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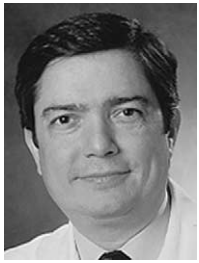
Preface
Endometriosis



Aydin Arici, MD
Guest Editor

Endometriosis is a leading cause of disability in reproductive age woman resulting in infertility and pelvic pain. It is the third leading cause of gynecologic hospitalization in the United States and remains one of the most enigmatic diseases in gynecology. Much has been accomplished over the last two decades in the understanding and treatment of endometriosis, but even more remains to be done.

This issue of the *Obstetrics and Gynecology Clinics of North America* is devoted to endometriosis, with the goal of providing the latest knowledge to the reader. A diverse group of internationally recognized experts have come together to discuss their clinical experiences and basic research in the field of endometriosis. I would like to express my gratitude to all the authors, who despite their other responsibilities, took the time to contribute to this issue.

Initial articles cover the epidemiology, genetics, and pathogenesis of endometriosis. Because the diagnosis of endometriosis involves many challenges to the clinician, these are discussed in articles devoted to adenomyosis, typical and atypical endometriosis, and noninvasive diagnostic tools for endometriosis.

Equally difficult is the establishment of guidelines for treatment of endometriosis. Medical, surgical, and combined medical-surgical treatments of endometriosis are discussed by experts in these fields, together with challenges of the current staging system for endometriosis.

This issue on endometriosis is concluded with articles discussing endometriosis related pain from patients' viewpoints and future directions in endometriosis research.

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I thank again my colleagues who contributed their time, effort, and expertise to this issue. I also greatly appreciate the support of Carin Davis and the staff at WB Saunders for their outstanding editorial competence. I hope that this issue will serve women and their physicians well in their ongoing efforts to confront this debilitating disease.

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30 (2003) 1–19

OBSTETRICS AND GYNECOLOGY CLINICS of North America

The epidemiology of endometriosis

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The third leading cause of gynecologic hospitalization in the United States, endometriosis is a common disorder that remains one of the most enigmatic gynecologic problems [1]. Endometriosis is defined as the presence of endometrial tissue external to the uterus, referred to as plaques or lesions. Symptoms arise from cyclical bleeding into the surrounding tissues, which results in inflammation and formation of scarring and adhesions. Lesions may be active or inactive and are present as white, red, clear, or bluish-black in pigment. Pain, however, may exist in the absence of these visible plaques. Signs and symptoms may include painful periods (dysmenorrhea), pelvic pain not associated with menses, painful intercourse (dyspareunia), painful urination (dysuria), and painful bowel movements (dyschezia) [2].

The effects of the disease can be physically and mentally debilitating, especially because misdiagnoses are common and the time between onset of symptoms to a confirmed diagnosis may average 6 years or more [3–6]. In an analysis of women who participated in the US Health Interview Survey, 50% of the women who reported endometriosis had required bed rest for at least 1 day because of their condition at some time during the past year, with the average number of days of bed rest being 17.8 [7]. Treatment options include hormonal suppression and surgery, with many women experiencing unsatisfactory results.

Despite the high personal morbidity and health care cost associated with endometriosis, to date, the incidence, prevalence, and risk factors of endometriosis

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remain uncertain. Few epidemiologic studies of endometriosis have been conducted primarily because it was perceived that such research would require unique study design techniques to overcome complex analytic problems specific to this disease. Despite a relative paucity of studies and diverse designs, however, some fairly consistent observations have emerged from studies completed to date—findings that the authors believe have led to a better understanding of the pathogenesis of endometriosis. The authors begin this article with a brief review of the theories of pathogenesis, followed by a discussion of the methodologic issues, and conclude with a summary of the epidemiologic findings as they fit with pathophysiologic models. The authors hope that this article will convince the reader that valid epidemiologic study of endometriosis is attainable. With creative and well-designed studies, epidemiology can play an important role in helping to devise strategies for the primary or secondary prevention of the disease.

A summary of pathogenic hypotheses

Retrograde menstruation

In 1927, Samson proposed the theory of retrograde menstruation, arguing that subsequent implantation and growth on extrauterine structures leads to the development of the disease [8]. This explanation was supported by clinical findings that lesions tended to be clustered around structures in close proximity to the distal ends of the fallopian tubes.

