectiveness of Metformin

Current evidence supports the idea that metformin appears to be most efficacious in women who are non-responders to clomiphene citrate; however, the data on ovulation rates, pregnancy, and live birth rates are contradictory in nature. In a 2008 meta-analysis including 17 studies (>1,600 women with PCOS), metformin did improve ovulation, especially in non-clomiphene-resistant women [74]. Of the two randomized controlled trials, The Reproductive Medicine Network revealed that metformin alone did not increase the odds of pregnancy, but in combination with clomiphene, overall pregnancy rates were increased. The combination of metformin and clomiphene was especially beneficial in clomiphene-resistant women. In contrast, a recent multicenter randomized study by Morin-Papunen et al. showed that metformin alone improved both pregnancy rates (PR) and live birth rates (LBR) compared to a control group (PR: 53.6 % vs. 40.4 %, LBR 41.9 % vs. 28.8 %) [75]. It is important to note that metformin alone and in combination with clomiphene helps reduce the number of multiple pregnancies compared to clomiphene alone [76].

Adverse Side Effects and Pregnancy Category of Metformin

The use of metformin most commonly causes gastrointestinal symptoms that include nausea, vomiting, and diarrhea. These entities may result in patient noncompliance; therefore, metformin is routinely given in a stepwise fashion starting at 500 mg daily and increasing at 1-week intervals up to either 1,500 or 2,000 mg daily. This helps limit the adverse side effects and increases the likelihood of effective dosing. Lactic acidosis is a rare but potential complication; therefore, baseline hepatic and renal function testings are recommended, followed by periodic evaluation of both liver and kidney function. Metformin is considered a pregnancy category B agent, with no demonstrable evidence of fetotoxicity or congenital malformations in either animal or human gestation.

Alternative Insulin-Sensitizing Agents

Other insulin-sensitizing agents, such as thiazolidinediones and chiro-inositol, have been used in small clinical trials to achieve ovulation in PCOS women with insulin resistance [77–81]. However, because of the higher safety profile, longer clinical experience, and lower cost of metformin, these agents should be reserved for cases resistant to metformin treatment [82].

Gonadotropins

Clomiphene citrate–resistant anovulatory women who ultimately require exogenous gonadotropins to achieve ovulation might benefit from a trial of sequential clomiphene citrate/gonadotropin therapy. Sequential clomiphene citrate and gonadotropin (hMG or FSH) therapy has become an increasingly utilized method of ovarian superovulation for patients who fail clomiphene citrate treatment [83–85]. The value of adding clomiphene citrate during ovarian superovulation is to decrease the FSH dose required for optimum stimulation. However, one must take care in monitoring, because clomiphene citrate use may be associated with peripheral antiestrogenic effects, offsetting the FSH dose reduction benefit [86].

An aromatase inhibitor may be used in conjunction with FSH injections in a sequential or overlap regimen to increase the number of preovulatory follicles that develop and to improve the outcome of treatment [84]. Giving an aromatase inhibitor together with FSH resulted in a significant reduction in the dose of FSH required for optimum ovarian stimulation, even in poor responders [87]. The additional effect of aromatase inhibitors to reduce the supraphysiologic levels of estrogen seen with the development of multiple ovarian follicles may also improve treatment outcome [50]. Some studies suggest the use of FSH and an antagonist (ganirelix) in intrauterine insemination (IUI) cycles may significantly increase pregnancy rates and reduce the incidence of premature luteinization when compared to FSH/IUI cycles alone [88].

WHO Group I individuals typically benefit most from these injectable medications, as both clomiphene citrate and aromatase inhibitors do not overcome the hypothalamic-pituitary-ovarian axis deficiencies seen in these individuals. Also, clomiphene-resistant women who have failed 3–6 cycles are candidates. In women with hypogonadotropic hypogonadism, the approximate likelihood of pregnancy is 25 % per cycle, compared to clomiphene-resistant anovulation, where rates are lower at 5–15 % [89].

Significant risks include an increased chance of multiple pregnancy and risk of OHSS. Therefore, pretreatment counseling is critical and careful monitoring is needed to decrease the chance of morbidity in these women. It is warranted to also discuss multifetal reduction in these individuals.

Laparoscopic Ovarian Drilling

Whether laparoscopic ovarian drilling (LOD) is a valid option for clomiphene citrate–resistant women with PCOS continues to be a matter of intense debate. The technique involves laparoscopic cautery, diathermy, or laser vaporization of the ovaries at multiple sites, the objective being to decrease circulating and intraovarian androgen levels by reducing the volume of ovarian stroma. LOD is reported to result in resumption of spontaneous ovulation and may restore sensitivity to clomiphene citrate treatment.

In a 2012 Cochrane Review, there was no evidence of a difference in clinical pregnancy, live birth rate, or miscarriage in women with clomiphene-resistant PCOS undergoing LOD compared to clomiphene citrate, gonadotropins, or aromatase inhibitors [90]. Consequently, ovarian drilling is perhaps best reserved for clomiphene citrate–resistant women in whom costs or logistic considerations effectively preclude alternative treatments (e.g., exogenous gonadotropins) [19].

Human Chorionic Gonadotropin

The administration of human chorionic gonadotropin (hCG) during clomiphene citrate treatment aims at either substituting (before the occurrence of endogenous LH surge) or supporting the endogenous LH surge. This may be particularly useful for timing insemination or recommending the optimum fecundity period for intercourse.

Adverse Effects and Concerns Regarding Oral Ovulation-Induction Agents

Multiple Gestations

The significant increase in the incidence of multiple births in most countries is almost entirely secondary to the use of gonadotropins and other agents for induction of ovulation [91]. The increased incidence of maternal and neonatal complications associated with multiple-gestation pregnancies has been well documented [92–94]. Review of data from the National Center for Health Statistics reveals that twins are 4 times more likely than singletons to die within the first month of life, and triplets are 10 times more likely to die in this time. Unfortunately, there is substantial pressure from patients in infertility programs to increase their success rates, and strategies include the administration of exogenous gonadotropins during ovulation induction or increasing the number of embryos transferred in cases of in vitro fertilization.

Ovarian Hyperstimulation Syndrome

The incidence of ovarian hyperstimulation syndrome (OHSS) in clomiphene citrate–treated women and in association with mild gonadotropin stimulation for IUI is difficult to determine, because diagnostic criteria vary widely among studies. Mild OHSS (moderate ovarian enlargement) is relatively common but also does not require active management. When clomiphene citrate–induction of ovulation proceeds in the recommended incremental fashion designed to establish the minimum effective dosage, the risk of severe OHSS (massive ovarian enlargement, progressive weight gain, severe abdominal pain, nausea and vomiting, hypovolemia, ascites, and oliguria) is remote [19]. This complication is discussed in more detail in another chapter of this book.

Ovarian Cancer

There remains an uncertain association of ovarian cancer with ovulation-induction treatment. A study consisting of eight pooled analyses showed that nulliparous women had an increased incidence of borderline serous tumors, but it did not show an increase in invasive ovarian cancer [95]. Certainly, no causal relationship between ovulation-inducing drugs and ovarian cancer has been established. Therefore, patients with concerns should be counseled that an increase in risk is possible but not established.

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15 ART: Clinical and Laboratory Aspects

Beth Plante, Gary D. Smith, and Sandra Ann Carson

Assisted Reproductive Technologies: Clinical Aspects

Assisted reproductive technologies (ART) can be defined as fertility treatment that involves removing eggs from a woman's ovaries and combining them with sperm in a laboratory. Methods used to achieve this result include in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), and zygote intrafallopian transfer (ZIFT). Currently, more than 150,000 cycles of human IVF and similar techniques are performed each year in the United States, resulting in the birth of over 60,000 babies. IVF, together with the much less commonly used techniques of GIFT and ZIFT, are collectively referred to as ART. In the early years of ART, success rates varied dramatically from center to center. Today, success rates are quite consistent nationwide, and ART procedures are responsible for over 1 % of all children born in the United States annually [1].

In Vitro Fertilization

The concept of IVF as a treatment for infertility is straightforward: obtain eggs from the ovaries, mix them with sperm in a dish containing culture medium, and transfer the eggs back to the woman after fertilization has occurred. However, this technique took over 100 years to develop.

The initial development of IVF in humans can be attributed directly to a team of two investigators, Drs. Patrick Steptoe and Robert Edwards. It was in 1969 that Dr. Edwards first stated, "Human oocytes have been matured and fertilized by spermatozoa in vitro. There may be certain clinical and scientific uses for human eggs fertilized by this procedure" [2]. This understated conclusion marked the first successful attempt to fertilize human eggs in a laboratory.

In 1959, successful IVF was reported using rabbits [3]. The first human birth to result from IVF was achieved in England in 1978 [4]. John and Lesley Brown had 9 years of infertility secondary to bilateral fallopian tube obstruction. Dr. Patrick Steptoe surgically retrieved a single mature oocyte from one of Lesley's ovaries during a natural cycle. Dr. Robert G. Edwards combined John's sperm with the oocyte in the laboratory and the resulting embryo was placed into Lesley's uterus a few days later. On July 25, 1978, Louise Joy Brown was delivered by cesarean section at approximately 37 weeks gestation and weighed 5 lb, 12 oz. In 2010, 32 years later, Robert G. Edwards was awarded the Nobel Prize for Physiology or Medicine "for the development of IVF" [4]. Today, most IVF is performed after ovarian stimulation so that multiple eggs can be retrieved transvaginally with a sonographically guided needle, followed by transcervical embryo transfer.

Ovarian Stimulation for IVF

As noted earlier, the first successful human IVF cycle utilized a natural menstrual cycle. Subsequently, higher pregnancy rates were achieved when ovarian stimulation was utilized. Monitoring the response to ovarian stimulation is accomplished with a combination of transvaginal ultrasonography and serum estradiol levels.

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Gonadotropins

Injectable gonadotropin FSH, with or without LH, circumvents the natural decline of FSH that occurs with development of the dominant follicle. In effect, continued FSH administration rescues oocytes that would be physiologically lost to atresia in a natural cycle, which selects only one dominant follicle. Human menopausal gonadotropins (a combination of FSH and LH) increased pregnancy rates when finally adopted for IVF cycles [5].

Human chorionic gonadotropin (hCG), 5,000–10,000 units, is typically used to mimic the LH surge and complete oocyte maturation in gonadotropin cycles. Egg retrieval is performed 34–36 h after the hCG injection. Urinary and recombinant hCG products appear equivalent [6].

Gonadotropin-Releasing Hormone Agonists

In the early years of IVF, more than one-quarter of stimulation cycles did not reach the stage of egg retrieval, primarily due to a premature LH surge [7]. Long-term administration of GnRH agonists initially stimulates LH and FSH release and within 2 weeks suppresses gonadotropins [8]. Initially, a GnRH agonist was reserved for patients who demonstrated a premature LH surge, but its popularity surged when high pregnancy rates were documented with routine use [9].

In the United States, the most popular GnRH agonist is leuprolide acetate, given subcutaneously at doses of 0.25–1.0 mg/day. The routine use of GnRH agonists reduces cycle cancellation rates and increases pregnancy per embryo transfer rates, probably because more eggs are obtained, giving a larger selection of embryos for transfer [10].

Gonadotropin-Releasing Hormone Antagonists

Antagonists of GnRH may be used instead of the agonist to prevent premature LH surges. The advantage of these drugs is the absence of a gonadotropin "flare." A multicenter IVF trial compared the GnRH antagonist ganirelix acetate with a long protocol using a GnRH agonist, leuprolide [11]. GnRH antagonist administration was required for only 4 days, compared to 19 days with the GnRH agonist. Fewer eggs, however, were obtained from subjects in the GnRH antagonist group.

In Vitro Maturation

Mammalian oocytes are maintained in meiotic arrest throughout most of follicular development; the resumption of meiosis I is induced by the preovulatory surge of LH, which is emulated during an IVF cycle by administration of hCG. Although the precise mechanisms that regulate the control of oocyte maturation remain obscure, it has been recognized for over 70 years that immature oocytes liberated from antral follicles may undergo spontaneous maturation in culture, termed "in vitro maturation" (IVM), without the need for hormonal stimulation [12].

With IVM, immature oocytes are typically obtained in the mid- to late-follicular phase of the menstrual cycle. Administration of hCG 36 h before egg collection appears to facilitate oocyte maturation [13]. Intracytoplasmic sperm injection is routinely performed on these oocytes to facilitate fertilization. If embryos are transferred back to the patient in the same cycle, endometrial preparation with estradiol and progesterone will synchronize the endometrial lining for embryo replacement.

To date, IVM has been most successful in young women with multiple antral follicles who typically have a high chance of pregnancy with conventional IVF. Despite this selection bias, IVM pregnancy rates remain lower than in stimulated IVF cycles [14]. In cancer patients, where the time and hormonal milieu associated with a traditional IVF cycle may adversely affect the patient's survival, there may be some advantage to the IVM technique. Likewise, patients with polycystic ovary syndrome (PCOS) who hyperstimulate with ovulation induction agents may be candidates for IVM [15]. As culture conditions for IVM are optimized and pregnancy rates improve, this technology may offer a safer, less expensive, more convenient alternative to stimulated IVF.

Oocyte Retrieval

Retrieval Techniques

Retrieval of oocytes is generally performed 34–36 h after administration of hCG in order to allow adequate oocyte maturation and avoid ovulation. Ultrasound-guided transvaginal retrieval techniques allow retrieval of oocytes without general anesthesia.

Transvaginal egg retrieval employs a needle guide mounted atop a high-frequency endovaginal ultrasound probe. The patient is placed in the dorsal lithotomy position and the perineum and vagina are irrigated with sterile saline irrigation. Preoperative antibiotic prophylaxis may be given, but pelvic infections are uncommon; patients with endometriomas, however, may be at higher risk despite the use of perioperative antibiotics [16]. Although transabdominal or periurethral oocyte retrievals have been performed in the past, the transvaginal egg retrieval has come to be the standard technique for egg retrieval in virtually all IVF programs.

Embryo Transfer

Embryos may be transferred into the uterus anytime during preimplantation development. The first successful IVF

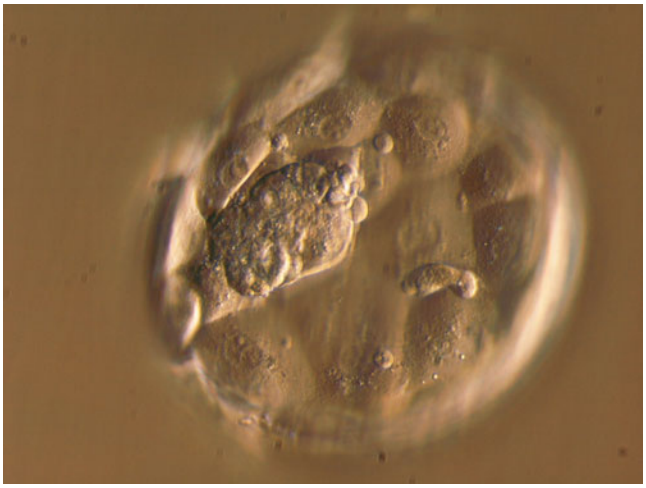

Fig. 15.1 Expanded blastocyst on day 5 of development. The inner cell mass (which ultimately develops into the fetus) is apparent (reproduced with permission from Steinkampf MP, Malizia BA. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

pregnancy occurred after transfer of a single blastocyst, but clinicians unable to duplicate this success turned to the transfer of embryos on day 2 or 3 after retrieval to overcome the limitations of their culture systems. At present, most IVF programs in the United States transfer embryos on day 3 after oocyte retrieval, but embryo transfers done on day 2 appear to give comparable results [1, 17]. Pregnancy can occur when the fertilized egg is transferred to the uterus 1 day after fertilization.

The transfer of one or two embryos on day 5 or 6 after fertilization (at the blastocyst stage) has been proposed as a way of minimizing the risk of high-order multiple pregnancies while maintaining satisfactory pregnancy rates. Blastocyst transfer has several potential advantages: (1) Delaying the embryo transfer to day 5 or 6 after fertilization allows for more detailed examination of embryo morphology (Fig. 15.1); (2) the embryonic genome is activated after about 2 days of development, and prolonged culture facilitates selection of the most robust embryos; and (3) preimplantation embryos do not reach the uterine cavity until day 4 or 5 in vivo [18]. The uterus provides a different nutritional milieu from the oviduct, and it has been postulated that the transfer of day-2 or day-3 embryos to the uterine cavity may reduce their potential for implantation [19].

Balancing the risks and benefits of multiple-embryo transfer remains one of the most vexing problems in the field. The transfer of more than one embryo increases the chance of pregnancy but also increases the risk of multiple gestations. The American Society for Reproductive Medicine recommends that no more than two embryos be transferred to women under 35 years of age who have a favorable prognosis for pregnancy and consideration should be given to transferring a single embryo (Table 15.1) [20]. A randomized, controlled trial comparing elective single-embryo transfer (eSET) and subsequent frozen embryo transfer vs. double-embryo transfer showed comparable live birth rates and a substantially lower rate of multiple gestations following eSET [21]. Several meta-analyses have demonstrated similar findings [22–24].

Table 15.1 Recommended limits on the number of embryos to transfer

Prognosis	<35 years	35–37 years	38–40 years	41–42 years
Cleavage stage				
Favorable	1–2	2	3	5
All others	2	3	4	5
Blastocysts				
Favorable	1	2	2	3
All others	2	2	3	3

Favorable = first cycle of IVF, good embryo quality, excess embryos available for cryopreservation, or previous successful IVF cycle

Luteal Phase Management

The ability to achieve pregnancy in ART cycles is impaired unless hormone supplementation is administered. Aspiration of granulosa cells during oocyte retrieval impairs corpus luteum function, as does the use of GnRH agonists, which limit the secretion of LH in the luteal phase. The result can be an iatrogenic luteal phase defect [25].

Luteal phase support with hCG or progesterone after IVF results in an increased pregnancy rate [26]. The use of hCG has not been shown to be better than intramuscular progesterone, but it is associated with a greater risk of ovarian hyperstimulation syndrome. Progesterone can be given orally, intravaginally, or intramuscularly and is usually initiated the day of or the day following egg retrieval. The route of administration does not influence pregnancy outcomes, but synthetic progesterone (intramuscular or vaginal) appears to be superior to oral micronized progesterone. The further addition of estrogen or hCG in the luteal phase does not further improve pregnancy outcomes over those seen with progesterone supplementation alone [26].

In patients who conceive, progesterone supplementation typically continues until at least 7 weeks gestation. This practice might not be necessary based on a randomized, controlled trial of over 300 IVF cycles that did not show a difference in the rate of successful pregnancies between women who continued progesterone in early pregnancy compared with those who stopped treatment after the first positive pregnancy test [27].

Outcomes of Assisted Reproductive Technologies

Success Rates

Many endpoints have been used to measure IVF success rates. Outcomes of interest include a positive pregnancy test, a sonographically visible pregnancy, appearance of fetal cardiac activity, and birth of a viable infant. Most patients are interested in the "take home baby rate"—that is, the chance of obtaining a healthy infant that they can bring home from the hospital after delivery. This statistic takes time to accumulate, however, and is reflective of a fertility clinic's success in years past as well as its selection of patients.

Choosing the denominator for calculating success rates is also problematic. Most commonly, all initiated cycles are included. Statistics are also calculated, however, using only cycles that have progressed to retrieval or those that have resulted in embryo transfer. The most meaningful calculations are made using the total number of initiated cycles, but cycles in which oocytes are retrieved or embryos are transferred are more likely to reflect the quality of the IVF laboratory.

Potential Adverse Outcomes

Known risks associated with ART include multiple gestation and ovarian hyperstimulation syndrome. Additionally, concern exists around a number of other issues, specifically adverse obstetrical events, congenital anomalies, genetic and epigenetic abnormalities, and developmental outcomes of IVF offspring. ART does appear to be associated with an increased risk of preterm birth, placental abnormalities, and low birth weight, even in singleton pregnancies [28, 29]. Similarly, ART is also associated with a slightly increased risk of congenital anomalies and major malformations. It is unclear, however, if any of these associations are related to the ART procedures themselves, the preceding ovarian stimulation, or the patients' underlying infertility [30, 31]. Intracytoplasmic spermatozoa injection (ICSI) also appears to be associated with an increased risk of birth defects, although there is disagreement about whether this risk is any greater than that seen with conventional IVF [32–34]. A limited number of studies also suggest that ART may be associated with an increased risk of imprinting disorders, theoretically due to laboratory manipulations that occur during meiosis [35]. Because imprinting disorders are quite rare, a causal relationship with ART is difficult to determine. Finally, data are limited in terms of motor and cognitive development of IVF offspring, but outcomes seem to be similar to those of naturally conceived children [36]. With all of these potential adverse outcomes, it is important to remember that the relative risk may be increased, but the absolute risk remains quite low. Consequently, the majority of ART pregnancies produce healthy offspring.

Ovum Donation

Donor oocyte programs are available in most IVF centers in the United States and abroad. Patients may wish to find their own donor in a sister, a relative, or a friend. Nevertheless, most egg donor IVF cycles employ an anonymous donor.

The Donor

The donors must undergo controlled ovarian hyperstimulation with its associated complications and are informed and educated about the treatment involved in oocyte donation.

The Recipient

The recipient's uterus must be primed and the endometrium prepared with exogenous estrogen and progesterone to receive the embryos fertilized, which requires significant coordination. Many regimens for endometrial preparation have been described, and successful pregnancies have also been reported in embryo transfer of donor oocytes in natural cycles.

Success Rates

The use of donor oocytes for IVF consistently results in high pregnancy rates when young, healthy, fertile women donate their oocytes, with pregnancy rates from 51 to 58 % per IVF cycle [37]. Many ethical issues surround donor oocyte programs, however. Controversies exist regarding directed donor oocyte programs, financial compensation for oocyte donation, and the methods used to recruit donors.

Assisted Reproductive Technologies: Laboratory Aspects

ART laboratories are considered to be "high-complexity laboratories" due to the level of knowledge, responsibility, and precision needed to perform the necessary procedures.

Collection-Specific Spermatozoa Evaluation and Processing

For most healthy men, masturbation is a straightforward method of semen collection. For men with ejaculatory dysfunction or azoospermia, however, other methods of spermatozoa recovery are necessary for fertility evaluation or therapeutic maneuvers. Men who are unable to ejaculate may require clinical spermatozoa collection, while men with azoospermia may be aided by surgical intervention.

Spermatozoa Washing

Spermatozoa washing is the most common method for the separation of spermatozoa from seminal plasma. Washing not only quickly and efficiently removes detrimental factors present within the seminal plasma but also concentrates spermatozoa within the ejaculate. Spermatozoa washing is accomplished by mixing the ejaculate with an appropriate, protein-supplemented medium and centrifuging and aspirating the supernatant. The pellet is resuspended. This procedure can be recommended for samples with good concentrations of highly motile spermatozoa with very few non-spermatozoa-contaminating cells.

Processing of Spermatozoa Collected Following Surgical Spermatozoa Collection

The management of severe male factor infertility has been dramatically changed with the introduction of ICSI. Since only one spermatozoon is needed to inject into each oocyte, few spermatozoa are needed to ensure fertilization of retrieved oocytes with ICSI. Advances in surgical techniques of spermatozoa retrieval combined with ICSI have allowed men with certain male infertility factors previously considered to be incompatible with fertility to father biological progeny. Spermatozoa may be retrieved from men with both nonobstructive and obstructive azoospermia.

Various surgical techniques have been refined to collect viable spermatozoa from the epididymis and testes. Since low-quantity and low-quality spermatozoa are usually recovered, specialized processing is required.

Oocytes: Preparation and Evaluation

Advances in culture medium composition have significantly influenced embryo quality and pregnancy rates over the years. All media for ART can now be purchased commercially. Prior to use, they must be tested for toxins and their ability to support growth and development of embryos. Media should only be opened in a laminar flow hood, with attention to maintaining sterility during the addition of protein.

Laboratory and Media Preparation

There are two basic kinds of media used for ART procedures: one used during the handling of gametes and embryos out of the CO_2 incubator, called "processing medium," and another used for culture while in CO_2 incubators, called "culture medium." Both consist of a combination of nutrients necessary to maintain early embryo metabolism and proper pH and osmolarity. A source of protein, such as albumin or synthetic serum, must be added in a percentage that can vary from 2 to 15 mg/L.

Media used for the culture of embryos are usually bicarbonate buffered and kept in an incubation chamber. It is very important that this medium is maintained at a stable pH and temperature, because embryos are extremely sensitive to variations of these two factors. Culture media, used for embryo development inside incubation chambers, should always be equilibrated inside the CO_2 environment prior to use. Overlaying of the medium with mineral oil is recommended to help avoid evaporation and increase stability of pH while the dish is temporarily out of the CO_2 environment. Oil overlay provides an effective barrier to atmospheric volatile organic compounds (VOCs), which can be embryotoxic if exposed directly to the culture medium.

Oocyte Collection and Evaluation

Oocyte Collection

The aspirated follicular fluid is collected by the physician into processing medium containing protein at 37°C and immediately transported to the laboratory. Test tubes containing processing medium plus heparin, to prevent clotting, should be prepared prior to the procedure. In the laboratory, aspirates are examined quickly for the presence of cumulus-oocyte complexes.

Oocyte Assessment

Immature oocytes are defined as being at a stage of meiosis prior to metaphase of meiosis II (MII). This includes oocytes in prophase of meiosis I, which are identified by the presence of a germinal vesicle or nuclear envelope in the cytoplasm, without any polar body present in the perivitelline space (Fig. 15.2). If present, cumulus and corona are commonly very tightly condensed. As prophase I resumes, the oocyte enters into metaphase of meiosis I (MI). This intermediate stage of maturation is recognized by the disappearance of the germinal vesicle and the absence of the first polar body (Fig. 15.3). For MI oocytes, the cumulus may be expanded, but the corona can still be compact. The extrusion of the first polar body marks the transition to a mature oocyte, which is now considered to be at MII (Fig. 15.4). Metaphase II oocytes

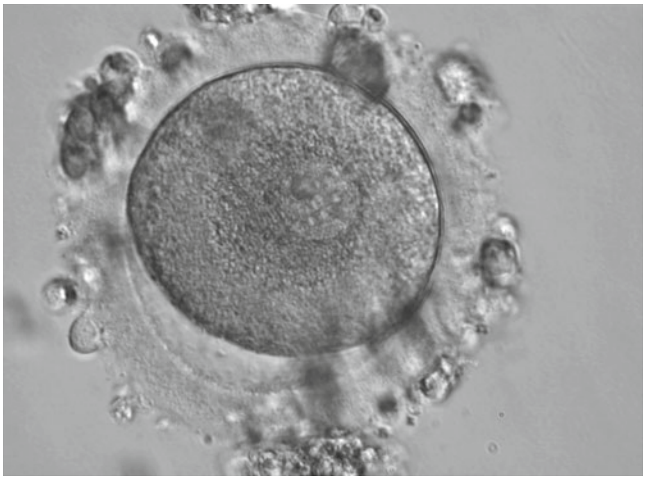

Fig. 15.2 Germinal vesicle intact representing an immature oocyte after cumulus removal (courtesy of Dr. Nina Desai, Cleveland Clinic)

Fig. 15.3 Metaphase I (MI) human oocyte without a polar body after cumulus cell removal (courtesy of Dr. Nina Desai, Cleveland Clinic)

Fig. 15.4 Metaphase II (mature) oocyte. Denuded oocyte showing presence of first polar body (courtesy of Dr. Nina Desai, Cleveland Clinic)

Fig. 15.5 Normal zygote showing presence of two polar bodies (only one seen in this view) and two pronuclei (courtesy of Dr. Nina Desai, Cleveland Clinic)

will usually have a fully expanded cumulus. Under normal circumstances, the oocyte will remain at MII until fertilization; when meiosis II resumes, the second polar body is extruded and the male and female pronuclei form (Fig. 15.5).

In Vitro Fertilization

Conventional Insemination

Oocytes are routinely inseminated 3–6 h after oocyte retrieval is performed, depending on oocyte maturity. Individual or groups of oocytes can be incubated and inseminated either in organ culture dishes, four-well dishes, or test tubes containing equilibrated medium, with or without oil overlay. Individual oocytes can also be inseminated in 30- to 50-μL drops of equilibrated medium in culture dishes with oil overlay, thus reducing the number of spermatozoa necessary for the insemination. Generally, concentrations range from 50,000 to 100,000 motile spermatozoa/mL. Spermatozoa concentrations that are too high can result in increased incidence of polyspermic fertilizations (more than one spermatozoon penetrating an oocyte). Concentrations that are too low may compromise fertilization rates.

Intracytoplasmic Sperm Injection

ICSI consists of insertion of a single spermatozoon directly into the oocyte cytoplasm. This technique was first successfully applied to human oocytes in 1992 and has since revolutionized the treatment of severe male factor infertility [38].

By injecting a spermatozoon into the oocyte cytoplasm, many steps of spermatozoa processing and developmental prerequisites are bypassed without compromising fertilization rates. There is currently debate regarding appropriate indications for ICSI.

Evidence strongly supports the following indications for the use of ICSI:

- Prior failed fertilization by conventional insemination
- Total motile spermatozoa concentration less than 10 million/mL
- Spermatozoa morphology less than 4 % normal forms based on strict criteria

Table 15.2 Cleavage-stage embryo two-step grading system

A	Minimum of 4 cells by 40 h post-insemination Minimum of 8 cells by 64 h post-insemination
B	Minimum of 2 cells by 40 h post-insemination Minimum of 4 cells by 64 h post-insemination
C	Minimum of 2 cells by 64 h post-insemination
D	No minimums (lowest possible grade). Do not make subtractions
Subtract from the grade for irregularities as follows:	
Spherical blastomeres with no fragmentation	No subtractions
Spherical blastomeres with ≤20 % fragmentation	Subtract 1 grade
Slightly irregular blastomeres with ≤50 % fragmentation	Subtract 2 grades
Irregular blastomeres with >50 % fragmentation	Subtract 3 grades

Preparation and Evaluation of Zygotes and Embryos

Culture Conditions

Traditionally, laboratories have cultured embryos at an atmospheric oxygen concentration of approximately 20 %. In contrast, the oxygen concentration in the fallopian tube and uterus is approximately 5 % [39, 40]. In animal models, high oxygen concentrations increase the production of reactive oxygen species. This increased oxidative stress may have deleterious effects on embryo quality [41]. Several studies suggest that culturing embryos at a lower oxygen concentration, about 5 %, may improve live birth rates with IVF and ICSI [42–45]. Additionally, there is no evidence to date that culturing embryos under low oxygen concentrations is associated with an increased risk of any adverse outcomes, such as multiple pregnancies, miscarriages, or congenital abnormalities [42].

Fertilization

The fertilization check of oocytes is performed 15–18 h after insemination for both IVF and ICSI procedures. It is necessary to examine the oocytes/zygotes within this time period to visualize the presence of pronuclei and polar bodies. Normal fertilization is characterized by the presence of two pronuclei, one male and one female, in the ooplasm and two polar bodies in the perivitelline space. If oocytes have undergone conventional IVF, the cumulus cells must be removed to clearly see the oocyte.

Abnormal fertilization may also be represented by oligopronuclear zygotes. The term applies to zygotes that express a single pronuclei. Only one pronuclei and the presence of two polar bodies may be observed in some cases when the oocyte undergoes parthenogenic activation or failure of the spermatozoa head to decondense. It is possible that a second pronuclei will developed later than the first one, and a repeat observation about 4 h after the first check is recommended. Failed fertilization is represented by absence of pronuclei and presence of one or two polar bodies that may be in the process of degeneration.

Embryo Assessment

Embryos can be assessed and graded daily while they are in culture. A standard morphologic method of grading is applied according to observations made on embryo development until their transfer to the uterus on day 3 (72 h post fertilization) (Table 15.2) or day 5 (blastocyst stage) [46, 47]. There are numerous scoring systems proposed for embryo development. Criteria for grading include the rate of division as judged by numbers of blastomeres, size, shape, symmetry, appearance of the cytoplasm, and presence of cytoplasmic fragments.

It is important to note that evaluation and scoring by morphology alone can be subjective and may not necessarily reveal embryos with the best developmental potential. Currently, there are numerous groups working on translational research focused on noninvasive means of assessing embryos for biomarkers that are indicative of embryonic developmental competence and implantation potential. Such evaluation, in concert with morphology and genetic analysis, has enormous potential to reshape the practice of embryo selection in the future.

Assisted Hatching

One of the most common unsolved problems in IVF is the fact that embryos with apparently good developmental potential do not always implant. It has been proposed that this may be due to defects of the zona pellucida, uterine receptivity, extensive fragmentation, modifications after freezing and thawing, or even suboptimal culture conditions. Potential defects in the zona pellucida may be overcome by assisted hatching (AH), a controlled incision of the embryo's zona pellucida. The true value of AH is still controversial [48].

Preimplantation Genetic Testing

Preimplantation genetic diagnosis (PGD) was originally seen as a means to detect and avoid congenital diseases by evaluation of individual blastomeres of preimplantation embryos. Initially, molecular PGD was employed for embryo sexing in couples at risk for X-linked diseases [49]. Technically, PGD consists of micromanipulation, biopsy, and DNA analysis of gametes and/or embryos.

Micromanipulation for the biopsy includes the mechanical opening of the zona pellucida and retrieval of one or two polar bodies (when performed on oocytes or zygotes) or one or two blastomeres (when performed on embryos). When embryos are tested, they are typically biopsied at the cleavage stage and then cryopreserved or placed in extended culture to develop to the blastocyst stage while genetic testing is underway. Alternatively, embryos can be biopsied at the blastocyst stage, when more cells can be analyzed and the embryo is less susceptible to biopsy-related injury. When biopsy is performed at this later stage, the blastocyst is typically cryopreserved until the genetic test results are available. For DNA analysis, there are currently two main techniques employed: polymerase chain reaction (PCR) for single gene disease detection and fluorescence in situ hybridization (FISH) for aneuploidy screening.

Single Gene Defects

PCR allows the amplification of a specific DNA sequence. This is a cyclic process in which at each cycle, the number of DNA fragments doubles, allowing the observation of specific genes or gene mutations through either radioactive labeling, ethidium bromide, fluorescent dye, or silver staining.

Aneuploidy Screening

For preimplantation genetic screening (PGS) (i.e., aneuploidy screening), FISH is often employed. This method utilizes direct-labeled probes, with the specific annealing of fluorescent nucleic acid probes to complementary sequences of DNA in a fixed specimen in which chromatin and/or chromosomes have been isolated [50–52]. Chromatin from polar bodies and/or blastomeres can be treated and fixed for FISH. In this procedure, cells are fixed on a slide in a manner that facilitates rupture of the cell, removal of the cytoplasmic material, and spreading and adhering of chromosomes. These chromosomes are then hybridized or annealed to the chromosome-specific fluorescent probes. Using a fluorescent microscope, one can count specific chromosomes and thus identify normal and/or abnormal chromosome numbers.

Limitations

Currently, panels of FISH probes vary, but the more comprehensive panels include chromosomes 8, 13, 14, 15, 16, 17, 18, 20, 21, 22, X, and Y. While FISH typically tests for the most common causes of aneuploidy, it is often criticized because it is not completely comprehensive. Additionally, early embryonic mosaicism limits the accuracy of FISH. To date, the majority of studies show no benefit to PGS on the live birth rate after IVF. In fact, for women of advanced maternal age, PGS appears to lower the live birth rate [53]. Newer screening methods, such as comparative genomic hybridization (CGH), will likely replace FISH in the future [54]. For all preimplantation genetic testing, couples should be counseled in advance that there is a chance of diagnostic error or inconclusive results. Additionally, conventional prenatal diagnosis is still recommended to confirm the accuracy of the preimplantation genetic testing results.

Cryopreservation

Cryopreservation of gametes and embryos maximizes success in any IVF program and prevents wastage of specimens. It is important to realize, however, that there are many ethical, religious, legal, and social implications involving embryo storage. Some countries, such as Germany, Austria, Switzerland, Denmark, and Sweden, have restricted or forbidden cryopreservation of embryos [46].

Technique

There are currently two primary categories of gamete/embryo cryopreservation strategies: slow-rate freezing and vitrification.

Slow-Rate Freezing

Slow-rate freeze protocols vary in permeating cryoprotectants, non-permeating cryoprotectants, and cooling and warming rates, thus making it difficult to generalize or compare cryopreservation results. The following is one general example of a cleavage-stage embryo slow-rate cryopreservation protocol.

Prior to cryopreservation, embryos that meet the program-specific freeze criteria are selected and assigned to cryopreservation. After washing embryos through processing media with 12–15 mg/mL of protein, they are exposed to the same media containing 1.5 mol/L of propylene glycol (propanediol) and then 1.5 mol/L propylene glycol plus 0.1 mol/L sucrose. Embryos are loaded into plastic straws or vials and placed in a programmable freezer, where they will be cooled at −2°C/min from room temperature down to −4 to −6°C. After a period of 5 min of holding the temperature, a supercooled object is pressed against the side of the container to induce "seeding." The hold is continued for a period of time, followed by continued temperature drop at a rate of −0.3°C/min until it reaches −32°C. At this point, the containers can be plunged directly into liquid nitrogen for storage.

Vitrification

Vitrification is a form of rapid cooling that utilizes very high concentrations of cryoprotectant that solidify without forming ice crystals. Ice crystals are a major cause of intracellular cryo-damage [55]. The vitrified solids contain the normal molecular and ionic distributions of the original liquid state and can be considered an extremely viscous, supercooled liquid. In this technique, oocytes or embryos are dehydrated by brief exposure to a concentrated solution of cryoprotectant before plunging the samples directly into liquid nitrogen. Application of vitrification for both oocytes and embryos is a current area of focus for many clinical, rodent, and domestic animal production laboratories [56].

Both slow-rate freezing and vitrification are being used extensively in the United States. For oocyte cryopreservation, vitrification appears to be superior to slow-rate freezing. For cleavage-stage embryos, both approaches seem to be equally successful. For freezing at the blastocyst stage, vitrification may offer more consistent results, although slow cooling is also quite efficacious [57]. With continued research, protocols for both techniques will likely be optimized.

Future Directions

Over the past 3 decades, few areas of medicine have experienced the rapid evolution that has occurred within the field of ART. Despite this progress, success rates have plateaued in recent years, and many new challenges and opportunities for improvement lie ahead. For instance, we may see a switch from daily FSH injections to long-acting FSH preparations [58]. FISH for aneuploidy screening will likely be replaced by CGH, microarrays, or quantitative PCR [54]. With growing pressure to decrease multiple gestations, noninvasive methods to improve embryo selection (such as metabolomic profiling or time-lapse imaging) will become increasingly important [59–61]. Finally, as a result of these innovations within our field, we will likely see a greater shift toward eSET in good-prognosis patients.

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Hysteroscopic Management of Intrauterine Disorders: Polypectomy, Myomectomy, Endometrial Ablation, Adhesiolysis, and Removal of Uterine Septum

16

Michelle G. Park and Keith B. Isaacson

Introduction

Hysteroscopy is a minimally invasive and highly accurate means of diagnosing and treating a multitude of intrauterine pathologies. During hysteroscopy, a telescope is inserted transvaginally through the cervix and into the uterine cavity. A distention media is used to expand the uterine cavity in order to visualize the endometrium and tubal ostia. Pathology can then be detected and treated with direct visualization.

As the technology has advanced, hysteroscopy has become more accessible to gynecologists both in the office and in the operating room. It has become an indispensable tool for treating fibroids, polyps, synechiae, and congenital uterine anomalies and for performing endometrial ablation procedures.

Indications

The most common indications for evaluation of the uterine cavity are abnormal uterine bleeding and infertility. Although hysterosalpingography (HSG) and modern ultrasonography are relatively sensitive in detecting intrauterine anomalies, diagnostic hysteroscopy remains the gold standard tool to visualize the cervical canal and uterine cavity. Today, there remain two schools of thought regarding initial evaluation of the uterine cavity. Many clinicians utilize HSG, transvaginal ultrasonography, and sonohysterography for initial evaluation. However, gynecologists can proceed directly to diagnostic hysteroscopy in the office or outpatient setting with little or no anesthesia for this purpose. Operative hysteroscopy allows for a visually directed biopsy of focal lesions and treatment of intrauterine lesions, including endometrial polyps, intracavitary leiomyomas, uterine septa, and uterine synechiae.

Basic Hysteroscopic Hardware

Types of Hysteroscopes

All hysteroscopes incorporate an optical system that includes mechanisms to deliver light into the uterine cavity and return the reflective image to an eyepiece or a camera. The most commonly used system is the rigid hysteroscope with an objective lens at the tip offset by 0, 12, 25, or 30° from the horizontal plane. Other systems include the fiber-optic diagnostic single-channel flexible hysteroscopes and digital flexible dual-channel hysteroscopes. All hysteroscopes include at least one channel for the inflow of distention media. Continuous flow hysteroscopes incorporate a second channel for the return of distention media and the placement of operative instruments.

Resectoscopes are all continuous flow systems that allow the attachment of operative instruments, including monopolar and bipolar electrodes in a variety of shapes. These are 7- to10-mm systems and are more typically utilized in the operating room setting. All resectoscopes incorporate a rod lens system for light delivery and image return.

Distention Media

The uterine cavity is a potential space that must be distended with either gas or fluid media in order to visualize the endometrium and intrauterine pathology in three dimensions. Each distention media has its own advantages and disadvantages.

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Low-Viscosity Fluids

Low-viscosity fluids are the most common distention media used today because they are suitable for both diagnostic and operative hysteroscopy, are relatively inexpensive, and are relatively low risk to use. The two groups of low-viscosity fluids are isotonic electrolyte-containing fluids and hypotonic nonelectrolyte media.

Isotonic electrolyte-containing fluids can be used for all operative procedures except those that require monopolar electrosurgery. Two commonly used fluids are 0.9 % sodium chloride and acetated lactated Ringers solution. Nonelectrolyte media is required for monopolar electrosurgery (i.e., resectoscopic) procedures.

When electrolyte-containing solutions are used (e.g., saline), the procedure should be discontinued when a fluid deficit of 2,500 mL is reached. The patient may require a diuretic if this fluid deficit is higher.

Hypotonic nonelectrolyte-containing fluids are required when the monopolar resectoscope is used, and several types are available. The most common fluids used are 5 % mannitol, 3 % sorbitol, and 1.5 % glycine. The theoretical advantage of 5 % mannitol is that it is isotonic, which, in theory, may reduce the risk of cerebral edema with excessive absorption [1]. Excessive absorption of all electrolyte-free media can lead to hyponatremia and its potentially life-threatening complications. Sodium levels fall 10 mEq/mL for every 1 L of electrolyte-free media absorbed.

When a fluid deficit of 1,000 cm^3 of nonelectrolyte solution is identified, electrolytes should be drawn and the case should be terminated. Consideration should be given to administer diuretics with close monitoring of electrolytes following surgery. Injection of 3–4 mL of dilute vasopressin (10 units in 200 mL saline) into the cervix prior to distention of the cavity can decrease both intraoperative bleeding and intravasation for at least 20–30 min [2].

Carbon Dioxide

Carbon dioxide is less commonly used today and is only appropriate for diagnostic procedures.

Dextran 70

One of the first fluid-distention media used was high-viscosity hypertonic dextran 70. A 32 % solution of dextran 70 in 10 % dextrose in water is a nonelectrolytic, nonconductive fluid with syrup-like consistency that can be used for both diagnostic and operative hysteroscopy. It is rarely used today as hysteroscopic distention media because of higher rates of allergic reactions and hypertonic-associated problems, including pulmonary edema [3].

Diagnostic Hysteroscopy

Diagnostic hysteroscopy is used to examine the intrauterine cavity. Patients should be on suppressive hormones or have the procedure timed to occur during the proliferative phase of their menstrual cycle in order to improve visualization. Diagnostic hysteroscopy can be performed in the office setting or the operating room, depending on the level of discomfort the patient experiences during the procedure.

Usually diagnostic hysteroscopy can be performed in the office setting without the need of any analgesia or anesthesia. Narrow, rigid, and flexible hysteroscopes generally have diameters <4 mm that can be inserted with minimal discomfort to the patient. If the patient experiences discomfort, oral analgesics may be required, and if cervical dilation is necessary, a paracervical block may also be placed.

It is important to insert the hysteroscope slowly and under direct visualization of the canal so that a false channel is not created and uterine perforation can be avoided. In order to allow for controlled insertion of the hysteroscope, some patients may require cervical softening prior to hysteroscopy. Misoprostol is most commonly used for cervical softening. Vaginal misoprostol (200–400 mcg) can be given 8–12 h before surgery, or oral misoprostol (400 mcg) can be given 12 and 24 h before surgery.