Lateral displacement of the cervix and uterosacral/cardinal ligament abnormalities are commonly found in women with endometriosis and may result from adhesions or scarring associated with the endometrial plaques [9–13]. Women with endometriosis are more likely to present with gross anatomic complications, such as a retroverted uterus or cervical stenosis, which, based on mathematical modeling of the fluid dynamics of menstrual flow, may increase the likelihood and volume of retrograde menstruation. Case reports identify endometriosis in the setting of müllerian anomalies, such as a noncommunicating uterine horn, in which the only menstrual egress is through the fallopian tube [14–17]. Similarly, surgical interventions that may alter the natural flow of menstrual fluid, such as cesarean section, cervical conization, or tubal ligation, also may be related to the risk of endometriosis [18,19].

Clinical work has shown that viable endometrial cells can be found in menstrual and peritoneal fluid [20], and endometrium has been implanted experimentally and grown within the peritoneal cavity [21]. Because laparoscopy during menses has shown that up to 90% of women exhibit retrograde menstruation, however, the question of why implantation rates differ between women remains unclear [22].

In addition to factors that modify the volume of retrograde menstruation, factors such as hormonal and immunologic factors may affect a woman's susceptibility to implantation [23].

Hormonal milieu

Strong circumstantial evidence indicates that endometriosis depends on circulating steroid hormones. The disease has not been reported in premenarchal girls, and the rare cases in postmenopausal women have been only in women who were exposed to hormone replacement therapy [24]. It has been hypothesized that conditions that alter estrogen status, such as age at menarche [25–27], body mass index [25,28,29], body fat distribution (as defined by waist-to-hip ratio, a measure of peripheral fat accumulation) [30,31], menstrual cycle characteristics [1,32], birth weight [33], cigarette smoking [1,32,34], and oral contraceptive use [1,35,36], may influence the incidence of endometriosis, perhaps by promoting the survival of extrauterine implants. It is not clear whether endogenous hormone levels influence the volume of retrograde menstruation or if it is involved in the promotion of extrauterine implant survival. Endometriosis plaques have been shown to have estrogen, progesterone, and androgen receptors, and they grow in the presence of estrogen but atrophy when exposed to androgens [5,7,37].

Immune system morbidity

For more than a decade, anecdotal and laboratory evidence has suggested that women with endometriosis have a higher prevalence of immune system morbidity as either an antecedent or consequential event. As an antecedent event, it is possible that women with endometriosis developed the disease because of immune system abnormalities that allow retrograde menstruation to establish lesions within the peritoneal cavity. Several immunologic studies have shown decreased natural killer cell activity in the peripheral blood mononuclear cells of women with endometriosis, although this finding has been inconsistent [5,7,38]. Other studies have shown an increased concentration of leukocytes (particularly macrophages, T lymphocytes, and natural killer cells) in the peritoneal fluid of women with endometriosis [4,5,7,38]. Halme et al compared the peritoneal macrophage secretions of women with and without endometriosis and found that cases demonstrated increased secretion of growth factors and proinflammatory cytokines [21]. This evidence, taken as a whole, suggests that the presence of endometriosis induces a local intrapelvic inflammatory reaction [39].

As a consequential event, it is possible that in the presence of endometriosis, the immune system is hyperstimulated by not having been able to clear the viable endometrial cells from the pelvic cavity [40,41], which increases the risk of autoimmune disorders. In the presence of an autoimmune disease, the immune system mistakenly attacks self, targeting the cells, tissues, and organs of a person's own body, which results in an inflammatory reaction [42]. Autoimmune diseases are defined by the organ or system that is being attacked. Although difficult to diagnose, to date, conditions believed to be autoimmune in origin include rheumatoid arthritis, in which the immune system predominantly targets the lining (synovium) that covers various joints; multiple sclerosis, the most common disabling disease of the nervous system in young adults, in which the immune

system targets nerve tissues of the central nervous system; systemic lupus erythematosus, more prevalent among African-American and Hispanic women, in which the immune system may attack and damage several organs, such as the kidney, brain, or lung; and autoimmune thyroid diseases, such as Hashimoto's thyroiditis or Grave's disease, that result from immune system destruction or stimulation of thyroid tissue and result in hypothyroid or hyperthyroid function [42].

The hypothesized link between endometriosis and autoimmune pathology was first presented more than 20 years ago by Weed and Arquenborg [43]. Hyperstimulation of the immune system, synergistic with the heightened inflammatory reaction associated with endometriosis, also may explain why women with endometriosis seem to be at greater risk of developing asthma, a type I hypersensitivity immune reaction [44].

Coelomic metaplasia

Another widely accepted hypothesis is that some plaques, particularly those that involve the ovaries, are generated by monoclonal tumors that arise from a somatic mutation of ovarian epithelium or pelvic peritoneal mesothelium. Pathways to initiation of coelomic metaplasia remain unclear, although it has been hypothesized that inflammatory response may play a role [4,45]. It is possible that what we currently clinically define as endometriosis may actually have heterogeneous origins, one that arises from retrograde menstruation and the other from metaplasia, with both potentially influenced by the hormonal milieu and immune system abnormalities.

Methodologic issues

Several methodologic issues have been problematic for epidemiologic studies of endometriosis, especially case-control studies [46,47]. First, the current clinical definition of the disease includes a wide spectrum of symptoms and pathologic findings. Second, no strategy for control selection seems entirely satisfactory, because factors that might influence which women come receive diagnosis of endometriosis could be related to exposures of interest. Third, whenever possible, incident rather than prevalent cases should be enrolled in epidemiologic studies; however, pinpointing the exact onset of disease is impossible when the pathologic changes specific to endometriosis are unclear and unobservable.

Traditionally, endometriosis has been defined as the presence of functional endometrial glands and stroma outside of the uterus (ectopic endometrium) but in the pelvic cavity. This definition allows women who have asymptomatic disease at the time of unrelated surgery (eg, tubal ligation) to be included as cases. From a public health standpoint, however, we are interested in disease that produces symptoms that result in morbidity that impacts the lives of women who are diagnosed. Some gynecologists have suggested that endometriosis be defined not only by the presence of ectopic endometrium but also by evidence that the lesions

are active cellularly or have affected normal physiology [48]. Examples of cellular activity or physiologic effect might include evidence that the lesions are deep (> 5 mm), manifest as ovarian endometriomas, or are associated with pelvic adhesions not attributable to other causes. Because it has been hypothesized that ovarian-related endometriosis may represent an underlying etiology that is distinct from peritoneal endometriosis, the authors suggest that when sample size permits, researchers should consider conducting subanalyses that distinguish between cases with and without a history of endometriomas.

Holt and Weiss have encouraged epidemiologists to operationalize these definitions in case selection, asserting that continuing the use of varied case definitions prevents comparison of study results [49]. At the very least, it seems that asymptomatic endometriosis found at tubal ligation should not be included in studies in which most cases were symptomatic. Zondervan et al argue that the relatively high prevalence of minimal or mild endometriosis among asymptomatic women may represent a normal physiologic process and that study cases should be limited to women who present with severe or at least symptomatic disease [47]. To reduce the magnitude of misclassification, analyses of incident diagnosis of endometriosis might be restricted to women who have laparoscopic confirmation of endometriosis, because this has long been considered the gold standard for endometriosis diagnosis [50,51]. Inclusion of cases diagnosed at hysterectomy also may be appropriate, but depending on the study design, comparison of the case and non-case groups may be confounded by indication for hysterectomy.

By limiting case definition to women with surgical confirmation of disease, we may be introducing selection bias. It is possible that patients with more frequent use of the medical system, women of higher socioeconomic class, or women with the most severe/aggressive disease may be more likely to undergo investigative laparoscopy. It is also possible that women with endometriosis whose symptoms are improved by less invasive, more generic treatments (eg, antiinflammatory medications, oral contraceptives) may never "need" an invasive, albeit confirmatory, diagnosis. In the few epidemiologic studies that were able to evaluate the distribution of severity among their cases, however, the severity of endometriosis among women with laparoscopic confirmation does not seem to be skewed to more extensive disease.