Once the uterine cavity is entered, visualization of landmarks is critical. A panoramic inspection of the endocervix, lower uterine segment, endometrial cavity, and tubal ostia should be performed as the hysteroscope is inserted. A careful, thorough survey is necessary so that pathology is not missed. Myomas of the endocervix and lower uterine segment can easily be missed with too rapid insertion of the hysteroscope.

Operative Hysteroscopy

For operative procedures, larger hysteroscopes and resectoscopes are used that require cervical dilation prior to insertion and are therefore used primarily in an operating room under sedation or with general or regional anesthesia.

Complications

The overall complication rate associated with hysteroscopy is reported to be 2.7 % [4]. The operative complications of hysteroscopy include uterine perforation, excessive hemorrhage, air embolus, pulmonary edema, pelvic organ injury secondary to thermal damage if electrode surgical systems are used, excessive fluid absorption, major vessel injury, intrauterine scar formation, and infection [5]. The different procedures described below carry all these risks to varying degrees, depending on the pathology being treated and the equipment being used. These complications are more or less likely depending on the procedure being performed. For example, perforation is more likely during adhesiolysis of severe Asherman's syndrome, and fluid overload is more likely in procedures that require incisions deep into the myometrium, exposing the uterine venous sinuses, as in hysteroscopic myomectomy.

Office Hysteroscopy

Office hysteroscopy is the gold standard tool for evaluation of intrauterine pathology. While it has been available for over 20 years, it has recently become more commonplace due to improved technology, improved reimbursements, and patients' desire to avoid general anesthesia and the operating room setting. Also, there have been advances in office-based hysteroscopic procedures such as tubal occlusion for sterilization that increased awareness of this valuable tool for providers and patients. Patients are able to undergo the procedure with minimal discomfort, most often with no or minimal oral and local analgesia. When performing any procedure in the office setting, the clinician must be especially cognizant of possible complications of hysteroscopy given the limited resources available to manage any complications that may be encountered.

Office hysteroscopy is an effective and well-tolerated alternative to day-surgery hysteroscopy. One study randomized 40 women requiring polypectomy into either office hysteroscopy or day-surgery hysteroscopy. The results demonstrated that the office hysteroscopy subjects experienced minimal pain during the procedure and had faster recovery times and lower postoperative analgesia requirements than the subjects who underwent day-surgery hysteroscopy. Ninety-five percent of women who underwent office hysteroscopy stated they would repeat outpatient hysteroscopy if their polyps recurred. Eighty-two percent of women who underwent day-surgery hysteroscopy stated that they would like to try office hysteroscopy if their polyps recurred [6]. Several studies such as these have demonstrated the safety and significantly lower cost of office hysteroscopy when compared to day surgery [6, 7]. Additionally, it seems that patients are well able to tolerate these hysteroscopic procedures in the office and they have the added benefit of avoiding general anesthesia. Given all of these benefits, clinicians should give serious thought to developing facilities for office hysteroscopy if they are lacking and promoting office hysteroscopy to their patients when appropriate.

Equipment

The two categories of hysteroscopes used in the office setting are rigid and flexible. Generally, the hysteroscopes used for office hysteroscopy range from 3 to 5 mm in diameter. Larger operative hysteroscopes that are 8–10 mm in diameter are generally reserved for the operating room because they require significant cervical dilation, which can be very uncomfortable for the patient. When diagnostic hysteroscopy is performed, the hysteroscopes are introduced without the operative sheaths so as to decrease the diameter of the hysteroscope and minimize trauma to the cervical canal. Rigid hysteroscopes may be used, which are 2.7–5 mm in outer diameter. Zero-, 15-, or 30-degree hysteroscopic lens are appropriate for diagnostic hysteroscopy. If the cervical canal is tortuous or the uterus is especially anteflexed or retroflexed, flexible hysteroscopes are generally superior to rigid hysteroscopes. They have the added advantage of having a tip that can deflect from 0° to 110° and curve with the natural course of the endometrial canal.

If operative procedures are planned in the office, operative sheaths must be used, which increase the diameter of the hysteroscope system to approximately 4–5 mm. These outer sheaths are needed to accommodate the instruments and provide an outflow of fluid, making the hysteroscope a continuous flow system with both an inflow and outflow channel. The inflow channel is essential for appropriate distention of the uterine cavity, and the outflow channel is used to flush out any blood and debris that may accumulate, thereby improving visibility.

The standard operative tools available in the office setting include cold scissors, biopsy cups, and graspers. Recent technologic advances have also made bipolar electrodes available for use in the office. These 5-Fr electrodes are small enough to pass through any standard operative port of the 5-mm office hysteroscope. Additionally, because they are bipolar electrodes, physiologic electrolyte-containing distention media can be used without dissipating the energy generated at the end of the electrode.

Technique

The traditional approach to office hysteroscopy involves the use of a speculum to visualize the cervix and a tenaculum to grasp the cervix and provide traction to straighten the cervical canal. Although this approach may be necessary for some patients when a difficult entry is encountered, in a vast majority of patients, the clinician may dispense with the speculum and tenaculum by traversing the vagina via vaginoscopy. Bettocchi et al. have shown that hysteroscopy can be performed very successfully with this technique, while decreasing the amount of pain experienced by the patient [8].

With this technique, the hysteroscope is placed just inside the vaginal canal and the distention media is instilled. The vaginal walls distend, allowing the clinician to advance the hysteroscope through the vaginal canal under direct visualization and without causing trauma to the walls. The hysteroscope is directed along the posterior wall of the vagina until the posterior fornix is reached. It is then pulled back slightly and angled upward until the cervical os is visualized. The hysteroscope can then be introduced into the cervical os, which then distends the endocervical canal. With the inflow distention media on low flow, the hysteroscope is guided through the canal under direct visualization so that the clinician may follow the natural course of the canal with minimal trauma to the surrounding tissue. Unless cervical stenosis is encountered, this procedure can be performed with minimal discomfort to the patient. In many cases, no analgesia or anesthesia is required.

Pain Management

With appropriate pain management, office hysteroscopy is successful in 90–95 % of cases [9, 10]. Many patients are able to tolerate hysteroscopy without any analgesics or anesthetics, especially when the vaginoscopic technique is used. However, if the patient experiences significant discomfort, the clinician should be armed with tools to manage the patient's pain so that the hysteroscopy can be completed successfully.

NSAIDs and Anxiolytics

Patients can be pretreated with nonsteroidal anti-inflammatory drugs (NSAID) so that prophylactic pain management can be initiated before the procedure has even begun. Patients should be told to take NSAIDs 1–2 h prior to their procedure. The patient may take this orally before arriving at the office, or it may be administered at the office in the form of IM ketorolac once they arrive. Anxiolytics may also be administered to calm the patient; Lorazepam is usually the drug of choice.

Topical Analgesia

Lignocaine spray of 30, 50, or 100 mg to the cervix and cervical canal was shown to decrease pain at the time of insertion of the hysteroscopy, and it was also shown to decrease vasovagal reactions [11]; 25 mg of lignocaine cream with 25 mg of prilocaine, administered to the cervix, was shown to have the same benefits. There have not been many studies in support of this method of analgesia. However, given the current data, topical analgesia is a reasonable option to reduce patient discomfort.

Paracervical Block

If cervical dilation is required, a paracervical block may be performed to minimize patient discomfort. In most studies, paracervical block was performed by placing 10 mL of mepivacaine or lidocaine via a 22-gauge spinal needle at 3, 5, 7, and 9 o'clock positions paracervically. The studies are conflicted regarding the efficacy of the paracervical block. Some studies showed no significant difference in pain scores between groups, while an equal number of other studies showed a statistically significant decrease in pain overall [12]. In one study, patients were randomized into injections of lidocaine or saline. There was improvement in pain at insertion of the hysteroscope in the group with lidocaine, but patients commented that the injections were as painful as the hysteroscopic procedure. Transcervical and intracervical blocks have not been shown to be effective [12].

Conscious Sedation

Conscious sedation is characterized by decreasing the patient's consciousness while allowing them to maintain their own airway. They are usually still able to respond to physical and verbal stimuli. Because the effects of the narcotics may be unpredictable, the patient's vital signs must be continuously monitored. Typical agents used for conscious sedation are fentanyl, propofol, and midazolam. Antiemetics may also be infused to counteract the gastrointestinal upset caused by the narcotics.

There are several requirements, outlined by the American Congress of Obstetricians and Gynecologists, which an office must fulfill in order to safely perform conscious sedation in the office setting [13]. The patient's oxygenation must be monitored continuously; this is most commonly done using pulse oximetry. If deeper sedation is administered, the patient's ventilatory function should also be monitored by direct observation, auscultation, or capnography. The patient's circulatory function should be monitored with a continuously displayed electrocardiogram, blood pressure and heart rate measurements every 5 min, and pulse plethysmography. Anesthesiologists or nurse anesthetists must be present to monitor the patient during conscious sedation. Gynecologists may administer conscious sedation after undergoing certification. However, they must keep in mind that an additional health-care professional must be present whose sole responsibility is monitoring and attending to the patient. The clinician must also be certified in ACLS, PALS, or BLS and must be sufficiently free after the procedure is performed to monitor the patient until stable for discharge.

Respiratory suppression is a significant concern when administering conscious sedation. The office must have all the equipment necessary to manage complications from respiratory suppression. An oxygen source must be present, as well as suction, resuscitation equipment including a defibrillator, and emergency medications. This equipment must be maintained and tested according to manufacture specifications. If any of these requirements are lacking, conscious sedation is not recommended.

Patient Selection

Several factors must be considered when determining if a patient is a reasonable candidate for office hysteroscopy. First and foremost, a thorough history must be taken of the patient. The patient should be screened for significant comorbid conditions compromising her ability to tolerate the stresses of hysteroscopy. If a history of cervical stenosis or severe anxiety is elicited, the patient should be taken for day-surgery hysteroscopy [14].

In terms of the pathology that can safely be treated in the office setting, clinicians should consider what the patient will be able to tolerate and be wary of what complications can potentially be encountered. Appropriate procedures include diagnostic hysteroscopy, endometrial biopsies, lysis of adhesions, small polyps, tubal occlusion, and global endometrial ablation. These procedures are appropriate because they are relatively short, so that the patients will be able to tolerate them, and they have very low risk of complications.

Any procedure requiring lengthy operative time (>15 min) is not suggested for an office-based procedure. The operating room is more appropriate in order for the patient to remain comfortable. Myomas or polyps larger than the internal os of the cervix (>1 cm) as well as extensive lysis of adhesion and large septum resections are more appropriately and safely done in the operating room.

Complications

Fluid Intravasation

Complications may be encountered regardless of the type of distention media used. A complication that may be seen, regardless of the type of distention media, is fluid overload, which occurs in approximately 0.2 % of cases [15]. This is seen when there is significant intravasation of the distention media. To prevent this from occurring, intrauterine pressure should be maintained at the lowest possible pressure while still maintaining adequate intracavitary visibility. Ancillary staff should be available to aid the clinician with the procedure and monitor the fluid deficit closely. If there is evidence of rapid intravasation of distention media, the procedure should be terminated. Clinicians should be especially cautious when performing lysis of adhesions, myomectomies, resection of septums, or any other procedures that may open vascular channels and potentiate intravasation.

For isotonic electrolyte-containing solutions, the maximum deficit in an office setting should not exceed 1,000–1,500 mL. If these deficits are surpassed, the patient should be monitored and the procedure terminated. There is no place to use electrolyte-free media in an office-based setting.

However, in most cases, procedures done in the office should be relatively quick, and fluid deficits should not be approaching maximum levels. Hysteroscopic procedures should be discontinued at lower fluid deficits than the thresholds described above, given that there is limited acute care available in the outpatient setting [14]. Therefore, if a long operative time is anticipated for a procedure, it may be better to schedule it for the operating room where the patient can be monitored closely as the fluid deficit increase.

Perforation

The most frequent reported complication of hysteroscopy is uterine perforation, which occurs at a rate of 14.2/1,000 procedures according to a survey conducted by the American Association of Gynecologic Laparoscopists in 1993 [15]. These perforations commonly occur during dilation of the cervix, as well as during the hysteroscopic portion of the procedure. Perforations of the lower uterine segment and fundus of the uterus may be seen. In most cases, no treatment is required. However, if there are signs of intra-abdominal bleeding, such as when there is a lateral perforation through major vessels, the patient should be transferred immediately for laparoscopic exploration with possible repair of the defect. Management of perforation with an activated electrosurgical device also warrants emergent surgical evaluation. These thermal injuries usually occur when clinicians are activating the electrodes while moving them away rather than towards themselves. Additionally, certain procedures such as lysis of adhesions and resection of septums have much higher rates of perforation than other hysteroscopic procedures [16, 17]. Therefore, clinicians should be especially cautious when performing these procedures in the office.

Vasovagal Reaction

A vasovagal reaction is not uncommon in office hysteroscopy. One study reported a rate of 0.72 % in patients undergoing hysteroscopy without analgesia [18]. Smaller hysteroscopes and improved pain control were shown to decrease the rates of vasovagal reaction [19]. A vasovagal episode is generally preceded by a feeling of light-headedness, nausea, diaphoresis, bradycardia, and/or pallor. If these symptoms are encountered, the procedure should be immediately terminated. Patient should be placed in Trendelenburg position or with her legs raised. Her vital signs should be monitored closely, and an intravenous fluid bolus may be necessary. In most cases, patients recuperate quickly with these interventions. If a patient has known history of vagal reactions, one can pretreat with 0.4 mg of atropine IM prior to the procedure.

Bleeding

Most bleeding encountered during hysteroscopy is self-limiting. However, persistent bleeding may be encountered.

This can be seen when the cervical canal is lacerated during dilation, after uterine perforation, or when vessels in the myometrium are transected, such as when clinicians are performing myomectomies or septum resections. If persistent bleeding is seen, electrocautery is most often ineffective. The primary treatment should be a Foley catheter placed into the cavity to tamponade the bleeding [20]. The Foley catheter is placed into the cavity and inflated with 15–30 mL of water until the bleeding stops [21]. If persistent bleeding is encountered despite these measures, more aggressive exploration in the operating room may be warranted.

Hysteroscopic Polypectomy

Symptomatic polyps are generally characterized by abnormal bleeding, postcoital staining, chronic vaginal discharge, or dysmenorrhea. Abnormal bleeding symptoms include intermenstrual spotting or heavier menstrual flow. There is good evidence that polyps can decrease fertility and that their removal will improve the chances of pregnancy [22].

It is obvious that symptomatic endometrial polyps should be removed. However, it is also important to remove asymptomatic polyps, particularly in postmenopausal women [23]. Although the vast majority of polyps are benign, endometrial cancer and hyperplasia will be found in approximately 2 % of endometrial polyps and are associated with coexisting malignancies elsewhere in the endometrium. In one study of over 1,400 polyps, endometrial cancer was found in 27 polyps (1.8 %) [23]. All but one of these women were postmenopausal, and only 26 % were asymptomatic.

Technique

Polyps can generally be removed with hysteroscopic scissors and pulled through the cervical canal intact. Larger polyps, with thick stalks, require resection with morcellation, most often with a resectoscope so that the tissue can be removed piecemeal. More recently, mechanical hysteroscopic morcellators have been increasingly used to manage both polyps and fibroids. A variety of morcellators are available, and each utilizes an electromechanical drive system to power a cutting blade inserted into a metallic cylinder. A vacuum is created that suctions the polyp into the cylinder, and the tissue is collected via the outflow tract.

Of the various methods described above for removing polyps, none have been shown to be more safe or effective than another, though there is a significant increase in the cost of the procedure if disposable devices are used. The choice of equipment should be based on the surgeon's preference when cost, safety, and efficacy are taken into account.

Hysteroscopic Myomectomy

Patients with symptomatic myomas generally present with abnormal uterine bleeding (i.e., menorrhagia), infertility, pelvic pain, or pressure. When determining the optimal surgical approach for their removal, it is important to determine the fibroid location in relation to the endometrial cavity. Fibroids can be described using the European Society of Hysteroscopy classification system, which groups fibroids into types based on the degree to which the myomas intrude into the endometrium and myometrium (Table 16.1).

Classification

Type 0 myomas are pedunculated, with the myoma lying completely within the endometrial cavity (Fig. 16.1). Type I myomas are described as "sessile" with <50 % intramural extension (Fig. 16.2). Type II myomas are submucosal in location, with >50 % intramural extension. These include transmural myomas, which extend from the submucosal to serosal edge. When viewed hysteroscopically, type II myomas form a bulge that can be seen in the endometrial cavity.

This system was originally designed to classify myomas exclusively on hysteroscopic appearance. However, this approach has significant limitations. During hysteroscopy, myomas can be compressed and recede into the myometrium as a result of the pressure of the distention media, thereby preventing full visualization of the myoma. For this reason, preoperative evaluation with ultrasonography is required to accurately determine how many myomas are present and how deeply the myomas penetrate the myometrium. Magnetic resonance imaging (MRI) can also be used for this purpose.

Surgical Approach According to Stage

For a successful surgical outcome, it is important to preoperatively identify the size, number, location, and intramural

Table 16.1 Hysteroscopic and sonohysterographic classification system for myomas encroaching upon the endometrial cavity

Hysteroscopic type [24]	Sonohysterographic class [25]	Description
Type 0	Class 1	Pedunculated myomas, where 100 % of the myoma lies within the endometrial cavity with no intramural extension
Type I	Class 2	Sessile myomas, with <50 % intramural extension
Type II	Class 3	Submucous myomas, with >50 % intramural extension

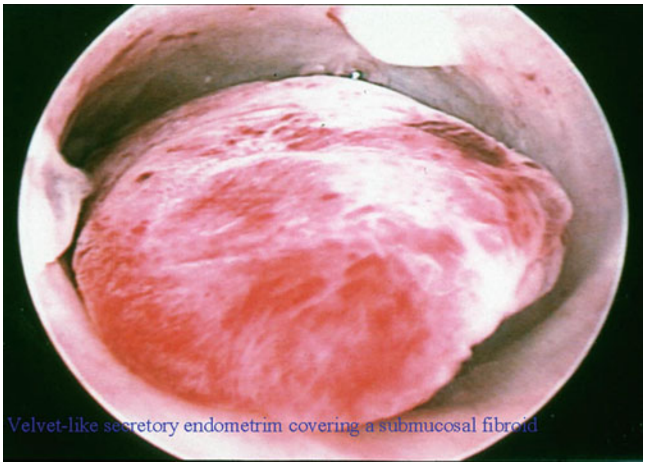

Fig. 16.1 Hysteroscopic view of a type 0 myoma. It is pedunculated, and the total myoma lies within 100 % of the endometrial cavity (reproduced with permission from Bradley LD. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Fig. 16.2 Hysteroscopic view of a type I myoma that involves less than 50 % of the myometrium (reproduced with permission from Bradley LD. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

extension of uterine myomas. These characteristics predict the surgeon's ability to completely resect the fibroids during one surgical procedure. Most often, when there is a large type II myoma, the procedure has to be terminated prior to completion due to excessive fluid absorption [26].

The degree of surgical difficulty and, thus, the risk to the patient are related to the depth of penetration and size of the myomas. Pedunculated hysteroscopic type 0 (class 1) myomas up to 3 cm in diameter can usually be easily removed hysteroscopically. Larger hysteroscopic type 0 myomas (>3 cm) and hysteroscopic type I (class 2) myomas can also be approached hysteroscopically. However, the risk of fluid intravasation increases as a result of increased surgical time and the opening of myometrial venous channels during resection. Additionally, there is poorer visibility with larger myomas given the more limited space within the uterus, inability to distend the cavity well, and the large amount of myoma "chips" that accumulate within the cavity. Often, incomplete removal of larger myomas requires two or more separate operative procedures. Only the most experienced hysteroscopist would attempt a hysteroscopic resection of an intracavitary myoma 5 cm or larger.

Likewise, hysteroscopic resection of type II (class 3) myomas should only be approached by high volume hysteroscopists. They are more commonly approached abdominally by laparoscopy or laparotomy. Hysteroscopic removal of type II myomas is also associated with a greater risk of fluid intravasation and uterine perforation and commonly requires two or more procedures for complete removal.

When patients have multiple intracavitary fibroids throughout the endometrial cavity, they would benefit from a "two-staged" operative hysteroscopic myomectomy, in which myomas are removed from only one uterine wall at a time. This is to decrease the risk of apposition of the uterine walls and the development of postoperative intrauterine synechiae.

Technique

There are various techniques for removing pedunculated and submucosal myomas, including avulsion, scissors, wire-loop resection with bipolar or monopolar equipment, morcellation, and laser vaporization. However, hysteroscopic wire-loop resection still remains the most popular method of removing myomas and will be the technique discussed in this section.

When monopolar energy is used for wire-loop resection, the current setting should be 60–80 W cutting current and requires an appropriately sized grounding pad. Higher settings may be necessary with very fibrous, dense, or calcified myomas. When the bipolar generator is used, it automatically adjusts the power to default settings.

Once the submucosal myoma is identified, the wire-loop electrode is advanced in clear view and retracted towards the surgeon behind the myoma. As the wire loop is drawn towards the surgeon, small, crescent-shaped "chips" or fragments of myoma are created. The whorled fibrous appearance of the myoma is clearly different from the fascicles of soft underlying myometrium. The fibrous tissue should be methodically resected until the border of the underlying myometrium is reached. However, increased bleeding from the myometrial bed and fluid intravasation may be encountered if the myometrium is breached. The resecting loop should stay within the pseudocapsule of the myoma and not cut this myometrium. If the hysteroscopist stays within the pseudocapsule, the likelihood of a uterine perforation will nearly be zero.

Myoma chips can remain free-floating until they interfere with visualization and are then removed with polyp forceps, Corson graspers, suction curette, or with the loop itself under direct visualization. All free-floating tissue fragments should be removed and sent for histologic examination. Removing all free-floating tissue also prevents delayed vaginal extrusion of this tissue material, malodorous discharge, adhesions, and infection.

Intermittently throughout the procedure, the intrauterine pressure should be lowered to 30 mmHg or the least amount of pressure that is possible while still maintaining visualization. This rapid reduction in intrauterine pressure will aid in enucleation of the myoma via a decompression mechanism that releases the encapsulated myoma from its myometrial bed. The myoma may appear to increase in size. In fact, more myoma protrudes into the endometrial cavity allowing a more complete resection without having to resect myometrium. False-negative views can occur during hysteroscopy because of the high intrauterine pressures. The "disappearing phenomena" refers to the flattening of endometrial polyps or fibroids, resulting in a falsely negative hysteroscopic study. This disappearing phenomenon can be avoided by decreasing the intrauterine pressure at the end of the procedure and reinspecting the endometrial cavity. As a general rule, the distention pressure within the uterine cavity should be the lowest pressure that gives the surgeon adequate flow and visualization. This will allow the myoma to protrude within the cavity and minimize unwanted fluid absorption.

Intraoperative Ultrasonography

Intraoperative ultrasonography guidance during operative hysteroscopy is useful for resection of myomas that are hard to define. Ultrasound guidance allows constant visualization of the uterine walls, as well as the hysteroscopic instruments. Therefore, the hysteroscopist may know when they are in danger of perforating the uterine wall. This added imaging allows for resection beyond the limit conventionally defined by hysteroscopy [27].

Fertility Preservation

If the patient desires fertility, overzealous resection of the myometrium must be avoided. Asherman's syndrome may occur when large portions of overlying endometrial tissue are resected with a sessile myoma. Patients who desire fertility and have multiple intracavitary myomas, especially those with myomas on opposite walls, may require resection in two separate occasions to minimize chances of intrauterine synechiae developing postoperatively.

Complications

Complications of hysteroscopic myomectomies include uterine perforation, bleeding, infection, and fluid intravasation. Uterine perforation most often occurs with cervical dilation with a blunt dilator. These patients can be observed in the recovery room and sent home when stable.

Major bleeding after a hysteroscopic myomectomy is rare. When excessive bleeding is encountered, it is generally secondary to myometrial bleeding. When the bleeding is excessive, it can be controlled with a 25-cm^3 catheter balloon left in place for 4–12 h.

Endometrial Ablation

Endometrial ablation was developed as a minor surgical procedure to treat women with intractable heavy menses unresponsive to medical management, who no longer desire fertility. Each year approximately 200,000 women undergo an endometrial ablation. Compared to hysterectomy, endometrial ablation offers the advantages of avoiding the morbidity and prolonged recovery associated with major surgery when patients fail medical management. However, the disadvantages include recurrence of bleeding over time. Up to 35 % of women who receive an endometrial ablation will receive a hysterectomy within 5 years of the procedure. Endometrial ablation should only be offered to women who are willing to accept eumenorrhea, hypomenorrhea, or cyclical bleeding rather than amenorrhea as a final clinical result. Only 40 % of women having an ablation will be amenorrheic.

Endometrial Ablation Techniques

There are several methods for endometrial ablation. The three "first-generation" hysteroscopic techniques include electrosurgical laser ablation, endomyometrial resection, and electrosurgical rollerball ablation. Second-generation techniques, also referred to as "global" methods, differ in that they do not require the use of the resectoscope to perform the ablation. Hysteroscopy is an integral part of only one of these systems.

First-Generation Hysteroscopic Endometrial Ablation

Mimicking the physiologic effect of Asherman's syndrome, the ultimate goals of endometrial ablation were to create severe endometrial scarification and secondary amenorrhea.

Endomyometrial resection utilizing a resectoscope was first reported by DeCherney and Polan in 1983 [28]. This technique utilizes unipolar electrocautery and is performed with hypotonic nonelectrolyte-containing distention media. This technique was the forerunner of hysteroscopic *rollerball endometrial ablation*, which has become the "gold standard" to which all emerging endometrial ablation technology is compared. Each of these devices destroys the basalis layer of the endometrium and is designed to result in hypomenorrhea or amenorrhea.

Technique: First-Generation Hysteroscopic Endometrial Ablation

The general concept of hysteroscopic endometrial ablation involves thorough destruction of the basalis layer of the cornua and lower uterine segment. For this reason, the patient should ideally be scheduled during the early proliferative phase. Otherwise, hormonal suppression of the endometrium is required to thin the endometrium and increase the chances of success of the ablation. Hormonal suppression also increases visualization by ridding the cavity of excess blood and tissue. Hormonal options for endometrial suppression include the use of depot leuprolide acetate, danacrine, oral contraceptive pills, or progesterone-only pills 4–8 weeks prior to surgery. Surgical preparation with suction or sharp curettage immediately prior to ablation has also been used with reported success.

Vasopressin

Vasopressin is used to decrease the risk of fluid absorption, fluid overload, and intraoperative bleeding [29]. A dilute solution of vasopressin (10 units in 50 mL saline) is injected as 5-mL aliquots into the stroma of the cervix at 12, 3, 6, and 9 o'clock positions. This causes intense arterial and myometrial wall contractions for 20–45 min. Vasopressin is not approved by the FDA for this purpose and should not be used in patients who are hypertensive.

Technique: Rollerball and Endomyometrial Resection

Several varieties and shapes of electrodes are available to perform hysteroscopic endometrial ablation, including ball, barrel, ellipsoid, and large-caliber loops. Most surgeons perform rollerball endometrial desiccation with a 3-mm rollerball probe, with the goal of systematically destroying the entire endometrium to the lower uterine segment and cornual region. The technique of endomyometrial resection is also a popular method for endometrial ablation and is performed with a 90-degree wire-loop electrode.

Following a systematic surgical plan ensures optimal clinical outcomes. Excellent visualization of the entire uterine cavity and endocervix is imperative. All intrauterine landmarks are clearly delineated hysteroscopically before initiating the procedure. Once a panoramic view of the endometrium is accomplished, the surgeon should determine if there is any previously unrecognized pathology. If a subtle lesion is discovered, then a directed biopsy with a wire-loop electrode is performed and the specimen labeled and submitted separately.

Once the surgeon visualizes all of the landmarks, the lower uterine segment is cauterized circumferentially to mark the endpoint and lowest level of endometrial ablation therapy. Ablation of the endocervix is avoided to minimize the risk of cervical stenosis. Cervical stenosis can result in cyclic pain, dysmenorrhea, and, in severe cases, hematometra.

After the lower uterine segment is identified and coagulated circumferentially, the cornua and fundal region are treated initially. With the rollerball, the electrode is advanced to the fundus and then directed at the cornua utilizing a "touch technique" to desiccate the cornua. It must be remembered that the thinnest region of the uterus is at the cornua. Extra care must be taken to avoid forward pressure, which could cause perforation. The most challenging part is the fundus, since the rollerball cannot truly be rolled against the fundus. The fundus should be addressed as the first step. The posterior wall should be resected next, followed by the lateral walls and anterior walls. Traditional technique utilizes direct tissue contact, such that one-half of the rollerball is buried in the endomyometrial juncture. The rollerball should only be activated when the electrode is moving toward the surgeon to avoid perforation. Perforation with the rollerball has the added risk of inflicting burns to the pelvic viscera beyond the uterine wall. Intermittently, the rollerball may need to be cleaned and debris evacuated to provide optimal visualization.

The endomyometrial resection with a wire loop follows the same principles. This loop is generally 3–4 mm deep. The loop should be buried into the endometrium just below the superficial level of the myometrium. The cut is performed using 60–80 W of cutting current. The loop is then advanced under direct view from the fundus to the lower uterine segment. Thus, the endomyometrial junction is shaved off, creating "crescent-shaped" tissue fragments.

At the conclusion of the endometrial ablation procedure, the intrauterine pressure is reduced in order to identify bleeding areas which may be treated with coagulation current.

Outcomes

The majority of patients who undergo endometrial ablation are satisfied with their clinical outcome, and at least 90 % will notice symptomatic improvement. However, 5–10 % of patients may ultimately be required to undergo additional interventions, such as repeat ablation or hysterectomy [30].

Hysteroscopic endometrial ablation is an outpatient procedure associated with a rapid return to work, minimal complications, and high patient satisfaction. Approximately 20–60 % of patients undergoing endometrial ablation with rollerball techniques will achieve amenorrhea, 65–70 % will become hypomenorrhic, and 5–10 % will fail. Approximately 35 % of patients treated by endometrial ablation will require a subsequent operation [30]. Women receiving appropriate preoperative counseling may find this attractive in treating menstrual disorders.

Second-Generation Endometrial Ablation Devices

Second-generation or "global" endometrial ablation refers to destruction of the entire endometrium with devices that require little or no hysteroscopic skills. Currently, FDA-approved second-generation devices available in the United States utilize intrauterine balloons, hot saline irrigation, cryosurgery, bipolar radio frequency, and microwave energy.

Second-generation technology offers the advantage of shorter procedure times while retaining the acceptable outcome rates similar to traditional rollerball ablation. However, these second-generation devices have limited or no ability to treat intracavitary pathology. For this reason, it is important for clinicians who routinely use these ablation devices to be skilled in operative hysteroscopy so that they will be equipped to treat any intracavitary pathology that they may encounter prior to performing the ablation procedures.

Complications of Endometrial Ablation

When energy using heat is used, the most concerning complication is perforation of the uterus with concurrent thermal damage to the surrounding viscera. Perforations of this kind require immediate laparoscopy to determine whether or not thermal injury to the pelvic organs has occurred. Thermal injury to the bowel that is not repaired may result in breakdown of the intestinal wall with spillage of bowel contents into the abdomen. When this occurs, it generally results in massive pelvic infections that may progress to disseminated intravascular coagulopathy. Other complications include skin burns with circulating hot saline, direct coupling vaginal burns from monopolar energy, and unwanted bladder or bowel thermal injuries from cryotherapy.

Hysteroscopic Resection of Adhesions

Most commonly, intrauterine adhesions form in the postpartum or postabortion period. Unfortunately, there is usually no way to avoid this complication during these critical periods, such as when a patient presents with postpartum hemorrhage and requires intrauterine procedures (i.e., D+C) for hemostasis. Early detection of intrauterine synechiae is a key preventative feature following intrauterine surgery, curettage, or spontaneous abortion. This is because early detection allows for identification of adhesions while they are still filmy, thin, and easily resected with prompt adhesiolysis [24, 31–34].

The incidence of Asherman's syndrome in a select group of women, especially after curettage for missed or incomplete abortion, is reported in the range of 17 %, but rates as high as 30 % are reported in the literature, the majority of which are mild in severity [35–38]. Furthermore, in at-risk women, such as those who undergo curettage postpartum, the rate is speculated to be even higher [5, 39].

Pathophysiology

Any intervention that destroys the endometrium may generate adhesions of the myometrium in the opposing uterine walls. The key predictive factor to intrauterine adhesions is the gravid uterus. The gestational changes noted with a gravid uterus soften the uterine wall, resulting in greater denudation of the basalis layer during surgical interventions. The basalis layer is crucial because it is the regenerative layer of the endometrium [40].

Classification of Intrauterine Adhesions

March Classification

Intrauterine adhesions can be characterized based on the extent of uterine involvement [25]. Minimal adhesions are defined as adhesions that involve less than one-fourth of the uterine cavity and are thin and filmy. The fundal and ostia areas are minimally involved or devoid of any adhesions. Moderate adhesions involve one-fourth to three-fourths of the uterine cavity but no agglutination of the uterine wall is seen. The tubal ostia and fundus are only partially occluded. Severe adhesions involve greater than three-fourths of the uterine cavity with agglutination of the uterine walls or thick bands with occlusion of the tubal ostia and upper uterine cavity. The March classification system is simple and easy to apply, but it is not prognostic [25] (Table 16.2).

American Society for Reproductive Medicine Classification

According to the 1988 ASRM (formerly the American Fertility Society) classification system, synechiae are classified in three stages, with stage III being complete obliteration of the uterine cavity (see Table 16.2) [41]. The ASRM classification system provides both an indirect and direct grading of intrauterine adhesions with HSG and hysteroscopy, respectively. The location of the adhesions is presumed to be prognostic for reproductive outcome given that most implantation occurs in the top fundal portion of the uterine cavity and cornual adhesions may cause tubal obstruction.

Table 16.2 Classification system for intrauterine adhesions[a]

Grade	Finding
Minimal	Less than 1/4 of the uterine cavity is involved
	Thin, filmy adhesions
	Fundus and ostia are clear of adhesions
Moderate	1/4–3/4 of the uterine cavity is involved
	No agglutination of uterine walls; only adhesions are present
	Upper uterine cavity and ostial areas are only partially occluded
Severe	More than 3/4 of the uterine cavity is involved
	Agglutination of uterine walls or thick adhesion bands
	Upper uterine cavity and ostial areas are totally occluded

[a]Adapted from [25]

Table 16.3 American Fertility Society intrauterine adhesions classification system

Extent of cavity involved	<1/3 1	1/3–2/3 2	>2/3 4
Type of adhesions	Filmy 1	Filmy and dense 2	Dense 4
Menstrual pattern	Normal 0	Hypomenorrhea 2	Amenorrhea 4
Prognostic classification		HSG[b] score	Hysteroscopy score
Stage I (mild)	1–4	__	__
Stage II (moderate)	5–8	__	__
Stage III (severe)	9–12	__	__

[a]Reproduced from American Fertility Society. The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, Mullerian anomalies, and intrauterine adhesions (Fertil Steril 1988; 49:944–55, with permission from the American Society for Reproductive Medicine)
[b]All adhesions should be considered dense

In addition, unlike the March classification system, the significance of endometrial sclerosis or atrophy is included in the ASRM system by ascertaining the menstrual pattern (Table 16.3).

Clinical Manifestations

The most common presentation of intrauterine adhesions is menstrual disturbance and/or reproductive disturbance (infertility and recurrent pregnancy loss). If conception occurs, it may be complicated by preterm labor or abnormal placentation such as placenta previa or placenta accreta. Menstrual disturbances are most often categorized by amenorrhea or hypo- or oligomenorrhea but can also be seen in eumenorrheic women.

The most common single presentation is infertility, representing 43 % of reported cases. Second most common is amenorrhea, which is seen in 37 % of cases [42]. The rate of abnormal placentation, although elevated in women with intrauterine adhesions, is the least common presentation reported in women with intrauterine adhesions.

Diagnosis

Sonohysterogram

A sonohysterogram is performed with transvaginal sonography (TVS) and can enhance the detection of intrauterine adhesions. Saline serves as a homogenous, echo-free contrast medium enabling better visualization of the uterine cavity than transvaginal ultrasonography alone. Alborzi et al. [43] published the largest series to evaluate the diagnostic accuracy of sonohysterogram compared to laparoscopy and hysteroscopy, which are the gold standards for diagnosis. The prospective study reviewed 86 women with infertility. In this study, sonohysterogram had a high diagnostic accuracy for the detection of Asherman's syndrome. Sonohysterogram accuracy in diagnosis was greater than that for hysterosalpingogram, with a sensitivity of 76.8 %, a specificity of 100 %, a PPV of 100 %, and a NPV of 97.7 %.

Transvaginal ultrasound should be performed in the late follicular or early luteal phase of the cycle, as the endometrium is thick enough to appear more echogenic than the myometrium and not too thick to obscure the midline echo. The classic appearance of the three-layer endometrium enables better imaging of uterine defects than the postmenstrual endometrium, which is thin, <3 mm. The typical appearance of uterine synechiae is focal, hyper-echoic, irregular, cordlike structures seen within the echo-free space between the basalis layers, which interrupt the continuity of the endometrial cavity. These structures can vary in size (2–6 mm) and/or in location within the cavity [44].

Hysteroscopy

Diagnostic hysteroscopy is the gold standard for the diagnosis of intrauterine adhesions, with demonstrated superiority to sonohysterogram and hysterosalpingogram, specifically in false-positive rates. Both radiologic techniques have high false-positive rates. Hysteroscopy has the added advantage of being able to assess intrauterine adhesions and classify them by location, shape, size, and nature.

Surgical Treatment

Hysteroscopic Surgery

Hysteroscopy has become not only an accurate tool for the diagnosis of adhesions but also the main method for their treatment. Hysteroscopic lysis of adhesions is indicated when the extent of adhesions is moderate to severe or access to tubal ostia is blocked. The significance of mild adhesions is still controversial, yet surgical treatment may be considered if all other causes of infertility or recurrent pregnancy loss have been excluded and/or successfully corrected and the patient still experiences persistent reproductive failure.

The basic technique involves resection of the intrauterine adhesions either by sharp and/or blunt dissection. Successful hysteroscopic resection can be accomplished by the use of sharp dissection using semirigid scissors, electrosurgery, and/or fiber-optic laser. Electrocautery is used by some clinicians. However, the disadvantage of its use in this context is possible thermal damage to the endometrium.

Adhesiolysis begins inferiorly and is carried out cephalad until a panoramic view of the endometrial cavity can be obtained and the tubal ostia are seen. The initiation of the adhesiolysis is from the internal os. The maintenance of adequate distention is key to the successful resection of intrauterine adhesions; distention provides traction to the scar tissue so that they may be more effectively resected with hysteroscopic scissors. In cases of severe disease, transabdominal ultrasound guidance with a full bladder is very helpful in preventing the creation of a false passage or uterine perforation.

Postoperative Adjunctive Therapy

Despite advances in the development of techniques for adhesiolysis, the two basic problems associated with poor outcome with these procedures still exist: the inability to treat extensive or severe adhesions and the lack of methods to prevent recurrence of the adhesions postoperatively. The use of Foley catheters, antibiotics, and high-dose estrogen postoperatively to prevent recurrence of adhesions is still widely debated, and no consensus exists [40]. A typical regimen is conjugated estrogen, 0.625–1.25 mg twice daily, or estradiol, 2 mg twice daily for 25 days, followed by 12 days of progesterone (10 mg is prescribed).

Another approach is to place a postoperative intrauterine stent. Either a pediatric catheter inflated with 15–20 cm^3 or a balloon uterine stent specifically designed for this purpose (Cook OB/GYN, Spencer, Indiana) may be inserted for 7–10 days to prevent the juxtaposition of the uterine walls.

A final approach is to perform office hysteroscopy within the first 7–14 days following extensive myomectomy to evaluate the endometrium for synechiae. If detected early, the adhesions are filmy and easily lysed with the distal tip of the hysteroscope. In some circumstances, hysteroscopic visualization every 7–10 days may be required until regeneration of the endometrium is confirmed and filmy adhesions treated. When performed too late, dense fibrous adhesions may be encountered, requiring repeat operative hysteroscopic adhesiolysis.

Complications

Complications after hysteroscopic adhesiolysis include all the standard risks seen with any operative hysteroscopy. The perforation risk is highest during hysteroscopic adhesiolysis [45]. The risk of postoperative infection after hysteroscopy in general is 1.42 %, but the risk of early onset endometritis is highest after lysis of synechiae compared to other hysteroscopic procedures including uterine septa [45].

Outcome

The success of surgery can be assessed by repeat hysteroscopy or imaging or simply by the presence of withdrawal bleeding, suggestive of adequate regeneration of the endometrium. Successful pregnancy outcome is also a parameter or measure of success in women trying to conceive and seems to be correlated with the severity of the intrauterine adhesions.

A number of series have been published reporting the outcome of hysteroscopic treatment of intrauterine adhesions. However, randomized clinical trials are lacking. A report of 40 consecutive women with recurrent pregnancy loss (24 women) or infertility (16 women) resulting from intrauterine adhesions showed excellent surgical results with mild or moderate disease [46]. Of the 40 women, 10 had mild adhesions, 20 had moderate adhesions, and 10 had severe adhesions, according to March classification system. Hysteroscopic adhesiolysis was performed with hysteroscopic scissors or monopolar electro surgery. Prophylactic antibiotics were used, and, postoperatively, a pediatric Foley was placed and estrogen was administered. All women with recurrent pregnancy loss conceived after adhesiolysis; 71 % were term or preterm with a viable pregnancy. Among the women with infertility, 62 % conceived, resulting in a 37.5 % live birth rate. Adhesion re-formation was absent or rare in women with mild or moderate adhesions, reported as 0–10 %. However, adhesion reformation was seen in 60 % of women with severe intrauterine adhesions, and none of the patients with severe adhesions conceived. Only one perforation was reported in a patient with severe adhesions.

Valle and Sciarra [16] reviewed 81 infertile women and reported a term pregnancy rate of 81 %, 66 %, and 15 %, respectively, in women with mild, moderate, and severe disease. Among these women with recurrent pregnancy loss, the term pregnancy rate was 94 %, 89 %, and 65 % in women who had mild, moderate, and severe adhesions, respectively. The literature is unified and quite clear that for women with severe intrauterine adhesions, the reproductive outcome remains poor even after hysteroscopic adhesiolysis [16, 46]. The recurrence rate of severe adhesions was 48.9 % and decreased to 35 % after repeat adhesiolysis [16].

The overall live delivery rate following adhesiolysis in women was 43.5 % during a mean follow-up period of 39.2 month (± 4.5 months). The live delivery rate based on the stage of adhesions was 33.3 %, 44.4 %, and 46.7 % for stages I, II, and III, respectively. The live birth rate among women who tried naturally was 61.9 % vs. 28 % after in vitro fertilization. Similar pregnancy rates were noted in women who conceived naturally whether the resectoscope or the coaxial bipolar system was used. The mean time to conception in these women was 12.2 months, and all pregnancies were achieved within 2 years post-adhesiolysis.

Increase in pregnancy complication rate was noted. Preterm rate was 50 %, and hysterectomy for abnormal placentation (placenta accreta) was seen in 2 of the 20 patients (10 %). In addition, Zikopoulos et al. [48] reviewed the literature of existing studies examining delivery rates in women undergoing hysteroscopic adhesiolysis. A large array of techniques were used in these studies. He identified seven published studies in the last decade. A total of 126 women were reported with an overall delivery rate of 38.1 % (48/126) among all the studies analyzed.

Pabuccu et al. [46] reported the highest success rate among women with recurrent abortion, with a delivery rate of 70.8 % vs. women with infertility with a 37.5 % delivery rate. The overall delivery rate was similar to that reported by Siegler and Valle [50] in 1988. They reviewed a series of studies that encompassed 775 subjects, of which 302 (38.9 %) achieved a term delivery.

The mainstay of diagnosis and treatment of intrauterine adhesions remains hysteroscopy. However, one cannot stress enough the need to exert due diligence and avoid forced or extensive interventions on the post-gravid uterus to minimize the development of intrauterine adhesions. Mild and moderate adhesions are associated with improved reproductive outcome post adhesiolysis, but severe intrauterine adhesions carry a very poor prognosis.