Selection of women who come to diagnosis on the basis of an evaluation for infertility may undersample those who are symptomatic with regard to pelvic pain and discomfort. Had such women not attempted to become pregnant, it can be assumed that most of these women would never have come to a laparoscopic diagnosis of endometriosis. When the cohort or case and control group are composed of infertile and non-infertile women, the authors suggest that analyses be conducted first with all women and then stratified by infertility status. When this stratification is not possible (ie, all cases have a history of infertility), researchers must remember that comparing infertile cases to a comparison or control group comprised of infertile women without endometriosis may yield results that differ not only in direction and magnitude of effect but also in interpretation from those that would be observed when comparisons are made

to fertile women without endometriosis [27]. This is particularly true when the exposures of interest, such as menstrual cycle characteristics or reproductive history, are correlated with endometriosis and infertility.

A prospective cohort study of endometriosis, in which women at risk for disease are enrolled and followed over time, is the ideal design. At baseline, the population should be restricted to women who are premenopausal and have intact uteri, because the occurrence of endometriosis after hysterectomy or in postmenopausal women is rare. Also, prevalent cases cannot be included in the study population and, depending on the minimum age of the cohort, may represent women who were diagnosed at a younger age because of more severe disease, greater access to medical care, or symptoms that were not improved by generic treatment. Cohorts that include healthy women who are young enough should be assembled to pick up women diagnosed with endometriosis at early ages.

When the design and conduct of a cohort study are not possible, a case-control study provides a valid estimate of the rate ratio—provided that the cases and controls are chosen validly [52]. The selection of controls, in particular, is key to assuring the validity of any case-control design. Controls must represent the exposure distribution of the population from which the cases arose, and sampling must be independent of that exposure. Any restriction or exclusion applied to cases must be applied to controls. To date, the debate over control selection has focused on preventing the inclusion of undiagnosed cases in the control group. As a result of the invasive nature of diagnosis, studies often have chosen controls from among groups of women who have had pelvic surgical investigation for other reasons (eg, tubal ligation, hysterectomy, or laparoscopy for reasons other than endometriosis). It is likely that these highly selected women represent a biased sample of women from the underlying population [46,47]. Detection bias also may exist, because the thoroughness of examination differs between cases identified during an evaluation for infertility or pelvic symptoms and controls that were declared to be free of endometriosis during a tubal ligation or other surgical procedure not initiated by symptoms [27]. Finally, to require that a control be matched to a case on the basis of symptoms and diagnostic procedures leads to a highly selected control group that would not allow many factors of potential interest to be studied because of overmatching.

Alternatively, purely population-based selection of controls also has been argued against, because women with undiagnosed disease may be selected, thus attenuating the association between exposure and disease. As Zondervan et al demonstrate, however, the likely community prevalence of severe/symptomatic endometriosis is less than 2% [47]. It does seem reasonable, however, to match on factors that influence the likelihood of receiving medical attention, such as availability of medical insurance and accessibility to gynecologic services. Cohort studies or case-control studies conducted within health maintenance organizations or in countries in which there is universal health care may be valuable in this respect but should not be made a prerequisite [46]. Researchers also may consider adjusting for health care use in analytic models, if only through a dichotomous proxy variable created from questions regarding frequency of

physical examinations, pap smears, pelvic examinations, or breast examinations conducted by a clinician.

Ideally, case-control studies should focus on incident rather than prevalent cases [47]. The proportion of women with long-term disease is greatest among prevalent cases, and self-reported or clinical data collected from cases after the onset of symptoms may represent changes in exposure or recall that occurred in response to disease. With chronic diseases such as endometriosis, however, which are typically diagnosed only after a threshold of symptoms is reached, it is impossible to know exactly when disease onset occurred. According to the Endometriosis Association's 1998 North American Member Survey (n = 4000), the average time to diagnosis is 9.3 years from onset of symptoms, with women waiting an average of 4.7 years to seek clinical help and then 4.6 years from first clinical visit to formal diagnosis [53]. Consequently, all analyses are really estimating the incidence of endometriosis diagnosis rather than incidence of disease onset, and the temporal relation between exposure and outcome may be inaccurately modeled. In any case, when prevalent cases are included a priori, it must be noted that the odds ratio is only a valid estimate of the underlying rate ratio if the outcome is rare—usually defined as a prevalence of 10% or less in the general population [52].

To date, all studies that evaluate the main effects of exposures that change overtime (menstrual cycle length and regularity, body mass index, and cigarette and alcohol use) have collected these data as current exposure rather than adolescent or early adulthood exposure. The authors argue that although these studies may aid in clarifying factors that are crucial to improved disease diagnosis, collection of data regarding earlier life exposures may model more accurately the temporal relation between these exposures and endometriosis, thus resulting in a more accurate approach to defining factors that are causally associated with disease rather than merely correlated with disease symptoms.