Uterine Septa

Etiology

Uterine septa are created when there is a failure of resorption of the midline septum between the Müllerian ducts. The etiology of a septate uterus remains to be elucidated. Sporadic case reports on family pedigrees suggest familial aggregation exists, but no clear genetic cause has been linked to the development of a septate uterus [28, 51]. In general, 92 % of women with congenital uterine anomalies have a normal karyotype, 46 XX, and approximately 8 % of women have an abnormal karyotype [52]. In rare cases, early in utero exposure to radiation, infection, such as rubella, and teratogens (diethylstilbestrol, thalidomide) has been implicated as the causal factor of the uterine anomaly.

Classification

A number of classification systems have been reported for Müllerian anomalies. However, the classification system proposed by the ASRM in 1988 is most commonly used to describe or define Müllerian defects. The classification system organizes uterine anomalies into six major uterine anatomic types or categories. In this classification system, a septate uterus is a class V anomaly. It is among the vertical fusion defects described by the ASRM classification system. Va is a complete septate uterus, and Vb is a partial septate uterus.

A septate uterus is characterized by a smooth external fundal contour with two uterine cavities. The extent of the septum or the degree of septation can vary from a small midline septum to total failure of resorption, resulting in a complete septate uterus with a longitudinal vaginal septum.

Incidence

The reported incidence of Müllerian or uterine anomalies is between 0.5 and 6 % of reproductive-age women and highest among women with poor reproductive outcome. The overall incidence of Müllerian defects reported in a series by Acien [53] was 5 % among women with normal reproductive history, 3 % among infertile women, and 5–10 % among women with first-trimester recurrent miscarriage and greater than 25 % in women with late first- or early second-trimester loss or preterm delivery. The most frequent to least frequent anomaly is bicornuate uterus, arcuate uterus, incomplete uterine septum, uterus didelphys, complete uterine septum, and a unicornuate uterus [53]. In this combined series of women, bicornuate uteri and complete or partial septums represented 74 % of the uterine anomalies.

In women with recurrent pregnancy loss, the relative frequency of a septate vs. bicornuate uterus is less clear. This is often attributed to old surgical data that often did not definitely differentiate a septate from a bicornuate uterus. In one of the largest studies of patients with recurrent pregnancy loss that were evaluated with either laparoscopy or sonohysterogram, the septate uterus was more prevalent in women with recurrent pregnancy loss than the controls [47]. A septate uterus is the most likely anomaly in patients with recurrent pregnancy loss.

Pathophysiology of Pregnancy Complications

The key presentation in women with a septate uterus is difficulty in maintaining a pregnancy and not a decreased ability to conceive (infertility). Additionally, a septate uterus is thought to impair normal reproductive performance by increasing the risk or incidence of early and late abortion, preterm delivery, and the rate of obstetrical complications [49].

Table 16.4 Diagnostic accuracy of HSG, TVS, and SHG for uterine malformations[a,b]

Examination	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HSG	75.0 (21.9–98.7)	95.1 (85.4–98.7)	50.0 (13.9–86.1)	98.3 (89.7–99.9)
TVS	0.0 (0.0–69.0)	95.2 (85.6–98.7)	0.0 (0.0–69.0)	95.2 (85.6–98.7)
SHG	75.0 (21.9–98.7)	93.4 (83.3–97.9)	42.9 (11.8–79.8)	98.3 (89.5–99.9)

HSG hysterosalpingogram; *SHG* sonohysterography; *TVS* transvaginal sonography; *PPV* positive predictive value; *NPV* negative predictive value
[a]Reprinted from Fertility and Sterility, 73/2, Soares SR, dos Reis MMBB, Camargos AF, Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine diseases, 406–11, Copyright 2000, with permission from Elsevier
[b]The numbers in parentheses are the limits of the 95 % confidence interval

The pathogenesis of pregnancy complications in women with a septate uterus has not been completely elucidated. The most widely accepted causal theory includes the inadequate vascularization of the fibroelastic septum and altered relations between the myometrial and endometrial vessels, thus exerting negative effects on fetal placentation.

The septum is primarily composed of avascular, fibromuscular tissue. Hence, it has been proposed that the endometrium lining the septum responds poorly to estrogen, resulting in irregular differentiation and estrogenic maturation [54]. Implantation on this poorly vascularized, fibrous septum leads to abnormal implantation, defective embryonic development, and subsequent abortion [55–57].

Diagnosis

Uterine imaging techniques used as a method of detection of uterine anomalies include HSG; TVS, with or without three- and four-dimensional technology; saline infusion sonohysterosonography (SHG); and MRI.

Hysterosalpingogram

HSG is a useful screening test and should be the first step in the evaluation of the uterine cavity. HSG is a simple, safe, relatively noninvasive radiological procedure performed under fluoroscopic guidance that enables visualization of the uterine cavity, but it is limited in differentiating between a septate and bicornuate uterus. Consequently, the limitations of HSG require additional evaluation.

Ultrasonography

Two-dimensional ultrasound and SHG can also be used to diagnose women suspected of Müllerian anomalies. The diagnostic accuracy of two-dimensional TVS and SHG compared to HSG is shown in Table 16.4 [58]. However, the presence of a uterine septum is best diagnosed with a three-dimensional ultrasound scan. One study examined 61 patients with a history of recurrent miscarriage or infertility. Subjects underwent a hysterosalpingogram, two-dimensional TVS, and three-dimensional TVS. The study demonstrated that three-dimensional ultrasonography was superior in the detection of arcuate uteri and major congenital anomalies. It facilitated visualization of the uterine cavity and myometrium, allowing easier diagnosis of septate uteri [59].

Magnetic Resonance Imaging

MRI can accurately predict the presence of uterine anomalies and has become the imaging method of choice to confirm inconclusive results from other methods. The clear advantages of an MRI include the ability to distinguish between myometrial and endometrium tissue, image the uterus in several planes, and define uterine contour [48, 60–62]. Because MRI can delineate the uterine contour, a septate uterus can be distinguished from a bicornuate uterus, unlike with other radiographic imaging modalities. Furthermore, the uterine septum can be further characterized by the absence of myometrial tissue and vascularization in the septum. Instead, a uterine septum is seen to have a fibrous consistency throughout its entire length. In a review of 23 cases of Müllerian anomalies, the correct diagnosis was made in 96 % of cases with MRI, compared to 85 % for TVS [63].

Another advantage of MRI is its ability to detect the associated anomalies in other organ systems typically seen with Müllerian anomalies such as renal or the urinary tract anomalies. The major disadvantage includes the lack of portability and higher costs compared with other imaging modalities.

After initial imaging has been performed with either HSG, TVS, or SHG, an MRI may be used to determine the contour of the uterine fundus to distinguish between a septate uterus and bicornuate uterus. There must be a less than 1 cm indentation at the fundus to qualify as a septate uterus. If there is any ambiguity in terms of the diagnosis, the gold standard for the accurate and proper classification for the diagnosis of a uterine anomaly is laparoscopy and hysteroscopy.

Surgical Treatment

Indication

The most common and accepted indication for surgical resection of a uterine septum is recurrent pregnancy loss in either the first or early second trimester. The goal of surgical repair of a uterine septum is restoration of a normal uterine cavity. However, normal surgical restoration of the uterine cavity does not necessarily imply good reproductive prognosis, as uterine vascularization may also be impaired.

Most studies would support the observation that primary infertility in the presence of a septate uterus is not an indication for hysteroscopic metroplasty. This procedure should only be considered after a comprehensive infertility evaluation. If no other etiologies are discovered, and the patient's infertility persists, this procedure may be undertaken. On the other hand, the simplicity and low morbidity of the hysteroscopic metroplasty has led many experts to recommend immediate removal prior to more comprehensive evaluation, especially in women with advanced age, given that uterine septums increase the rate of miscarriage.

Hysteroscopic metroplasty is the method of choice, with benefits that include lower morbidity, faster recovery, and lower risk of infection, hemorrhage, and adhesions than with metroplasty via laparotomy or laparoscopy. Additionally, by avoiding large incisions into the myometrium, hysteroscopic metroplasty does not result in a recommendation for cesarean delivery in future pregnancies.

Hysteroscopic Technique

Operative hysteroscopy is usually performed under general anesthesia using either an operative hysteroscope or resectoscope. The basic technique usually involves simple incision of the septum rather than removal or resection. However some large, broad-based septums are sometimes excised partially.

Micro-scissors are the method of choice for surgical resection of the septum. However, one limitation of the scissors is increased difficulty in dissecting and cutting broad-based septums. In these instances, electrocautery with the wire loop can be used. Generally, electrocautery is avoided secondary to theoretical concern that the use of electrocautery will cause thermal damage on the endometrium and myometrium, with potential risk of uterine rupture with a subsequent pregnancy.

Hysteroscopic metroplasty is performed ideally in the early follicular phase of the menstrual cycle when the endometrium is thin, hence, not requiring any preparation of the endometrium. Classical teaching has described the use of a laparoscope to visualize the uterine fundus while it is transilluminated with the hysteroscope. This allows for assessment of myometrial thickness, which is important to optimally resect the septum while avoiding uterine perforation. However, this is not necessary in most cases. More recent data suggest that a transabdominal intraoperative ultrasound can be used both safely and adequately to prevent perforation.

Resection or horizontal incision of the septum is carried out from the lower margin of the septum and continued cephalad toward the tubal ostia, always staying in the midline and horizontal plane of the septum. Incision into the myometrium should be minimized. One can safely assume that the base of the septum has been reached if increased bleeding is noted.

The surgical technique used for a complete uterine septum includes placement of a plastic uterine dilator, balloon hysterosalpingogram catheter, or Foley balloon through the contralateral cervix to indent the septum wall. It also functions to prevent the loss of distention medium through the second cervical opening. The hysteroscope is then inserted into the opposite cervix, and resection is initiated over the indented septum wall, enabling a safe resection. It is important to identify the point above the cervix at which the resection can be initiated. Once the passage is created, resection is then completed as described above, while sparing the cervical tissue. Limited studies exist, but the recommendation is to spare the cervical portion and preserve the septum below the internal os in order to minimize the risk of cervical incompetence in subsequent pregnancies [4, 64, 65].

The endpoint of resection can be characterized by a number of parameters. Visualization of pink, vascular myometrium, distinct from the white, avascular tissue of the septum, is important. It is also important to examine the relationship of the resection to the tubal ostia and the proximity of the resection to the uterine serosa, which can be assessed via the laparoscope or ultrasound. A successful resection of the septum is defined when the tubal ostia are seen together with no separation in between, an enlargement of the uterine cavity is demonstrated, and an improvement in the uterine shape is accomplished.

Abdominal Metroplasty

Abdominal metroplasty is at times performed if the uterine septum cannot be resected hysteroscopically. This entails either a wedge resection at the fundus that removes the septum, as in the Jones procedures, or opening the uterus in the midline and removing the septum, as in the Tompkins metroplasty technique. Abdominal metroplasty is rarely performed and should only be undertaken by skilled surgeons.

Postoperative Care

Postoperative estrogens or intrauterine contraceptive devices have been used. However, randomized studies in women undergoing hysteroscopic resection of their uterine septums have noted no difference in the postoperative intrauterine adhesion rates, when followed up with HSG or hysteroscopy, despite the use of either agent [66, 67]. Studies have also not shown any added value with use of prophylactic antibiotics.

Complications

The complications associated with the hysteroscopic metroplasty can be viewed in two major categories: those intrinsic to operative hysteroscopy and those related to the technique and instruments used for septum resection.

The major concern with the use of the electrosurgical systems is uterine rupture at the actual site of the septum with a later pregnancy secondary to weakening of the uterine wall from thermal damage. However, vaginal delivery is still recommended unless extensive damage has occurred through thermal injury or a fundal perforation has occurred. The rate of uterine perforation is lower with resection of a uterine septum than it is for intrauterine adhesiolysis. It is reported to be less than 1 %.

Outcome

Many studies exist that report both presurgical and postsurgical outcomes in women with hysteroscopic metroplasty. However, to date there are no published randomized clinical trials that compare pregnancy outcomes in treated vs. untreated groups of symptomatic women. Hence, surgical outcomes after treatment of septate uteruses are based on retrospective studies evaluating the reproductive outcome of women, often using patients as their own control. The overall reported rate of successful pregnancy after hysteroscopic metroplasty is 85–90 % [50, 68–70].

Hickok et al. wrote a small retrospective series of 40 women with uterine septums. Preoperatively, they observed a miscarriage rate of 77.4 %, delivery rate of 22.6 %, and uncomplicated delivery rate of 6.5 %. After hysteroscopic metroplasty, the miscarriage rate was seen to be 18.2 %, delivery rate was 81.8 %, and uncomplicated delivery rate was 77.3 % [71]. Kupesic reviewed the reproductive outcome from 13 studies in women with untreated septate uterus and reported on 1,304 pregnancies. They observed a miscarriage rate of 81.9 % and a preterm delivery rate of 9.6 % [72]. But the authors caution that the group of women reviewed may represent a biased group of women; women with a septate uterus and normal reproductive outcome may have been excluded. Kupesic also reported a review of the existing literature with regard to the reproductive outcome before and after hysteroscopic metroplasty for the septate uterus. In 388 patients, 1,059 pregnancies were achieved before metroplasty and 362 pregnancies after surgery. The miscarriage rate and preterm and term delivery rates before and after were 87.8 %, 9.0 %, and 3.2 % vs. 14.6 %, 5.2 %, and 80.1 %, respectively [72]. These and other studies demonstrated an improvement in fertility after metroplasty. The chance of pregnancy was not affected by maternal age, number of previous pregnancy losses, and method of septal resection (micro-scissors, resectoscope, or laser) nor the type of septum present, partial or complete [58, 73].

However, other studies demonstrate that there is no improvement in outcome in women with recurrent pregnancy loss after metroplasty. Kirk et al., in a series of 146 women, showed no increase in the number of living children after metroplasty [74]. However, there was also no negative effect of hysteroscopic metroplasty on fertility potential in women with recurrent pregnancy loss.

The possible adverse effect of the presence of a septate uterus on the outcome of assisted reproductive technology is still debated. The existing studies do not demonstrate any impairment on ovarian response to stimulation nor implantation rates in the presence of Müllerian anomalies to include a septate uterus. However, the studies do report a higher rate of abortion and preterm delivery if the septum is uncorrected [72, 75]. Although the hysteroscopic metroplasty is not intended to enhance fertility, it may be indicated for the improvement of their pregnancy outcome, especially after multiple treatment failed assisted reproductive cycles.

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17 Gynecologic Laparoscopy

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Tommaso Falcone, and William W. Hurd

Introduction

During the last 3 decades, the discipline of gynecologic surgery has seen significant advancement in the use of minimally invasive surgery. The laparoscopic technique itself was revolutionized by the continuous fine tuning of the traditional instruments and the addition of new ones. The last decade has witnessed the introduction of robotic assistance and the laparoendoscopic single-site surgery (LESS) as new enhancements to the field. In addition, laparoscopy has become one of the most common surgical procedures performed in the United States and worldwide. It became the gold standard for many gynecologic procedures, such as removal of ectopic pregnancies and the treatment of endometriosis. For other procedures, including laparoscopically assisted hysterectomy and treatment of gynecologic cancers, the relative risks and benefits of the laparoscopic approach are still being determined.

This chapter will give an overview of the history and modern use of laparoscopy. Laparoscopic complications and specific laparoscopic techniques are considered in subsequent chapters.

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History

Hippocrates described the first example of an endoscope, an early rectal speculum, in Greece between 460 BC and 375 BC. The ruins of Pompeii, Italy (70 AD), provided the next example, a three-bladed vaginal speculum, similar to a modern-day speculum. Next, Philipp Bozzini in Germany (1773–1809) developed a light conductor that he called "Lichtleiter," which directed light into the patient's body and then reflected the image back to the eye of the surgeon. John D. Fisher (1798–1850) described an endoscope to inspect the vagina, and he later modified it to examine the bladder and urethra. In 1853, Antoine Jean Desormeaux pioneered the first functional endoscope, which was mainly used for urologic cases. This instrument had mirrors and a lens with a lamp flame as the light source, which burned a mixture of alcohol and turpentine.

The first experimental laparoscopy ("celioscopy") was performed in Berlin in 1901, by Dr. Georg Kelling, who placed a cystoscope into the abdomen of dogs to evaluate the ability of insufflated air to stop gastrointestinal hemorrhage [1]. Dr. Hans Christian Jacobaeus of Sweden published the first description of "laparothoracoscopy" in 1910 as a technique to evaluate patients with peritoneal tuberculosis. However, laparoscopy made little headway into clinical practice until after World War I. It took until the 1960s for laparoscopy to be accepted in the United States and Europe as a safe and valuable surgical procedure.

For many years, gynecologic laparoscopy was performed almost exclusively for diagnostic purposes and for sterilizations. By the 1970s, the role of laparoscopy had expanded to include lysis of adhesions and treatment of endometriosis [2]. The technology and equipment advanced over the next 4 decades such that laparoscopy is now used for a wide variety of procedures ranging from treatment of ectopic pregnancies and ovarian cysts to hysterectomy, incontinence procedures, and management of gynecological malignancies.

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General Techniques for Laparoscopy

Primary Trocar Placement

For many years, the standard techniques used for creating a pneumoperitoneum and placing a laparoscopic port into the abdomen were either a closed technique or an open approach. In the last decades, multiple alternative approaches and locations have been reported. The four most common approaches are as follows:

- Standard closed technique (Veress needle insufflation followed by primary trocar insertion)
- Direct trocar insertion (no insufflation prior to trocar insertion)
- Open laparoscopy
- Left upper quadrant (LUQ) insertion technique

Both reusable and disposable instruments are commonly used. The ultimate safety of many of the newer techniques and instruments has yet to be determined.

Standard Closed Technique: Veress Needle and Primary Trocar Insertion

The standard closed technique was used almost exclusively for decades and continues to be widely used today. Both the Veress needle and primary trocar are blindly placed through a periumbilical incision into the peritoneal cavity. Using this approach with reusable instruments, the combined risk of injuring retroperitoneal vessels, bladder, or bowel has been found to be less than 1 in 1,000 cases [3]. This approach has become the "gold standard" against which all other techniques are judged.

For the standard technique, the patient is placed in a horizontal position, and the abdominal wall is elevated by manually grasping the skin and subcutaneous tissue. This is done to maximize the distance between the umbilicus and the retroperitoneal vessels. An alternative method used to elevation the abdominal wall is to place penetrating towel clips at the base of the umbilicus.

In a woman of ideal weight (body mass index [BMI] <25 kg/m^2) or only slightly overweight (BMI 25–30 kg/m^2), the lower anterior abdominal wall is grasped and elevated, and the Veress needle is inserted toward the hollow of the sacrum at a 45° angle (Fig. 17.1) [4]. In the thinnest patients in this group, the retroperitoneal vessels are much closer to the abdominal wall and the margin for error is reduced, with as little as 4 cm between the skin and these vessels. In the obese patient (BMI >30 kg/m^2; weight usually >200 lb) a more vertical approach, approximately 70–80°, is required to enter the peritoneal cavity because of the increased thickness of the abdominal wall. It is important to avoid subcutaneous tunneling of the Veress needle and/or the trocars prior to puncturing the fascia of the anterior abdominal wall.

Verification that the Veress needle tip is in the peritoneal cavity is done by a number of methods, including the "hanging drop test," injection and aspiration of fluid through the Veress needle, and close observation of intra-abdominal pressure during carbon dioxide insufflation. After a pneumoperitoneum has been created, the Veress needle is removed and the primary port trocar (most commonly 5 or 10 mm in diameter) is placed at an angle identical to that used for the Veress needle.

Direct Trocar Insertion

Direct trocar insertion is a technique whereby the primary trocar is inserted without having previously inserted the Veress needle and insufflating the abdomen with carbon dioxide [5]. This could be achieved blindly or via the optical-trocar-assisted

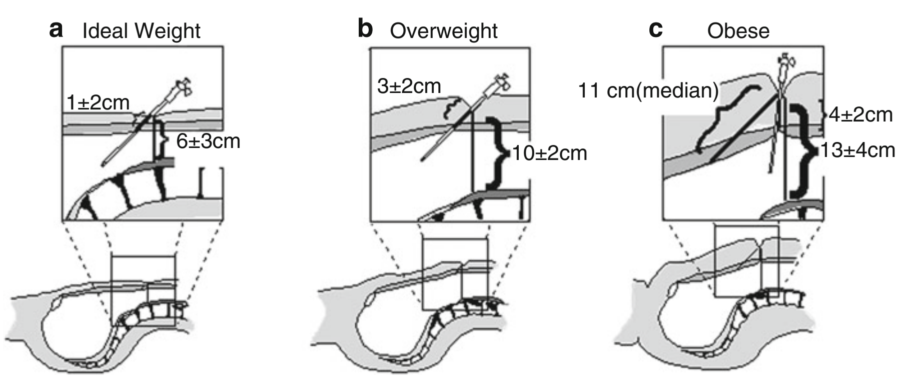

Fig. 17.1 Changes in the anterior abdominal wall anatomy with weight. Diagram of representative sagittal views derived from magnetic resonance and computed tomographic imaging for patients in three groups: (**a**) Ideal weight (body mass index [BMI] <25 kg/m^2). (**b**) Overweight (BMI 25–30 kg/m^2). (**c**) Obese (BMI >30 kg/m^2). An 11.5-cm Veress needle is superimposed on each view for comparison (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

technique. The direct primary trocar is inserted at an angle similar to that described above for the closed technique. The peritoneal cavity is then insufflated with carbon dioxide through the umbilical port.

The optical trocar insertion allows visualization of the layers that are being penetrated during entry via a laparoscope in the cannula. It is assumed that this approach could reduce the risk of injury since the technique is no longer blind. However, vascular and visceral injuries are reported with this approach. On the other side, seeing the injury as it happens will allow prompt recognition and repair, nullifying the consequences of delayed diagnosis and management.

This technique decreases the risk of extraperitoneal insufflation by allowing the surgeon to confirm intraperitoneal placement of the primary trocar before insufflation. Although small randomized studies have not demonstrated an increased risk of injuries, some series suggest that this technique might increase the risk of bowel injury [5, 6]. Further large studies are required.

Open Laparoscopy

Open laparoscopy, first described by Dr. Harrith Hasson in 1971, refers to creating a small incision in the abdomen and placing the port through the incision without using a sharp trocar [6, 7]. The skin and anterior rectus fascia are incised with a scalpel, and the peritoneal cavity is bluntly entered with a Kelly or Crile forceps. A laparoscopic port with a blunt-tipped trocar is then placed into the peritoneal cavity. For the "Hasson" technique, fascial sutures are used to assist subsequent closure and help maintain a pneumoperitoneum [6]. This method almost eliminates the risk of retroperitoneal vessel injury and is preferred by many laparoscopists for this reason. Although open laparoscopy does not entirely avoid the risk of bowel injury, many laparoscopists use this approach in an effort to decrease this risk in patients with previous abdominal surgery suspected of having adhesions.

Left Upper Quadrant Technique

This approach was developed for use in patients with previous abdominal surgery with suspected or known periumbilical bowel adhesions, during pregnancy, and with large pelvic masses. It is performed by using a LUQ site to place both the Veress needle and primary laparoscopy port into the abdomen. This point, sometimes referred to as Palmer's point, is in the mid-clavicular line beneath the lower rib margin (Fig. 17.2).

It is important to know the anatomy of the LUQ before using this technique. The most important organs that are closest to this site are the stomach and left lobe of the liver [8]. Although a small series has shown the risk of complications to be small, the relative risk of complications with this technique remains to be demonstrated by a large study [9].

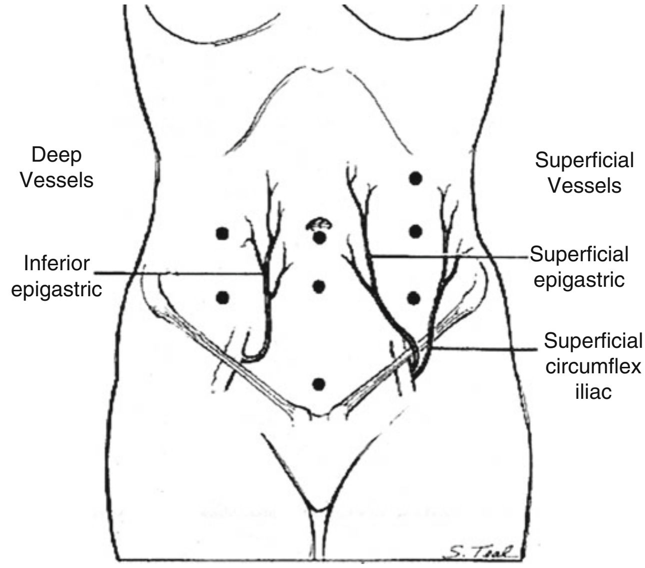

Fig. 17.2 Ideal port sites in relation to the deep and superficial vessels of the anterior abdominal wall (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Placement of Secondary Ports

Secondary ports are required to perform most gynecologic laparoscopy procedures today. After identifying the epigastric vessels by transillumination and visualizing them intra-abdominally through the laparoscope, 1–4 secondary ports are placed, depending on the procedure [10]. A midline port is often placed 3–4 cm above the pubic symphysis. Lateral ports are placed approximately 8 cm from the midline and 5 cm above the pubic symphysis to avoid the inferior epigastric vessels (see Fig. 17.2) [11]. This lateral site corresponds to McBurney's point in the right lower quadrant and is approximately one-third the distance from the anterior iliac crest to the pubic symphysis (Fig. 17.3). Additional lateral ports for the principal surgeon are required for most operative laparoscopy cases. The site chosen is typically at the level of the umbilicus lateral to the rectus muscle. This site offers the surgeon a comfortable use of both hands and allows access to most areas of the pelvic or abdominal cavity.

Secondary ports are placed with sharp trocars under direct laparoscopic visualization to avoid injuring intraperitoneal structures. These trocars should be placed directly into the peritoneal cavity without tunneling. After removal, the intra-abdominal gas pressure is reduced to observe for signs of hemorrhage indicative of abdominal wall vessel injury. If the port diameter is ≥10 mm, the fascia and peritoneum should be closed with a full-thickness suture to reduce the risk of subsequent herniation.

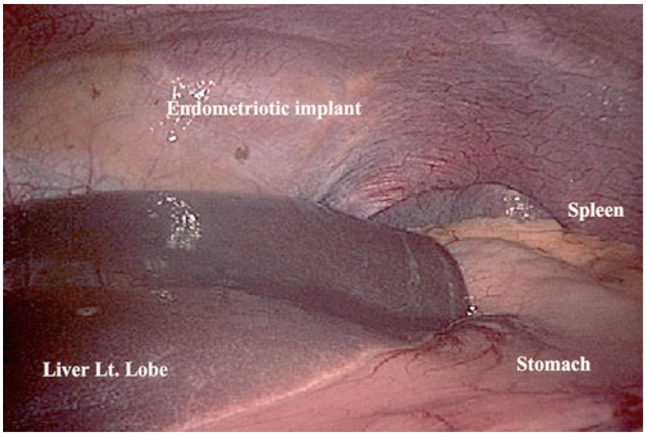

Fig. 17.3 Anatomy of the left upper abdomen (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Removal of Ports and Port-Site Closure

At the conclusion of the procedure, port removal should be performed in a way to minimize patient risk. Secondary ports should be removed under direct visualization to detect any bleeding that might have been masked by the port or the intra-abdominal pressure. All carbon dioxide used for pneumoperitoneum should be allowed to escape prior to removal of the umbilical port to minimize postoperative shoulder pain and avoid pushing bowel into the incision sites as residual gas escapes.

Multifunctional Laparoscopic Instruments

Traditionally, power instruments were used during laparoscopy because suture ligation, the most common hemostatic method used during laparotomy, is difficult to perform laparoscopically. **Electrocoagulation** was perhaps the first power instrument used during laparoscopy. This instrument is heated by passing electrical current through the tip of a grasping instrument, which is then used to coagulate tissue.

In the last 4 decades, other methodologies have been developed, most notably electrosurgery. **Unipolar electrosurgery** passes current through the patient to cut or coagulate tissue. **Bipolar electrosurgery** was developed in an effort to minimize the risk of inadvertent injury to adjacent tissue, particularly the bowel. Bipolar electrosurgery offers an increased margin of safety because the electrical current is confined to the tip of the instrument, but the cutting ability is reduced. **Lasers** offer a precise, rapid, and accurate method of thermally destroying the tissue; however, hemostatic effects are less and lasers are costly. The **ultrasonic scalpel** is an ultrasonically activated instrument that moves longitudinally at a rate of 55,000 vibrations per second and is able to cut tissue and coagulate small vessels without heat or electrical energy. Tips available for this instrument include grasper/scissors, a hook blade, and a ball tip.

Over the past decade, significant improvements in the design and functionality of these instruments were achieved. The most important refinement was the additional cutting following coagulation. This technology uses the combination of pressure and energy to create the seal by melting the collagen and elastin in the vessel walls and reforming it into a permanent seal. Subsequently, the tissue is then divided using an internal blade. The technology reduces thermal spread to 2 mm. Controlled coagulation and cutting are achieved by a wide variety of commercially available instruments including LigaSure, LigaSure Advance, Gyrus, Harmonic Scalpel, and EnSeal.

Laparoscopic Procedures

Diagnostic Laparoscopy

Laparoscopy has been used effectively as a valuable diagnostic tool for a wide variety of abdominal and pelvic pathologies. It has been used for the assessment of acute or chronic pain, suspected ectopic pregnancy, endometriosis, adnexal torsion, or other extragenital pelvic pathologies. In most cases, the laparoscope is placed through an infraumbilical port, and a probe is placed through a second suprapubic port to manipulate the pelvic organs, if only a diagnostic laparoscopy is performed. However, for operative laparoscopy other than the simplest procedures, the suprapubic port is not useful and is quite uncomfortable. If operative laparoscopy is performed, the accessory trocars should be placed in the right and left lower quadrants. For advanced laparoscopy, an accessory trocar at the level of the umbilicus lateral to the rectus muscle will allow the principal surgeon to operate comfortably and have access to the pelvis. If tubal patency is a concern, a dilute dye can be injected transcervically, a procedure termed chromopertubation.

Before initiating any surgery, the peritoneal cavity should be thoroughly inspected using a systematic approach. With the surgeon controlling the movement of the laparoscope, each quadrant of the abdomen and then the pelvis should be carefully inspected. Care should be taken to inspect the appendix, omentum, peritoneal surfaces, stomach, surface of the bowel, diaphragms, and liver (Figs. 17.4 and 17.5) [12]. The spleen is usually difficult to see except in thin women (see Fig. 17.3). If any suspicious lesions are observed, fluid should be obtained for cytology (pelvic washings) prior to biopsying the lesion for frozen section.

Laparoscopic pelvic assessment is often performed in a non-standardized fashion depending on the surgeon's

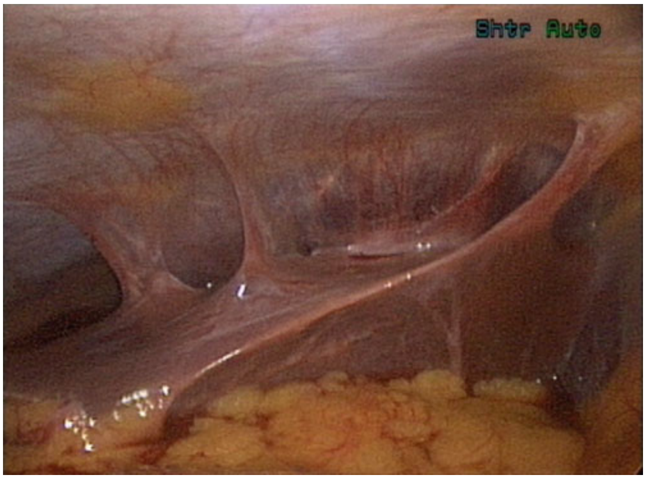

Fig. 17.4 Sub-diaphragmatic adhesions of Fitz-Hugh-Curtis syndrome. These two physicians, Dr. Curtis in 1930 and Dr. Fitz-Hugh in 1934, described the relationship with gonococcal infection (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

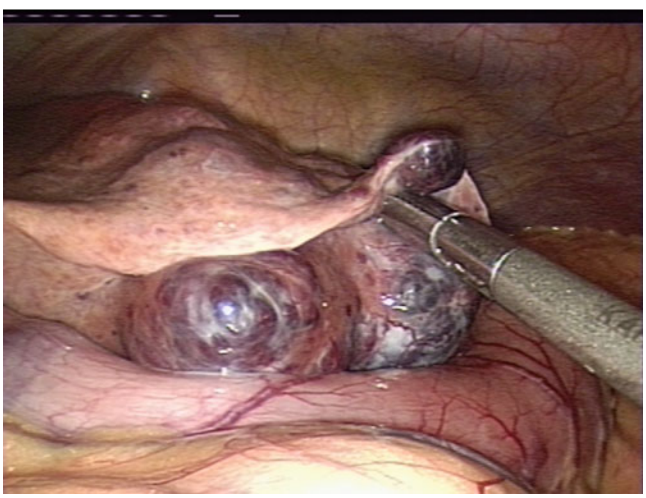

Fig. 17.5 Liver hemangioma (reproduced with permission from Hurd WW, Duke J, Falcone T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

discretion. Reporting positive or negative findings is random and lesions in atypical locations such as anterior and posterior cul-de-sac, deep inguinal rings, and ovarian fossa may be missed, and patient care would be less than optimal. We proposed a method for systematic pelvic assessment based on anatomical landmarks [13].

In this system, the pelvis was topographically divided into two midline zones (zones I and II) and two paired (right and left) lateral zones (zones III and IV). Zone I is the area between the two round ligaments from their origin at the uterine cornua to their insertion in the deep inguinal rings. Zone II is the area between the two uterosacral ligaments from their origin from the back of the uterus to their insertions in the sacrum posteriorly. Zone III is the area between the uterosacral ligament inferiorly and the entire length of the fallopian tube and the infundibulopelvic ligament superiorly. Zone III contains the tubes and the ovaries. Zone IV is the triangular area lateral to the fallopian tube and the infundibulopelvic ligament and medial to the external iliac vessels up to the round ligament. This system was validated in a retrospective study and prospective evaluation is ongoing [13].

Tubal Sterilization

Tubal sterilization is one of the most commonly used methods of birth control. Laparoscopy is one of the most common techniques used for permanent sterilization in the world. Original laparoscopic techniques used electrocautery or electrosurgery to coagulate the midportion of the tubes. Other techniques, including clips and silastic bands, have gained popularity. The pregnancy rates vary by age of the patient, ranging from 1 to 3 % after 10 years [14].

Lysis of Adhesion and Tubal Reconstructive Surgery

Adhesions are frequently encountered pelvic pathology. They are usually the result of previous pelvic infections secondary to PID or a ruptured appendix, endometriosis, or previous surgery. These adhesions may contribute to infertility or chronic pelvic pain. Lysis of adhesions is performed bluntly or by sharp dissection using scissors or a power source. Extreme caution should be used if adhesions <1 cm from ureter or bowel are lysed using unipolar electrosurgery because of the unpredictable nature of current arcing. The other power techniques, such as the ultrasonic scalpel, may be a better choice for adhesiolysis near bowel for surgeons that do not have experience with unipolar cautery.

Tubal reconstructive surgery is still performed even in the era of in vitro fertilization (IVF) and is almost exclusively performed laparoscopically. Fertility-enhancing procedures include adhesiolysis, fimbrioplasty, and terminal neosalpingostomy. Prior to and during these procedures, **chromopertubation** is carried out to document proximal tubal patency by injecting dilute indigo carmine dye through the cervix using a cannula. Laparoscopic surgery is performed using the principles of microsurgery to avoid tissue damage, including delicate handling of tissues and minimal use of electrosurgery for hemostasis.

Patients with mild tubal disease and preservation of fimbria have excellent pregnancy rates after laparoscopic surgery. Although these patients remain at risk for subsequent ectopic pregnancy, the risk of multiple gestations associated with IVF is avoided for patients who subsequently achieve a viable intrauterine pregnancy.

Unfortunately, adhesions often reform after lysis. Multiple techniques have been used in an effort to decrease reformation. Gentle tissue handling and good hemostasis also appear to be important. Barrier methods have been shown in clinical trials to decrease adhesions but have yet to be proven to improve pain relief or future fertility.

Fulguration of Endometriosis

Laparoscopy is the primary surgical approach used to treat endometriosis. Endometriosis lesions may be resected or ablated, using scissors or any of the power instruments. These treatment approaches have been shown in randomized controlled trials to improve fertility and decrease pelvic pain.

Ectopic Pregnancy Treatment

Laparoscopy has become the surgical approach of choice for most ectopic pregnancies [15]. The embryo and gestational sac are removed either through a longitudinal incision (linear salpingostomy) or by removing the tube (salpingectomy). Even a ruptured tubal pregnancy can be treated laparoscopically, as long as the patient is hemodynamically stable.

Ovarian Cystectomy and Oophorectomy

Ovarian pathological conditions, including cysts, commonly result in gynecologic complaint such as pain. The underlying pathology ranges from physiologic and self-limiting functional cysts to ovarian torsion and other benign conditions, to ovarian malignancy. Ovarian cysts are usually characterized ultrasonographically and treated when necessary by laparoscopy or laparotomy, depending upon the size of the cyst and the level of suspicion for malignancy [16]. The most important concept in adnexal surgery is to avoid spilling the cyst content whenever possible.

Myomectomy

Many women with symptomatic fibroid uterus prefer a myomectomy over hysterectomy to preserve fertility or the uterus [17]. In some cases, myomectomy can be performed laparoscopically. The challenges in the case of intramural myomas are related to hemostasis, effective closure of the resulting myometrial defect, and removal of the specimen from the abdomen. Vasopressin can be injected into the uterus to help maintain hemostasis. The excised fibroid can be removed by morcellation or colpotomy. Power morcellators are available to expedite the process. Barrier techniques may be used to decrease subsequent adhesion formation. Some early case series have reported increased risk of subsequent uterine rupture during pregnancy after laparoscopic myomectomy compared to those performed by laparotomy [18]. However several randomized clinical trials have shown no increased risk in expert hands [18]. A totally laparoscopic approach should be attempted only by gynecologists skilled in laparoscopic suturing.

Laparoscopic Management of Pelvic Pain

Many women have severe dysmenorrhea that is unresolved despite medical management but wish to maintain future childbearing potential. In these patients, two laparoscopic approaches have been attempted with some success. Laparoscopic uterosacral nerve ablation (LUNA) is performed by stretching and dividing each uterosacral ligament using electrosurgery or laser alone or in combination with scissors. Care must be taken to avoid injuring the ureters. This procedure has been shown to have some temporary success, but a recent Cochrane review has questioned the validity of this procedure [19].

Laparoscopic presacral neurectomy (LPSN) is a second approach for central pain. This technically challenging procedure is performed by careful retroperitoneal dissection between the common iliac artery on the right and the inferior mesenteric artery where it crosses over both left common iliac artery and vein on the left. The superior hypogastric plexus, which includes the presacral nerves, is dissected from the left common iliac vein and periosteum of sacral promontory and a 2–3-cm segment is resected. Surgical risks include vascular complications, and long-term risks, such as constipation, are more common than with LUNA. Although both LUNA and LPSN appear to give some patients at least temporary relief from central pain, many clinicians believe that there is insufficient evidence to recommend the use of nerve interruption in the management of dysmenorrhea, regardless of the cause [19].

Hysterectomy

Laparoscopy hysterectomy, first described by Dr. Harry Reich in 1992, is commonly performed today [20]. The three basic laparoscopic approaches for hysterectomy are laparoscopic-assisted vaginal hysterectomy (LAVH), laparoscopic hysterectomy, and laparoscopic supracervical hysterectomy (LSH). Although the basic techniques for each of these approaches are fairly standardized, controversy exists over the risks, benefits, and most appropriate indication of each.

Laparoscopic-Assisted Vaginal Hysterectomy

LAVH is the most commonly employed and technically straightforward of the three. Using 3–4 ports, the peritoneal cavity is surveyed and lysis of adhesions is performed if necessary. Then the infundibulopelvic or utero-ovarian ligaments are occluded and divided, depending on whether the ovaries will be removed. The round ligament is divided, the utero-vesicle peritoneum is incised, and the bladder dissected from the anterior uterus. This step results in an increased risk of bladder injury compared to either abdominal or vaginal hysterectomy. At this point, the uterine arteries laparoscopically are sometimes occluded and divided, although this is associated with an increased risk of ureter injury compared to either abdominal or vaginal hysterectomy. Finally, the posterior cul-de-sac is incised.

The surgeon proceeds vaginally for the remainder of the case, dissecting the vesicovaginal septum anteriorly to enter the anterior cul-de-sac, ligating the uterine vessels if not previously done, removing the uterus and ovaries if appropriate, and closing the vaginal cuff.

Laparoscopic Hysterectomy

Laparoscopic hysterectomy (LH), the common second approach, is performed initially like the LAVH, except that the entire hysterectomy is performed laparoscopically. This approach is often used when there is little or no uterine descent, which makes the vaginal approach unfeasible.

After the infundibulopelvic (or utero-ovarian) and round ligaments are occluded and divided, the bladder is dissected away from the anterior uterus. The ureters are identified, and the uterine vessels and uterosacral ligaments are occluded and divided. The posterior cul-de-sac is incised, the vagina is circumferentially separated from the cervix, and the specimen is removed vaginally. The cuff is closed laparoscopically or vaginally.

Supracervical Hysterectomy

The LSH is a third common laparoscopic approach to hysterectomy for benign indications [21]. The technique begins in a manner identical to LAVH and LH. However, prior to reaching the level of the uterine arteries, the fundus is transected at the uterocervical junction. In order to minimize residual cyclic vaginal bleeding and decrease the risk of developing cervical dysplasia or cancer, the glandular tissue endocervix is cored out or cauterized. The uterine specimen is removed through a 12-mm port abdominal using a power morcellator.

This approach eliminates both the vaginal and abdominal incision, thus decreasing the risk of infection. The risk of ureteral injury is also decreased, since the procedure stops above the level of the uterine artery. However, a risk of subsequently developing cervical dysplasia or cancer remains due to the presence of the cervical stump. For this reason, routine Pap smears are required, and some patient will require additional surgery related to cervical abnormalities. Furthermore, at least two randomized clinical trials have failed to show superior results in bladder function or sexual function [22, 23]. These studies did show a higher reoperation rate for bleeding and prolapse.

Although small trials have tried to assess the value of laparoscopic hysterectomy, a large multicenter, randomized trial that compared laparoscopic with abdominal hysterectomy and laparoscopic with vaginal hysterectomy has provided insight into the role of this procedure [24]. The study confirmed that the laparoscopic approach offers no advantage over the vaginal approach. It also confirmed that the laparoscopic approach is associated with less postoperative pain, shorter hospital stay, and faster convalescence compared with the abdominal approach. It demonstrated that the laparoscopic approach was associated with a slightly higher risk of urinary tract injury. The shorter length of hospitalization with laparoscopic hysterectomy offsets some of the additional costs incurred by longer operating room times and the expense of disposable instruments [25].

Oncologic Procedures

Laparoscopy originally was used in gynecologic oncology for second-look procedures after surgical and chemical treatment of the malignancy. More recently, laparoscopy has been used for the initial staging of gynecologic cancer, including hysterectomy, peritoneal washes with biopsy, partial omentectomy, and pelvic and periaortic lymphadenectomy. Techniques have also been developed for laparoscopically assisted radical vaginal hysterectomy.

The laparoscopic approach to gynecologic cancer remains controversial. There is some concern that laparoscopy might increase the risk of intraperitoneal spread of ovarian cancer. Until the risk, benefits, and the effect on long-term prognosis have been shown to be equal to laparotomy, the laparoscopic approach will remain under close scrutiny.

Robotically Assisted Laparoscopic Surgery

Robotic technology has attempted to address the limitations of conventional laparoscopic surgery. The use of a remotely controlled robot has the potential to facilitate these procedures by allowing the surgeon to be seated comfortably while providing the surgeon a three-dimensional view with improved dexterity and access.

The most commonly used robotic system is the “da Vinci system” (Intuitive Surgical, Mountain View, CA, USA). The FDA approved it for use in abdominal surgeries in 2000. There are three main components: the surgeon console, the surgical cart, and the vision cart. The surgeon sits at a console separate from the surgical field. Movement of handles at the console results in movement of surgical instruments at the operative field. In this system, the surgeon looks into a console that has a dual lens system within the 12-mm laparoscope. The system provides true binocular 3D vision that is similar to looking into a microscope that enables the surgeon to see fine structures up to a tenfold magnification. Movement of the laparoscope is accomplished through the movement of the handles at the console.

The most impressive part of the system is the intra-abdominal articulation of the microinstruments 2 cm from the tip. This articulation serves the same function as a human wrist, mimicking the movements of a hand. This articulating wrist has 7 degrees of freedom of the instruments, providing an opportunity for better suturing, dissection, and reconstructing tissue by allowing the surgeon access to deep pelvic structures. The movement of the instrument tip is intuitive and requires minimal training.

The cart contains the instrument arms and camera arm. The vision cart allows all members of the surgical team to visualize the procedure. Not only does this system provide visual advantages for more precise surgery, improved dexterity, surgeon comfort with less hand fatigue, and improved instrument articulation but also it eliminates unintentional hand tremors.

There are some limitations with the use of robotic technology. One is the initial system cost, maintenance costs, and expense of disposable instruments. Another is the lack of tactile feedback during the procedure, requiring the use of visual cues to properly carry out surgical tasks. For appropriate docking of the robot, it is imperative that a dedicated staff specifically trained on the device is available during all procedures.

Another limitation of the robotic system is its bulky size. Increased surgical operation time is a main limitation of the robotic system. This is attributed to the time required for robot preparation and docking as well as console time Sait reported an operative time of 92.4 min for a laparoscopic hysterectomy, compared to 119.4 min for a hysterectomy with robotic assistance. However, they also showed a significant learning curve, shortening the length of operation times with increasing robotic experience [26].

Cost is an important limitation that should be considered. Robotic surgical systems are very costly, adding approximately $3,500 per procedure and approximately $2.5 billion nationally per year. This is a huge expense considering little evidence of improved outcomes over standard laparoscopy. Added to these costs, Medicare and most US private insurers do not pay additional fees for use of robotics. To overcome this, hospitals most likely will increase charges for procedures or diagnoses for which robots are used [27–29]. The reality is that robotics overall is more costly than laparoscopy, but if it allows more surgeons to perform MIS, then maybe in the end it will end up being less costly. The advantages and the disadvantages of the robotic systems are summarized in Table 17.1.

Table 17.1 Advantages and disadvantages of the da Vinci robotic system

Advantages	Disadvantages
3D visualization	Initial system cost
Improved ergonomics	No tactile feedback
Improved dexterity	Lack of research on efficiency
7 Degrees of freedom	Insufficient cases to train residents
Elimination of fulcrum effect Motion scaling	Large size of systems
Improved suture capabilities and knot tying	

Table 17.2 Current uses of robotics in reproductive surgery, gynecologic oncology, and reconstructive pelvic surgery

Reproductive surgery
Simple hysterectomy
Myomectomy
USO, BSO
Tubal reanastomosis
Resection of endometriosis
Ovariopexy
Gynecologic oncology
Radical hysterectomy
Pelvic and para-aortic lymphadenectomy
Appendectomy
LAVH
USO, BSO
Sentinel lymph node biopsy
Omentectomy
LARVH
Ovarian cystectomy
Radical parametrectomy
Radical vaginal trachelectomy
Radical cystectomy
Reconstructive pelvic surgery
Bladder repair
Hysterectomy
Vesicovaginal fistula repair
Sacrocolpopexy

Robotic Gynecologic Surgery

Robotic systems have the potential to convert surgical procedures that we presently perform by laparotomy to laparoscopy and are currently utilized in the fields of reproductive endocrinology and fertility, gynecologic oncology, and female pelvic medicine/reconstructive surgery (Table 17.2). It has been used in robotically assisted tubal anastomosis.

Robotically Assisted Tubal Reanastomosis

For a variety of reasons, sterilization reversal is an alternative to IVF, particularly for patients younger than the age of 35. The immediate and the long-term postoperative outcomes were compared with laparoscopic tubal anastomosis without robotic assistance [30]. The operative times were longer with the use of the robot. The tubal patency rates and clinical pregnancy rates were not significantly different. The major difficulty with laparoscopic tubal anastomosis, with or without robotic assistance, is the limited needle angles to the tubes due to operating through fixed ports. It has been reported that robotic technology is successful in facilitating laparoscopic tubal anastomosis using the da Vinci system. All of the tubal anastomoses were performed with the use of three or four robotic arms, three or four robotic instruments, and one assistant trocar. While the use of robotics prolonged surgical and anesthesia times as well as increased cost, there was no significant difference in pregnancy outcomes compared to a laparotomy technique. Additionally, patients were able to return to normal activities faster than after a laparotomy [31, 32].

Robotically Assisted Myomectomy

Myomectomy remains the best choice of treatment of symptomatic fibroids in patients desiring to preserve their fertility, even with the new modalities such as uterine artery embolization [18, 33] Open myomectomy used to be the treatment modality until the emergence on minimally invasive technique. Laparoscopy yielded better cosmesis and shorter postoperative pain and hospital stay. However, this procedure was very challenging. A limitation included needing to precisely dissect the fibroid without unnecessary breaching of the endometrial cavity. Since laparoscopic suturing is a difficult skill to master, it is complicated to suture the fibroid beds in layers with precise approximation of edges, which is needed to prevent uterine rupture during labor. These challenges limited the enthusiasm and acceptance of this technique.

Many studies demonstrated the feasibility of robotically assisted myomectomy [18, 34]. Most recently, the operative and immediate postoperative surgical outcomes of robotically assisted laparoscopic myomectomy, standard laparoscopic myomectomy, and open myomectomy were compared. Blood loss, operative time, and hospital stay were lower for the robot-assisted group. These results showed an association of robotic-assisted myomectomy with decreased blood loss and length of hospital stay compared with traditional laparoscopy and to open myomectomy [35].

Robotically Assisted Resection of Endometriosis

Nezhat et al. compared robotic treatment of stage I or II endometriosis to conventional laparoscopy in a retrospective cohort controlled study in 2010. Forty patients were treated for endometriosis by robot-assisted laparoscopy, and 38 patients were treated by standard laparoscopy. There were no significant differences between these groups in blood loss, hospitalization, or complications, but the mean operative time with the robot was 191 min (135–295) compared with 159 min (85–320) during standard laparoscopy. Since both treatments have excellent outcomes and the robotic technique required a longer operative time, it was concluded that the robot has no added value for the treatment of early stage endometriosis [36].

Most recently, we reported on the safety and feasibility of robotic surgical treatment of advanced pelvic endometriosis. Fifty women underwent a robotic procedure for advanced endometriosis. Twenty-one (42 %) had stage III and 29 (58 %) had stage IV endometriosis. The median total operative time was 209 (range: 97–368) min, including patient positioning, robot docking, performing surgery, and closure of the port sites. Median actual operative time was 154 (range: 67–325) min, and both total OR time and actual operative time were comparable between the two groups. There was no difference between the two groups regarding estimated blood loss and uterine weight. Pathological evaluation confirmed the endometriosis diagnosis in all patients [37].

Clinical Applications in Gynecologic Oncology

The traditional approach to gynecologic oncology surgeries involves a total hysterectomy, bilateral salpingo-oophorectomy, and dissection of both pelvic and para-aortic lymph nodes. These surgeries and others are now being conducted with the use of robotic surgical systems in select patients.

The specific advantages of robotic-assisted endoscopic procedures in gynecologic oncology arise from the da Vinci's enlarged operative field without the need for large fascial incisions. This allows for more easily identifiable pelvic anatomy while patients experience decreased postoperative morbidity and faster recovery to permit rapid initiation of adjuvant radiotherapy or chemotherapy. The safety profile of the da Vinci utilized for gynecologic oncology applications appears reassuring, with less blood loss and a low complication rate in managing ovarian, endometrial, and cervical cancers, respectively [38–40].

Clinical Applications in Female Pelvic Medicine and Reconstructive Surgery

In the literature, robotics have been utilized in the repair of both vesicovaginal fistulas and in the treatment of post-hysterectomy vaginal vault prolapse with sacrocolpopexy [41]. It has been shown that the involvement of obstetrics and gynecology and urology residents has no effect on the surgical outcome of robotic-assisted sacrocolpopexy (RASCP) [42]. The question remains that although the use of robotics combines the outcomes of an open procedure, the benefits of minimally invasive surgery and easy adoptability, does it outweigh the increased cost and time [43]?

Single-Port Laparoscopy

The concept of natural orifice surgery has been recently revisited. Advancements in surgical instruments, optics, and ports have allowed the development of single-port laparoscopy or LESS. LESS can be used for salpingostomy or salpingectomy to treat tubal ectopic pregnancy [44].

Recent studies indicate that the procedure has low rate of complications and similar surgical outcomes compared to conventional laparoscopy. LESS has also been found to be associated with a reduction of gas leakage. The use of LESS has the advantages of reduced postoperative pain, earlier return to daily activities, reduced incidence of port-site hernias and hemorrhage, and improved cosmesis and patient satisfaction. However, data on long-term effectiveness are lacking [45].

LESS is now being used to treat benign and malignant adnexal disease and for hysterectomy. For adnexal disease, LESS can be used to remove ovarian cysts, for salpingo-oophorectomy, to remove endometriosis and to remove malignant masses. Single-port access total hysterectomy is more commonly used now, with various advancements in place to overcome the limited free movement and technical difficulty. Combining LESS with the da Vinci robot system allows further benefits, including better cosmesis, reduced morbidity from injury during trocar placement, a reduced incidence of postoperative wound infections and hernia formation, and improved dexterity [45].

Laparoscopic Complications

Overall, laparoscopy has a relatively favorable complication profile compared to the same procedure performed via laparotomy. In addition to the procedure-related complications, laparoscopy is associated with uncommon but significant complications related to trocar insertion. These injuries involve primarily blood vessels, bowel, and bladder. Given its mostly blind nature, insertion of the Veress needle and primary trocar for initial entry by trocar insertion remains the most hazardous part of laparoscopy, accounting for 40 % of all laparoscopic complications and the majority of the fatalities. Despite decades of research and development to find safer methods for initial laparoscopic entry, major vessel injuries have been reported using virtually all types of trocar insertion methods [46]. The following is a brief discussion of avoidance and manage of these complications.

Retroperitoneal Vessel Injury

Techniques used to place primary and secondary laparoscopic ports into the peritoneal cavity are often accompanied by a small but unavoidable risk of injury to blood vessels located in the anterior abdominal wall and the major blood vessels located in the retroperitoneal space. Injury of major abdominal blood vessels is a rare but treatable life-threatening complication of laparoscopy, which occurs in approximately 3 per 10,000 laparoscopies [47]. These injuries most commonly occur during insertion of the Veress needle or primary trocar.

Prevention

The majority of retroperitoneal vessel injuries during laparoscopy occur during blind placement of the Veress needle or primary trocar through a periumbilical incision [48]. To minimize this risk, surgeons need to be aware of anatomic considerations so that they can determine the most appropriate direction and angle of insertion for each patient, as discussed above. The different approaches for primary prevention of vessel injuries are discussed in the following sections.

Awareness of the Patient's Position

For greatest safety, the surgeon should make sure they are aware of the patient's position in relation to horizontal prior to laparoscopic instrument placement. Most laparoscopic surgery is performed in the Trendelenburg position to keep bowel away from the operative field in the pelvis. If the patient is placed in Trendelenburg position with the feet elevated 30° relative to the head prior to instrument insertion, instruments inserted at 45° from horizontal will actually be placed at 75° from the horizontal plane of the patient's spine, which is likely to increase the risk of major vessel injury, particularly in slender patients [49].

High-Pressure Entry

Another technique used in conjunction with closed laparoscopy in an effort to decrease the risk of major vessel injury is "high-pressure entry." Rather than inserting the primary umbilical trocar after obtaining intra-abdominal pressure of 18–20 mmHg, many surgeons increase the pressure to 25–30 mmHg. The rationale is to make the anterior abdominal wall stiffer such that the downward pressure exerted by trocar insertion does not decrease the distance of the umbilicus to the retroperitoneal vessels [50]. Although no controlled studies large enough to demonstrate an advantage have been published, large series including more than 8,000 cases suggest that the risk of major vessel injury using this technique is approximately 1 in 10,000 cases (0.01 %), compared to a risk of 4 in 10,000 cases (0.04 %) reported using standard pressures [51].

Verify Veress Needle Location

Use of the Veress needle used to insufflate the peritoneal cavity is associated with a small risk of intravascular insufflation and venous gas embolism, reported to occur in approximately 1 in 100,000 laparoscopic procedures [52].

Several methods have been used to demonstrate the intraperitoneal location of the Veress needle tip. First, the Veress needle should be placed with the valve open, so that entering a high-pressure arterial blood vessel will immediately result in extrusion of blood through the needle. Second, after needle placement, a syringe should be used to aspirate the Veress needle, to verify that a low-pressure venous blood vessel has not been entered. This is often followed by the "hanging drop test," wherein a drop of saline is placed at the open end of the Veress needle hub. When the abdominal wall is elevated, the drop often disappears into the shaft if the tip is located in the relatively low-pressure peritoneal cavity but will usually not disappear if the tip is preperitoneal or embedded in some other structure.

The "Waggle test" is another maneuver used by some to verify that the needle has not entered the retroperitoneal space. After the needle is placed in the proper position, the hub is moved from side to side using gentle lateral pressure. Lack of lateral mobility suggests that the tip is anchored in the immovable retroperitoneal space, and the needle should be slowly withdrawn until lateral movement is possible. This technique is difficult to interpret in obese patients because the abdominal wall itself can limit lateral movement of the Veress needle, even if it is placed through the base of the umbilicus at the proper angle.

It is recommended that at least one of these methods be used when placing a Veress needle into the abdomen [53]. However, none of these methods absolutely verify intraperitoneal placement of the needle tip. Once insufflation is begun, the strongest predictor of intraperitoneal placement appears to be an initial filling pressure of <10 mmHg.

Other Laparoscopic Entry Methods

Multiple insertion methods and instruments have been developed in an effort to decrease the risk of trocar complications. Although each method has theoretical advantages compared to the traditional closed techniques, none has completely eliminated the risk of major vessel injury.

Open Laparoscopy

Open laparoscopy is a widely used alternative technique for placement of the primary laparoscopic port. The Hasson technique is fundamentally a minilaparotomy incision followed by placement of the primary port directly into the peritoneal cavity [54]. Open laparoscopy almost completely prevents the risk of major vessel injury, decreasing the rate to 0.01 %, compared to a rate of 0.04 % associated with closed techniques using a Veress needle [51].

Direct Trocar Insertion

Direct trocar insertion is a laparoscopic entry technique wherein the primary trocar is placed without prior insufflation, with or without elevation of the anterior abdominal wall manually or with towel clips. This approach is slightly faster than standard closed laparoscopy and avoids the risks of Veress needle placement. Unfortunately, this technique might increase this risk of major vessel injury. Large series (>10,000 cases) report a major vessel injury risk of 0.06–0.09 % compared to 0.04 % using a standard closed technique [53]. This risk of major vessel injury might be one reason why direct trocar insertion is one of the least frequently used techniques by gynecologists.

Left Upper Quadrant

LUQ insertion of the Veress needle and primary trocar through a site in the LUQ is recommended by some surgeons to decrease the risk of complications associated with bowel adhesions in women with prior abdominal surgeries. The LUQ insertion site (Palmer's point) is located 3 cm below the middle of the left costal margin, and instruments are routinely inserted perpendicular to the patients' skin.

Major vessel injuries have not been reported using this technique. Anatomic studies indicate that the abdominal wall is uniformly thin in this location and the distance from the skin to the retroperitoneal structures is >11 cm in most patients [13]. However, because this distance can be <7 cm in many slender patients, it is recommended that, in slender patients, instruments placed through Palmer's point be directed 45° caudally relative to the patient's spine [55].

Alternative Primary Trocar Design

Alternative primary trocars have been developed, including shielded disposable trocars, optical trocars, and radially expanding trocars [53]. Unfortunately, their use does not prevent major blood vessel injuries. Currently, there is no evidence of benefit of one technique or instrument over another in terms of preventing major vascular injury.

Treatment

Major vessel injuries are a rare but unavoidable laparoscopic complication associated with the closed entry techniques. Every laparoscopic surgeon that uses a closed technique should develop a plan of action for major vessel injury. The surgeon should also become familiar with the availability of laparotomy instruments, blood products, vascular clamps, and surgical consultants. This is especially important when these procedures are performed in a free-standing outpatient surgical facility.

When a major vascular injury is suspected, the following steps should be taken without delay. The nursing personnel should prepare for emergency laparotomy, and anesthesia personnel should consider placing additional intravenous lines and calling for blood products and additional assistance. The surgeon should immediately perform a laparotomy via a midline incision, and blood loss should be minimized using direct pressure over the injury site. When the injury occurs in a medical center, a trauma surgeon or vascular surgeon should be called in to identify and repair the vascular injuries.

The treatment approach is different when a major vessel injury occurs in a facility where vascular surgery personnel and equipment are not available. In these instances, a laparoscopic surgeon without experience in vascular surgery should not attempt to open the retroperitoneal area to repair the vessel [53]. This approach can further injure the vessels, and resultant lack of circulation to the lower extremities can have catastrophic results. Rather, the abdomen should be packed tightly with dry laparotomy pads, and the abdomen quickly closed with either running full-thickness sutures or towel clips [56]. The patient should then be transported by the most expedient method to the nearest fully equipped trauma center.

Abdominal Wall Vessels Injury

Anterior abdominal wall vessels at risk for injury can be divided into two groups: superficial and deep [11]. The superficial vessels consist of the superficial epigastric and circumflex iliac arteries, which are located in the subcutaneous tissue. The deep vessel at risk is the deep inferior epigastric artery, which is located beneath the rectus abdominus muscles immediately above the peritoneum.

Damage to the superficial vessels is often asymptomatic at the time of surgery, whereas damage to a deep vessel usually leads to immediate and rapid blood loss. If unrecognized, damage to either type of vessels can result in postoperative hemorrhage or hematoma.

Prevention

The primary method for avoiding injury to any of these vessels is to visualize the vessels via transillumination and direct laparoscopic visualization prior to lateral trocar insertion. Transillumination of the anterior abdominal wall with the laparoscopic light source is an effective way to visualize the superficial vessels in almost 90 % of patients [10]. The inferior epigastric vessels cannot be seen by transillumination since they lie beneath the rectus abdominus muscle and fascia but can be directly visualized laparoscopically immediately beneath the peritoneum in the majority of patients where they lie between the insertion of the round ligament at the inguinal canal and the medial umbilical fold. Since both the deep and superficial vessels are located an average 5.5 cm from the midline, risk of vessel injury can be minimized by placing secondary trocars 8 cm lateral to the midline and 8 cm above the pubic symphysis [11].

Treatment

When a superficial vessel is found to be bleeding after the port is removed, the most effective approach is to grasp the vessel with a Crile "hemostat" forceps, followed by cautery or ligation. In cases where the injured vessel cannot be grasped, a pressure dressing is often sufficient.

When an inferior epigastric vessel is injured, the result is immediate and brisk bleeding from the port site into the peritoneal cavity. Anesthesiology personnel should be alerted because additional intravenous lines and blood products might be required if the patient becomes hemodynamically unstable. If another port is available, an attempt should be made to occlude the injured vessel with a laparoscopic bipolar electrosurgery instrument above and below the injury. If another port has not yet been placed or electrosurgery is not effective, the bleeding can be temporarily slowed by placing a Foley catheter through the port site into the peritoneal cavity. After the bulb is inflated with saline, the catheter is retracted to hold the bulb tightly against the peritoneal surface and a Kelly forceps used to cross-clamp the catheter on the skin side to maintain traction.

If bipolar electrosurgery is unsuccessful, precisely positioned sutures can be placed above and below the injury using port-site closure instruments. These sutures should be tied deep to the skin above the fascia.

If hemostasis cannot otherwise be achieved, the incision should be widened and the injured vessels individually ligated. The port-site incision should be enlarged transversely to at least 4–6 cm, the fascia of the anterior rectus sheath incised, and the lateral edge of the rectus abdominus muscle retracted medially. The bleeding vessels can be grasped with hemostatic forceps and selectively ligated above and below the injury.

Delayed bleeding can occur when the abdominal pressure decreases after removal of the carbon dioxide, especially if the method used to occlude an injured vessel becomes loose as the patient awakes from anesthesia and is moved [57]. Signs of hemodynamic instability in the recovery room necessitate a return to surgery because uncontrolled bleeding from a lacerated inferior epigastric artery can be life-threatening.

Gastrointestinal Injury

Despite the continued development of both laparoscopic instruments and techniques, gastrointestinal injury continues to be a common, yet potentially avoidable complication of laparoscopy. In the last 4 decades, the risk of this complication appears to have increased from approximately 3 per 10,000 procedures to as high as 13 per 10,000 procedures [47, 58]. Most bowel injuries occur during placement of the Veress needle or primary trocar and usually when bowel is adherent to the anterior abdominal wall from previous surgery [59]. Other gastrointestinal injuries result from operative procedures including adhesiolysis, tissue dissection, devascularization injury, and thermal injury.

It is essential to minimize morbidity related to gastrointestinal injuries both by prevention and early recognition. Despite an increasing awareness of these risks, gastrointestinal injuries continue to be the most lethal type of injuries associated with laparoscopy, with a mortality rate reported as high as 3.6 % [58].

Preventive Measures

No method has yet to be discovered that completely prevents gastrointestinal injuries during laparoscopic port placement [60]. However, it is well established that patients with previous abdominal surgery are at increased risk of gastrointestinal injury during laparoscopy since adhesions to the anterior abdominal wall occur in approximately 25 % of these patients. For this reason, certain measures have been used in an effort to decrease the risk of gastrointestinal injuries in these patients.

Two commonly used techniques for high-risk patients are open laparoscopy, as first described by Hasson, and a LUQ closed technique utilizing Palmer's point [60, 61]. Unfortunately, neither of these techniques has been shown in prospective comparison studies to decrease the risk of intestinal injury relative to the open technique [60, 62, 63].

Another alternative approach is the use of an optical-access trocar. These devices are designed to increase safety by visualizing each layer of the abdominal wall during port placement. Unfortunately, these devices have been shown to decrease the risk of gastrointestinal injuries [64].

Recognition and Treatment

Veress Needle Injuries

The spring-loaded tip of the 14-gauge Veress needle does not prevent perforation of adherent bowel or bowel with limited excursion related to physiologic attachments, such as the transverse colon [65]. Most bowel perforations caused by the Veress needle do not need to be repaired as long as the puncture is not actively bleeding or associate with a tear [66]. Even in the case of colonic puncture, nonoperative management with copious irrigation appears to be sufficient [67].

Stomach Injuries

Injury to the stomach during laparoscopy is relatively uncommon and was reported to occur in less than 3 in 10,000 cases in the earlier days of laparoscopy [66]. Risk factors include a history of upper abdominal surgery and difficult induction of anesthesia, as a gas distended stomach can be below the level of the umbilicus. Routine decompression of the stomach with a nasogastric tube prior to Veress needle or trocar placement has virtually eliminated this risk, even when a LUQ approach is used.

Trocar injury to the stomach requires surgical repair, either via laparotomy or laparoscopy [68]. The defect should be repaired in layers with a delayed absorbable suture by a surgeon experienced in gastric surgery. The abdominal cavity should be irrigated, being careful to remove all food particles as well as gastric juices. Nasogastric suction is maintained postoperatively until normal bowel peristalsis resumes.

Small Intestine Injuries

Intraoperative injuries to the small intestine often go unrecognized during surgery. Injury should be suspected whenever multiple anterior abdominal wall adhesions are present. When the primary trocar and sleeve penetrate completely through both walls of bowel adherent near the umbilicus, the injury will not be visible. Whenever the routine 360° survey of the abdominal cavity reveals bowel adherent near the point of insertion, a 5-mm laparoscope should be placed through a lower quadrant port to view the umbilical port site and search for injury. An injury to nonadherent bowel with the Veress needle or a trocar during initial port placement or during lysis of adhesions may fall out of view into the abdomen. If such an injury is suspected, the bowel should be run with laparoscopic bowel graspers or manually using a laparotomy incision until an injury is satisfactorily excluded.

Postoperatively, unrecognized trocar injuries to the small intestine usually presents with symptoms of nausea, vomiting, anorexia, abdominal pain, peritoneal signs, and possibly fever on the second to fourth postoperative day. Although the bacterial load of the small intestine is low, the contents are not sterile, and sepsis is a common result of undiagnosed injuries.

A full-thickness injury to the small intestine of 5 mm or greater should be repaired in two layers, sewing perpendicular to the long axis of the intestine to avoid stricture formation. This can be accomplished with an initial interrupted layer of 3-0 delayed absorbable suture to approximate the mucosa and muscularis. A serosal layer of 3-0 delayed absorbable suture is commonly placed in an interrupted fashion. This is usually performed by laparotomy or by minilaparotomy at the umbilical site, where the injured bowel loop is pulled through to the skin surface and repaired. Laparoscopic repair has also been reported by surgeons with advanced gastrointestinal surgical skills [69]. If the laceration to the small bowel exceeds one-half of the diameter of the bowel lumen, segmental resection is recommended.

Large Intestine Injuries

Trocar injuries to the large intestines are reported to occur with frequency of approximately 1 per 1,000 cases [70]. Due to the high concentration of coliform bacteria in the large intestine, unrecognized injuries can result in serious intra-abdominal infections that can quickly become life-threatening.

Whenever a large intestine injury is suspected, the area should be carefully inspected using atraumatic bowel graspers. If adhesions or anatomy make laparoscopic inspection difficult, laparotomy is reasonable. An occult injury to the rectosigmoid colon may be detected using the “flat tire test,” in which the posterior cul-de-sac is filled with normal saline and air is injected into the rectum using a proctosigmoidoscope or a catheter-tipped bulb syringe [71]. Visible bubbles indicate a large intestine injury.

The management of large intestine injuries depends upon size, site, and time between injury and diagnosis. In general, once the diagnosis of colonic injury is made, broad-spectrum antibiotics should be administered and consultation should be sought with a surgeon experienced with these types of injury. In the case of a small tear with minimal spillage of bowel contents, the defect is closed in two layers with copious irrigation. When a larger injury has occurred or the injury involves the mesentery, a diverting colostomy is sometimes necessary. In the case of delayed (postoperative) diagnosis, tissue inflammation usually makes a diverting colostomy necessary.

Port-Site Hernia

For the first 2 decades of laparoscopy, ports were placed almost exclusively in the midline, where the anterior and posterior rectus fascia fuses. These midline ports usually consisted of a 10-mm port at the umbilicus and a 5-mm suprapubic port. Port-site hernias at these locations are rare, and those reported are usually limited omental herniation through the umbilical site.

The use of lateral ports for more complex operative laparoscopy has resulted in a dramatic increase in the risk of port-site herniation. In one retrospective review, port-site hernias occurred in 5 of 3,500 (0.17 %) procedures, with all hernias occurring where ports with diameters ≥10 mm were placed lateral to the midline [72]. Since the rectus fascia splits laterally to form both anterior and posterior sheaths below the arcuate line, bowel herniation can occur between these two fascial layers in what has been called a “Spigelian hernia.”

Prevention

To minimize the risk of port-site herniation, both the anterior and posterior fascial sheaths should be closed after removal of all ports 8 mm and larger. This closure is usually performed with the aid of one of a number of commercially available devices or needles that incorporate the peritoneum as well as both fascial layers. Unfortunately, port-site herniation is not completely prevented by careful fascial closure [73].

Recognition and Treatment

Trocar-site hernias usually present as a palpable mass beneath a lateral trocar-site skin incision that manifests during a Valsalva maneuver. Ultrasonography can distinguish herniated bowel from a hematoma. A persistent mass associate with pain indicates an incarcerated hernia and represents a surgical emergency.

Herniated bowel can often be reduced laparoscopically, followed by careful inspection of the affected segments. Although simple repair of the peritoneal and fascial defects is all that is required in most healthy patients, in some cases synthetic mesh may be needed.

Bladder Injuries

Injury to the bladder related to laparoscopic port placement is relatively uncommon and usually related to insertion of the primary trocar in the presence of a distended bladder or insertion of a suprapubic midline trocar in a patient whose bladder dome had extended cephalad secondary to previous surgery [74].

Prevention

The risk of trocar injuries to the bladder can be decreased by draining the bladder with a catheter prior to primary trocar placement. In patients with prior lower abdominal surgery, it seems prudent to place the suprapubic trocar above any previous transverse skin incisions. In all patients, an attempt should be made to visualize the superior bladder margin laparoscopically prior to suprapubic trocar placement [10]. In cases where the superior margin of the bladder cannot be seen, the bladder can be filled with 300 mL to better define its margin. An alternative approach is to use a lateral port site rather than a midline suprapubic site, although the decreased risk of bladder injury may be offset by an increased risk of vessel injury.

Recognition

Laparoscopic bladder injuries are often difficult to recognize intraoperatively. Visible leakage of urine at the time of injury is unlikely in patients with a Foley catheter in place. A common sign of bladder injury is significant bleeding from a suprapubic port site placed in the relatively avascular midline. Frank hematuria suggests a full-thickness injury. An uncommon, but pathognomonic, sign of bladder injury during laparoscopy is insufflation of the Foley catheter bag with carbon dioxide [75].

If bladder injury is suspected during laparoscopy, an indigo carmine solution can be instilled retrograde through a urethral catheter to detect small leaks. Cystoscopy or, less commonly, intentional cystotomy may be used to inspect the bladder mucosa in questionable cases, or to determine the extent of a known injury and to insure that there is no ureteral involvement.

Postoperative recognition of a bladder injury can likewise be difficult. Whenever a patient returns within days of laparoscopy with significant abdominal findings, the possibility of an occult bladder injury should be considered [74]. Bladder injury should be included in the differential diagnosis in the presence of painful urination and microscopic hematuria. Elevation of blood urea nitrogen (BUN) and a serum creatinine suggests intra-abdominal spill of urine with transperitoneal reabsorption. Drainage from a suprapubic incision can be evaluated further by instillation of a dilute indigo carmine solution into the bladder.

Treatment

When a bladder injury is diagnosed in the postoperative period, a retrograde cystogram should be performed to determine the extent of the injury. If surgery is indicated because of peritoneal signs of uncertain etiology, cystoscopy prior to laparotomy may be extremely helpful in determining surgical approach.

Small, uncomplicated, and isolated injuries of superior portion of the bladder can be treated with catheter drainage alone [76]. A retrograde cystogram should be performed after 10 days of continuous drainage and will document spontaneous healing in 85 % of patients with small injuries. Primary surgical repair is required for larger injuries and those that involve the dependent portions of the bladder, including the trigone, especially if there is a risk of concomitant injury to the urethra or ureter. Closure should be performed using a watertight, multilayered repair with absorbable suture. Laparoscopic repair may be performed by those with adequate surgical expertise as long as there is adequate exposure and the ureters and bladder neck are not compromised [77].

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Uterine Leiomyomas

18

Shahryar K. Kavoussi, Layne Kumetz, and Gregory M. Christman

Introduction

Leiomyomas are benign monoclonal tumors of smooth muscle cell origin, the vast majority of which are found within the uterine corpus, although they can occur throughout the body in any structure containing smooth muscle. Leiomyomas are uncommonly malignant but are responsible for a large number of surgical interventions, including hysterectomy. Although symptoms usually correlate with size, large tumors can be asymptomatic and small tumors can be symptomatic. This chapter will review the pathophysiology and management of leiomyomas.

Prevalence

Uterine leiomyomas (also known as fibroids or myomas) are the most common benign pelvic tumors found in women. As many as 50 % of reproductive-age women have clinically apparent uterine leiomyomas and 25 % of women have symptomatic leiomyomas. On pathological examination, 77 % of hysterectomy specimens contain one or more uterine leiomyomas [1].

Although benign, uterine leiomyomas are associated with significant morbidity, including abnormal uterine bleeding, chronic pelvic pain, impaired fertility, and recurrent pregnancy loss. In the United States, leiomyomas are cited as the primary indication for hysterectomy in over 250,000 cases per year and account for over $5 billion in health-care costs annually [2].

Classification

Uterine leiomyomas are typically classified into subgroups by their position relative to the layers of the uterus. **Subserosal myomas** occur near the serosal surface of the uterus. They may have either a broad or pedunculated base and can extend between the folds of the broad ligament. **Intramural myomas** originate within the myometrium and may enlarge enough to distort the uterine cavity or the serosal surface. **Submucosal myomas** develop just below the endometrium and, with progression, protrude into the uterine cavity. Subserosal and submucosal myomas can also be either **pedunculated** or **broad-based**. **Cervical myomas**, on the other hand, are derived from cells in the cervix as opposed to the uterine corpus (Fig. 18.1).

Leiomyomas can occur as a solitary mass but are most often present as multiple masses within a uterus. They vary greatly in size from microscopic to multi-lobulated tumors that can weigh as much as 50 lb and give the patient the appearance of a term gravid uterus. On physical exam, the size of a uterus containing leiomyomas ("a fibroid uterus") is frequently described in terms of weeks comparable to a gravid uterus.

Clinical Impact

Although at least 50 % of uterine leiomyomas are asymptomatic, many women have significant symptoms that impact their quality of life and warrant treatment. The major clinical manifestations of uterine leiomyoma can be roughly classified into three categories: increased uterine bleeding, pelvic pressure or pain, and reproductive dysfunction.

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Fig. 18.1 Hysterectomy specimen demonstrating the presence of intramural, submucosal, and subserosal leiomyomas

Abnormal Uterine Bleeding

Abnormal uterine bleeding is the most common symptom reported by women with leiomyomas. The most common types of leiomyomas associated with heavy bleeding are intramural or submucosal myomas. The typical bleeding patterns are menorrhagia or hypermenorrhea. Intermenstrual bleeding can indicate the presence of an intracavitary myoma or specific endometrial pathology; therefore, a more detailed evaluation of the uterine cavity is warranted in these cases. Heavy vaginal bleeding can lead to problems such as iron-deficiency anemia, which can be severe enough to require blood transfusions, and frequent changes of sanitary protection can cause significant distress in work or social situations [3].

Chronic Pelvic Pain

Pelvic pain or pressure is the second most common complaint and is frequently described as analogous to the discomfort associated with uterine growth during pregnancy. The pain can occur both during and between bleeding episodes. Posterior leiomyomas may give rise to low back pain, whereas anterior leiomyomas may compress the bladder. Leiomyomas that become large enough to fill the pelvis may potentially interfere with voiding and defecation or cause dyspareunia. Very large leiomyomas can, on occasion, outgrow their blood supply, leading to tissue ischemia and necrosis clinically manifested as acute, severe pelvic pain. Pedunculated leiomyomas can suffer torsion, which can lead to ischemia and acute pain. During pregnancy, leiomyomas have been known to undergo "red degeneration," where hemorrhage occurs within the myoma, leading to acute pain [3].

Reproductive Function

Uterine leiomyomas are believed to influence reproduction in several ways; however, their direct effect on fertility is still a subject of much debate. The incidence of infertility and uterine leiomyomas increase with advancing maternal age, and no specific data exist to ascertain if the proportion of infertile women with leiomyomas is greater than the proportion of fertile women with leiomyomas.

Yet the indirect evidence is substantial. In one review, pregnancy rates among women with leiomyomas distorting and not distorting the uterine cavity were 9 % and 35 %, respectively, as compared to 40 % among controls with no leiomyomas [4]. Furthermore, the multiple reports of successful pregnancies among infertile women after myomectomy strongly suggest a connection [5–7].

Though exact physiologic mechanisms for reproductive dysfunction are unclear, many plausible theories exist. There is a potential for reduced fecundity if a myoma occurs in the cornual region of the uterus due to mechanical occlusion of a fallopian tube [3]. It is possible that large leiomyomas may impair the rhythmic uterine contractions that facilitate sperm motility [8]. It has further been documented that endometrial histology varies in relation to the location of the leiomyoma. Submucosal leiomyomas may be associated with localized endometrial atrophy as well as alterations in the vascular blood flow, which may impede the implantation of an embryo, the delivery of hormones or growth factors involved in implantation, or interfere with the normal immune response to pregnancy [9–11]. Submucosal leiomyomas, which distort the uterine cavity, are associated with first-trimester pregnancy loss, preterm delivery, abnormal presentation in labor, and postpartum hemorrhage [12].

In regard to the effectiveness of assisted reproductive technology, leiomyomas are generally thought to reduce the effectiveness of assisted reproductive procedures. Early evidence demonstrated that both pregnancy and implantation rates were significantly lower in patients with intramural or submucosal leiomyomas [13, 14]. In one study, the presence of an intramural leiomyoma decreased the chances of an ongoing pregnancy by 50 % following in vitro fertilization [15]. Evidence suggests that patients with subserosal leiomyomas have assisted reproductive technology outcomes consistent with patients without leiomyomas [14, 16, 17].

Epidemiology of Uterine Leiomyomas

The diagnosis of uterine leiomyomas increases with age throughout the reproductive years, with the highest prevalence occurring in the fifth decade of a woman's life. African-American women have a two- to threefold greater relative risk of leiomyomas compared to Caucasian women and tend to be diagnosed at an earlier age and have more severe disease (larger leiomyomas and greater incidence of anemia) as compared to Caucasian women [18, 19].

Nulliparous women have higher rates of leiomyomas than multiparous women, and the risk of developing leiomyomas decreases consistently with each subsequent term birth [20]. Early age at menarche is associated with a two- to threefold increased risk of developing leiomyomas [21].

Leiomyomas clearly demonstrate their hormonal responsiveness in the fact that they form after puberty, have the potential to enlarge during pregnancy, and regress after menopause. However, studies of exogenous hormone treatments, including oral contraceptives and hormone replacement therapy, reveal conflicting data, and no clear association can be inferred [22].

Twin and family studies suggest a familial predisposition to developing leiomyomas, though further research in genetics of leiomyomas has yet to be done [22]. These studies are hampered by the extremely high incidence of leiomyoma formation in the general population.

According to some studies, an increase in body mass index has been found to increase the risk for uterine leiomyomas by a factor of 2–3, and the evidence suggests that it is adult-onset obesity rather than excessive weight in childhood that infers this risk. However, other studies have not observed similar associations with increased BMI [21].

The majority of epidemiologic studies find that cigarette smokers are at a 20–50 % reduced risk for the development of uterine leiomyomas through an unclear mechanism and that the inverse association was independent of BMI. It is unclear whether this relationship varies as a function of pack years. No clear relationship has been shown between leiomyomas and specific dietary factors or physical activity [21].

Pathology and Pathophysiology

Genetics

Leiomyomas are defined as monoclonal proliferations of benign smooth muscle [23]. Each monoclonal myoma may be associated with various chromosomal translocations, duplications, and deletions [24]. Many, but not all, myomas contain nonrandom cytogenetic abnormalities, while the myometrium has a normal karyotype. Most of the mutations occur in genes that are involved in cellular growth or are responsible for architectural transcription.

Two hereditary disorders have been reported in which uterine leiomyomas are part of a syndrome complex that demonstrates the potential genetic contribution to myoma formation. The first is hereditary leiomyomatosis and renal cell cancer complex. This is an autosomal dominant syndrome with smooth muscle tumors of the uterus, skin, and kidney. The second is a syndrome of pulmonary leiomyomatosis and lymphangiomyomatosis (LAM) that is the result of mutations in one of the two genes responsible for tuberous sclerosis, a syndrome that results in multiple hamartomas.

Pathology

Grossly, myomas usually appear as discrete, round masses that are lighter in color than the surrounding myometrium, with a glistening, pearly white appearance. Histological features include smooth muscle fibers that form interlacing bundles, with fibrous tissue in between the bundles.

Endocrinology

The influence of steroidal hormones is central to the theory of clonal expansion of leiomyomas. Myomas are responsive to estrogen and progesterone and are therefore more likely to increase in size and cause associated symptoms in women of reproductive age. Serum concentrations of circulating estrogen or progesterone have not been found to be increased.

Tumor initiators and yet undetermined genetic factors are involved in key somatic mutations that facilitate the progression of a normal myocyte into a leiomyocyte responsive to estrogen and progesterone. Estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor (EGFR) are integral in the development of myoma [25]. Studies have shown that, in comparison with the normal myometrium, myomas have an increased concentration of ER and PR [26, 27].

Aromatase p450 is overexpressed by leiomyomas [28, 29]. Therefore, in addition to circulating estrogen acting on the ER, the local conversion of circulating androgens to estrogens may be important in potentiating the actions of estrogen in the leiomyocyte (Fig. 18.2) [30].

Traditionally, estrogen was thought to be the primary hormonal mediator of myoma growth. Although progestins have been applied for the treatment of bleeding from symptomatic myomas, recent studies have shown that progesterone may play a much greater role as a mediator of myoma growth than previously thought [31]. The antiprogestin RU486 (mifepristone) has been shown to decrease the size of myomas [32, 33], and another study showed that myomas in the secretory phase have increased mitotic counts compared to those in the proliferative phase [34].

Growth of neoplastic tumors is the result of accelerated cellular proliferation that outpaces the inhibitory effect of apoptosis. Apoptosis has been shown to be inhibited in uterine

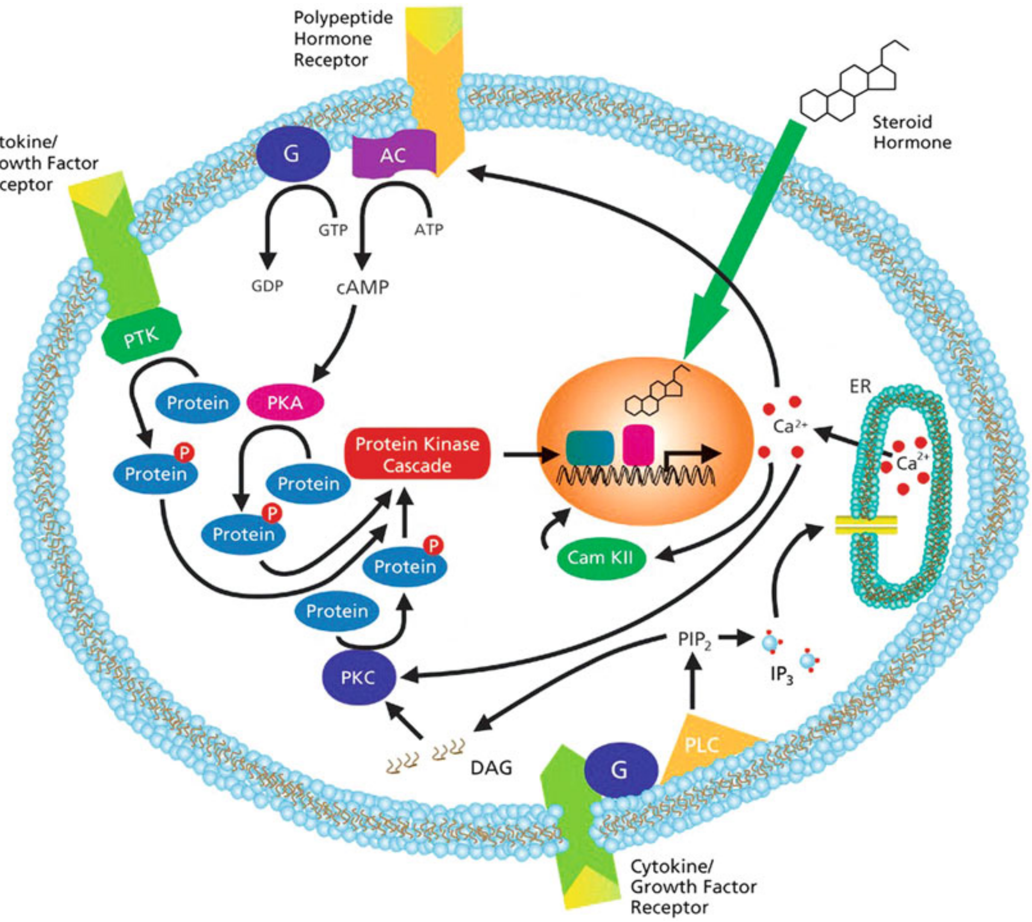

Fig. 18.2 Sex steroid hormone action. Estrogen and progesterone exert action through binding of specific receptors, which then bind to DNA at specific response elements. Binding of estrogen and progesterone at a variety of genes has different effects in various cells. Figure provided to the public via the Internet by Fisher Scientific, Inc.

leiomyomas. Progesterone has been shown to increase the antiapoptotic protein, bcl-2 [35]. Therefore, the stimulation of myoma expansion may be a function of the suppression of apoptosis by progesterone. It has been observed in vitro that the addition of progesterone to cultured leiomyoma cells increased the expression of bcl-2 when compared to controls [35]. Normal myometrium did not express increased levels of bcl-2 in the presence of progesterone.