Validation of key exposure variables (eg, menstrual cycle length, time to regularity, alcohol use) may not be possible through comparison with corroborating information, such as medical records, that would allow one to assess the potential for misclassification. In prospective analyses, collection of this information from the patient before disease diagnosis at least decreases the likelihood of recall bias, although as discussed previously, it is possible that women who are symptomatic but as yet undiagnosed could answer differentially. With any study design, systematic within-person errors regarding reporting of weight and "negative" behaviors, although unrelated to disease status, may attenuate effect estimates.

In conclusion, although it is fair to say that the ideal epidemiologic study has not yet been performed, it is worthwhile to review the picture that has developed to date (Table 1).

Epidemiologic findings

Few well-designed epidemiologic studies of risk factors for endometriosis exist. Eskenazi et al conducted a review of more than 100 published studies and

Table 1
A summary of risk factors for endometriosis

Risk factor	Direction and consistency of effect
Menstrual and reproductive factors	
Earlier age at menarche	↑↑, consistent
Shorter menstrual cycle length	↑↑, consistent
Heavier menstrual volume	↑, limited study
Irregular cycle duration	—, inconsistent
Tampon use	—, inconsistent
Oral contraceptive use	—, inconsistent
Greater parity	↓↓, consistent
Body habitus	
Greater height	↑, inconsistent
Greater weight	↓, inconsistent
Greater body mass index	↓, consistent
Greater waist-to-hip ratio	↓, limited study
Red hair	↑, limited study
White race	↑↑, limited study
Lifestyle and environmental factors	
Regular exercise	↓, limited study
Cigarette smoking	↓, inconsistent
Alcohol use	↑, limited study
Caffeine intake	↑, limited study
PCB, dioxin exposure	↑, consistent in primates but inconsistent in women
Immune disorder comorbidity	
Diagnosis with an autoimmune disorder	↑↑, extremely limited study

Arrows indicate the approximate magnitude of the relation: ↑, slight to moderate increase in risk; ↑↑, moderate to large increase in risk; ↓, slight to moderate decrease in risk; ↓↓, moderate to large decrease in risk; —, no association.

found that only 6 (1 cohort and 5 case-control studies) included a surgically confirmed case group, provided clear criteria for control selection, and considered potential confounding factors in the analysis. The other publications were case series reports, did not have a well-defined comparison group, or did not control for any confounders [1]. Five years later, Zondervan et al conducted a similar review and found just two additional case-control studies that met these minimal criteria [47].

Prevalence and incidence

Prevalence estimates of endometriosis vary by mode of diagnosis. Among women who seek tubal ligation, the prevalence of endometriosis was found in two studies to range from 2% to 18% [54,55]. The prevalence within infertile populations has been reported to range from 5% to 50% [4,56–60]. The range for endometriosis prevalence for women admitted to a hospital because of pelvic

pain is 5% to 21% [56–59]. A group that seems to be at considerable risk for endometriosis includes adolescents with intractable dysmenorrhea or pelvic pain, in whom approximately 50% are found to have the diagnosis [61,62]. This observation suggests that adolescents with severe dysmenorrhea (generally defined as requiring analgesics and bed rest) have a high likelihood of having endometriosis and require particular attention for efforts at early detection or prevention. No autopsy data for any age group have ever been published.

Incidence data in the general population are less readily available. Houston et al reported that the incidence rate of histologically confirmed endometriosis among white, 15- to 49-year-old women in Rochester, Minnesota from 1970 to 1979 was 160/100,000 woman-years [24,63]. Incidence increased with age from 17/100,000 woman-years among women aged 15 to 19 to 285/100,000 woman-years among women aged 40 to 44. The incidence rate fell to 184/100,000 woman-years among women aged 45 to 49. A more recent study based on hospital discharges found endometriosis as a first listed diagnosis in 1.3 per 1000 discharges in women aged 15 to 44 [64]. Endometriosis seems confined to the childbearing and immediate postmenopausal years, which provides demographic support for the idea that pathogenesis involves the estrogen milieu.