The complex process of apoptosis involves not only the bcl-2 family but Fas/FasL and Rb-1 [36]. Martel et al. have described the various apoptotic pathways deficient in leiomyomas and potential corresponding targets for therapy of myomas. The role of apoptosis in the pathogenesis of myomas is a promising area for future research with a great potential for clinical application.

The synergistic interplay between estrogen and progesterone signaling in the pathophysiology of myoma growth has been observed as well. The increase in progesterone receptors as a result of increased estrogen has been well established. An in vitro study showed that progesterone upregulates the expression of EGF, and estrogen also increases the expression of EGFR [25].

Pregnancy and Leiomyomas

The influence of the pregnancy-induced endocrine milieu on a leiomyoma is complex. There are many reports of dramatic leiomyoma growth in pregnancy; however, all prospective studies have shown that most leiomyomas demonstrate no change in diameter from the first trimester to delivery. Essentially, it is impossible to predict which myoma will grow.

The main potential complications in pregnancy are pain and pregnancy wastage. Pain can be the result of myomatous degeneration. This phenomenon can be the consequence of necrosis from decreased blood supply to a subserosal or pedunculated myoma. Usually there is localized pain and a cystic or heterogeneous pattern on ultrasound.

Pregnancy wastage is often due to retroplacental myomas that may cause abruptio placenta, bleeding, and premature rupture of membranes. Lower uterine segment myomas may increase the probability of Cesarean section due to obstruction or malpresentation.

Diagnostic Imaging and Leiomyomas

Imaging has become an integral aspect of the evaluation of leiomyomas. Myoma size and location can be assessed to varying degrees, depending on the imaging technology applied to the evaluation process. Ultrasonography, hysterosalpingography, and magnetic resonance imaging (MRI) are currently the modalities most commonly utilized to image myomas.

Ultrasound

Traditional ultrasound is a cost-effective technology for assessing uterine leiomyomas. The transvaginal approach is more accurate than abdominal ultrasound. However, abdominal ultrasound may be a useful adjunct to transvaginal ultrasound, if a large uterine size warrants such an approach [22]. The presence of myomas may be detected by ultrasound as evidenced by uterine enlargement or a nodular contour of the uterus. They may also appear as discrete, focal masses within the myometrium [37, 38]. Myomas can appear hypoechoic or heterogeneous when compared with the appearance of the myometrium on ultrasound, and they may be characterized by calcification and posterior shadowing [37, 39]. Sagittal and axial views aid in providing information on the location and size of myomas.

Additional information regarding intracavitary masses, such as submucous myomas, may be obtained by means of saline infusion sonohysterography. This imaging technique consists of real-time transvaginal ultrasound during which sterile saline is injected into the uterine cavity. The saline is injected transcervically via a catheter of small caliber. As the uterine cavity is distended by the saline, intracavitary masses may be visualized as echogenic structures against the echolucent background of the distending media [40]. Intramural myomas within close proximity of the endometrial cavity may also be assessed by sonohysterography. In addition, entities such as endometrial polyps and uterine anomalies such as adhesions may also be detected. Sonohysterography can be used not only to diagnose submucous myomas but also to assess the potential access to surgical intervention [41].

Three-dimensional ultrasound [42] and color Doppler ultrasound [43] are increasingly being applied to the evaluation of myomas for imaging. Color Doppler ultrasound highlights vascular flow, which is usually increased at the periphery of myomas and decreased centrally [42, 43].

Hysterosalpingography

Hysterosalpingography is a screening test for intracavitary anatomic defects and entails injection of iodine contrast dye transcervically, via a catheter, into the uterine cavity with radiologic assessment under fluoroscopy. Hysterosalpingography is performed in the follicular phase of the menstrual cycle in order to avoid interfering with ovulation and/or a potential pregnancy. Since the hysterosalpingography instillation medium contains iodine, an iodine-allergic patient would require premedication with glucocorticoids and antihistamines prior to the procedure [44].

Hysterosalpingography allows visualization of submucous myomas, as the uterine cavity is distended by the contrast medium. The size and contour of the uterus may be altered by submucous myomas. Intramural myomas may enlarge the uterine cavity in a globular manner, and fundal myomas may enlarge the space between the cornua. Subserosal myomas are not typically noted on hysterosalpingography; however, if large enough, they may be detected as a mass effect on the uterine cavity [37]. In cases where a submucous myoma must be differentiated from an endometrial polyp on hysterosalpingography, hysteroscopy and sonohysterography play roles as complementary, potentially confirmatory adjuncts.

Magnetic Resonance Imaging

MRI is increasingly being utilized for imaging leiomyomas. The location of myomas can be accurately documented, more so with MRI than with ultrasound. It is often used to evaluate the precise location for surgical planning or prior to uterine artery embolization mapping.

Disadvantages of MRI include cost, limited availability, and an inability to perform the procedure in patients with morbid obesity or claustrophobia. Traditionally, cost had been more of a disadvantage; however, as the expense of MRI decreases, it is more commonly employed in clinical and presurgical evaluation. MRI is contraindicated in patients with pacemakers, defibrillators, metallic foreign bodies, and in rare cases of allergy to gadolinium [45].

In T2-weighted images, the endometrial stripe is visualized as a central, high signal; the junctional zone is a low signal; and the myometrial areas are an intermediate signal [46]. Leiomyomas are represented by variable signal density. Most of the time, they appear as hypodense, well-demarcated masses; however, increased cellularity [47] and degeneration may be seen as high signal intensity [46].

There is less distinction of the endometrial lining, junctional zone, and myometrium in T1-weighted images. These components are usually homogeneous and, consequently, obscured in appearance. Fatty or hemorrhagic degeneration may be represented by a high signal intensity [48].

Treatment of Leiomyomas

Treatment of leiomyomas has traditionally been interventional. In patients with a menstrual abnormality, the birth control pill has been used successfully, and the presence of a leiomyoma is not considered a contraindication. If the oral contraceptive fails, typically surgery is offered. However, new medical therapy may potentially change this approach.

The important concept in the management of leiomyomas is that intervention is not required in asymptomatic women. No longer acceptable reasons for surgical intervention include previous suggested indications, such as nonpalpable adnexa and preemptive intervention for asymptomatic fibroids in order to circumvent a potentially more difficult surgery in the future. Rapid growth of a leiomyoma traditionally was considered a potential sign of malignancy. However, this sign in isolation of other manifestations is not considered prognostic of a sarcoma.

Surgery is indicated with a history of pregnancy complications. Surgical treatment specifically for infertility is indicated if there is distortion of the uterine cavity. Surgical removal of uterine fibroids is sometimes considered in patients with longstanding infertility and when no other identifiable cause is found, although the latter indication is strongly debated.

Medical Treatment of Leiomyomas

Medical treatment of leiomyomas is indicated for the treatment of pain or menstrual dysfunction. Medical therapy has not been investigated for the management of infertility or pregnancy-related complications.

Gonadotropin-Releasing Hormone Agonists

Gonadotropin-releasing hormone (GnRH) agonists are an effective means of medically treating patients with symptomatic leiomyomas. After affecting an initial flare of LH and FSH, GnRH agonists down regulate the hypothalamic-pituitary-ovarian axis via action on pituitary receptors. The flare effect is due to an initial stimulation of FSH and LH owing to the binding of pituitary receptors, after which these receptors are desensitized, with a subsequent decrease in FSH and LH secretion [49]. This results in decreased estrogen production.

GnRH agonists have been shown to directly inhibit local aromatase p450 expression in leiomyoma cells [50], thereby presumably resulting in decreased local conversion of circulating androgens to estrogens within the leiomyocyte. Several studies have concluded that GnRH agonists can directly induce apoptosis and also suppress the cellular proliferation of myomas presumably via action on peripheral GnRH receptors.

Maximum reduction of the mean uterine volume occurs within 3 months of GnRH agonist administration. The decrease in volume is usually in the range of 40–80 %. However, after the discontinuation of GnRH agonists, myomas will rapidly grow back to their pretreatment size, usually in the span of several months [51].

Advantages of GnRH agonists include their use in the perimenopausal transition with add-back therapy for the goal of avoiding hysterectomy. Additionally, laparoscopic myomectomy may be made more feasible with GnRH-agonist pretreatment, and GnRH agonists can also be beneficial in a patient who is to undergo hysterectomy to facilitate a vaginal approach rather than an abdominal incision. In a randomized clinical trial comparing the study group (patients receiving GnRH agonist and iron) to a control group (iron alone), preoperative hematologic parameters were improved [52].

Although decreased tumor bulk and a decrease in associated symptoms are attained, the potential for unwanted long-term side effects exists; therefore, treatment with GnRH agonist is recommended for no more than 6 months. Common side effects include hot flashes, vaginal dryness, headache, and mood swings. Most importantly, in terms of bone health status, there is a recognized decrease in bone mineral density during therapy [53]. Although add-back doses of steroidal hormones can be used with the aim of decreasing this bone loss; the long-term use of GnRH agonists with add-back is impractical and not recommended, especially in younger patients.

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are compounds that bind to the ER and confer an agonist or antagonist effect, depending on tissue specificity. They have been applied to the treatment and prevention of estrogen-responsive breast cancer; examples include the use of tamoxifen and raloxifene. Tamoxifen, a triphenylethylene, has antagonist activity in the breast and displays a desirable agonist activity in the bone and the cardiovascular system as well as a mild agonist activity in endometrial tissue [54, 55]. Raloxifene, a benzothiophene, has a similar profile and the added benefit of not acting as an agonist in the endometrium [56].

In animal models, SERMs have been shown to be effective in decreasing the growth of myomas. Eker rats are a rat strain with a tuberous sclerosis 2 (TS-2) gene defect that can spontaneously develop leiomyomas. In studies, the administration of SERMs was associated with the inhibition of leiomyoma formation in Eker rats [57, 58]. Guinea pigs require long-term exposure to estrogen in order for leiomyoma

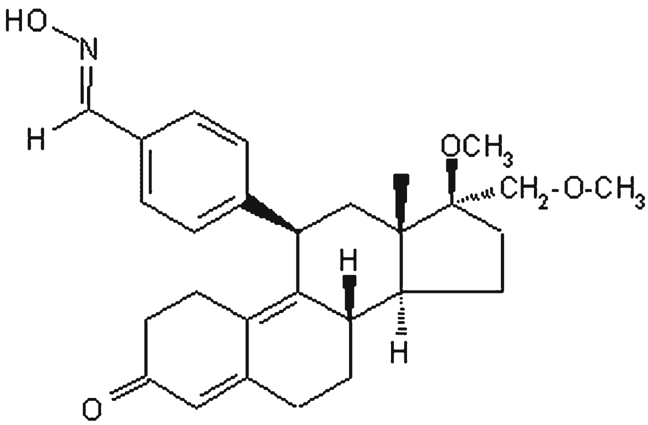

Fig. 18.3 Chemical structure of asoprisnil

formation to occur. Two groups of oophorectomized guinea pigs, an estrogen-only group and an estrogen plus raloxifene group, were compared for myoma formation. A decrease in the size of the induced myomas was observed in the estrogen plus raloxifene group [59].

In humans, raloxifene appears effective in decreasing the size of myomas in postmenopausal women [60], but beneficial effects were not significant in premenopausal women [61]. A recent study has demonstrated that a combination of raloxifene and GnRH agonist is more effective in reducing leiomyoma volume [62] and preventing a decrease in bone mineral density [63] than the use of GnRHa alone. A Cochrane review of three studies showed no consistent evidence from a limited number of studies that SERMs reduce fibroid size or improve clinical outcomes and suggested that further studies be conducted to establish benefit [64].

Selective Progesterone Receptor Modulators

This class of compounds has either agonist or antagonist effects on the progesterone receptor, based on tissue specificity [65]. Asoprisnil is a selective progesterone receptor modulators (SPRM) that, along with its major metabolite, J912, has high affinity for the PR, moderately binds growth hormone receptor, and has a very low binding potential for androgen receptors (Fig. 18.3). Asoprisnil has virtually no affinity for ER or mineralocorticoid receptors [66]. It differs from the long-term effect of progesterone on the endometrium in that amenorrhea is rapidly established without breakthrough bleeding [67].

The oral SPRM ulipristal acetate has successfully completed phase III clinical trials [68]. A randomized trial of ulipristal acetate vs. placebo for fibroid treatment before surgery has shown that treatment for 13 weeks effectively controlled excessive bleeding and reduced the size of fibroids [69]. Further clinical research is necessary to study long-term effects of SPRMs on health and reproduction. As SPRMs are studied and tested further in clinical trials, there may be a practical use for this class of medications in the treatment of leiomyomas, especially in women with menorrhagia who are interested in avoiding surgery or maintaining future fertility.

Aromatase Inhibitors

Aromatase inhibitors (AI) were originally used for the treatment of breast cancer, and this class of medications is FDA-approved for this purpose. In recent years, the use of AI has expanded within the field of reproductive medicine, and its application in the potential treatment of uterine fibroids has been investigated in several studies. The rationale behind AI is due to the finding that aromatase p450 enzyme concentrations are elevated at the local level in leiomyoma tissues [28, 29]. Interestingly, aromatase expression has been shown to be highest among African-American women (83-fold) when compared to that of Caucasian (38-fold) and Japanese women (33-fold) [70]; this higher expression may be one underlying mechanism that may explain the higher prevalence of uterine fibroids among African-American women when compared to women of other ethnic backgrounds.

In a prospective study by Gurates et al., a 3-month course of the AI letrozole at 5 mg per day was found to significantly decrease uterine and leiomyoma volume without changes in lumbar spine BMD or biochemical markers of bone metabolism; in addition, heavy menstrual bleeding associated with leiomyoma was improved [71]. Furthermore, an RCT that compared the effects of AI treatment with GnRHa on myoma volume and hormonal status in premenopausal women with leiomyomas showed promise as well. Treatment duration for both groups was 12 weeks. Leiomyoma volume was significantly reduced in the AI and GnRHa groups without a difference between groups. The AI group did not have a significant change in hormonal milieu from baseline in contrast to the GnRHa group. The authors concluded that uterine leiomyomata may be successfully managed by the use of AI, which may be most useful in the setting of pretreatment for surgery. Advantages of AI over GnRHa may include rapid onset of action as well as the avoidance of the GnRHa flare [72].

Surgical Therapy of Leiomyomas

Surgical treatment is the mainstream therapy for leiomyomas. Hysterectomy represents the only definitive curative therapy; however, myomectomy, endometrial ablation, and myolysis are increasing in frequency as alternative therapeutic procedures. Indications for surgical intervention include failure to respond to medical treatment, worsening vaginal bleeding, suspicion of malignancy, or treatment of recurrent pregnancy loss. In postmenopausal women with an enlarging pelvic mass and abnormal bleeding, surgery should be strongly considered. In this population, the incidence of a sarcoma is still uncommon but higher than the incidence found in the premenopausal population, about 1–2 % [73].

Hysterectomy

Hysterectomy has been described as the definitive management for symptomatic leiomyomata. When employed, it is highly effective for the carefully selected patient with symptomatic leiomyomata who does not desire future fertility. Among hysterectomies performed abdominally vs. vaginally for uterine leiomyomas, the abdominal route has been chosen approximately 75 % of the time based on data from the late 1980s and early 1990s [74].

Vaginal hysterectomy is associated with a lower complication rate and decreased need for blood transfusion. Another advantage of vaginal hysterectomy is the association with shorter operating times [75]. Myoma size and location, total uterine size, and the surgeon's skill are factors that may determine the feasibility of vaginal hysterectomy.

Laparoscopy-assisted vaginal hysterectomy, total laparoscopic hysterectomy, and laparoscopic supracervical hysterectomy are minimally invasive surgical methods that are associated with decreased postoperative pain and recovery time in comparison to vaginal and abdominal hysterectomy [76]. These surgical modalities may incur increased hospital costs in terms of equipment and operating room time, but they have the recognized advantages listed above. In addition, these options provide the ability to assess the pelvis if the patient also complains of pelvic pain.

Myomectomy

Hysterectomy has long been considered the definitive treatment for symptomatic uterine leiomyomas. Yet as more and more women delay childbearing, the incidence of uterine leiomyomas among patients suffering with infertility increases, and hysterectomy becomes an unacceptable management option. Therefore, abdominal, laparoscopic, and hysteroscopic myomectomies have become increasingly common treatment modalities for women with leiomyomas and infertility.

Myomectomy is the surgery of choice for treating women with symptomatic myomas in those who desire to preserve fertility or to otherwise keep their uteri. It is most useful for subserosal, especially pedunculated subserosal, myomas as well as intramural leiomyomas. Myomectomy entails the surgical removal of myomas by enucleation. It is preferable to use as few incisions as possible to remove myomas from the uterus, in order to minimize adhesion formation as well as to minimize any compromise of the myometrial integrity. The surgeon must be diligent regarding the orientation of the uterus, especially during the repair, in order to preserve the integrity of the endometrial cavity. Several techniques have been described to decrease blood loss with an abdominal myomectomy and include the use of tourniquets around the lower segment of the uterus to occlude the uterine arteries and the use of dilute vasopressin.

Methods employed to minimize postoperative adhesion formation include the use of permanent or absorbable barriers and good surgical technique minimizing trauma to the tissues, use of nonreactive suture material, and the avoidance of tissue desiccation or aggressive cautery. Due to the potential risk of uterine rupture, a trial of labor after myomectomy is not recommended by the American College of Obstetricians and Gynecologists (ACOG) [77].

Laparoscopic Myomectomy

Laparoscopic myomectomy offers many advantages for this minimally invasive surgery technique to remove myomas. This procedure, however, requires appropriate training and advanced endoscopic skills from the surgeon. It is most useful in cases in which myomas are easily visualized and readily accessible.

Several studies have shown advantages of the laparoscopic approach to myomectomy. Mars et al. randomized 20 patients undergoing myomectomy to laparoscopy and 20 patients to laparotomy. The laparoscopy group had lower postoperative pain as well as a greater number of patients that were analgesia-free on postoperative day 2, discharged home by postoperative day 3, and fully recovered on postoperative day 15 [78]. Among a group of 131 patients randomly assigned to myomectomy via laparoscopy or laparotomy, Seracchioli et al. found that laparoscopic myomectomy is associated with lower intraoperative blood loss and shorter postoperative length of stay in the hospital. Moreover, no significant difference in subsequent fecundability, spontaneous miscarriage rate, preterm delivery rate, and Cesarean section rate was found between the groups. A lower incidence of postoperative febrile morbidity was yet another advantage found in this study [73].

Adhesion formation was evaluated in a retrospective study of 28 patients who underwent laparoscopy or laparotomy for myomectomy followed by a second-look laparoscopy [79]. A lower incidence of adhesion formation was noted in patients that initially underwent a laparoscopic approach to their myomectomy. Similar observations were made in two other studies [80, 81].

Disadvantages of laparoscopic myomectomy include the lack of opportunity to palpate the uterus intraoperatively, resulting in the potential for a higher rate of subsequent

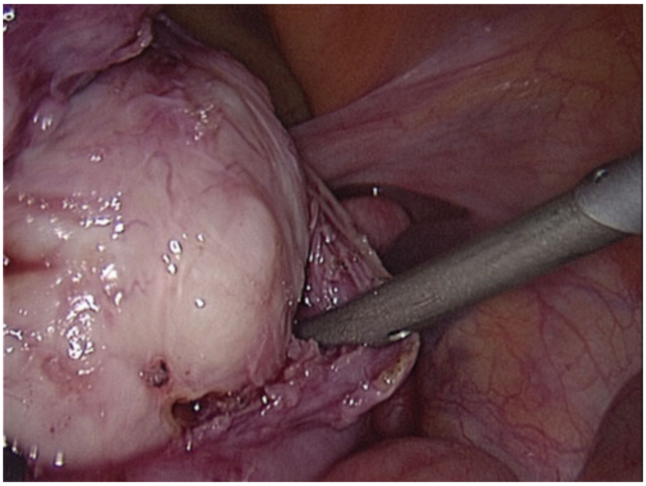

Fig. 18.4 Dissection of the myoma bed is usually accomplished bluntly, as in open cases. Picture provided by Dr. T. Falcone

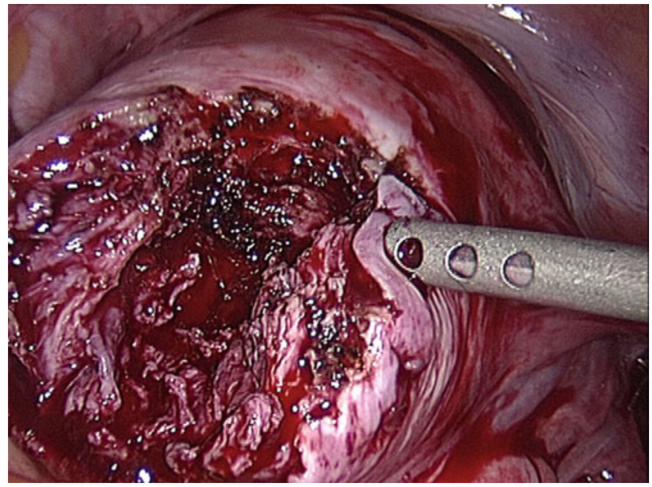

Fig. 18.5 A large myometrial defect is left behind. This requires closure with two to three layers of delayed absorbable sutures. Picture provided by Dr. T. Falcone

recurrence of myomas [82, 83], which is supported by several studies and refuted by others [84]. An additional disadvantage is operator dependent and involves the technical difficulty in repairing the enucleated leiomyomas, with a potential risk for uterine rupture during future pregnancies if improperly closed.

Robotic-Assisted Myomectomy

The advantages of robotic-assisted myomectomy (RAM) compared with conventional laparoscopic myomectomy include the potential for greater technical feasibility of removing uterine fibroids via a minimally invasive surgical approach [85] due to three-dimensional vision, ergonomic considerations, seven degrees of freedom in terms of wrist movement with distal aspect of the robotic arms, and no fulcrum effect [86]. RAM has been associated with decreased intraoperative blood loss as well as a shorter length of hospital stay as compared with laparoscopic as well as abdominal myomectomy [86]. A large, retrospective, multicenter study showed that women who underwent RAM by experienced robotic surgeons had similar pregnancy outcomes as those who underwent laparoscopic myomectomy; in addition, 11 % were found to have pelvic adhesions at subsequent Cesarean section [87].

Technical Considerations

Laparoscopic myomectomy and RAM are usually performed with the standard three to four ports. Dilute vasopressin, although not approved by the FDA for this indication, is diluted 20 units in 100 mL of normal saline and injected into the myoma. An incision is then made into the myoma and enucleated bluntly (Fig. 18.4). After enucleation, the defect (Fig. 18.5) is closed in two to three layers, typically a deep layer and then a seromuscular layer. Intra- and extracorporeal knot tying are acceptable. This step is the most critical part of a laparoscopic myomectomy, and the defect should be closed tightly (Fig. 18.6).

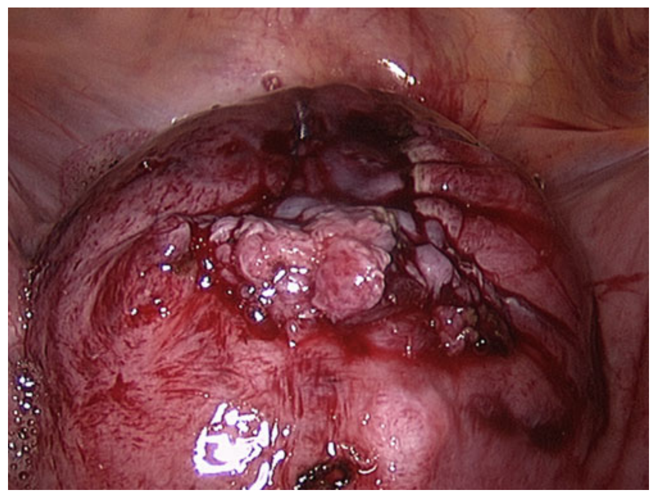

Fig. 18.6 The myometrial defect is tightly closed. Picture provided by Dr. T. Falcone

Uterine Artery/Leiomyoma Embolization

Uterine artery embolization (UAE), also referred to as uterine fibroid embolization (UFE), is a minimally invasive alternative to hysterectomy and myomectomy that has been applied to the treatment of symptomatic leiomyomas. UAE was initially developed for the control of pelvic hemorrhage and has been utilized for the primary treatment of leiomyomas since 1995 [88]. It has been associated with positive clinical outcomes and a high rate of patient satisfaction. Success rates of over 90 % have been reported [89, 90]. Decreases in the size of the uterus and dominant leiomyomas of 45 % have been reported [39]. The ACOG has found that UAE is safe and effective [91].

Pre-embolization imaging with MRI aids in precisely locating the position of leiomyomas prior to the procedure. Embolization of the uterine arteries with polyvinyl alcohol or tris-acryl gelatin microspheres involves femoral artery catheterization under fluoroscopic guidance. Typically, uterine fibroid embolization is performed with the patient under conscious sedation [45].

Post-embolization follow-up consists of clinical evaluation as well as a follow-up MRI in order to assess and monitor the final resulting volume of leiomyomas and the uterus. The degeneration and devascularization of leiomyomas can be visualized on MRI as increased signals on T1-weighted and decreased signals on T2-weighted images [92–94]. After uterine fibroid embolization, the size of leiomyomas can continue to decrease for 1 year or more. The high rate of patient satisfaction [89] is due to the improvements in menorrhagia and pressure symptoms as well as pain relief experienced by a high percentage of women undergoing uterine fibroid embolization.

A systematic review and meta-analysis of 54 studies with a total of over 8,000 patient examined the complication rates and effectiveness of UAE in the treatment of uterine fibroids. There were no reported deaths, and rate of major complications was 2.9 %. During a 0.25–2-year follow-up, clinical symptomatic improvement ranged from 78 to 90 % [95]. A Cochrane review of six RCTs that totaled 732 women found moderately good evidence that patient satisfaction rates at 2 and 5 years were similar between UAE and either hysterectomy or myomectomy. UAE was also found to be associated with a shorter length of hospital stay and a quicker return to daily activities. However, UAE is associated with high rates of minor complications and surgical reintervention [96].

It is important to note that successful pregnancies have occurred following this procedure [97, 98]. There is a recognized risk of inducing premature ovarian failure in older women. Several studies have shown an increased risk of intrauterine growth restriction and placentation problems in pregnancies following uterine fibroid embolization. A systematic review showed that after UAE, there were higher rates of miscarriage, Cesarean section, and postpartum hemorrhage as compared with controls [99]. There are currently no studies that establish a successful pregnancy rate after UAE [100]. Although there are confounding variables in the existing studies and a Cochrane review showed very low evidence suggesting better fertility outcomes with myomectomy over UAE [97], short-term reproductive outcomes favor myomectomy over UAE [101]; for women seeking pregnancy, myomectomy is favored over UAE. Complications of uterine fibroid embolization include angiography-related problems [45], allergic reactions [102], perforation of the uterus [103], and infection [94, 102]. If the collateral blood supply to the ovary is embolized, then infertility, amenorrhea, and premature menopause are potential risks [90, 104]. Sciatic nerve injury leading to buttock claudication is a recognized potential complication of uterine fibroid embolization [103]. Deep venous thrombosis and pulmonary embolus are rare risks, as is death [103, 105, 106], which has a reported 3/10,000 rate with uterine fibroid embolization compared with the 1/1,000 rate from hysterectomy [107].

Post-embolization syndrome is relatively common occurrence and consists of nausea, vomiting, pain, and a temporary increased WBC count following the procedure. This syndrome affects most patients to some extent in the first 48 h after the procedure but is severe in approximately 15 % of those who undergo uterine fibroid embolization [108].

MRI-Guided Focused Ultrasound Therapy

Over the last decade, high-intensity focused ultrasound surgery (HIFU), with either MRI or US for guidance, has been used for treating symptomatic uterine fibroids in a noninvasive manner. The therapeutic HIFU component focuses thermal energy in order to ablate leiomyoma tissue in a precise and controlled fashion. By placing an ultrasound transducer on the abdomen of a patient and focusing the ultrasound energy at a specific, controllable depth and position, leiomyoma tissue is destroyed within the focal zone. The therapeutic ultrasound effect is often monitored by MRI, which precisely records the temperature elevation from the heat generated over time. Once the temperature reaches 57 °C for 1 s, tissue is rapidly destroyed within the focal zone. Tissue within 2–3 mm of the focal zone is unaffected owing to the very precise demarcation between normal and destroyed tissue.

MRI-guided focused ultrasound surgery (MRgFUS or MRgHIFU) is considered superior to US guidance due to increased soft tissue resolution as well as capability of tissue temperature mapping; in addition, ultrasound-guided HIFU is not FDA-approved, whereas MRgFUS as a therapy for leiomyomata received FDA approval in October 2004 [109]. The ExAblate System (ExAblate 2000, Exablate 2010; InSightex, Haifa, Israel) is the first medical device approved for the treatment of leiomyomas as its primary indication. General patient selection criteria include leiomyomas between 4 and 10 cm, maximum depth of subcutaneous tissue to the leiomyoma <12 cm, completion of childbearing, premenopausal status, and leiomyomas that can be clearly visualized on MRI. Based on results of a 6-month follow-up study, the mean leiomyoma reduction in volume after HIFU was 13.5 cm^3, and the mean volume of nonperfused tissue was 51.2 cm^3. Furthermore, 79.3 % of patients reported a greater than 10-point reduction in symptom scores and improvement in quality-of-life measures on the questionnaire used in the study [110]. Adverse events included minor skin burns in 4 % of patients, worsening menorrhagia in 4 % of patients, hospitalization for nausea in only 1 % of patients, and nontargeted sonication of the uterine serosa in 1 % of patients [110].

Two other studies have assessed longer-term clinical outcomes. In a 24-month follow-up study, improvement in symptoms and moderate volume reductions in types of uterine fibroids with lower MRI image intensity than the myometrium were observed after MRgFUS [111]. In a 12-month study of patients who underwent MRgFUS, relief of symptoms was reported in 86 %, 93 %, and 88 % of patients at 3-, 6-, and 12-month follow-up, respectively [112]. Reintervention rates are low and comparable to those with UAE [112]. There are no randomized clinical trials that have compared this technology with surgical or other radiologic treatment.

The effect that MRgFUS may have on fertility is not clear; however, there have been reports of successful pregnancies after MRgFUS. A published series of all pregnancies after MRgFUS reported to the manufacturer and the FDA, as part of post-approval device monitoring, showed that normal pregnancy outcomes and deliveries are possible. The series reported a 41 % live birth rate, 20 % ongoing pregnancy rate, 11 % rate of elective termination, and 28 % spontaneous abortion rate after MRgFUS for the treatment of uterine fibroids [113]. Furthermore, in a retrospective study, seven women who unintentionally became pregnant after ultrasound-guided HIFU continued their pregnancies without complications [114].

Cryomyolysis

Cryomyolysis of uterine leiomyomas has been performed by laparoscopy, and in recent years, MRI-guided cryomyolysis has been devised as a less invasive approach. Cryomyolysis involves placement of a 2-cm diameter cryoprobe, directly into a uterine leiomyoma. After the cryoprobes are advanced and placed into position, the probes are then cooled by liquid nitrogen instillation or by differential gas exchange, reducing the local temperature within the leiomyoma to less than −90 °C, resulting in a 3.5–5-cm ice ball, ultimately resulting in tissue necrosis Because the temperature at the edge of the ice ball is 0 °C and not destructive to surrounding tissue, the imaging of the ice ball can predict the limits of targeted tissue [115]. Laparoscopic cryomyolysis for women with leiomyomas as well as a combination of abnormal uterine bleeding, pelvic pain/pressure, and/or urinary frequency was shown to be effective in a study of 20 patients [116].

MRI-guided cryomyolysis was devised as a less invasive and more precise approach. MRI can be employed to accurately visualize ice-ball formation, which eventually encompasses the leiomyomas appearing black due to the slow or absent hydrogen ion spins of water molecules in the ice.

A report of MRI-guided cryomyolysis used to treat leiomyomas in 10 patients showed MRI evidence of marked uterine volume reduction between 48 and 334 days after the procedure. The mean volume reduction was 65 %. All patients reported improvement of symptoms, whether they were due to bleeding and/or pressure symptoms. One patient had uterine bleeding for 2 months post-procedure, with subsequent spontaneous resolution. Another patient had a residual submucosal leiomyoma that had to be resected hysteroscopically at a later date. Complications included a patient with laceration of a serosal vessel covering a leiomyoma, which required a laparotomy and open myomectomy for repair. Another complication was peroneal nerve involvement and a mild foot drop that resolved over several months. Nausea and mild abdominal discomfort that was relieved by NSAIDs were reported as minor complications [117].

Another study of 14 women evaluated the efficacy of 2 months of pretreatment with GnRH agonist prior to laparoscopically directed cryomyolysis [118]. The GnRH agonist was discontinued immediately prior to the procedure. Four months after cryomyolysis, the follow-up MRI showed a mean volume decrease of 10 % among the frozen leiomyomas, whereas other uterine tissue returned to their size prior to GnRH agonist treatment.

Studies have shown that cryomyolysis can be an effective, minimally invasive means to treat symptomatic uterine fibroids [118, 119]. Myoma volume was reduced by 50 %, along with a corresponding reduction in symptoms, 6 months after the procedure [93]. Follow-up data have shown that at 12 months, further myoma shrinkage up to 62 % from baseline occurred and heavy menstrual bleeding was reduced [120]. Because long-term outcomes are necessary and the effects of cryomyolysis on fertility are unknown, although a small series of nine women showed fertility may remain preserved [121], this technique is considered experimental at the present.

Laparoscopic Uterine Artery Ligation

Laparoscopic uterine artery ligation is yet another potential option for women who opt to preserve their uteri. A study comparing this technique to uterine artery embolization suggests that that the uterine volume was slightly reduced at 3 months and stabilized at 6 months, with an average volume reduction of 58.5 % [122]. The authors concluded that both laparoscopic uterine artery ligation and UAE are reasonable alternatives to hysterectomy.

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Tubal Disease and Ectopic Pregnancy

19

Rebecca Flyckt and Jeffrey M. Goldberg

Introduction

Tubal factor infertility remains a frequently encountered entity in gynecologic practice. Analysis of data from the World Health Organization indicates that tubal factor accounts for approximately 35 % of female infertility cases worldwide, with a much higher prevalence in areas such as Africa [1]. The overall incidence of tubal disease in Europe or the United States may be lower. For example, one population-based study of over 700 couples indicated that approximately 14 % of couples evaluated by an infertility specialist had tubal disease [2]. This figure remained stable in a more recent survey of a similar cohort [3]. The most common causes of this condition include pelvic infection, endometriosis, and sequelae of ectopic pregnancy. Although the incidence of advanced ectopic pregnancies and some reportable sexually transmitted diseases is decreasing in the United States [4], early and accurate recognition in the diagnostic workup of the infertile couple is essential.

Fortunately, tubal infertility is often amenable to surgical intervention. With increasing success rates of assisted reproductive technologies (ART), patients are opting more frequently for in vitro fertilization (IVF) to circumvent damaged fallopian tubes. However, surgical management is still recommended in cases of proximal occlusion, mild hydrosalpinges, and reversal of tubal ligation.

In this chapter, the etiology of tubal factor infertility will be discussed, with a focus on pelvic inflammatory disease (PID) and ectopic pregnancy. In addition, the assessment of tubal status with hysterosalpingogram (HSG) will be reviewed. Finally, the surgical approaches to the treatment of tubal disease will be addressed, with an emphasis on tubal reconstructive techniques and management of hydrosalpinges. The treatment of tubal disease with ART does not differ from other conditions and is discussed separately.

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Mechanisms of Tubal Damage

The fallopian tubes are delicate organs which function in sperm transport, the pickup and fertilization of oocytes, and the transport of embryos to the uterus. The ciliated endothelium is particularly susceptible to damage by infection. However, abnormalities of the fallopian tubes can also follow tubal pregnancy, endometriosis, developmental exposure to teratogens, or iatrogenic causes (Table 19.1).

The most common cause of tubal infertility is PID, which can arise after ascending infection with *N. gonorrhoeae* or *C. trachomatis* [5, 6]. Other infectious agents thought to be deleterious to tubal structure and function include *Mycoplasma* species and tuberculosis; however, a causal relationship has not been substantiated [7]. It is known that the chance of post-inflammatory tubal damage rises with repeat infections and the successful function of the fallopian tubes is directly related to the severity of the damage [5, 8]. According to these landmark studies from Sweden involving thousands of women, the incidence of tubal infertility after laparoscopically diagnosed PID was 10–12 % after one infection, 23–35 % after two infections, and 54–75 % after three infections [8]. Longitudinal data from multiple centers within the United States confirm a twofold increased risk of infertility after recurrent PID [9].

It appears that many women with tubal disease or seropositivity for chlamydial antigen have not had any documented or reported history of prior infection [10]. Therefore, it must be concluded that salpingitis and resulting tubal damage can result even after asymptomatic or subclinical infections [7]. A recent study of women with subclinical PID based on endometrial histology demonstrated decreased

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Table 19.1 Causes of tubal infertility

• Pelvic inflammatory disease
• Prior ectopic pregnancy
• Surgery/trauma to fallopian tubes
• Endometriosis/adhesions
• Septic abortion/endometritis/salpingitis
• Ruptured appendix
• Inflammatory bowel disease
• Iatrogenic
• DES

fertility compared to controls [7]; these findings were despite treatment for uncomplicated lower genital tract infections.

As might be expected, a close association also exists between PID and ectopic pregnancy. Women with an episode of PID have approximately a 10 % chance of developing an ectopic pregnancy in their first pregnancy following documented salpingitis [5]. In one retrospective cohort study examining sequelae of [11] chlamydial infection, the authors found that women with two episodes of infection were twice as likely, and women with three of more episodes of infection were greater than four times as likely, to be hospitalized for ectopic pregnancy [11].

Imaging of the Fallopian Tubes

Whatever the cause of tubal damage, recognizing tubal factor infertility is essential in the diagnostic workup of the infertile couple. HSG has been the standard initial test for assessing the uterine cavity and fallopian tubes since 1925 [12], although laparoscopic chromopertubation remains the gold standard for diagnosing tubal disease. HSG is a radiographic imaging procedure to image the uterine cavity and demonstrate tubal patency by injecting radiographic contrast media through the cervix. The technique is easy to learn and perform, is relatively low cost, has an acceptable radiation exposure, and has few complications. Indications and contraindications for HSG are listed in Tables 19.2 and 19.3. Risks, benefits, and alternatives of the procedure are outlined in Table 19.4. The most significant additional benefit of HSG is an enhanced post-procedure pregnancy rate. Alternative methods for evaluating tubal condition (such as sonohysterosalpingography or "hystero-contrast-sonography," transvaginal hydrolaparoscopy, falloposcopy, and salpingoscopy) are used far less often.

HSG should be considered a screening test and should have a high sensitivity, so as not to miss the opportunity to treat an abnormality, but a low false-positive rate to prevent unnecessary additional testing and treatments. The accuracy of an HSG is highly dependent on technique and interpretation. The technical quality of the HSG is important to limit misinterpretations (i.e., eliminating air bubbles that may be confused with a polyp or myoma or using inadequate contrast volume or injection pressure to demonstrate tubal patency).

Table 19.2 Indications for hysterosalpingogram (HSG)

• Basic infertility workup
• Recurrent pregnancy loss
• Evaluation after uterine or tubal surgery
• Suspected uterine anomaly
• Confirming post-procedure tubal occlusion

Table 19.3 Contraindications to HSG

• Active pelvic infection
• Cervicitis
• Severe iodine allergy
• Bleeding/menstruation
• Known or suspected endometrial carcinoma
• Pregnancy

Table 19.4 Risks, benefits, and alternatives to HSG

Risks/complications • Vasovagal reactions • Post-procedure infection • Granuloma formation with oil-based contrast • Oil embolism with oil-based contrast
Benefits • Guides infertility treatment management • Fertility enhancement
Alternatives • Chlamydia antibody testing • Sonohysterography or sonohysterosalpingography • Magnetic resonance imaging • 3D ultrasonography • Radionuclide HSG • Laparoscopy • Hysteroscopy • Transvaginal hydrolaparoscopy • Salpingoscopy and falloposcopy

Diagnosing Uterine Cavity Abnormalities

HSG has a high sensitivity but a low specificity for the diagnosis of uterine cavity abnormalities [13]. HSG and diagnostic hysteroscopy performed on 336 infertile women showed that HSG had a sensitivity of 98 % but a specificity of only 35 % due to difficulties distinguishing between polyps and myomas [14]. Thus, HSG fulfills the requirements as a good first-line screening test for revealing abnormalities of the uterine cavity, though any abnormalities found will likely need further evaluation to make a definitive diagnosis. A uterine septum and a bicornuate uterus cannot be differentiated on an HSG. Evaluation of the external fundal contour by laparoscopy, MRI, or 3D ultrasonography is required to make a definitive diagnosis. Other conditions visualized on HSG are adhesions, diethylstilbestrol (DES) changes, and adenomyosis.

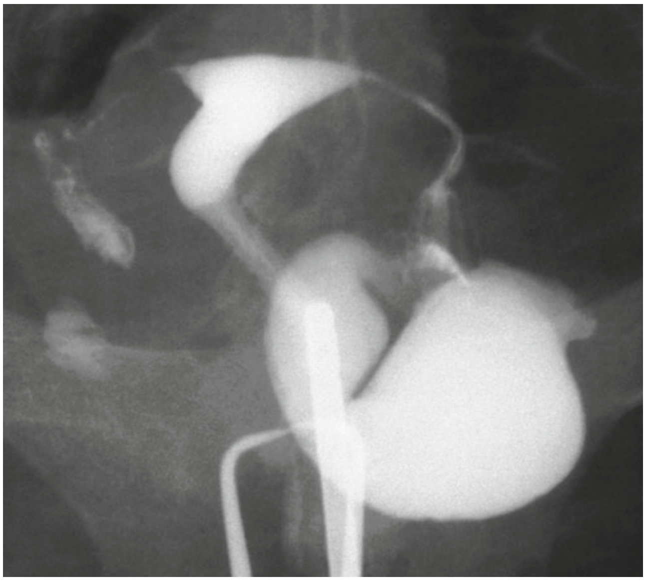

Fig. 19.1 Hydrosalpinx

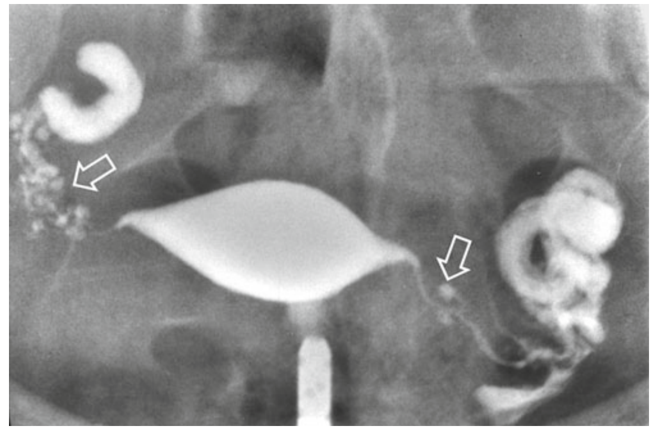

Fig. 19.2 Salpingitis isthmica nodosa

Diagnosing Tubal Abnormalities

HSG appears to be a highly valid and accurate test for assessing tubal patency in subfertile couples; however, its reliability for diagnosing tubal occlusion is questionable. Tubal blockage on HSG is not confirmed by laparoscopy in up to 62 % of patients. Laparoscopy is needed to confirm or exclude tubal occlusion on HSG [15]. Even laparoscopy is imperfect, as 2 % of patients with bilateral tubal occlusion subsequently conceived spontaneously [16]. One study noted that 60 % of patients with proximal tubal occlusion (PTO) on HSG showed patency on repeat HSG 1 month later [17].

Surprisingly, hydrosalpinges may also be poorly diagnosed by HSG. When detected, they may be only mildly dilated with preservation of mucosal folds or massively dilated with complete loss of the normal intratubal architecture (Fig. 19.1). HSG can also diagnose salpingitis isthmica nodosa, represented by diverticuli from the mucosa into the muscularis (Fig. 19.2). HSG is also not an ideal test for diagnosing pelvic adhesions because it detects them in only one-half of the cases in which they are present [12]. Adhesions are usually diagnosed on HSG by the presence of loculated spill of contrast.

Technical Considerations

If possible, the gynecologist should perform the study; patients are comforted by having their physician present during this stressful test. At a minimum, the films should be obtained for review as the radiologists' reports vary greatly depending on training and experience. The procedure should be performed during the window between the end of menses but before ovulation. Although most patients experience uterine cramping during the HSG, the duration of the procedure is short and the discomfort generally resolves rapidly. Several studies have noted that NSAIDs do provide significant analgesia for HSG [18–20]. Proponents of water-soluble media (WSM) vs. oil-soluble media (OSM) have been arguing the relative merits of their favored contrast for decades and as yet there is no clear winner. WSM offers better image quality as the higher density OSM tends to obscure fine details in the uterus and tubal mucosal folds. Also, since WSM dissipates quickly, there is no need for delayed films, whereas 1–24-h delayed films are necessary with OSM. OSM also carries increased risks for oil embolism and granuloma formation. OSM has been claimed to have higher post-procedure pregnancy rates, although this finding has been questioned in the current Cochrane analysis [21].

Technique and Troubleshooting

The HSG cannula is attached to a 20-cc syringe filled with contrast media, which is flushed to expel air and prevent air bubble artifacts. The acorn is advanced so that it is about 1 cm from the end of the cannula. The cannula tip should not go beyond the internal os. Using a lubricated, open-sided bivalve speculum, the cervix is cleansed with an antiseptic solution, a single-tooth tenaculum is applied to the anterior lip, and the cannula tip is inserted into the cervical canal. Gentle upward pressure on the cannula while pulling downward on the tenaculum will seal the cervix and straighten the uterine axis.

Contrast media is then injected slowly. If a filling defect is suspected to be an air bubble, the patient can be rotated to her side (defect side down). A polyp or myoma will remain stationary, whereas a bubble will rise to the elevated side. Only 5–10 mL are usually required to complete the study.

Patients should be observed for several minutes afterwards for bleeding and signs of vasovagal or allergic reactions.

While bilateral PTO is usually indicative of anatomical pathology, unilateral PTO is frequently transient due to spasm of the uterotubal ostium, plugging by mucus, debris, or air bubbles. Unilateral PTO is found in 10–24 %, but 16–80 % are patent on repeat HSG or laparoscopy with chromotubation [22]. Increasing the hydrostatic pressure and rotating the patient may establish patency during HSG showing PTO. The use of antispasmodic agents such as glucagon to prevent PTO has also been recommended, but this practice is supported only by limited anecdotal reports in the literature.

Surgical Management of Tubal Disease

Tubal disease may be present at multiple sites; however, tubal damage or blockages are often categorized as proximal, mid-tubal, or distal. The success of surgical management will depend of the location and degree of the damage in addition to nonsurgical factors, such as patient age and ovarian reserve. In general, patients with extensive tubal damage will be best served by IVF, whereas those without widespread disease may be good surgical candidates.

Minimally invasive surgical techniques are most appropriate for tubal surgery, but this approach requires some advanced training as well as meticulous dissection and hemostasis to prevent adhesions or further damage to the tubes.

Proximal Tubal Occlusion

PTO is most often caused by salpingitis isthmica nodosa, chronic pelvic infection, intratubal endometriosis, mucus plugging, or anatomic malformations [23]. Persistent PTO can be treated by therapies such as selective salpingography, tubal cannulation under fluoroscopy, hysteroscopic cannulation, or tubal resection and reanastomosis with a high degree of success.

Selective salpingography is performed by injecting contrast media through a transcervical catheter positioned at the tubal ostia hysteroscopically or under fluoroscopic guidance (Fig. 19.3). Patency is confirmed by laparoscopy or fluoroscopy, respectively. Often, the hydrostatic pressure will relieve an obstruction. If unsuccessful, this is immediately followed with the introduction of a smaller catheter with an atraumatic guidewire through the selective salpingography catheter. The inner catheter and guidewire are advanced through the tubal ostia into the proximal isthmus. Chromotubation is then performed though the catheter to demonstrate patency through the fimbria. Although there is a high success rate of 75–95 % in terms of achieving patency, reocclusion rates average 30 % [23–25]. Despite similar tubal patency rates between hysteroscopic and fluoroscopic tubal cannulation, pregnancy rates are significantly higher with the former technique, 48.9 % vs. 15.6 % [23]. Tubal perforation occurs 2–10 % of the time but is innocuous. Although microsurgical resection and reanastomosis can be used when tubal cannulation fails or when PTO is due to salpingitis isthmica nodosa, IVF is the preferred treatment.

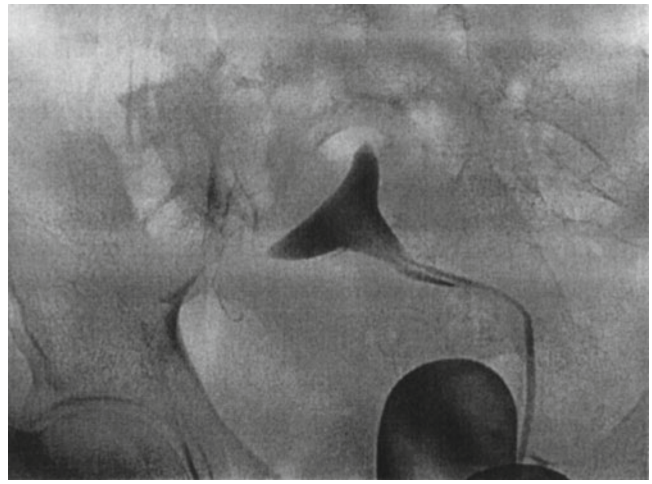

Fig. 19.3 Selective salpingography (reproduced with permission from Al-Fadhli R, Tulandi T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Sterilization Reversal

Tubal sterilization is the most common contraceptive used by women worldwide [26]. Although it should be considered a permanent birth control option, 5–20 % of women experience regret [27], and 1–2 % of women seek reversal of sterilization [28]. In a large prospective, multicenter cohort study of over 11,000 women, regret appeared most strongly associated with younger age at the time of sterilization [27]. Compared with IVF, tubal anastomosis offers patients desiring sterilization reversal the advantage of a one-time, minimally invasive treatment with high success rates. In addition, some patients prefer the ability to attempt conception each month as well as to conceive more than once as well as the avoidance of the inconvenience and risks of IVF. The disadvantages are the possibility of bleeding, infection, inadvertent injury to other organs, and anesthetic complications inherent with any surgery. There is also a higher risk for ectopic pregnancy following tubal reanastomosis. Other factors involved in the decision are the IVF program's success rates, surgeon's ability, presence of other infertility factors, cost, and patient preference. A retrospective cohort study reported significantly higher cumulative pregnancy rates for tubal

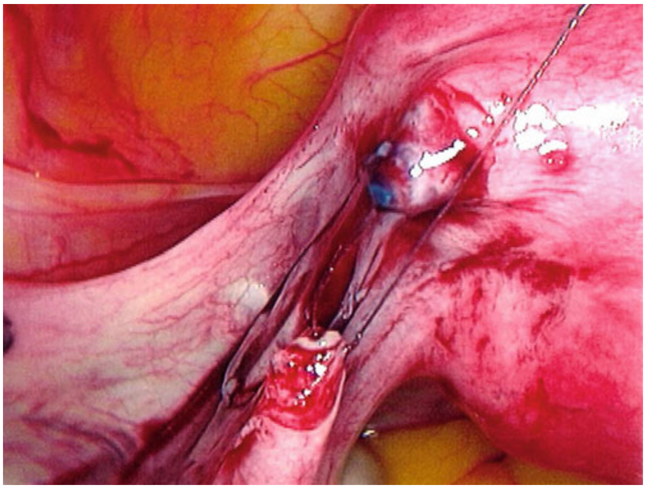

Fig. 19.4 Approximation of tubal lumen (reproduced with permission from Al-Fadhli R, Tulandi T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

anastomosis compared to IVF for women less than 37 years of age, but there was no significant difference in women aged 37 years or older [29]. Furthermore, the average cost per delivery for tubal anastomosis was almost half that of IVF.

The procedure is performed by first mobilizing and opening the occluded tubal ends. A stitch is then placed in the mesosalpinx beneath the tubal ends to align them and relieve tension on the anastomosis. The anastomosis is accomplished with interrupted sutures through the tubal muscularis (Fig. 19.4). The serosa is also repaired with interrupted stitches. Transcervical chromotubation is used to confirm tubal patency. An HSG is recommended if the patient has not conceived within six cycles postoperatively. Tubal anastomosis has usually been performed utilizing an operating microscope by laparotomy with an overnight hospitalization, but outpatient minilaparotomy achieves the same success rate with less cost and discomfort.

Cumulative pregnancy rates after tubal reanastomosis in women under age 40 range from 70 % to over 90 % [28, 30–32]. Even women ages 40–45 years have good success rates of 13–70 %, with subsequent ectopic risks of 2–10 % [28, 30, 32–35]. Most of the pregnancies occur within the first year of surgery. High success rates were also reported with laparoscopic tubal anastomosis, though few possess the skills to perform this [28, 31]. Robotic surgery is a technique that facilitates laparoscopic tubal reanastomosis. Two small studies comparing tubal anastomosis by robotic-assisted laparoscopy vs. laparotomy or minilaparotomy found no difference in pregnancy rates, but robotic surgery took significantly longer and cost more, even compared with laparotomy with overnight hospitalization, though recovery was quicker after robotic surgery [36, 37].

Distal Tubal Disease

The most frequent site for tubal infertility is the distal tube, which manifests as hydrosalpinges and fimbrial phimosis (agglutination of fimbria leading to a narrowed tubal opening). As described earlier, these most often follow PID but can also be preceded by peritonitis from any cause, trauma from previous surgery, or endometriosis. The decision to attempt repair of distal tubal disease is often made at the time of surgery, and categorizing patients as either favorable or poor prognosis may be helpful in guiding management. Tubal reconstruction should not be considered in patients with both proximal and distal occlusion or for women with severe disease, and patients should be counseled preoperatively about the possibility of neosalpingostomy/fimbrioplasty vs. salpingectomy depending on the surgical findings. According to American Fertility Society classifications, a favorable-prognosis patient generally has minimal adnexal adhesions, tubal dilation <3 cm, preservation of normal tubal walls, and endosalpinx [38]. In contrast, poor-prognosis patients may have dense tubal adhesions; significantly dilated tubes; thickened, fibrotic tubal walls; and damaged mucosa.

For favorable-prognosis patients, a neosalpingostomy or fimbrioplasty can be attempted to open up a hydrosalpinx or narrowed tubal opening (Fig. 19.5). This is accomplished laparoscopically, with the release of adhesions and incision at the distal end of the occluded or narrowed tube. The mucosa and fimbria are everted and attached to the serosa using fine suture or electrosurgery. Transcervical chromopertubation is used to confirm patency at the completion of the procedure. Depending on the severity of the disease, pregnancy rates can range from 58 to 77 % in favorable-prognosis patients, with ectopic rates of 2–8 % [39]. As expected, for poor-prognosis patients, pregnancy rates fall to 0–22 %, with ectopic rates of 0–17 % [39]. It should be noted that although patency can often be achieved surgically in both favorable- and poor-prognosis patients, the irreversible damage caused by pelvic infection to the endosalpinx may account for compromised tubal function after surgery.

Hydrosalpinx and IVF

The harmful effect of hydrosalpinx on IVF outcomes has been well documented [40–43]. This observation may be explained by toxic effects of the hydrosalpinx fluid on the embryo, flushing of the embryo from the endometrium by hydrosalpinx fluid, or impaired endometrial receptivity. A meta-analysis of over 5,500 women showed that the implantation and delivery rate per transfer is halved and the miscarriage rate is increased in women with untreated hydrosalpinx undergoing IVF [44]. Several prospective randomized

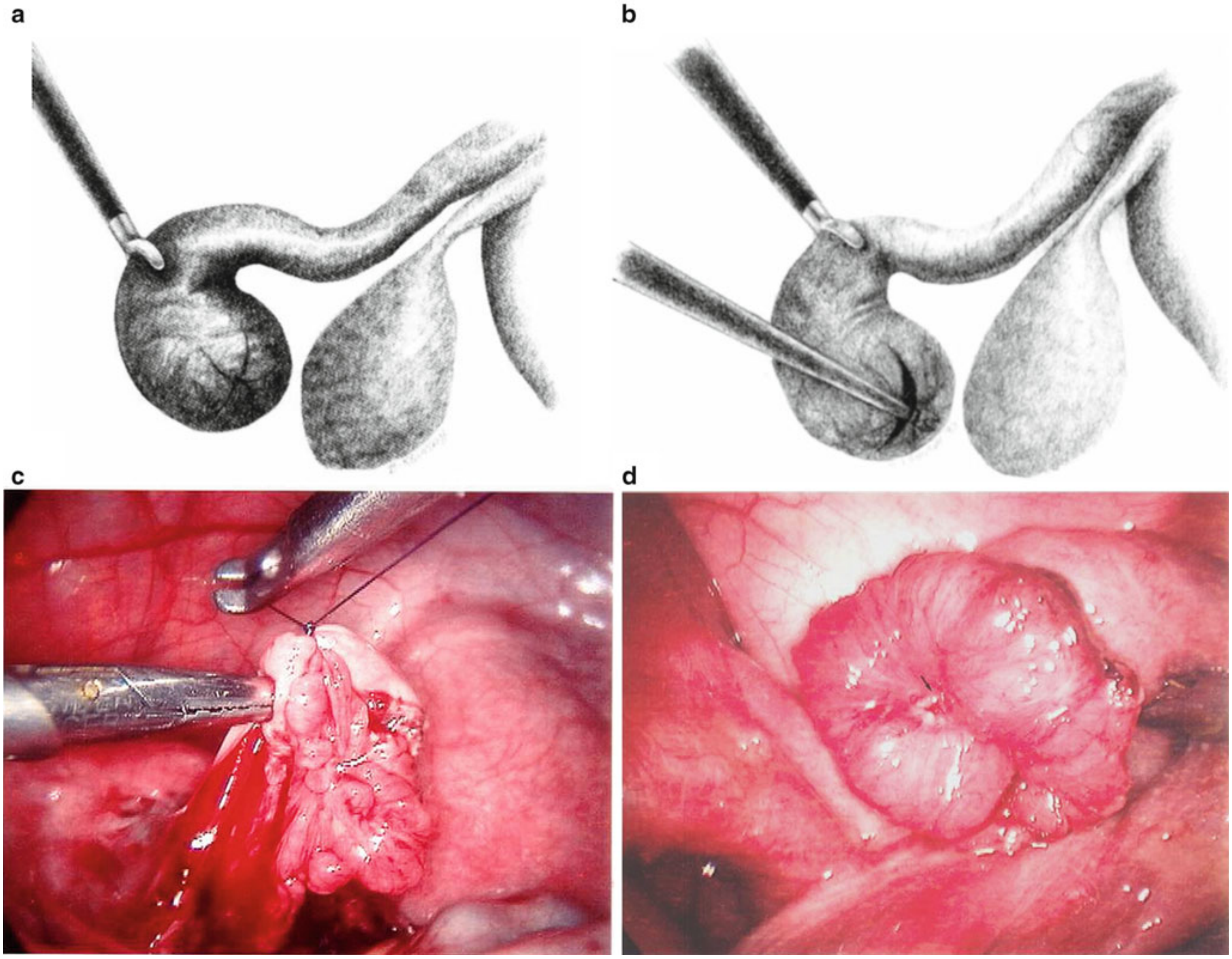

Fig. 19.5 (**a**–**d**) Technique for neosalpingostomy (reproduced with permission from Al-Fadhli R, Tulandi T. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

trials have demonstrated that hydrosalpinges treated with salpingectomy prior to IVF result in restoration of comparable pregnancy rates to controls [45–47]. This finding has been replicated even in patients with unilateral salpingectomy for unilateral hydrosalpinx [48]. A large hydrosalpinx visualized by ultrasound should be removed prior to IVF, as these appear to be associated with the poorest outcomes [44, 45, 49]. An area of greater controversy is whether less pronounced hydrosalpinges (such as those identified by HSG or on laparoscopy) should also be removed in this circumstance.

The gold standard for treatment of hydrosalpinx prior to IVF is laparoscopic salpingectomy; however, other techniques such as proximal tubal ligation or occlusion, ultrasound-guided drainage, or salpingostomy have been suggested. Salpingectomy prior to IVF should be performed laparoscopically, taking care to remain close to the tube to preserve the ovarian blood supply and maintain ovarian reserve. PTO has been demonstrated to be an effective alternative to salpingectomy in cases where salpingectomy is technically difficult or surgery is contraindicated [47, 50]. Pregnancy rates following tubal occlusion appear comparable to treatment with salpingectomy [51]. Most recently, several small case series have demonstrated the effectiveness of using tubal inserts to hysteroscopically occlude hydrosalpinges [52, 53]; however, persistent coils within the endometrial cavity may theoretically limit the success of subsequent ART cycles.

Ectopic Pregnancy

Women with a history of tubal adhesions are at an increased risk of ectopic pregnancy. Ectopic pregnancy is defined by the abnormal implantation of an embryo outside of the endometrial cavity. These pregnancies represent approximately 1.55–2 % of pregnancies and most frequently affect the

Table 19.5 Risk factors for ectopic pregnancy[a]

- Strong associations
  - Tubal surgery
  - Pelvic inflammatory disease
  - Prior ectopic pregnancy
- Weaker associations
  - Infertility
  - Cigarette smoking
  - Increasing age
  - More than one lifetime sexual partner
  - Abdominal or pelvic surgery
  - Sexually transmitted diseases (gonorrhea and/or chlamydia)
  - Intrauterine device use
- No clear association
  - Oral contraceptive use
  - Prior spontaneous miscarriage
  - Prior elective termination of pregnancy
  - Cesarean section

[a]Reproduced with permission from Seeber B, Barnhart K. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

fallopian tube (>90 %), although they can uncommonly involve sites such as the abdomen, ovary, cervix, or cesarean scar [54]. Further, within the tube itself, ectopic pregnancies have a predilection for the ampullary portion of the tube where fertilization occurs (70 %); an additional 10 % occur in the isthmus, 10 % in the fimbria, and 2 % in the uterine cornu or interstitium [55]. Although rates of ectopic pregnancy in the United States appear to have peaked and plateaued in the 1990s [56], the true incidence is difficult to estimate because these pregnancies are increasingly managed as outpatients and therefore may not be included in hospital databases. It does appear that enhanced awareness and improved detection methods have resulted in more favorable outcomes. Nevertheless, ectopic pregnancies remain the leading cause of maternal deaths in the first trimester [57] and must be recognized and managed promptly. Failure to do so can result in fallopian tube rupture, intraperitoneal hemorrhage, shock, and even death.

Abnormal implantations are thought to result mainly from inflammation or blockage within the tubal lumen. As with infertility, most tubal damage that precedes ectopic pregnancy is caused by infection with *N. gonorrhoeae* or *C. trachomatis*. As many as half of women with ectopic pregnancies will have no identifiable risk factors [58]. Known risk factors for ectopic pregnancy include infection, prior ectopic pregnancy, and prior tubal surgery. Although IUD use does not increase the overall risk of ectopic pregnancy, a positive pregnancy test in an IUD user warrants suspicion for ectopic, as the location of the gestation is most likely extrauterine [58]. A complete list of maternal risk factors is shown in Table 19.5. It has also been theorized that chromosomally abnormal embryos may have a higher rate of inappropriate implantation. However, more recent studies with larger patient numbers are not consistent with earlier case reports showing high percentages of abnormal karyotypes from ectopic pregnancies [59, 60].

Of special consideration is the relationship between ART and extrauterine pregnancies. The ectopic risk is increased in patients undergoing treatments ranging from ovulation induction to IVF, perhaps due to preexisting tubal pathology or the effects of supraphysiologic hormonal levels on tubal motility. Ectopic risk after oocyte retrieval and embryo transfer is 4.5 %, significantly higher than what is observed in the general population [61]. The incidence of heterotopic pregnancy (i.e., simultaneous intrauterine and extrauterine pregnancies) is similarly increased in ART patients. In contrast to the classic rate of 1:30,000 pregnancies or even more recent rates of 1:4,000 pregnancies, heterotopic pregnancies in ART patients are estimated to occur as frequently as 1:100 pregnancies [62, 63].

Presentation and Diagnosis

Over the past few decades, increased reports of ectopic pregnancy are likely due to earlier and better methods of detection. Most diagnoses are made based on abnormally rising levels of beta-hCG or characteristic ultrasonographic findings. In recent years, the development of the radioimmunoassay for hCG quantification and the introduction of the specific antiserum to the beta subunit of hCG has improved the clinician's ability to monitor the rise and fall of this hormone in early pregnancy [64]. Similarly, ultrasound technology has progressed to allow higher resolution transvaginal images assisted by Doppler flow to evaluate the adnexa and endometrium.

Although diagnostic methods have recently improved, the typical presentation of ectopic pregnancy outside of the ART setting has remained extremely consistent over time. Pain (99 %) and vaginal bleeding (56 %) are the hallmarks of confirmed ectopic pregnancies [65]. Pain is thought to result from tubal distention and/or peritoneal irritation from hemoperitoneum. However, the sensitivity and specificity of these clinical indicators is low, and clinicians should be aware that these complaints may be intermittent or absent. An important observation is that neither presenting complaints nor beta-hCG levels correlate well with risk of tubal rupture. Studies have shown that low (<100 IU/L) or even decreasing beta-hCG levels can still be associated with ruptured ectopic pregnancy [66, 67].

The physical exam for ectopic pregnancy should begin with vital signs; the combination of hypotension and tachycardia signifies acute blood loss and the need for prompt

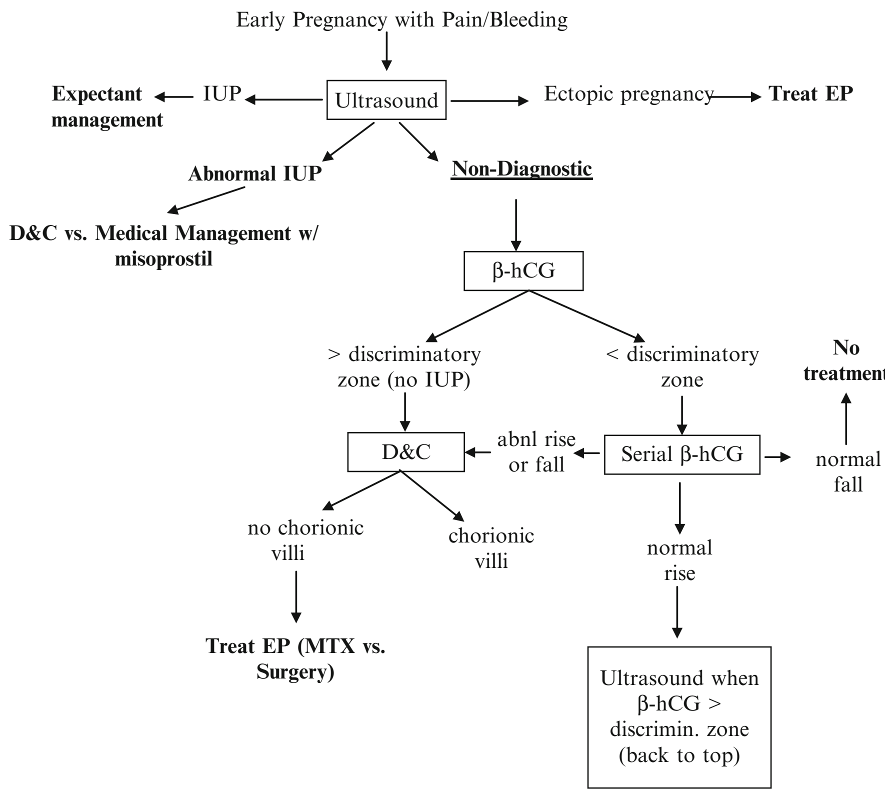

Fig. 19.6 Diagnostic algorithm flow chart (reproduced with permission from Seeber B, Barnhart K. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [78])

surgical intervention and fluid resuscitation. The abdominal exam may reveal signs of peritoneal irritation (e.g., rebound or guarding) or tenderness to palpation. A normal physical exam cannot be used to exclude ectopic pregnancy. The speculum exam should document the presence of blood or products of conception from the cervical os. The adnexa should be gently palpated on bimanual exam; excessive pressure can increase the risk of tubal rupture.

Although suspicion for ruptured ectopic may necessitate a rapid bedside transabdominal ultrasound to verify hemoperitoneum, most ART patients and nonurgent non-ART patients can be investigated further with standard transvaginal ultrasound techniques and beta-hCG levels. An algorithm for diagnosing ectopic pregnancies based on ultrasound and laboratory findings is outlined in Fig. 19.6. A key concept in the evaluation of ectopic pregnancies is the "discriminatory zone." This cutoff is defined as the beta-hCG level above which a single intrauterine gestational sac should be visualized, if present, by transvaginal ultrasound [68]. The discriminatory zone varies by institution but is generally between 1,500 and 2,500 mIU/mL. Care must be taken to differentiate a "pseudosac" (endometrial cavity distended by bleeding from decidualized endometrium) from a true gestational sac. In the presence of multiple gestation, as may be suspected after ART, the discriminatory zone may not be valid; twins and higher-order multiples may have beta-hCG levels above the cutoff value before ultrasound detection is possible [69].

In cases where the beta-hCG is lower than the discriminatory zone, serial beta-hCG measurements will be helpful. Although older gynecologic dogma suggested that beta-hCG levels should rise by 66 % in 2 days, newer data indicate that beta-hCG rise in viable gestations may be considerably slower than previously reported [70]. According to these data, an increase in beta-hCG of less than 53 % in 48 h confirms an abnormal early pregnancy with 99 % specificity. Adhering to this model can minimize the risk of intervening during a potentially viable pregnancy. Serum progesterone levels have limited utility in ectopic pregnancy due to the common scenario of an equivocal progesterone level as well as the long turnaround time for the test at many centers [71]. When an abnormally rising hCG is documented and the distinction cannot be made between failing intrauterine pregnancy and ectopic pregnancy, uterine cavity sampling to look for presence or absence of chorionic villi may be required.

Medical Management of Ectopic Pregnancy

Methotrexate (MTX) is an antimetabolite that has been used for decades in the treatment of ectopic pregnancy and gestational trophoblastic diseases. MTX acts as a folic acid

Table 19.6 Absolute contraindications to methotrexate therapy[a]

• Breastfeeding
• Overt or laboratory evidence of immunodeficiency
• Alcoholism, alcoholic liver disease, or other chronic liver disease
• Preexisting blood dyscrasias (bone marrow hypoplasia, leukopenia, thrombocytopenia, significant anemia)
• Known sensitivity to methotrexate
• Active pulmonary disease
• Peptic ulcer disease
• Hepatic, renal, or hematological dysfunction

[a]Reproduced from Seeber B, Barnhart K. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007; adapted from [77]

antagonist that inhibits cellular DNA and RNA synthesis. MTX arrests mitosis in rapidly dividing tissues such as trophoblast, bone marrow, and orogastric/intestinal mucosa. Side effects such as gastrointestinal symptoms (nausea, vomiting, indigestion, abdominal pain, or stomatitis), as well as more unusual severe effects such as gastritis, enteritis, or pneumonitis [68], are uncommon with regimens used for treating ectopic pregnancy. Hepatotoxicity and bone marrow suppression have also been rarely described. A baseline complete blood count and liver function testing should be obtained prior to MTX treatment. More commonly, abdominal pain and cramping after MTX administration is often reported 2–7 days after the injection and typically represents tubal distention or abortion of necrotic trophoblastic tissue. Without signs of tubal rupture or acute bleeding by laboratory or ultrasonographic criteria, these symptoms can be managed expectantly with reassurance for the patient.

Initial treatment with MTX is appropriate for patients with confirmed or highly suspected unruptured ectopic pregnancies that desire conservative management and are reliable for follow-up. Absolute medical contraindications to MTX therapy are listed in Table 19.6. Relative contraindications for MTX are the subject of some debate; however, many clinicians would restrict MTX treatment to patients with adnexal masses <3.5 cm, no fetal cardiac activity, and beta-hCG beneath a predetermined limit [72]. Although prior hCG thresholds were in the 5,000–15,000 mIU/mL range, a recent systematic review has reported a significant and substantial increase in failure rates (14.3 % vs. 3.7 %) with single-dose MTX when the hCG level exceeds 5,000 mIU/mL [73]. A multi-dose approach or surgical treatment may be preferable in this scenario.

Several regimens have been outlined for the treatment of ectopic pregnancy with intramuscular MTX. Protocols for the single-dose, two-dose, and fixed multi-dose MTX regimens as outlined by the American Congress of Obstetricians and Gynecologists are shown in Table 19.7 [68]. Under the single-dose and two-dose regimens, MTX is administered at a dose of 50 mg/m^2 based on body surface area, which is calculated as follows:

$$\text{BSA}\ (\text{m}^2) = \{[\text{Height (cm)} \times \text{Weight (kg)}]/3{,}600\}^{1/2}$$

A fixed multi-dose protocol adds "rescue" with leucovorin (i.e., folinic acid). For all protocols, a beta-hCG decrease of less than 15 % between days 4 and 7 represents treatment failure and the need for additional management [68]. Although the single-dose regimen is the least complicated and costly and minimizes side effects, a meta-analysis of multiple case series has shown that the fixed multiple-dose regimen is five times more effective than a single-dose regimen [74]. It should be noted that, regardless of treatment regimen, the overall success for treatment with MTX was high (89 %). MTX therapy can be safely pursued in appropriately selected patients; two-dose or fixed multiple-dose regimens should be considered in more advanced gestations or when MTX therapy is pursued in the setting of relative contraindications. All regimens must be followed until beta-hCG levels are <5 mIU/mL to ensure complete resolution of the trophoblastic tissue.

Surgical Management of Ectopic Pregnancy

Ectopic pregnancies can be treated with either chemotherapeutic agents or surgery. Both strategies have high success rates and maintain the potential for future pregnancies. Indications for surgical management of ectopic pregnancy are listed in Table 19.8. The traditional surgical approach for ectopic pregnancy was exploratory laparotomy with salpingectomy. Today, laparoscopy with tubal preservation (i.e., salpingostomy) is usually possible. Exploratory laparotomy is still used when patients are unstable following tubal rupture, when the laparoscopy would be complex (significant adhesions, extratubal gestation) or contraindicated, or when clinical proficiency in laparoscopy is lacking.

Laparoscopic salpingectomy can be accomplished with either a combined coagulation-cutting device or by sequential use of bipolar cautery and laparoscopic scissors. Care should be taken to stay close to the tube to avoid compromising the ovarian blood supply. The more conservative approach via linear salpingostomy involves an incision on the antimesenteric surface of the tube using the unipolar needle or scissors, laser, or ultrasonic scalpel. Prior to that, dilute vasopressin is injected in the mesosalpinx beneath the ectopic to improve hemostasis and to minimize tubal damage from excessive cauterization. After tubal incision, the trophoblastic tissue is then either gently teased or irrigated from the tube, taking into consideration that the majority of tissue is usually located on the more proximal aspect of the incision. Failure to remove all trophoblastic tissue can lead to persistent ectopic pregnancy or post-ectopic tubal obstruction; a Cochrane review concluded that persistent ectopic rates after

Table 19.7 Methotrexate protocols for ectopic pregnancy[a]

Treatment day	Laboratory tests	Intervention
Pretreatment	*B*-hCG, CBC with differential, LFTs, creatinine, type and screen	Rule out SAB RhoGAM if Rh-negative
Single-dose protocol		
1	*B*-hCG	MTX 50 mg/m^2 IM
4	*B*-hCG	
7	*B*-hCG	MTX 50 mg/m^2 IM if *B*-hCG <15 % decrease day 4 to 7
Two-dose protocol		
Pretreatment	*B*-hCG, CBC with differential, LFTs, creatinine, type and screen	Rule out SAB RhoGAM if Rh-negative
0	*B*-hCG	MTX 50 mg/m^2 IM
4	*B*-hCG	MTX 50 mg/m^2 IM
7	*B*-hCG	MTX 50 mg/m^2 IM if <15 % decline day 4 to 7. If >15 % stop treatment and start surveillance
11	*B*-hCG	MTX 50 mg/m^2 IM if <15 % decline day 7 to 11. If >15 % consider surgical treatment
Multi-dose protocol		
1	*B*-hCG	MTX 1.0 mg/kg
2		LEU 0.1 mg/kg
3	*B*-hCG	MTX 1.0 mg/kg if <15 % decline day 1 to 3. If >15 % stop treatment and start surveillance
4		LEU 0.1 mg/kg
5	*B*-hCG	MTX 1.0 mg/kg if <15 % decline day 3 to 5. If >15 % stop treatment and start surveillance
6		LEU 0.1 mg/kg
7	*B*-hCG	MTX 1.0 mg/kg if <15 % decline day 5 to 7. If >15 % stop treatment and start surveillance
8		LEU 0.1 mg/kg
Surveillance every 7 days (until *B*-hCG <5 mIU/mL)		

β(beta)-hCG β(beta)-human chorionic gonadotropin; *CBC* complete blood count; *LFTs* liver function tests; *MTX* methotrexate; *SAB* spontaneous abortion
[a]Adapted from [26]

Table 19.8 Indications for surgical management of ectopic pregnancy

- Hemodynamic instability
- Signs of tubal rupture
- Simultaneous intrauterine pregnancy
- Unable to adhere to follow-up plan
- Contraindications to MTX (see Table 19.6)
- NB: Surgery may also be considered with ectopic size >3.5 cm, presence of fetal cardiac activity, or elevated beta-hCG due to higher risk of MTX failure

salpingostomy can be reduced when coupled with a prophylactic single injection of MTX [75].

When choosing between salpingostomy and salpingectomy, the most conservative approach possible (i.e., salpingostomy) is advised in patients wishing to preserve their fertility. However, in cases complicated by severe tubal damage, adhesions, or rupture, large ectopic size (>5 cm), or bleeding from the incision site, a salpingectomy may be the only feasible strategy. Additional factors such as previous ectopic in the same tube, prior tubal reanastomosis or other tubal surgery, or undesired future fertility are also important when determining a surgical approach. In a multi-study review, linear salpingostomy was associated with a 15 % recurrent ectopic rate and 60 % successful postoperative pregnancy rate [64]. The risk of persistent ectopic pregnancy with tube-sparing surgery was 3–20 %. For salpingectomy, this same review cited a recurrence risk of 10 % and a subsequent pregnancy rate of 38 %. When possible, tubal conservation is recommended because it may increase future fecundity; however, patients must be carefully followed owing to the concern for persistent ectopic, recurrent ectopic, and post-ectopic tubal obstruction. Serial beta-hCG levels should be performed weekly until they reach non-detectable values to ensure resolution of the ectopic pregnancy after salpingostomy.

Expectant Management

Increased monitoring and early pregnancy detection now prompt the clinician to intervene when ectopic pregnancy is suspected. However, prior to sensitive hCG assays and high-resolution ultrasonography, it is likely that many early tubal pregnancies resolved spontaneously without adverse outcomes. With close and careful follow-up, patients with low and decreasing beta-hCG levels may be appropriate for expectant management, but these patients must be counseled and accept the risk of tubal rupture and its associated morbidities. In one prospective observational study of over 100 women, spontaneous resolution rates were 88 % when the first hCG level was <200 IU/L [76].

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20 Endometriosis

Dan I. Lebovic and Tommaso Falcone

Introduction

Endometriosis is an incorrigible disease that is responsible for a multitude of hospital admissions and operative procedures [1]. This disease is typically a chronic, progressive disorder, with pelvic pain and infertility as the hallmark symptoms. Owing to the recognition of more subtle forms of endometriosis and the more liberal use of diagnostic laparoscopy, the prevalence has been increasing.

Prevalence of Endometriosis

Endometriosis affects nearly 10 % of reproductive-age women between the ages of 12 and 80 years, with the average age at diagnosis around 28 years. Several conditions are associated with higher rates of disease: up to 50 % of those with subfertility or chronic pelvic pain and nearly 70 % of adolescents with nonresponsive pelvic pain. The incidence of ovarian cystic endometriosis (endometriomas) increases with age [2], but its incidence and progression seem to stabilize in patients who are older than 35 years. Based on a study that involved repetitive laparoscopy in baboons, we know that endometriosis appears spontaneously and that new lesions seem to appear and disappear spontaneously [3]. Thus, endometriotic lesions in humans most likely are in continuous evolution as well [4].

Diagnosis of Endometriosis

The definitive diagnosis of endometriosis can only be made surgically. The classic symptoms are dysmenorrhea, noncyclic pelvic pain, dyspareunia, and infertility. Specific physical signs indicative of advanced endometriosis are listed in Table 20.1. Physical examination during menstruation is more sensitive in the detection of pelvic disease. There are no laboratory tests that can identify patients with endometriosis with a high degree of sensitivity and specificity. The search for biomarkers of this disease is quite active [5], although further studies are required before clinical use can be recommended.

The microscopic definition of endometriosis implies the presence of endometrial glands and stroma outside the endometrial cavity and uterine musculature. The various typical lesions of endometriosis are referred to as clear, white, red, polypoid, flame-like, powder-burn, brown, blue-black, brown, or yellow lesions (Fig. 20.1). Defects in the peritoneum or peritoneal windows may contain these lesions. The R-AFS scoring system for endometriosis is widely used clinically but has significant intra- and interobserver variation (between 38 and 52 %) [6].

The positive predictive value of laparoscopic visualization of endometriosis is considered to be approximately 50 %. Classic red or black lesions have a visual accuracy between 90 and 100 %. White lesions are associated with endometriosis less often. The main pathologies that can be confused with endometriosis are endosalpingiosis, fibrosis, mesothelial hyperplasia, carbon deposits from previous surgery, and malignancy. Hemangiomas, adrenal rests, and splenosis can also rarely be confused with endometriosis. For this reason, diagnostic laparoscopy should be accompanied with biopsy for lesions that are not clearly endometriosis.

Ovarian endometriosis, as opposed to non-ovarian disease, can be predicted with a high degree of accuracy with ultrasonography. There may be a role for magnetic resonance imaging (MRI) in the identification of deeply infiltrating

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Table 20.1 Signs and symptoms of endometriosis

Signs
Adnexal mass
Adnexal tenderness
Uterine tenderness
Fixed retroversion
Lateral cervical displacement
Cul-de-sac
Tenderness
Nodularity
Mass
Uterosacral ligament
Tenderness
Nodularity
Vaginal lesions
Cervical lesions
Symptoms
Reproductive tract
Infertility
Dysmenorrhea
Dyspareunia
Noncyclic pelvic pain
Gastrointestinal
Diarrhea and/or constipation
Tenesmus
Abdominal cramps
Cyclic rectal bleeding
Urinary
Low-back pain

Fig. 20.1 Typical *black* and *white* lesions of the pelvic peritoneum with dense fibrosis and retraction

lesions that involve the cul-de-sac or lesions in uncommon locations such as the sciatic nerve. Diagnostic tests for patients with gastrointestinal symptoms such as colonoscopy or barium enema radiography are typically normal or may occasionally show stricture. Patients with significant urinary symptoms should have a urologic evaluation to rule out interstitial cystitis or potentially endometriosis of the bladder wall.

The more common differential diagnoses of patients with chronic pain and potential endometriosis are adhesions, chronic pelvic inflammatory disease, interstitial cystitis, irritable bowel syndrome, and musculoskeletal problems such as myofascial pain or neuralgias.

Classification of Endometriosis

The classification of endometriosis has been an evolving process. In 1978, the American Fertility Society (AFS, now called the American Society for Reproductive Medicine) classified four stages and used an arbitrarily weighted point score that included assessment of the extent of endometriosis in two dimensions and the presence and extent of adhesions in the peritoneum, ovaries, and tubes. It also took into account whether the endometriosis was unilateral or bilateral. The size of the endometrioma was considered along with the presence of filmy vs. dense adhesions. In 1985 and again in 1996 (R-AFS), further revisions were made to the original AFS classification [7] (Fig. 20.2). An endometrioma that is larger than 3 cm in diameter is at least stage 3 disease. Despite the improvements, the correlation between the stage of endometriosis and the likelihood of pregnancy or degree of pain is poor, and future improvement in the classification is both warranted and likely.

Associated Disease Processes

Surveys of endometriosis patients report increased incidence of atopic disease and other autoimmune phenomenon such as thyroid disease, fibromyalgia, and chronic fatigue syndrome. Endometriosis is associated with increased incidence of non-Hodgkin's lymphoma, dysplastic nevi, and melanoma. Ovarian cancer (endometrioid and clear cell) is higher, although the overall lifetime risk is quite low: 1.5 % compared to 1 % in the general population.

Anatomic Sites of Endometriosis

Endometriosis is most commonly found in the posterior pelvis compartment [8]. The following are the most common locations, in descending order: ovaries, cul-de-sac, broad ligament, and uterosacral ligaments. The left hemipelvis is the most common location—64 % compared to the right hemipelvis. More endometriosis is found on the left as opposed to the right ovary, possibly because the sigmoid colon alters intraperitoneal fluid movement.

The bowel is the most common extragenital location of endometriosis. Bowel locations, in decreasing order of frequency, are the sigmoid colon (>65 % of cases), rectum, terminal ileum, appendix, and the cecum. Most bowel lesions

Stage I (Minimal) - 1-5
Stage II (Mild) - 6-15
Stage III (Moderate) - 16-40
Stage IV (Severe) - >40
Total__________

Laparoscopy______ Laparotomy______ Photography______
Recommended Treatment______

Prognosis______

	ENDOMETRIOSIS	<1cm	1-3cm	>3cm
PERITONEUM	Superficial	1	2	4
	Deep	2	4	6
OVARY	R Superficial	1	2	4
	Deep	4	16	20
	L Superficial	1	2	4
	Deep	4	16	20

	POSTERIOR CULDESAC OBLITERATION	Partial	Complete
		4	40

	ADHESIONS	<1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
OVARY	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
TUBE	R Filmy	1	2	4
	Dense	4*	8*	16
	L Filmy	1	2	4
	Dense	4*	8*	16

*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.

Denote appearance of superficial implant types as red [(R), red, red-pink, flamelike, vesicular blobs, clear vesicles], white [(W), opacifications, peritoneal defects, yellow-brown], or black [(B) black, hemosiderin deposits, blue]. Denote percent of total described as R___%, W___% and B___%. Total should equal 100%.

Fig. 20.2 ASRM revised classification of endometriosis

are superficial and limited to the serosa. Occasionally, transmural involvement of the bowel occurs, which may cause cyclic diarrhea, rectal bleeding, abdominal distension, and, rarely, bowel obstruction. The urinary tract is involved in only 1 % of cases, and it most often affects the bladder (84 %). Symptoms for vesical endometriosis are similar to those associated with recurrent cystitis. Endometriosis of the urinary tract should be suspected if cyclical urinary symptoms, such as urgency, frequency, and suprapubic pain with or without hematuria, occur.

Ovarian Endometriosis

Ovarian endometriosis or endometriomas increase with age and are generally associated with a more advanced stage of the disease [2]. This form of endometriosis can be diagnosed with a high level of accuracy by serial ultrasounds. They may be confused with a hemorrhagic corpus luteum, which will disappear over the course of a few months. These ovarian forms of endometriosis often have associated peritoneal implants (85 % of the time), although only a small percentage of patients with peritoneal implants will eventually develop an endometrioma. Endometriomas can be uniloculated or multiloculated. They are more commonly localized in the left ovary, as with peritoneal implants, likely due to the natural peritoneal fluid flow subsequent to menstrual regurgitation.

Deep Endometriosis

Invasion of endometriotic cells deeper than 5 mm has been associated with increased pain [9]. A rectovaginal exam during the menstrual period in the office setting or a thorough exam under anesthesia prior to laparoscopy may alert the surgeon to the presence of these types of lesions. In a study looking at 93 women with deep infiltrating peritoneal endometriosis, 61 % had concomitant superficial implants and 51 % had endometriomas. Deep nodules were the only form of the disease in just 7 % of the women.

Extra-Pelvic Endometriosis

Cutaneous endometriosis has been reported in abdominal scars following cesarean sections, hysterectomy, appendectomy, and laparoscopy. Rare lung cases of endometriosis leading to cyclical hemoptysis or even pneumothorax have been reported and imply that hematogenous and/or lymphatic dissemination of endometrial cells is possible. This mechanism can also explain the possible spread to rare locations, such as the brain, liver, pancreas, kidney, vertebra, and bones.