Menstrual and reproductive factors

In addition to their relation to the hormonal milieu, menstrual cycle characteristics and reproductive history may influence the total "bulk" of endometrial cells released into the peritoneal cavity [65]. Dysmenorrhea is strongly associated with risk for endometriosis but generally has been interpreted as a symptom of disease [25,27,32]. Other menstrual risk factors related to endometriosis are age at menarche and cycle length. The risk of endometriosis seems to be increased in the presence of reproductive factors that are associated with increased exposure to menstruation, such as shorter menstrual cycle length, longer duration of flow, greater menstrual volume of flow, and reduced parity [1]. Most epidemiologic studies that address the topic have found that an early menarche, often defined as age 11, increases the risk for endometriosis [19,27,32,66–68]. Most of these same studies and others have found that risk is also increased with a shorter cycle length, often defined as 27 days [19,28,32,66,68,69]. Because early menarche and short cycles seem to be such consistent risk factors for endometriosis, it seems reasonable to predict that if one better understands their physiologic determinants, then one might better understand the pathophysiology of endometriosis.

In a hospital-based case-control study of 286 women with primary infertility and 3794 women who had delivered a live-born infant, cases were more likely than controls to have shorter menstrual cycle lengths ($\leq$27 days versus $\geq$38 days) (OR = 2.1, 95% CI 1.5–2.9). A nonsignificant increase in risk was found with cycle irregularity [32]. Less consistent evidence is related to duration and heaviness of menstrual flow and tampon use. Darrow et al reported that women under the age of 30 compared with friend controls of the same age were significantly more likely to have menstrual flow 6 or more days per month, heavy

flow, severe cramps, increasing symptoms, and to have used tampons for more than 14 years [25]. Cramer et al, however, observed no correlation with exclusive tampon use [32]. A recent study reported that women who used tampons exclusively are less likely to have endometriosis; however, this may be because case women experience greater volume and must use a combination of tampon and feminine napkin [70,71]. This study also suggested that women with endometriosis are less likely to engage in intercourse during menses, and avoiding this behavior might "protect" against the disease. The authors, however, concur with other critics who argue that women are likely to experience chronic pelvic pain and dypareunia that peaks during menstruation and are less likely to report having sex at this time [71].

A study based on endometriosis found at tubal ligation—despite previous comments about its general lack of value—did suggest that higher parity is associated with a lower risk for observing endometriosis [28]. Because infertility may be a consequence of the disease and may be part of case definition in any particular study, it can be problematic to study pregnancy history as a risk factor for endometriosis. Although the relation with parity may be related to the prevalence of infertility among women with endometriosis and must be investigated through studies designed to take this into account, the authors hypothesize that among parous women, the more often a woman is pregnant, the lower her risk of developing endometriosis. Because each pregnancy decreases the lifetime number of months during which a woman is exposed to menstrual fluid, the mechanism of risk may be similar to that of women with longer menstrual cycles who are exposed to the sloughing of endometrial tissue less frequently during any given calendar period as compared to women with shorter cycles. It is also possible that the extreme dilation of the cervix during childbirth improves menstrual outflow once postpartum menses resume.

Finally, the association between oral contraceptives and endometriosis has been difficult to sort out. In several studies, current oral contraceptive use has been shown to have a protective effect on endometriosis, whereas former use seems to increase risk [1,28,72]. Biologic hypotheses compatible with protection include the possibility that oral contraceptives decrease risk by stopping ovulation and decreasing the volume of menstrual flow, which decreases the likelihood of retrograde menstruation or monoclonal mutation. Hypotheses related to increased risk would point out that even low doses of estradiol and progesterone increase risk by increasing the likelihood of implantation and lesion growth [1,37]. Because oral contraceptives are often prescribed as a first line of treatment when patients present with menstrual cycle irregularity, heavy menstrual flow, or dysmenorrhea, however, it is impossible to tell if women who are taking oral contraceptives and are subsequently diagnosed with endometriosis developed the disease before or after the exposure. This intractable confounding by indication makes interpretation of these analyses difficult. It is probable that diagnosis of endometriosis is delayed in oral contraceptive users, because oral contraceptives have been shown to decrease symptoms in the short term, but symptoms remerge once use is discontinued or the disease progresses in severity [1]. Concern has been raised about whether women

who have had hysterectomy and oophorectomy for endometriosis should receive unopposed estrogen therapy, but definitive studies are lacking.