Predisposing Factors for Endometriosis

Endometriosis is mainly present during the reproductive years (average age of 28) and regresses during menopause, suggesting that the development and growth of endometriosis is estrogen dependent. Accordingly, the Nurses' Health Study prospectively assessed predisposing factors for endometriosis and observed an association with early age of menarche, shorter length of menstrual cycles during late adolescence, and nulliparity. Furthermore, women with low estrogen levels and low body mass index, who use alcohol, who are infertile smokers, and who exercise intensely appear to be at decreased risk [10].

Heredity is an important predisposing factor for endometriosis since the prevalence is increased sevenfold among first-degree relatives. In monozygotic twins, the prevalence increased 15-fold. Exposure to pollutants, especially endocrine-disrupting compounds such as dioxins or polychlorinated biphenyls (PCBs), might also play a role in the predisposition to endometriosis.

Data from the Nurses' Health Study II suggests that specific dietary fat consumption may influence the risk of developing endometriosis—long-chain omega-3 fatty acids were protective, whereas *trans*-unsaturated fats led to a greater risk.

Pathophysiology

Managing endometriosis receives the lion share of attention, although investigation into the genesis of the disease does not lag far behind. In fact, it is likely that only with the discovery of the true pathogenesis of endometriosis will more efficacious therapy emerge as well as preventative measures for younger women. The three different endometriosis entities—endometriomas, implants, and rectovaginal disease—could develop along distinct routes, but overlapping mechanisms are probably at play for at least some of these. The disease has multitudinous theories for pathogenesis, yet only a handful continue to be proffered as valid: (1) retrograde menstruation (Sampson's theory), (2) metaplastic transformation (Meyer's theory), (3) lymphatic or hematogenous embolization (Halban's theory), and (4) tissue relocation (i.e., iatrogenic surgical displacement of endometrium during laparoscopy or cesarean section).

Over the years each theory has received indirect corroboration. Retrograde menstruum from the fallopian tubes into the pelvis and beyond has been supported by identifying menstrual tissue refluxing from the fallopian tubes during surgery and the identification of fresh endometrial lesions during menstrual phase laparoscopy. In addition, the baboon model of endometriosis is, in effect, iatrogenic retrograde menses invariably leading to the development of scattered lesions [11]. Lastly, a greater frequency of lesions in the right subphrenic region and left hemipelvis/ovary supports retrograde menses, since these locations follow the natural tendency of intra-abdominal peritoneal flow and obstruction via the falciform ligament. Unclear, however, is why endometriosis is not then found in all women, given the ubiquitous nature of retrograde menstruation.

Metaplasia of the coelomic epithelium seems equitable given that both peritoneal and endometrial tissues emanate from coelomic cells. Zheng et al. have shown histologic, morphologic evidence of transitioning ovarian surface epithelium into endometriotic cells, corroborating a metaplastic process [12]. A corollary to this postulate is that of the embryogenetic theory or müllerianosis: misplaced endometrial tissue during the embryologic period of organogenesis. Signorile et al. demonstrated the presence of ectopic endometrium in 9 % of 101 human female fetuses [13]. With endogenous estradiol stimulation later in life, this tissue could grow and thus present as ectopic implants. Theoretically, deep rectovaginal lesions could derive from such abnormal embryogenesis.

Newer, exciting pathophysiology theories borrow from the traditional theories and, most significantly, build upon these premises in order to better grasp the true etiology. For instance, stem cells originating from the bone marrow (Meyer's theory) have been found to populate eutopic endometrium (Halban's theory) that may then be shed (Sampson's theory) into the peritoneal cavity. Vercellini et al. provided another concept for the development of endometriomas when they described how a hemorrhagic corpus luteum may progress to an endometrioma [14]. If this were truly an endometrioma, then retrograde menses (Sampson's theory) would be a prerequisite to populate the cyst contents with endometrial cells.

Underlying virtually all of these theories is the molecular underpinnings of the disease and, in particular, the inherent immune dysfunction that could at once promote and sustain endometriosis [15]. The aberrant immune factors found in women affected with endometriosis could explain why some develop the disease while others do not. The chronic inflammatory milieu can impair normal clearance of endometrial tissue and encourage adherence/invasion, angiogenesis, and nerve fiber innervation [16].

Mechanism of Infertility

Even though there is a purported association between infertility and endometriosis, the mechanism of this association remains complex and is not completely understood [17]. The following factors may explain a diminished fecundity:

- *Anatomical changes*. Endometriosis, when moderate or severe, will often lead to peritubal or periovarian adhesions, thus compromising tubal motility and ovum capture.
- *Immunological factors*. The peritoneal fluid of women with endometriosis has an abnormal level of cytokines, prostaglandins, growth factors, and inflammatory cells, which are likely to participate in the etiology and or sustenance of endometrial implants. These alterations negatively affect sperm motility, oocyte maturation, fertilization, embryo survival, and tubal function.
- *Effect on embryo development and implantation*. Patients with stage I and II endometriosis have high levels of anti-endometrial antibodies, which may reduce implantation. IL-1 and IL-6 are elevated in the peritoneal fluid of patients with endometriosis and are embryotoxic. Expression of HOXA10 and HOXA11 genes, which are usually upregulated during the secretory phase of the menstrual cycle, is not upregulated in patients with endometriosis. These genes regulate the expression of α(alpha) vβ(beta)3 integrin, which plays a crucial role in the embryo's ability to attach to the endometrium. A decrease in αvβ3 and L-selectin expression has been reported in patients with endometriosis, which might explain the decrease in implantation.

Mechanism of Pain

Pain associated with endometriosis is quite complex. Pain associated with advanced disease can be caused by extensive adhesions, ovarian cysts, or deeply infiltrating endometriosis. Expression of nerve growth factor is associated with endometriosis pain. Sensory nerve fibers have been found more frequently in the functional layer of the endometrium of women with endometriosis than those unaffected by the disease. Finally, discrete changes in the central pain system (i.e., regional gray matter volume) may contribute to chronic pain in women with endometriosis.

Even patients with early-stage disease (few scattered implants) can experience severe pain. This pain can be explained in part by the increase in prostaglandins. In contrast to the normal endometrium (referred to as eutopic endometrium), ectopic endometrium (endometriosis) is the site of at least two molecular aberrations that result in the accumulation of increasing quantities of estradiol and prostaglandin E2 (PGE2). With the first aberration, activation of the gene that encodes aromatase increases, leading to increased aromatase activity in endometriotic tissue. This activation is stimulated by PGE2, which is the most potent inducer of aromatase activity in the endometriotic stromal cells. The second important molecular aberration in endometriotic tissue is the increased stimulation of COX-2 by estradiol, which leads to increased production of PGE2. This establishes a circular event leading to accumulation of PGE2 in the endometriotic tissue.

Treatment of Endometriosis in the Infertile Couple

It is estimated that in an infertile couple with stage 1 or 2 endometriosis, the monthly fecundity rate is 3 % per cycle. Medical suppressive therapy with an oral contraceptive agent or gonadotropin-releasing hormone agonist (GnRHa) does not improve the pregnancy rate prior to trying nonassisted reproductive technology.

Surgical Treatment

Surgical treatment of minimal or mild endometriosis improves the spontaneous pregnancy rate; however, the absolute amount is small. A meta-analysis of the two randomized clinical trials investigating this question showed a mild improvement with a number needed to treat (NNT)—that is, the number of persons that would need to be treated surgically to achieve an extra pregnancy—of 12 (95 % CI, 7, 49).

Postoperative suppressive medical therapy after surgical treatment does not improve fertility. The only value of medical suppressive therapy (i.e., GnRHa) appears to be before in vitro fertilization (IVF) [18]. In these cases, the use of GnRHa for 3–6 months prior to IVF increases the clinical pregnancy rate by a factor of 4 (OR 4.28, 95 % CI, 2, 9.15). What is not clear for the moment is if a specific disease severity may have a better response to such suppressive therapy.

If the patient does not wish surgical therapy, then the next step is either IVF or treatment with clomiphene citrate and intrauterine insemination (IUI) followed by gonadotropins and IUI. There is insufficient evidence to recommend surgery prior to IVF.

In advanced disease, surgical management improves fertility. However, this surgery is complex and requires meticulous dissection. If an initial surgery for advanced disease fails, subsequent surgery is less successful than IVF in establishing a pregnancy and should be reserved for patients who require management of pain.

In Vitro Fertilization

In a retrospective cohort study, the diagnosis of endometriosis (without endometriomas) was associated with similar IVF pregnancy rates [19] compared with tubal factor infertility. IVF offers the best fecundity rate for those with endometriosis. Admittedly, cost may be a limiting factor.

Endometriomas

Endometriomas larger than 4 cm should be removed to confirm they are benign. In a randomized trial, excision of the endometrioma was associated with less recurrence and higher spontaneous fertility rates than fenestration and bipolar coagulation [20]. IVF outcomes have been reported to be similar in patients with and without endometriomas. However, the number of oocytes, fertilization rates, and the number of embryos obtained were decreased in women with endometriomas compared with those without endometriomas. The preponderance of evidence suggests that in symptomatic women, one can safely remove endometriomas without compromising ovarian function or the success of assisted reproduction. Accidental puncture of an endometrioma during oocyte retrieval may cause infection or contamination of follicular fluid. Histologic confirmation of the benign nature of a large endometrioma may be prudent.

Interestingly, in a retrospective chart review, the higher the rate of endometrioma recurrence, the greater the antral follicle count in the affected ovary. This may be a consequence of less ovarian trauma at the time of cystectomy [21]. Simple drainage of an endometrioma has been shown to be ineffective.

Treatment of Patients with Chronic Pelvic Pain

Medical Management for Pain

Several classes of drugs have traditionally been used to manage pain associated with endometriosis (Table 20.2). Progestins or combined oral contraceptives and nonsteroidal anti-inflammatory drugs are used as first-line therapy for chronic pain associated with endometriosis. In a prospective, randomized controlled trial comparing combined oral contraceptives with GnRHa, both treatment arms led to similar pain relief [22].

Progestins

Subcutaneous medroxyprogesterone acetate (Depo-subQ provera 104, Pfizer, New York, NY, USA) administered every 12–14 weeks subcutaneously was approved by the US Food and Drug Administration (FDA) for the treatment of endometriosis-related pelvic pain. The bone loss seems to be less

Table 20.2 Drugs used for the treatment of endometriosis[a]

Class	Drug	Dosage
Androgen	Danazol[b]	100–400 mg PO twice a day
		100 mg per vagina daily
Aromatase inhibitor	Anastrozole[c]	1 mg PO daily
	Letrozole[c]	2.5 mg PO daily
Estrogen–progestin combinations	Monophasic estrogen/progestin[b]	Low ethinyl estradiol dose continuously
Gonadotropin-releasing hormone agonist	Goserelin[b,c]	3.6 mg SC monthly (10.8 mg IM every 3 months)
	Leuprolide depot[b,c]	3.75 mg IM monthly (11.75 mg IM every 3 months)
	Nafarelin[b,c]	200 μg intranasally twice a day
Gonadotropin-releasing hormone antagonist	Cetrorelix	3 mg SC weekly
Progestin	Depo-subQ provera 104[b]	104 mg/0.65 mL SC every 3 months
	Dienogest	2 mg PO daily[d]
	Etonogestrel-releasing implant	1 for 3 years
	Levonorgestrel-releasing IUS	1 for 5 years
	Medroxyprogesterone acetate	30 mg PO daily for 6 months, followed by 100 mg IM every 2 weeks × 2 months, then 200 mg IM monthly × 4 months
	Norethindrone acetate[b]	5 mg PO daily

SC subcutaneously; *IM* intramuscularly; *IUS* intrauterine system
[a]Adapted from [4]
[b]FDA approved for endometriosis
[c]With add-back therapy, that is, norethindrone acetate 5 mg daily + vitamin D 800 IU daily + calcium 1.25 g daily
[d]Dienogest is a 19-nortestosterone derivative that is approved in the European Union for the treatment of endometriosis. It is not available in the United States as a separate drug. It is only available in the oral contraceptive Natazia (Bayer Pharmaceuticals, Montville, NJ, USA) (estradiol valerate/dienogest), which is a newer four-phasic pack that contains dienogest

pronounced than with the use of GnRHa without add-back therapy. However, there are no data yet with the prolonged use of Depo-subQ, and the recommendation is not to use the drug for more than 2 years unless other methods are unacceptable. Note that the rate of abnormal vaginal bleeding while on Depo-subQ was 17 %.

There are several other progestins that have been used for the treatment of endometriosis-associated pelvic pain. The FDA also approved norethindrone acetate (NETA) 5 mg daily with a GnRHa. Through NETA's estrogenic activity, there is a beneficial effect on both bone mineral density and vasomotor symptoms; 5 mg of NETA is equivalent to 20–30 μg of oral ethinyl estradiol. The levonorgestrel intrauterine system has been successfully used for symptomatic endometriosis. Trials have demonstrated pain relief and decreased menstrual blood loss.

Gonadotropin-Releasing Hormone Agonists

GnRH agonists can be given via an intramuscular (leuprolide acetate), subcutaneous (goserelin), or nasal (nafarelin) route. After an initial increase in gonadotropins during the first 10 days, there is a decrease in pituitary secretion secondary to GnRH receptor downregulation. These drugs are typically given for an initial 6-month course. The majority of patients (75–80 %) in clinical trials responded. However, many patients will have recurrence of pain within 5 months. A main concern is the progressive loss in bone mineral density. Menstrual periods return between 2 and 3 months after the last monthly injection, but recovery of bone mineral density takes more time.

Add-back therapy with estrogens or progestins was introduced as a way to reduce the hypoestrogenic side effects of GnRHa, especially the loss in bone mineral density, but also vasomotor symptoms and atrophic vaginal mucosa. Other symptoms such as insomnia, mood disorders, and cognitive dysfunction may occur. The mean decrease in bone mineral density after 1 year of GnRHa treatment without add-back is between 3 and 7 %.

Add-back therapy has been proposed for both short-term (less than 6 months) and long-term use (more than 6 months). Many studies have shown that the efficacy is not reduced. Norethindrone (5 mg orally daily) is the most commonly used add-back regimen. Low-dose estrogen (conjugated equine estrogen 0.625 mg) can also be added to the norethindrone without loss of benefit in symptom control. Higher doses of estrogen (i.e., combined oral contraceptives) are associated with diminished efficacy in relieving pain symptoms.

Aromatase Inhibitors

Aromatase inhibitors have been successfully used in a limited number of cases of persistent disease after hysterectomy and bilateral oophorectomy as well as in patients with intact pelvic organs. The putative mechanism is by suppression of locally produced estrogens from aromatase activity expressed by the endometriotic cells. Typically, an aromatase inhibitor such as letrozole 2.5 mg or anastrozole 1 mg daily is given with NETA (5 mg daily) to prevent ovarian cyst formation and a possible decrease in bone mineral density.

Surgical Management for Pain

Two prospective randomized controlled studies have clearly shown that surgical therapy is superior to no treatment for relief of pain from endometriosis [23]. One randomized controlled trial with only 16 women did not show a statistically significant difference in pain relief [24].

Several observations can be surmised from these studies: (1) surgery is more effective than simple diagnostic laparoscopy in the treatment of pain associated with endometriosis; (2) there is a significant placebo effect associated with surgery, especially early on (3 months), that persists in approximately 20 % of patients; (3) between 20 and 40 % of patients will not respond to surgery and will continue to experience pain; (4) surgery is least effective for early-stage disease.

It is unclear if excision of endometriosis is superior to simple ablation by cautery or laser. Postoperative treatment with suppressive therapy with a GnRHa for 6 months may delay recurrence of symptoms.

Neurectomy

Interruption of the cervical and uterine sensory nerves by transection of the uterosacral ligaments, a uterosacral nerve ablation, has been shown not to have any long-term benefit [25]. A presacral neurectomy has been shown to be beneficial in patients with chronic pelvic pain and endometriosis when performed with surgical treatment of the endometriosis lesions [26]. The procedure is associated with greater relief of midline pain rather than lateral pain. The success of the procedure is dependent on excision of the superior hypogastric plexus (presacral nerve) before extensive branching has occurred. The most common postsurgical complications are constipation and urinary urgency.

Hysterectomy

Hysterectomy with or without bilateral salpingo-oophorectomy can be considered in patients whose disease fails to respond to conservative management and who do not desire future fertility. Most studies have shown significant pain relief from definitive surgery. Caution should be used in recommending oophorectomy in women less than 40 years of age [27].

Post-hysterectomy Recurrence

Endometriosis can recur in 5–10 % of patients after hysterectomy and bilateral salpingo-oophorectomy. The role of hormone replacement therapy after surgical castration is controversial. There is a possibility of symptom or disease recurrence (3.5 %). On the other hand, there is the real possibility of severe vasomotor symptoms and osteoporosis. Hormone replacement therapy is not contraindicated, and the risks and benefits should be discussed with the patient.

Rectovaginal Endometriosis

The management of rectovaginal endometriosis is extremely difficult and usually involves the rectosigmoid. Patients usually have severe symptoms that may involve the gastrointestinal tract such as constipation, diarrhea, and painful bowel movements. However, some patients with rectovaginal endometriosis are asymptomatic. These patients do not need treatment.

Management of Endometriosis on Extragenital Organs

Gastrointestinal Endometriosis

Although gastrointestinal symptoms are quite common in women with endometriosis, the overall incidence of bowel involvement is reported to be around 5 %. Endometriosis of the gastrointestinal system typically involves the rectum or rectosigmoid. Recurrence of disease after hysterectomy and oophorectomy more commonly involves the bowel. Excision of disease or intestinal resection can be performed by laparotomy or by laparoscopy. Rectovaginal fistula and abscess formation are the most serious complications reported.

Respiratory System

Diaphragmatic endometriosis can be asymptomatic and noted incidentally at diagnostic laparoscopy (Fig. 20.3). Symptomatic patients often report right-sided chest pain or shoulder pain in association with menstruation that occasionally radiates into the neck or arm and dyspnea. Asymptomatic diaphragmatic lesions do not need treatment. Electrosurgery, laser, or surgical excision should be performed carefully because the thickness of the diaphragm ranges between 1 and 5 mm.

Thoracic endometriosis most commonly presents as a right-sided catamenial pneumothorax but can also be manifested by hemothorax, hemoptysis, or pulmonary nodules. The typical symptoms are chest pain and dyspnea. Approximately 30 % of these women have pelvic endometriosis at the time of surgical management of the thoracic disease. A chest CT scan may demonstrate pulmonary or pleural nodules, especially if performed during a menstrual period. Chemical pleurodesis is associated with a lower recurrence rate of catamenial pneumothorax than hormonal treatment. However, initial treatment with hormonal therapy is indicated.

Genitourinary System

Endometriosis often involves the peritoneum over the ureter. However, direct ureter involvement is uncommon and has been reported in less than 1 % of patients; it is predominantly left-sided (63 %) when this is observed. Ureteral involvement can be the result of extrinsic compression from extensive endometriosis that surrounds the ureter with significant fibrosis. The majority of the patients have significant involvement of

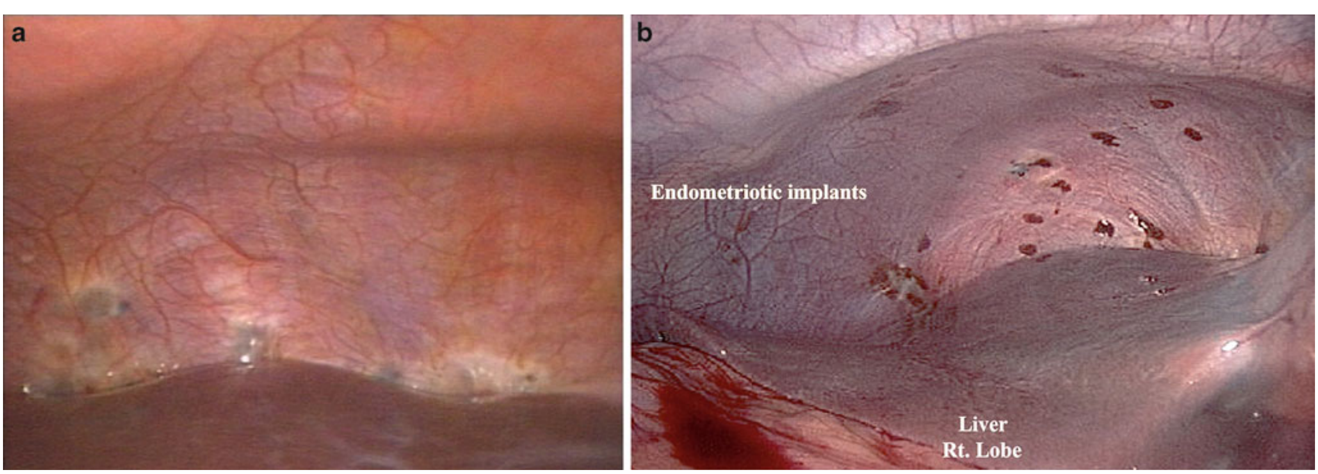

Fig. 20.3 (**a**) This figure shows fibrotic-type endometriosis involving the right hemidiaphragm. The lesions are seen above the liver. Most of the lesions are obscured by the liver. (**b**) Hemorrhagic-type endometriosis lesions of the right hemidiaphragm

the rectovaginal septum with nodules that are often greater than 3 cm. Preoperative imaging studies such as MRI with contrast should be used to evaluate the renal system preoperatively in patients with rectovaginal disease. Medical therapy has been used successfully in a limited number of patients. Most cases of ureteral endometriosis can be treated with excision of the periureteral fibrosis and active lesions without ureter resection.

Sciatic Nerve Involvement

Patients with endometriosis of the sciatic nerve can present with hip pain, which is usually localized to the buttock. The pain radiates down the back of the leg, and numbness occurs in areas innervated by the sciatic nerve. The symptoms typically occur in association with a menstrual period but then extend into other times of the cycle. Progressive leg and foot muscle weakness with electromyogram studies showing denervation can be demonstrated. MRI typically shows a lesion infiltrating the sciatic nerve. CT-guided biopsy can be used to confirm the diagnosis. Two-thirds of cases are localized to the right side. Most cases have pelvic endometriosis associated with this disease. Treatment with a GnRHa and add-back therapy have been shown to reverse the neurologic abnormalities.

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21 Surgical Techniques for Management of Anomalies of the Müllerian Ducts

Marjan Attaran

Introduction

Malformations of the Müllerian ducts and the external genitalia can have significant impact on both reproductive potential and sexual function. When a patient presents with such an abnormality, it is important to put significant thought and time into determining the correct diagnosis and subsequent treatment.

The literature reports rates of female congenital anomalies between 0.2 and 0.4 % of the general population [1]. But the prevalence of female congenital anomalies may be as high as 7 % when using some of the newer diagnostic methods [2]. These rates are much higher when looking at subgroups of patients with recurrent pregnancy loss and infertility. However, in many instances these anomalies are asymptomatic.

This chapter will begin with a review of the diagnostic and presurgical evaluation of these anomalies. The majority of the chapter will deal with the basic principles of surgical techniques used to correct these anomalies.

Classification

The classification of Müllerian anomalies helps with both the diagnosis and the comparison of outcomes after various modes of management. However, there is no single classification that encompasses all anomalies that have been reported in the literature [3–5]. Although the direct cause of the majority of these anomalies is not known, on the basis of our embryological knowledge, the pathogenesis of most of these anomalies can be understood. On the basis of pathophysiology, Müllerian anomalies can be broadly classified into problems according to the developmental mechanism whose failure gave rise to the malformation. Anomalies can usually be classified as being related to (1) agenesis, (2) vertical fusion defects, or (3) lateral fusion defects [6].

Agenesis of the uterus and vagina is a relatively common abnormality. Agenesis of other Müllerian structures is extremely rare. Vertical fusion defects are usually the result of abnormal canalization of the vaginal plate and result in defects such as a transverse vaginal septum and imperforate hymen. Lateral fusion defects can be symmetrical or asymmetrical and include septum of the uterus and vagina, as well as unicornuate and bicornuate uteri and related abnormalities.

There are endless variations to Müllerian and vaginal anomalies. It is impossible to note these variations effectively in any one classification system. Consequently, many investigators are still searching for that elusive classification system that can not only encompass all the anomalies noted in the vagina, cervix, uterus, and adnexa but also translate into in-sync comprehension and visualization of the defect by other colleagues [7, 8].

The most accepted classification of uterine anomalies, published by the American Society of Reproductive Medicine (ASRM), places uterine anomalies into distinct groups based on anatomic configuration (Table 21.1) [9]. Since vaginal anomalies are not included in this classification, they must be described along with the uterine anomaly. This classification does not give insight into pathophysiology but is an effective way to communicate observations for purposes of treatment and prognosis.

Müllerian Agenesis

Clinical Presentation

Müllerian agenesis (i.e., Mayer-Rokitansky-Kuster-Hauser syndrome) was first described in 1829. Its incidence is reported to be 1 in every 5,000 newborn females [10].

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Table 21.1 ASRM classification of Müllerian anomalies

I. Hypoplasia/agenesis	a. Vaginal b. Cervical c. Fundal d. Tubal e. Combined
II. Unicornuate	a. Communicating b. Noncommunicating c. No cavity d. No horn
III. Didelphys	
IV. Bicornuate	a. Complete b. Partial
V. Septate	a. Complete b. Partial
VI. Arcuate	
VII. DES related	

Because the vagina and associated uterine structures do not develop with this disorder, it is an ASRM Class IA Müllerian anomaly. Patients typically present during their adolescent years with complaint of primary amenorrhea. As a cause of primary amenorrhea, Müllerian agenesis is second only to gonadal dysgenesis [11].

Patients with Müllerian agenesis will present with normal onset of puberty and appropriate secondary sexual characteristics but apparently delayed menarche. They do not complain of cyclic pelvic pain like patients with obstructive Müllerian anomalies. The external genitalia appear completely normal, with normal pubic hair growth and normal-sized labia minora, which is in contrast to patients with complete androgen insensitivity syndrome. Hymeneal fringes may be evident but the vaginal opening is absent. No pelvic masses suggestive of hematocolpos will be evident, which is in contrast to cases of complete transverse septum.

Since these patients have a 46XX karyotype, normal ovaries will be present in the pelvis. Ovulation can be documented as a shift in basal body temperature. These patients' hormonal levels are normal, and their cycle length, based on hormonal studies, varies from 30 to 34 days [12]. In addition, they may experience the monthly pain (mittelschmerz) that is indicative of ovulation.

Associated Anomalies

Some, but not all, anomalies described can be extrapolated from the embryology [13–17]. Hearing difficulties have been reported in patients with Müllerian agenesis [14, 15]. A higher rate of auditory defects has been noted in general in patients with Müllerian anomalies compared to those with normal Müllerian structures [17].

Müllerian agenesis is associated with renal and skeletal system anomalies. Renal abnormalities are noted in 40 % of these patients. These include complete agenesis of a kidney to malposition of a kidney to changes in renal structure [18]. Skeletal abnormalities are noted in 12 % of patients and include primarily spine defects followed by limb and rib defects [19]. Patients with Müllerian agenesis should be actively assessed for these associated anomalies.

Etiology

The etiology of Müllerian agenesis remains unknown. It appears to be influenced by multifactorial inheritance, and rare familial cases have been reported. It does not appear to be transmitted in an autosomal dominant inheritance pattern, since none of the female offspring of women with Müllerian agenesis (born via in vitro fertilization and surrogacy) have shown evidence of vaginal agenesis [13].

Diagnosis

Imaging

The diagnosis of Müllerian agenesis is confirmed via imaging techniques. Abdominal ultrasonography will demonstrate the lack of uterus and existence of ovaries. The presence of a midline mass consistent with a blood collection usually indicates an obstructive Müllerian anomaly. The distinction between Müllerian agenesis and obstruction is extremely important since an incorrect diagnosis can seriously jeopardize appropriate management.

With the advent of magnetic resonance imaging (MRI), laparoscopy is no longer considered necessary to make this diagnosis. Typical findings in the pelvis include normal ovaries and fallopian tubes and usually small Müllerian remnants attached to the proximal portion of the fallopian tubes that may be solid or have functioning endometrial tissue.

Direct communication with the radiologist about the differential diagnosis prior to imaging studies is important. On occasion, the unsuspecting radiologist may interpret the small uterine remnants as a uterus. Careful attention to the very small dimensions of this structure will alert the physician to this possibility.

Explaining the Diagnosis to the Patient

The diagnosis, usually made in early adolescence, must be explained to the patient with great sensitivity. At a time when being like her peers is extremely important, knowledge of this diagnosis can be psychologically devastating. Each patient must be reassured that her external genitalia appear normal and that they will be able to have a normal sex life after the creation of a normally functioning vagina. Although usually not voiced, the inability to subsequently bear children is a major disappointment to teenagers. Fortunately, with ART procedures, including IVF and surrogacy, having her own genetic child will be an option for many of these young women.

Creation of a Neovagina

The first goal of treatment of Müllerian agenesis is the creation of a functional vagina to allow intercourse. Frank first proposed vaginal dilatation with the use of a dilator as a means of creating a neovagina in 1938 [20]. However, the surgical techniques of vaginoplasty remained the preferred methods for many years. The success of any technique depends in large part on the emotional maturity of the patient. Pretreatment counseling and continued support during treatment are important.

Fig. 21.1 Examples of vaginal dilators of different sizes (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Vaginal Dilation

The simplicity and ease of vaginal dilation and its significantly lower complication rate than surgical techniques dictate its use as an initial form of therapy for most patients with Müllerian agenesis. The American College of Obstetrics and Gynecology has released a committee opinion that recommends nonsurgical management of Müllerian agenesis as the first mode of treatment [21].

Frank's technique of dilation involves actively placing pressure with the dilators against the vaginal dimple (Fig. 21.1). Not only the patient is in an awkward position but the hand applying the pressure can become tired. In 1981, Ingram proposed the concept of passive dilation, where pressure is placed upon the dilator by sitting on a bicycle seat [22].

Roberts reported a success rate of 92 % in women who dilated the vagina via the Ingram technique for 20 min 3 times a day [23]. The average time of the creation of a functional vagina was 11 months. This series demonstrated that an initial dimple <0.5 cm was all that was necessary to achieve adequate dilation. Interestingly, failure of this technique was not related to the length of vaginal dimple but rather more closely associated with the patient's youth. Failure of this technique was more common in patients younger than 18 years of age.

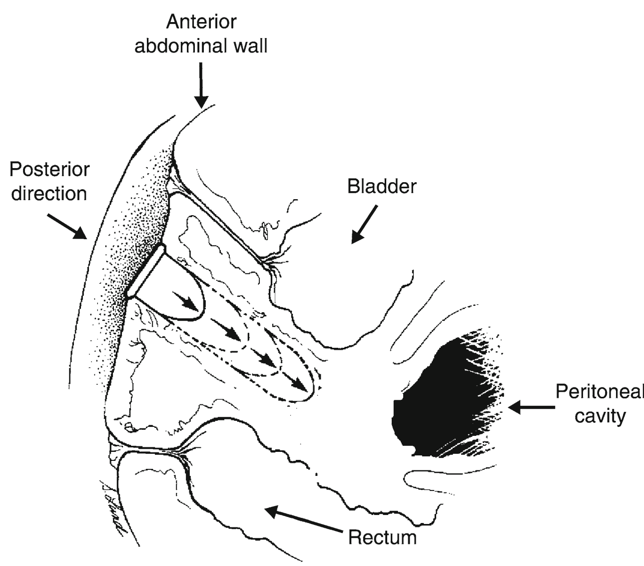

Fig. 21.2 Schematic drawing of angle of dilator placement. The patient is viewed in lithotomy position, and the axis is directed away from the bladder (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Procedure

When the patient expresses a desire to proceed with therapy, she is shown the exact location of her vaginal dimple. The axis of dilator placement is also demonstrated (Fig. 21.2). The process is initiated by placement of the smallest dilator against the dimple. Pressure is kept upon the distal aspect of the dilator by sitting upon a stool while leaning slightly forward. When the dilator fits comfortably, she moves to the next size dilator. The patient is instructed to use this technique a minimum of 20 min a day, 2–3 times a day. In motivated patients, a functional vagina can be created in as short as 12 weeks.

Counseling and psychological support is integral to successful treatment [24–26]. Patients are requested to return to the office frequently so that the physician can monitor their progress, and provide guidance and an opportunity to answer questions. Intercourse may be attempted when the largest dilator fits comfortably.

Multiple types of graduated dilators, made of various materials, are present on the market. None have been found superior to the others. Patients may stop and reinitiate the dilation at any time without any negative long-term sequelae. Although most patients appear interested in initiating this therapy the summer before college, when they are mature enough and motivated to create the vagina, the timing of therapy is purely dependent on the patient's desires. The median age of starting treatment is 17 years of age. For appropriately selected motivated patients, the reported success rates are as high as 90 % [27].

Vecchietti Procedure

Giuseppe Vecchietti described this method of creating a neovagina in 1965 [28]. Similar to vaginal dilatation, this method avoids the use of a graft. The Vecchietti procedure is a one-step procedure in which a neovagina is created in 7 days by continuous pressure on the vaginal dimple using an acrylic "olive" connected by retroperitoneal sutures to a spring tension device on the lower abdomen. Although the original description of the Vecchietti procedure utilized laparotomy, this technique is currently performed laparoscopically [29, 30].

Procedure

The first step in this procedure is to use a sharp ligature carrier (similar to a Stamey Needle) to insert one end of a suture through the retrohymeneal dimple into the peritoneal cavity between the bladder and the rectum under laparoscopic guidance. The other end of this number-2 polyglycolic acid suture is placed through a 3-cm acrylic olive and likewise inserted through the dimple into the peritoneal cavity using the sharp ligature carrier.

The next step is to use a curved blunt ligature carrier to burrow retroperitoneally from a lateral suprapubic laparoscopic port site to the peritoneal fold between the bladder and the uterine rudiment. One end of the "olive" suture is placed through the eye of this curved ligature carrier and pulled retroperitoneally back through the abdominal wall port site incision. This procedure is repeated on the opposite side with the other end of the suture. After the laparoscope is removed and the abdominal wall incisions are closed, the ends of the suture are attached to a suprapubic Vecchietti spring traction device located externally on the abdomen [29].

Table 21.2 Surgical methods of creating a neovagina

Dissection of a perineal space	Split-thickness skin graft (McIndoe) Full-thickness skin graft Peritoneum (Davydov) Tissue engineering Muscle and skin flap Adhesion barriers Tissue expansion
Bowel vaginoplasty	Sigmoid colon
Vulvovaginal pouch	Williams vaginoplasty
Traction on retrohymeneal fovea	Vecchietti

Postoperatively, constant traction is applied to the perineal olive by daily readjustment of the tension of the sutures and traction device. The neovagina lengthens by 1–2 cm per day, such that a 10–12-cm vagina is created in 7–9 days. After creation of the vagina, the olive (now located at the apex of the neovagina) is removed and the patient is sent home with a vaginal mold in place. After surgery, it is important to initiate regular sexual intercourse or routinely use dilators to maintain vaginal length.

Vecchietti reported a series of over 500 patients with a success rate of 100 % and only nine complications, including one bladder and one rectal fistula [31]. Several smaller studies have subsequently been reported by other surgeons with similar outcomes [32, 33]. A 3-year follow-up study assessed the functional and psychological outcome in five patients [34]. All five subjects reported having a functioning vagina, allowing satisfactory intercourse and improvement in general well-being.

Vaginoplasty Techniques

The traditional surgical management of vaginal agenesis is to create a vaginal space followed by placement of a lining to prevent stenosis. Multiple tissues and at least one man-made material have been used to line this cavity with varying degrees of success in preventing subsequent stenosis of the neovagina (Table 21.2).

McIndoe Procedure

The most widely used surgical technique for the creation of a neovagina is the McIndoe operation. The first step of the procedure is obtaining the split-thickness skin graft. The plastic surgery team typically acquires the skin graft from the buttock area, a location usually covered by clothing. The patient is placed in the prone position and the site is cleansed with an antiseptic solution and then soaked with an epinephrine saline gauze to allow vasoconstriction of small punctuate bleeding sites. Mineral oil is applied to the donor site and the skin is held taught while the electrodermatome device is used to obtain a thick split-thickness skin graft. The skin

Fig. 21.3 Skin graft is sutured around a mold (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

graft should be 0.015–0.018-in. thick. After application of antiseptic solution, the donor site is covered with OpSite, which is fixed in place by several stitches. Within 2–3 weeks the area heals with acceptable scarring.

The skin graft is placed through a 1:5 ratio skin mesher. The purpose of meshing the skin is not to stretch the skin but rather to allow escape of any underlying blood clots or serous fluids. This skin graft is sutured around the mold with 4-0 absorbable suture (Fig. 21.3). The mold is covered completely because any uncovered sites, whether due to lack of enough tissue or a gaping hole in the line of suture, tend to result in the formation of granulation tissue. Thus, great care must be taken to obtain sufficient amount of graft for this procedure.

The patient is placed in the dorsolithotomy position. A transverse incision is made in the vaginal vestibule, between the rectum and urethral openings (Fig. 21.4). In a patient who has not had prior surgery or radiation in the area, areolar tissue is now encountered. This tissue is easily dissected with either fingers or a Hegar dilator on either side of a median raphe (Fig. 21.5). The dissection is continued for at least the length of the mold without entering the peritoneal cavity. By cutting the median raphe, the two channels are then connected. If the dissection is performed in this manner, minimal amount of bleeding is encountered. Any bleeding sites must be controlled meticulously to avoid lifting of the graft from the newly created vaginal wall and subsequent nonadherence and necrosis.

After creation of the vaginal space, the mold covered with the skin graft is placed inside the cavity (Fig. 21.6). At the introitus, the skin graft is attached with several separate 3-0 absorbable stitches. To hold the mold in place, several loose, nonreactive sutures, such as 2-0 silk, are used to approximate the labia minor in the midline.

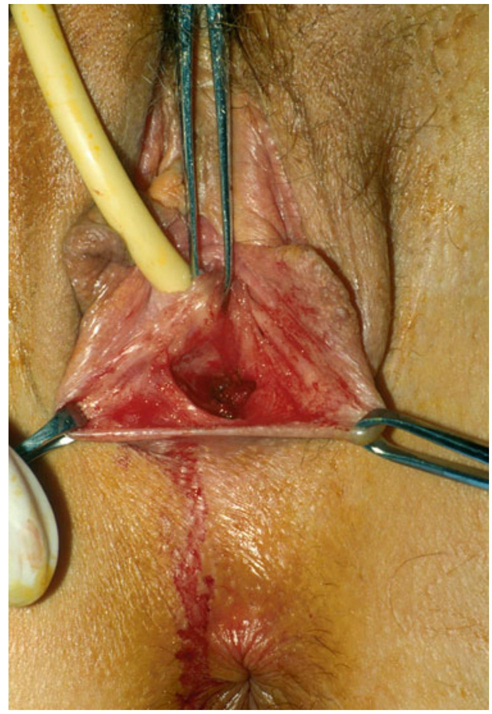

Fig. 21.4 The initial transverse cut was made on the fibrous tissue and an initial space developed (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

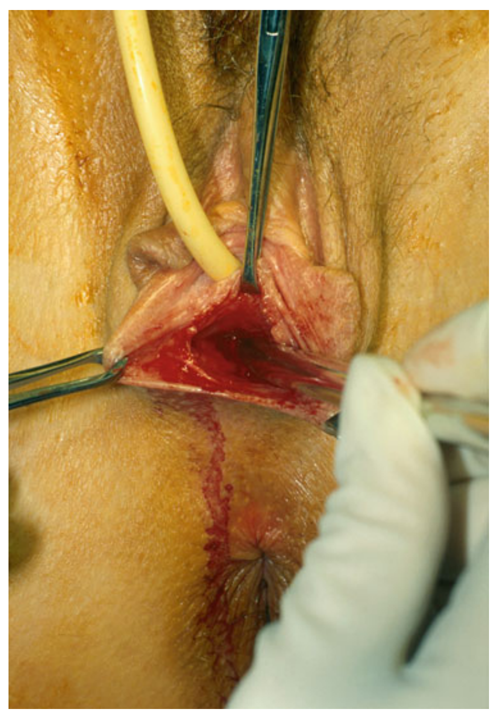

Fig. 21.5 Placement of Hegar dilator to create space for the graft. The direction of the Hegar dilators is posterior (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

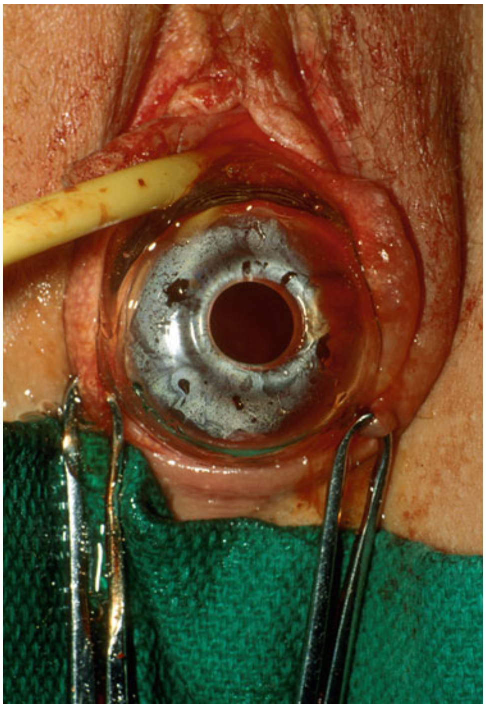

Fig. 21.6 The mold with the graft in placed in the space. Notice that the space to be created must accommodate the mold completely (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

During the ensuing week, the patient is maintained on bed rest, broad-spectrum antibiotics, a low-residue diet, and an agent to decrease bowel motility. She also has an indwelling urinary catheter. Upon return to the operating room in 1 week, the mold is carefully removed. The graft site is carefully assessed for any signs of necrosis or underlying hematoma after the vaginal cavity is irrigated with warm saline. Another soft mold is then reinserted and kept in place for the next 3 months except during defecation and urination. Nighttime usage of the mold is recommended for the next 6 months. To prevent contracture of the vagina, the patient is instructed to reinsert the mold during extended times of sexual inactivity.

Difficulty in dissecting the neovagina and increased probability of bleeding and fistula formation are encountered in the patient with a prior surgical procedure. Other problems that may be encountered include narrow subpubic arch, strong levators, shorter perineum, prior hymenectomy, and congenitally deep cul-de-sac [35, 36].

Because of concern regarding tissue necrosis from mold pressure and subsequent fistula formation, both rigid and soft molds have been used for this procedure (Figs. 21.7 and 21.8). Theoretically, soft molds decrease the risk of fistula formation that can result from avascular necrosis. A soft mold can be created by covering a foam rubber block with a condom [36]. The foam is able to expand and fit the neovaginal

Fig. 21.7 Hard glass mold (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

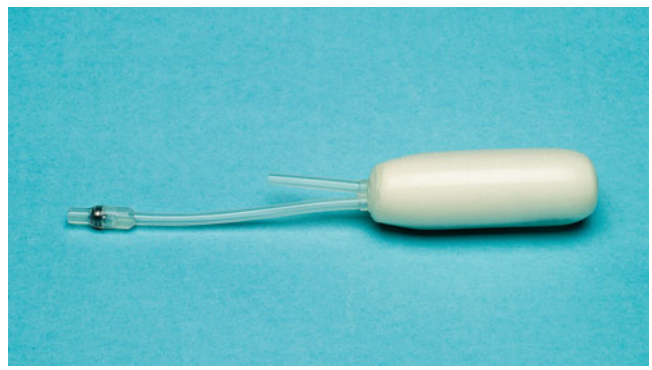

Fig. 21.8 Adjustable vaginal mold by Mentor, Minneapolis, MN (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

space, thereby providing equal pressure throughout the canal. However, a report on the use of a rigid mold on 201 patients who underwent the McIndoe operation demonstrated a fistula formation rate of less than 1 % [37]. There is no study comparing the outcomes of soft vs. rigid molds in this operation. Typically, a rigid mold is used initially, but the patient is sent home with a soft mold in place.

An 80 % success rate has been reported with this procedure [38]. Since success rates are highest in those patients who have not undergone prior vaginoplasty, patients must be counseled extensively prior to surgery regarding the need for prolonged use of the mold. Indeed, part of the presurgical assessment involves determination of patient maturity and motivation concerning the use of dilators. Lack of compliance with the postoperative use of dilators will lead to contracture and diminishment of vaginal length.

Surgical complications include postoperative infection and hemorrhage, failure of graft and formation of granulation tissue, and fistula formation. In general, the incidence of complications is low: rectal perforation rate of 1 %, graft infection of 4 %, and graft-site infection of 5.5 % [37]. In a review of 50 patients, two rectovaginal fistulas and one graft failure were reported [35]. Five patients required an additional operative procedure. Eighty-five percent of these patients considered their surgery to be a success.

Long-term data on the McIndoe procedure, while limited, consistently indicate an improvement in quality of life. In a series of 44 patients who underwent a surgical procedure to create the vagina, 82 % achieved a functional satisfactory postoperative result [39]. Vaginal length varied from 3.5 to 15 cm. In another long-term study of women who underwent a McIndoe procedure, 79 % of the patients reported improved quality of life, 91 % remained sexually active, and 75 % regularly achieved orgasm [40].

The newly created vagina must be inspected at the time of the yearly pelvic examination. Hair growth has been reported to be a problem with some skin grafts. Transformation to squamous cell carcinoma from skin graft has been described [41, 42].

Peritoneal Graft: Davydov Procedure

Use of the peritoneum to line the newly created vaginal space was popularized by Davydov, a Russian gynecologist, and first described by Rothman in the United States in 1972 [43–45]. In his original description, a laparotomy is performed after creation of the vaginal space as described above with the McIndoe operation.

A cut is made on the peritoneum overlying the new vagina. Long sutures are applied to the anterior posterior and lateral sides of this peritoneum. The sutures are then pulled down through the vaginal space, thus pulling the peritoneum to the introitus. The edge of the peritoneum is then stitched to the mucosa of the introitus. Closing the peritoneum on the abdominal side then forms the top of the vagina. Several investigators have also described the laparoscopic modification of this procedure [46–48].

This procedure may have several advantages compared to the traditional McIndoe. Contrary to skin grafts that leave visible scarring at the donor site, there is no outward sign of using a graft in the Davydov procedure. There appears to be no danger of lack of graft takes and no problem with hair growth.

In Davydov's first reported series, sexual intercourse was initiated within several weeks of surgery in all but 1 of his 30 patients. On follow-up, the length of the vagina was noted to be 8–11 cm. In a series of 18 patients who underwent the laparoscopic modification of this procedure, 85 % reported being sexually satisfied during an 8–40-month follow-up. Although there was one report of a rectovaginal fistula 18 months after the surgery, there was no evidence of vault prolapse or enterocele formation. Minor granulation tissue was noted at the vaginal cuff, but the vault was primarily covered with squamous epithelium tissue.

Adhesion Barrier Lining

Jackson first described the use of an adhesion barrier to line the neovagina in 1994 [49]. Oxidized regenerated cellulose (INTERCEED; Johnson and Johnson Patient Care Inc., New Brunswick, NJ) forms a gelatinous barrier on raw surfaces and thus prevents adhesion formation. After creation of the vaginal space, sheets of cloth-like oxidized regenerated cellulose are wrapped around the mold and placed in the vagina in a manner similar to the McIndoe. The neovaginal space must be free of any bleeding. Epithelialization is noted to occur within 3–6 months. Small areas of granulation tissue may be seen at the apex of the vagina and resolve after application of silver nitrate. Average vaginal depth ranges from 6 to12 cm. Continuous use of mold is encouraged until complete epithelialization has occurred.

A case series assessed the outcome of this technique on 10 patients with vaginal agenesis [50]. Complete squamous epithelialization was noted within 1–4 months. When compared to a normal vagina, fern formation was noted and the vaginal pH was always acidic. However, none of the women complained of vaginal dryness or foul-smelling discharge. Patients who were sexually active did not report any problems.

The advantages of the use of oxidized regenerated cellulose include avoidance of any scars, readily available product, and low expense. In addition, the surgical procedure is simplified into a one-stage procedure. Although the reported data appear encouraging, confirmatory studies are required before the use of oxidized regenerated cellulose can be recommended without any reservations.

Tissue Engineering

The first case in which in vitro cultured vaginal tissue was utilized to line the neovagina was published in 2007 [51]. A 1-cm^2 biopsy was performed from the vulvar vestibule. Autologous keratinocyte cultures were created from this biopsy. The McIndoe procedure was utilized to create the vaginal space, and the autologous in vitro cultured tissue was used to line the cavity. The length of the vagina is reported to be normal, as is its depth. Of course, long-term data are currently lacking, as is information on vaginal stenosis. But if proven to be effective, this method of creating a vagina may lead to the increasing popularity of the McIndoe procedure, given the lack of concomitant scars on the skin.

Muscle and Skin Flap

These approaches are not procedures of choice for women with vaginal agenesis. However, they may be used for those who require vaginal reconstruction after exposure to radiation or multiple surgical procedures. The advantage of using a full-thickness skin flap is that it avoids the problem of contracture encountered with split-thickness grafts.

The use of gracilis myocutaneous flaps and rectus abdominus myocutaneous flaps for vaginal reconstruction has been reported [52, 53]. This approach has been associated with a conspicuous scar and a higher failure rate. Wee and Joseph in Singapore designed flaps that maintained good blood supply and innervation [54]. Known as a "pudendal-thigh flap vaginoplasty," this technique has been particularly successful in patients with vulvar anomalies [55]. In one study of patients with Müllerian agenesis, 100 % success in creating a functional vagina was reported [56].

The patient's own labia majora and labia minora have also been used to create a vagina [57]. Tissue expansion has also been advocated to create labiovaginal flaps, which are then used to line the neovagina [58, 59]. Other modifications of this procedure have been reported [60, 61].

Bowel Vaginoplasty

This is not a procedure of choice in women with vaginal agenesis. For this procedure, also known as a colocolpopoiesis, a portion of large bowel with its preserved vascular pedicle is sutured into the neovagina. In recent years, sigmoid colon use has been recommended.

Continuous use of dilators is not considered necessary, although constriction has been noted when ilium has been used. Success rates of up to 90 % have been reported. Reported complications include profuse vaginal discharge, prolapse, introital stenosis, bowel obstruction, and colitis [62, 63]. Finally, there is a report of a mucinous adenocarcinoma arising in a neovagina lined with the sigmoid colon [64].

A laparoscopic modification of this procedure has also been described [65, 66]. Given the increased complication rates, it seems appropriate to reserve this treatment modality for complex situations in which a prior vaginoplasty technique has failed or when there are multiple urogenital malformations.

Obstructed Rudimentary Uterine Bulbs

Patients with Müllerian agenesis commonly have Müllerian remnants noted on MRI or during a laparoscopy. The MRI has the added value of determining if any endometrial tissue exists within these remnants (Figs. 21.9 and 21.10). Patients with functional endometrial tissue may present after many asymptomatic years with cyclic pelvic pain secondary to monthly endometrial shedding, and development of endometriosis has been reported in these patients. Symptomatic Müllerian bulbs should be removed either via laparotomy or laparoscopy.

Surgical Technique

The procedure is started by placing traction on the ipsilateral uterine bulb. The round ligament is grasped and cut and the peritoneum incised anteriorly, thereby creating a bladder flap. The retroperitoneal space is entered, the ureters identified, and the utero-ovarian ligaments transected. The dissection continues with identification and coagulation of the uterine arteries. Finally, the uterine remnants and the fibrous tissue connecting them are incised.

Cervical Agenesis

Cervical agenesis is a rare Müllerian anomaly whose true incidence remains unknown despite many case reports in the literature [67] (Fig. 21.11). Various degrees of cervical abnormalities, ranging from dysgenesis to agenesis, have been described [68]. The vagina may or may not be present

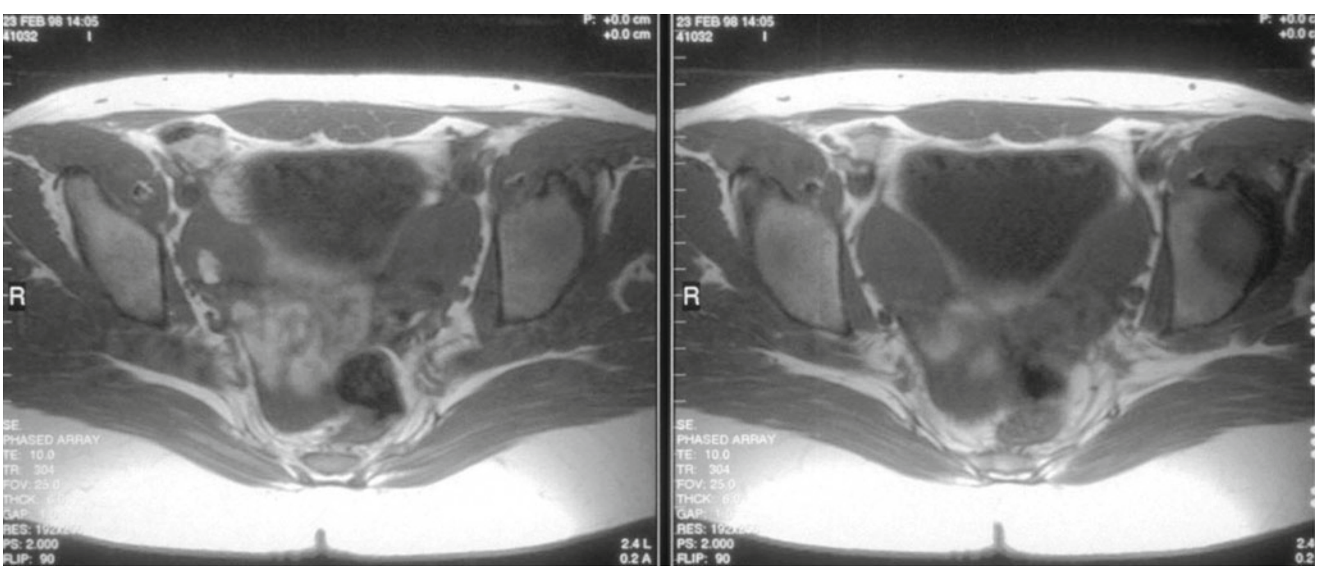

Fig. 21.9 Magnetic resonance image of functioning rudimentary bulbs (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

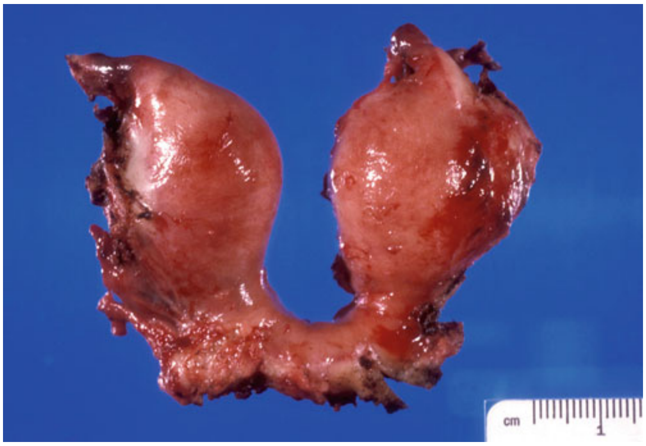

Fig. 21.10 Pathologic specimen of the extirpated rudimentary bulbs (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

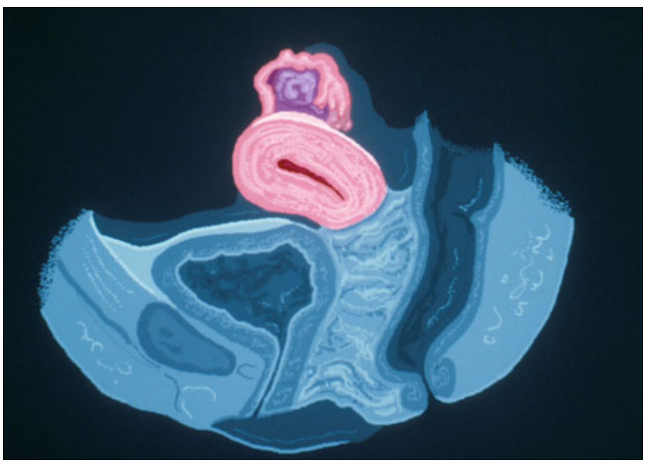

Fig. 21.11 Schematic representation of cervical agenesis (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

in patients with cervical agenesis. In a series of 58 patients with cervical atresia, 48 % had isolated congenital cervical atresia with a normal vagina [69]. The rest of the patients had either a vaginal dimple or complete atresia.

Diagnosis

Unlike some of the other Müllerian anomalies, patients with cervical agenesis present very early in adolescence. Typically, patients present between the ages of 12 and 16 with a primary complaint of pelvic pain secondary to obstruction of flow from the uterus. Initially, the pain is cyclic, but it may evolve with time into continuous pain. It is not uncommon for such patients to have been evaluated by their pediatricians for other causes of abdominal pain. Although these girls have

Fig. 21.12 Magnetic resonance image of cervical agenesis. The uterine cavity is distended with clots and no cervix is identified (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

amenorrhea, this symptom fails to raise a red flag since the patients are so young at presentation that lack of menses is not concerning. Continuing menstruation in an obstructed uterus forms a hematometra and possibly hematosalpinx, endometriosis, and adhesions in the pelvis.

Imaging of such a pelvis can easily lead to a misdiagnosis. Such patients have been taken to surgery for pain thought to be secondary to a pelvic mass, only to find that they have a congenital anomaly. While ultrasound may be helpful in looking for a cervix, one's clinical suspicion must be communicated directly to the radiologist performing the procedure. MRI is very helpful in visualizing the cervix and can accurately determine its presence or absence [16, 70, 71] (Fig. 21.12).

Patients with cervical agenesis who lack a vagina must be differentiated from those with a high obstructing vaginal septum. MRI is very helpful in making this differentiation by showing accumulation of blood in the upper vagina and a cervix in patients with a high transverse septum. Lack of a hematocolpos helps make the diagnosis of cervical agenesis or dysgenesis. Theoretically, MRI should detect absence of the cervix, but is unable to clearly differentiate among the various degrees of cervical dysgenesis.

Management

Pain Control

Pain control should be the first goal of treatment. Although analgesia may be required, severe pain will resolve within several days. Suppressive therapy is used to prevent further endometrial shedding until definitive surgery can be performed. Agents that are commonly used to achieve this suppression are continuous oral contraceptive pills, norethindrone acetate, depo-medroxyprogesterone acetate, and GnRH agonists or antagonists.

Most adolescents are neither emotionally ready nor equipped to decide on a surgical course of action, which may be as invasive as hysterectomy. Thus, if suppressive therapy with oral contraceptives or depo-medroxyprogesterone has provided pain relief, many may choose to hold off on definitive surgical therapy until they can fully understand the possible consequences. In addition, this alleviates the burden placed upon parents to make a decision regarding their daughter's reproductive future.

Surgical Approach

There are no specific guidelines in the literature to determine the correct surgical procedure. It is clear, however, that each patient should be assessed individually. The definitive and safest treatment of cervical agenesis is a total abdominal hysterectomy. A hysterectomy would diminish continued physical pain and discomfort. In addition, with the advent of surrogate pregnancy, an early hysterectomy would potentially preserve more ovarian tissue, which may be used to achieve a pregnancy via surrogacy and in vitro fertilization. On the other hand, it is a daunting decision to have a hysterectomy at a young age.

The other surgical options for management of cervical dysgenesis are cervical canalization or uterovaginal anastamosis. There are many reports in the literature of cervical canalization and stent placement [72–74]. Although success has been reported with establishment of menses, many patients will require reoperation secondary to fibrosis of the cervical tract and obstruction. In addition, pregnancy rates are very low. In a review of patients with cervical agenesis, 59 % of those that underwent cervical canalization achieved normal menstruation. Four of the 23 who achieved cervical patency required multiple surgeries [69]. The task is even more daunting if there is also a vaginal anomaly that requires reconstruction.

There are several forms of cervical dysgenesis that are sometimes confused with agenesis [75]. A hysterectomy may be considered in those patients with no evidence of a cervix, but canalization/uterovaginal anastamosis may be considered in those with a vagina and an obstructed cervix. Although preserving fertility is ultimately the goal of canalization procedures, sepsis and death have been reported subsequent to canalization [76]. In addition, subsequent pregnancy rates are very low [73, 77].

The poor pregnancy rates may be attributed to several factors. Prolonged delay in diagnosis can lead to extensive endometriosis and scarring in the pelvis. Also, while canalization and stent placement may maintain an open pathway for menstruation, the lack of epithelialization of the fistula not only increases risk of fibrosis but also may impede sperm migration into the uterus. The only pregnancy that was achieved in Rock's series was one in whom a full-thickness skin graft was used in the canualized area [75]. More recently, the use of bladder mucosa to line the newly created cervical canal was reported [72].

After preoperative assessment and formation of operative plan, the surgeon must be prepared to proceed with a concomitant vaginoplasty, if indeed the patient suffers from vaginal atresia. The patient should have been preoperatively counseled regarding the need for prolonged use of a mold following surgery.

Advances in artificial reproductive techniques have led to reports of pregnancies in patients with cervical agenesis. Thus, many patients are likely to choose effective continued endometrial suppression over a surgical solution in hopes of keeping a glimmer of reproductive hope. With passage of time and attainment of adulthood, such patients may be better able to accept the diagnosis and its consequences.

Uterine Fusion Defects

Uterine fusion defects include septate, bicornuate, and didelphic uteri. Patients with these isolated uterine anomalies are asymptomatic. The diagnosis is usually made during evaluation of infertility, recurrent pregnancy loss, or an obstetric complication.

Correct diagnosis of these anomalies is of utmost importance, since their management varies significantly [78]. The diagnosis is typically made based on evaluation of anatomy via imaging techniques including ultrasonography, hysterosalpingography, and MRI or via laparoscopy and hysteroscopy. Although radiologic guidelines exist to differentiate these entities, the multitude of varieties of these malformations can pose a significant challenge in making the correct diagnosis.

Septate Uterus

Reproductive difficulties are encountered far more commonly in women with a septate uterus than any other uterine fusion defect. The septate uterus is associated with the highest spontaneous abortion rate of uterine fusion defects.

The management of uterine septum detected after an evaluation for recurrent pregnancy loss is hysteroscopic resection of the septum. However, the management of a septum found during an infertility investigation is less straightforward [78]. While the septum does not appear to cause infertility, the concern regarding possible spontaneous abortion after undergoing infertility treatment may be enough to justify hysteroscopic removal of the septum prior to infertility treatment.

On a historical note, in the distant past a uterine septum was sometimes removed along with the midline section of the uterus via laparotomy using a Jones or Tompkins metroplasty procedure. These relatively extreme approaches have been completely supplanted by the hysteroscopic septoplasty described.

Bicornuate Uterus

The most frequently diagnosed uterine malformation is the bicornuate uterus [79]. This anomaly is usually discovered incidentally during an investigation for infertility or recurrent pregnancy loss.

It is important to differentiate a bicornuate from a septate uterus. Hysterosalpingogram (HSG) alone cannot differentiate these entities, because this imaging approach cannot evaluate the external contour of the uterus. While laparoscopy was used primarily for this purpose in the past, modern imaging techniques including 3D ultrasonography and MRI can adequately differentiate these two entities.

Imaging criteria to differentiate septate and bicornuate uteri have been developed [80, 81]. A septate uterus has a flat or convex fundus or a fundal indentation <10 mm. The septum should be relatively thin such that the angle between the medial borders of the hemicavities is <60°. A bicornuate uterus has two distinct fundi with an intervening fundal indentation of at least 10 mm. In most cases, the angle between the medial borders of the hemicavities will be >60°.

On MRI, a septate uterus will fail to show an intervening myometrium between the T2-hypointense septum that separates the endometrial cavities [80, 81]. In contrast, a bicornuate uterus will show two T2-hyperintense endometrial cavities, each with a junctional zone and myometrial band of intermediate signal intensity.

Conception does not appear to be a problem in women with bicornuate uterus [78]. However, higher rates of preterm delivery (19 %) and spontaneous abortion (42 %) have been reported [82]. Uteroplacental insufficiency and cervical incompetence may play roles in the higher obstetric complications. Thus, the treatment options for bicornuate uterus may include the Strassman metroplasty and cervical cerclage. Since the benefit of metroplasty has never been established in a controlled study, it is typically considered only after multiple pregnancy losses and complications [83].

Surgical Technique: Strassman Metroplasty

The Strassman metroplasty is the procedure of choice in the uncommon case where unification of a bicornuate uterus is indicated. Via laparotomy, a transverse incision is made across the fundus of the bicornuate uterus. The opened cavity is then repaired in an anteroposterior fashion. Since the subsequent length of gestation appears to increase after each pregnancy loss in patients with a bicornuate uterus (due to myometrial stretching or unknown factors), metroplasty is always the procedure of last resort.

Didelphic Uterus

The didelphic uterus is defined as two completely separate uteri and cervix (Fig. 21.13). It accounts for 10 % of all uterine anomalies [79]. On ultrasonography, the two bulbs of the uterus are distinctly noted and can be followed down to their individual cervices.

Surgical correction is not indicated. In a long-term follow-up of 49 patients with didelphic uteri, 89 % of those desiring pregnancy had at least one living child [84]. The spontaneous miscarriage rate was 21 %, and only one ectopic pregnancy occurred. The most common problem was preterm delivery, which occurred in 24 % pregnancies. Fortunately, only 7 % of the infants weighed <1,500 g at birth. Breech presentation was noted in 51 % of the infants; thus, the cesarean section rate is increased.

Unicornuate Uterus and Rudimentary Horn

A unicornuate uterus may be associated with a communicating or noncommunicating rudimentary horn. In either case, patients will have monthly regular periods. If a rudimentary horn is a communicating or a noncommunicating horn but nonfunctioning, the patient is unlikely to have severe dysmenorrhea. The diagnosis in these patients is usually made at the time of investigation for infertility and obstetric problems (including recurrent pregnancy loss) or at the time of cesarean section. In contrast, if a noncommunicating rudimentary horn is functioning, most patients will have severe dysmenorrhea unresponsive to medical therapy (Fig. 21.14).

Diagnosis

Pelvic examination may reveal a deviated uterus or an adnexal mass. Ultrasonography will be consistent with a unicornuate uterus on one side, while the other side may be interpreted as a rudimentary horn, a pedunculated leiomyoma or an ovarian endometrioma. An HSG will show a unicornuate uterus and will often, but not always, demonstrate the presence of a communication with the rudimentary horn when present. Both 3D ultrasonography and MRI are often useful in making a definitive diagnosis.

Management

Management depends on whether the rudimentary horn is functional and/or communicating. A nonfunctioning, noncommunicating rudimentary horn does not need to be removed, as it will be asymptomatic and not put the patient at any risk. In contrast, a functioning, noncommunicating rudimentary horn should be removed upon diagnosis to alleviate

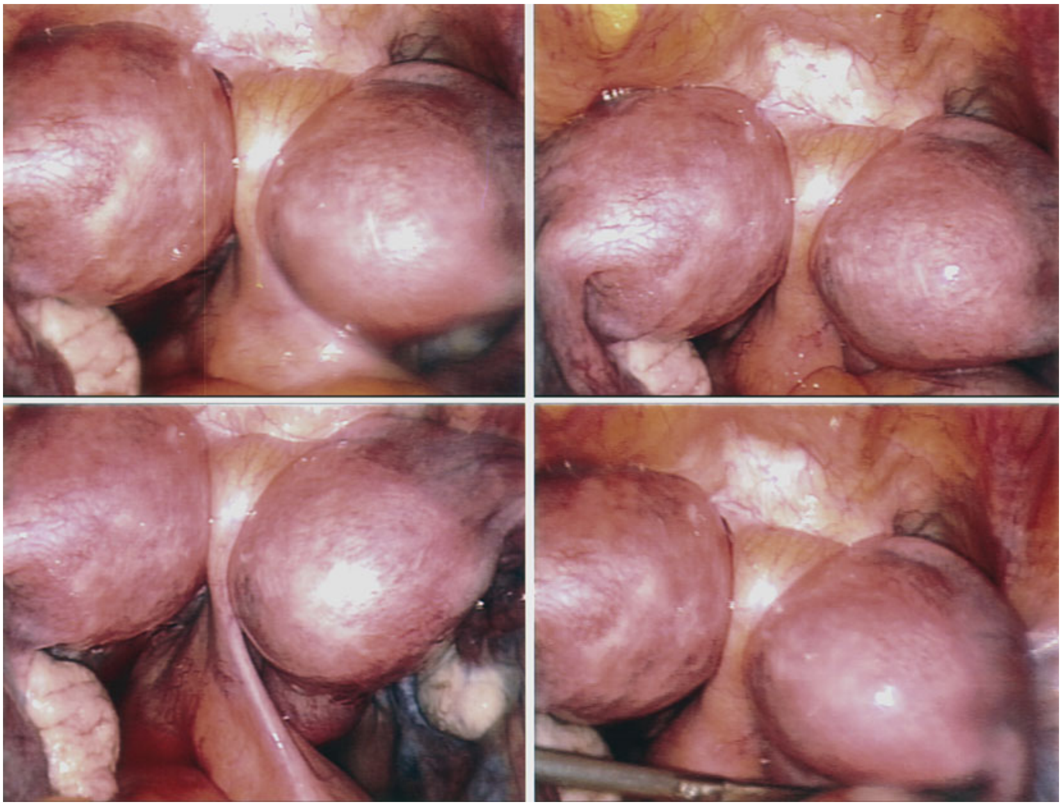

Fig. 21.13 The appearance of didelphic uteri at laparoscopy. Note the peritoneal band in between the two horns (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

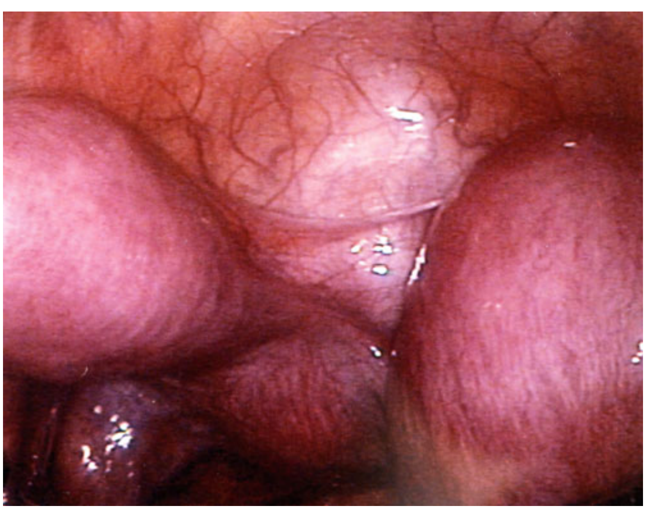

Fig. 21.14 Noncommunicating uterine horn seen at laparoscopy (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

the patient's often severe dysmenorrhea and to avoid the risk of rupture, should a pregnancy occur in this horn [85]. It would seem that a functioning, communicating rudimentary horn could be left in situ, since it is unlikely to be symptomatic for the patient. However, there is a risk of developing a pregnancy in such a horn, leading to subsequent rupture if undiagnosed [86]. Therefore, surgical removal is recommended prior to attempting pregnancy.

Surgical Technique: Removal of a Rudimentary Horn

A rudimentary uterine horn can be removed via laparotomy or laparoscopy using similar techniques, depending on surgical experience [87]. After gaining access to the pelvis, the round ligament of the rudimentary horn is identified, ligated, and divided. Access is gained into the retroperitoneal space, the ureter is identified, and the bladder is dissected off the lower border of the rudimentary horn.

The rudimentary horn should be removed together with the corresponding fallopian tube to avoid a future ectopic pregnancy in a blind residual tube via sperm transmigration. After disconnecting the tube from the mesosalpinx, the utero-ovarian ligament is transected so that the ovary can be spared.

The rudimentary horn may share myometrial tissue with the unicornuate uterus or be attached by a band of fibrous tissue [87, 88]. In cases where the uterine horn is attached to the uterus with fibrous band, the blood supply is found within

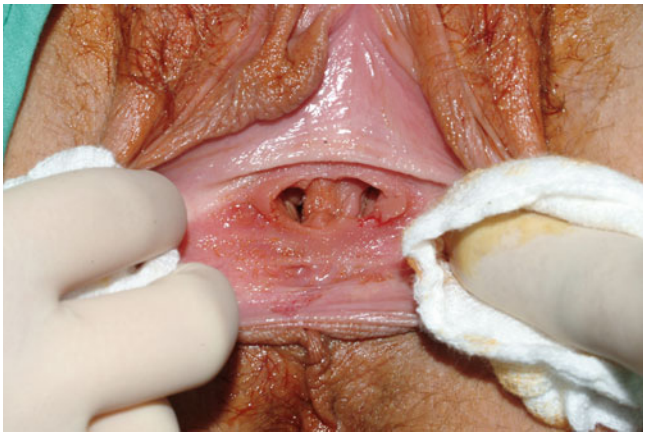

Fig. 21.15 Nonobstructing longitudinal vaginal septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

this band. Coagulation and transection of the band are all that is required.

In cases where the rudimentary horn is attached to the uterus via shared myometrium, the blood supply cannot be easily identified and thus the uterine artery ascending beneath the rudimentary horn should be identified and ligated. It may be difficult to find a plane of dissection between the horn and the uterus, but care must be taken to avoid entry into the cavity of the unicornuate uterus or compromising the integrity of the myometrial thickness. After this dissection, the myometrial defect should be carefully re-approximated with interrupted or continuous sutures to minimize the risk of uterine rupture during a subsequent pregnancy.

Longitudinal Vaginal Septum

A longitudinal vaginal septum can be either non-obstructing or obstructing. A non-obstructing vaginal septum is often asymptomatic and discovered at the time of a pelvic exam or childbirth. A woman with an obstructing vaginal septum often presents with an increasingly severe dysmenorrhea and a unilateral vaginal mass.

The Non-obstructing Longitudinal Vaginal Septum

Non-obstructing longitudinal vaginal septa account for 12 % of the malformations of the vagina. Although most are asymptomatic, some patients complain of continued vaginal bleeding despite placement of tampon, difficulty removing a tampon, or dyspareunia. These septa may be complete or partial and can exist in any portion of the vagina (Fig. 21.15). The communication can be extremely small, and a septum can easily be missed during physical examination, especially if there is a dominant vaginal canal.

Once the diagnosis is made, both the uterus and renal anatomy should be assessed for associated anomalies. In one study, 60 % of patients with longitudinal vaginal septa were found to have a bicornuate uterus [89]. Other investigators have noted a predominance of didelphic uteri in such cases [84].

A longitudinal vaginal septum should be removed in patients with complaints of dyspareunia and those who desire to be able to effectively use a tampon. In cases of didelphic uteri, removal of the septum may be necessary to allow sufficient access to each cervix for pap smears.

Some obstetricians advocate the removal of a longitudinal vaginal septum prior to delivery to avoid potential dystocia and laceration of the septum [89]. The number of patients with vaginal septa who have had successful vaginal deliveries remains unknown. However, emergent resection of a vaginal septum at the time of delivery to resolve dystocia has been reported [90]. It seems reasonable to remove a thick longitudinal septum prior to pregnancy, or prior to delivery, if discovered during pregnancy.

Surgical Technique

The goal of surgery is the removal of a wedge of tissue without damaging the cervix, bladder, or rectum. A Foley catheter is placed in the bladder. Since longitudinal vaginal septa are well vascularized, the anterior border of the septum, followed by the posterior border, are removed using unipolar electrosurgery. Care must be taken not to remove the septum too close to the vaginal wall, as this will leave larger mucosa defects. The edges of these mucosal defects are re-approximated with 2-0 absorbable suture. Postoperative use of a vaginal mold is not necessary.

The Obstructing Longitudinal Vaginal Septum

Women with an obstructing longitudinal septum usually present with normal-onset menarche and increasingly severe dysmenorrhea. These patients are most likely to have a didelphic uterus. One of the uteri has a patent outlet, and the other is obstructed (Fig. 21.16).

If the obstruction is low in the vagina, eventually a bulge may be noted upon examination of the lower canal. However, a higher obstruction may be completely missed with just visual inspection, which is frequently the case in an adolescent. Digital examination may reveal a tense bulge in the vaginal wall (Fig. 21.17). In many instances, the bulge is towards the anterior portion of the vagina between the 12 o'clock and 3 o'clock positions or 9 o'clock and 12 o'clock positions due to the rotation of the two cervices.

Ultrasonography of the pelvis will usually show a pelvic mass, which can be misleading unless a vaginal septum is

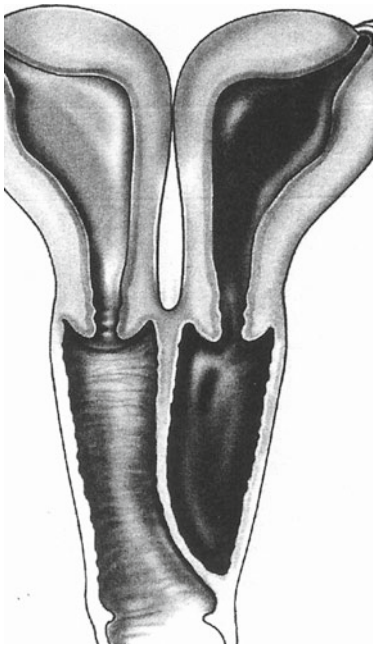

Fig. 21.16 Schematic of obstructing longitudinal septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Fig. 21.17 An obstructing longitudinal septum usually presents as a bulge in the vagina (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

considered in the differential diagnosis. MRI is the best imaging mode for definitively diagnosing this abnormality. Like other Müllerian anomalies, a longitudinal vaginal septum is associated with renal abnormalities, including absent kidneys, pelvic kidneys, and double ureters [91].

Some longitudinal septa will be found to be only partially obstructing and a small opening in the septum can be found during menses with close inspection. Symptoms may vary from irregular and prolonged bleeding to profuse vaginal discharge. Occasionally, the pinpoint opening provides a pathway for organisms to access the obstructed vagina leading to pelvic infection and pyocolpos. Physical examination is unlikely to reveal a tense bulge, but a slight fullness may sometimes be appreciated in the paravaginal area.

Surgical Technique

Accurate delineation of anatomy is a prerequisite for surgical excision of a noncommunicating longitudinal vaginal septum. The first step is to place a needle into the bulging vaginal wall to identify the correct plane of dissection. Once blood extrudes from the needle, the adjacent tissue is incised with electrosurgery to gain access into the obstructed vagina. Allis clamps are placed on the edges of this incision and the cavity is assessed. When removing the medial border of this septum, care must be taken to avoid damaging the urethra. The septum should be removed in its entirety to allow easy access to the second cervix for pap smears. The raw mucosal edges are approximated with 2-0 absorbable suture. Use of a vaginal mold after surgery is not necessary, since post-resection stenosis is rare. In difficult cases, use of either a resectoscope or hysteroscope to remove longitudinal vaginal septum has been described [92, 93].

The previously hidden cervix and obstructed vaginal canal will often appear abnormal. The cervix is usually flush with the vaginal fornix and often appears erythematous and glandular. Histologically, the obstructed vaginal canal and septum on its obstructed side will have columnar epithelium and glandular crypts [91]. Some patients may complain of profuse vaginal discharge after removal of the septum. Metaplastic transformation of the vaginal mucosa to mature squamous epithelium can take many years.

Simultaneous laparoscopy during removal of a vaginal septum is not recommended unless the diagnosis is unclear on MRI or imaging studies indicate concomitant pelvic masses. As in all cases of obstructive Müllerian anomalies, endometriosis is frequently encountered, even if the septum is only partially obstructing [94, 95]. With the possible exception of endometriomas, excision of the endometriosis is not recommended, since these lesions will regress after removal of the obstruction [94].

The obstetric outcome of such patients is similar to that reported for patients with simple uterine didelphys. Pregnancy rates of 87 % and live birth rates of 77 % have been reported [91].

Transverse Vaginal Septum

The incidence of transverse vaginal septum appears to be between 1 in 21,000 and 1 in 72,000 [68]. A transverse vaginal septum may be located in the upper (46 %), middle

Fig. 21.18 Complete transverse septum. Notice that there is no bulge with a Valsalva maneuver that would be seen with an imperforate hymen (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

(40 %), and lower (14 %) third of the vagina [96, 97]. A transverse vaginal septum may be complete or incomplete and varies in thickness (Fig. 21.18).

Presenting Symptoms

Patients with a complete transverse vaginal septum generally present with a complaint of primary amenorrhea in early to mid-puberty. Pelvic pain is a common, but not universal, presenting complaint. Patients with high transverse vaginal septa are most likely to experience pelvic pain, and the pain will manifest earlier than in patients with septa located lower in the vagina. This is believed to be secondary to decreased space for the hematocolpos that ensues after initiation of menses.

Patients with an incomplete transverse vaginal septum may complain of profuse vaginal discharge, dyspareunia, inability to insert tampon, or tear during intercourse with resultant bleeding. If asymptomatic, it may not be discovered until a routine gynecologic examination.

Very rarely a transverse vaginal septum may be detected in an infant or young child. In such instances, a mucocolpos can present as an abdominal mass [68]. If large enough, this mass may cause ureteral obstruction with secondary hydronephrosis. Compression of the vena cava and cardiopulmonary failure have also been reported.

Diagnosis

Manual and speculum examinations provide the most important information for diagnosis of a transverse vaginal septum. If the septum is very low, a vaginal opening may not be appreciated on evaluation of the external genitalia. A low transverse vaginal septum can usually be differentiated from an imperforate hymen by visual inspection. By increasing the intra-abdominal pressure and increasing the bulge of the imperforate hymen, the Valsalva maneuver may further assist in this differentiation.

If an opening to the vagina is noted, a manual or speculum exam may reveal a higher location of the septum. A rectal exam is very helpful in detecting a hematocolpos, since the bulge is readily palpable.

Transperineal and transabdominal ultrasonography can sometimes diagnose and determine the thickness of a transverse vaginal septum. However, in most cases an MRI of the pelvis will be required to differentiate a transverse vaginal septum from other Müllerian anomalies such as cervical agenesis.

These patients should also be evaluated for associated anomalies, including aortic coarctation, atrial septal defects, urinary tract anomalies, and malformations of the lumbar spine [96].

Surgical Technique

Surgical removal of a transverse vaginal septum is recommended as soon as practical after diagnosis to avoid continued retrograde menstruation. Endometriosis is common in patients with a transverse vaginal septum. However, removal of endometriosis lesions is not recommended, since relief of the obstruction leads to their spontaneous resolution.

Delay in detection or treatment of a transverse vaginal septum may lead to impaired fertility secondary to irreversible pelvic adhesions, hematosalpinges, and endometriosis. In one long-term follow-up study of 19 patients with transverse septa, 47 % became pregnant [96]. However, a small study in Finland showed a significantly higher live birth rate in women who had under gone very early diagnosis and management of their transverse vaginal septa [98].

The unfortunate consequence of very early surgical management is an increased rate of subsequent vaginal stenosis. This is most likely due to inconsistent use of vaginal dilators by young adolescents, which are a necessary part of treatment of a thick vaginal septum (see below).

An alternative to early surgery for very young patients is medical termination of monthly endometrial shedding using depo-medroxyprogesterone to postpone surgery [99]. Later, when she is emotionally ready for the surgery, the patient can be instructed to dilate the distal vagina to stretch the distal vaginal mucosa, potentially decreasing the need for a graft, and to prepare her for postoperative use of the dilator [86].

The thickness and location of the septum will determine the best approach to surgery. Thin, low transverse vaginal septa are much easier to repair than thick, usually high, septa.

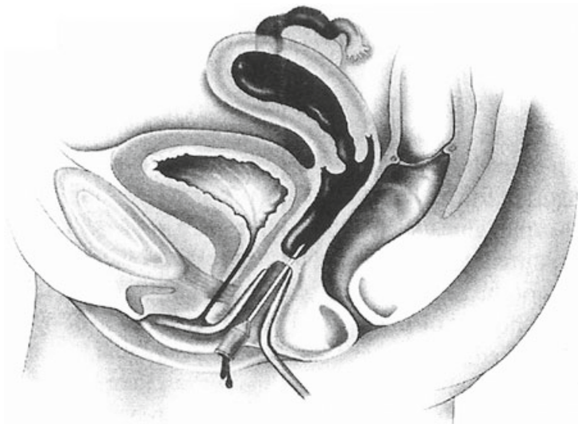

Fig. 21.19 Placement of angiocath into the transverse septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

Surgical Technique: Thin Transverse Vaginal Septum

Transverse septa that are thin and low in the vagina can usually be excised without difficulty. If visually a slight bulge cannot be appreciated on examination, an angiocath needle is placed through the septum (Fig. 21.19). With return of thick blood through the angiocath, the plane of dissection becomes clear. Access is gained into the upper vaginal cavity by perforating the transverse septum with unipolar electrosurgery or scissors.

The septum is excised in its entirety and the upper vaginal mucosa is re-approximated to the lower vaginal mucosa using 2-0 absorbable suture (Fig. 21.20). In most instances, to prevent stenosis of the vagina, continual use of a mold is recommended for several weeks after surgery.

Surgical Technique: Thick Transverse Vaginal Septum

Managing thick transverse septa can be quite challenging. Before surgery, the patient must be prepared for prolonged use of mold and possible split-thickness skin graft to line the vagina. The main concern is potential bowel injury; therefore, a bowel preparation is recommended.

During surgery, a bulge will not be seen in the presence of a thick transverse septum. The correct angle of dissection can be determined by inserting an angiocath needle into the hematocolpos under ultrasound guidance. In difficult cases, the septum can be approached transfundally via the uterus using laparoscopy and laparotomy [68].

The dissection is carried out, taking care to protect the bladder and rectum. A Foley catheter is placed into the bladder. As the loose areolar tissue is being dissected, the rectum is frequently examined to ensure appropriate angle of dissection. If there is inadvertent entry into the bladder or the rectum, the procedure should be stopped and completed at a future date.

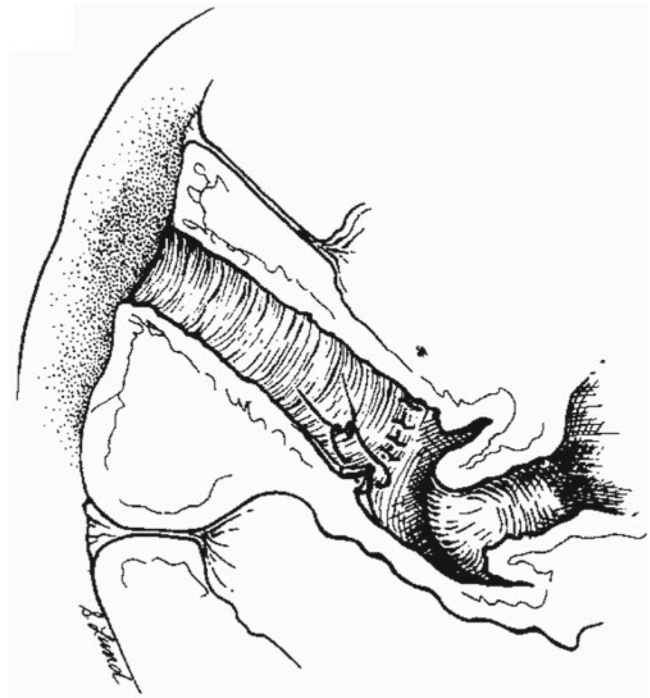

Fig. 21.20 Re-approximation of distal and proximal vagina mucosa after excision of the septum (reproduced with permission from Attaran M, Gidwan G, Ross J. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007)

After the cervix is visualized, the goal is to re-approximate the upper mucosal tissue to the lower vaginal mucosa.

Z-Plasty Technique

If a thick septum is completely incised, the distance between the vaginal mucosal of the proximal and distal portions of the vagina may be so great that the edges cannot be re-approximated without tension. For this reason, a z-plasty technique, as first described by Garcia and colleagues, should be considered for the correction of thick transverse vaginal septa or when the vagina is short [100].

For this technique, four lower mucosal flaps are created by making oblique crossed incisions through the vaginal tissue on the perineal side of the transverse septum, taking great care to avoid injuring either the bladder or rectum. Four upper mucosal flaps are created by making oblique crossed incisions through the vaginal tissue on the hematocolpos side of the transverse septum. The upper and lower mucosal flaps are separated by sharp and blunt dissection and sutured together at their free edges to form of a continuous z-plasty.

Excellent results have been noted on 13 patients who underwent this procedure [101]. A vaginal mold must be used for 5–8 weeks after the procedure to avoid vaginal stenosis. If the girl is not sexually active, a dilator should be used at night for 6–8 additional months. The patient should be instructed in self-examination and should return if she notices any signs of early stenosis.

In cases of a thick septum where a z-plasty technique is not used, a skin graft may be required. The technique utilized is similar to that described for the McIndoe procedure. Prolonged use of a mold is required postoperatively.

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