Body habitus

Weak inverse associations between endometriosis and weight and body mass index (kg/m^2) have been found [25,27,32]. An inverse relation between body mass index and incidence of breast cancer among premenopausal women has been seen consistently in recent prospective studies and in metaanalysis of case-control and cohort studies. Women with higher body mass index have more irregular menstrual cycles and increased rates of anovulatory infertility. In this group, a lower risk of breast cancer has been identified and may be the result of fewer ovulatory cycles and less exposure to ovarian hormones [73]. The same hypothesis supports a decrease in risk of endometriosis. Conversely, an increased risk with taller height has been reported for endometriosis [27,32]. Taller women may have higher follicular-phase estradiol levels [74].

A study of laparoscopically confirmed endometriosis cases compared to friend controls matched on age ($n = 88$ cases, 88 controls) reported that for women aged 30 or younger, the odds of endometriosis were inversely related to waist-to-hip ratio (OR = 6.18 for women with a ratio of 0.61–0.72 compared to women with a ratio of 0.76–1.01, 95% CI = 2.01–19.01) [31]. A high waist-to-hip ratio reflects peripheral fat accumulation that is associated with a high estrogen-to-progesterone androgen profile. Women who had a high birth weight, which suggests exposure to greater levels of estrogens in utero as compared to infants of average and normal birth weight, are at lower risk of cardiovascular disease and type 2 diabetes mellitus but higher risk of breast cancer. Birth weight may be associated with the risk of endometriosis [33,75,76].

In addition to these anthropometric risk factors, the literature contains data that support hypotheses that endometriosis may be associated with red hair [77]. A few studies have suggested that Asian women are at higher risk of endometriosis compared to women of other races, whereas African-American women are at lower risk [1,24,28]. It has been argued, however, that the relation with African-American ancestry is spurious because of misclassification of the outcome, because African-American women are often misdiagnosed as having pelvic inflammatory disease rather than endometriosis. Conduct of studies that adequately account for access to health care and quality of diagnosis may shed light on the truth behind this controversy.

Lifestyle and environmental factors

Cigarette smoking is known to have an affect on the hormonal milieu. Studies of the effect of smoking on endometriosis have produced conflicting results [25,32,68,72]. Cramer et al [32] reported an inverse association with cigarette smoking in heavy smokers (one pack or more per day) who had begun before the age of 17 (OR = 0.5, 95% CI = 0.3–0.9). In a cohort study of 17,302 women who

attended family clinics, Vessey et al found no association [72]. Although these contradictions may be caused by noise in the epidemiologic data, they also could result from the fact that although smokers are relatively estrogen deficient, they are also exposed to higher levels of exogenous estrogen in the form of dioxin. Although data vary by tobacco source, it is estimated that a person who smokes one pack of cigarettes per day takes in approximately 4.3 pg of polychlorinated dibenzodioxins/kg body weight/day [3].

Several studies of endometriosis also have suggested a relation with caffeine and alcohol consumption. Endometriosis patients have higher scores on the Michigan Alcoholism Screening Test compared to controls and tend to consume more alcohol on a yearly basis [3]. Grodstein et al [78] reported that among women with infertility solely caused by endometriosis and excluding women with additional causes of infertility (n = 158 cases, 3833 controls), the odds ratio for endometriosis was 1.7 (95% CI = 1.2–2.5) for moderate drinkers and 1.8 (95% CI = 1–3.2) for heavy drinkers compared to women who did not drink, after adjusting for age, number of sexual partners, cigarette smoking, and caffeine intake [78]. Although it is possible that the women were self-medicating because of the painful symptoms of endometriosis, the relation reported by Grodstein was unchanged when analyses were restricted to women who experienced no pain symptoms. Alcohol intake consistently has been shown to increase the risk of breast cancer [73]. Moderate intake has been shown to increase total and bioavailable estrogen levels. Hypotheses that relate alcohol consumption to endometriosis also may include disruption of a critical pathway of tissue containment/immune response during the menstrual cycle in adults or a critical pathway of physiologic development during adolescence.

Regular exercise, which may lower estrogen levels [79], has been associated with a reduced risk for endometriosis [27,32,69]. Valid study of this relation may be complicated by the effect of disease symptoms on physical activity. Physical activity may reduce risk of the disease, but once symptoms begin, women may be less likely to exercise, hiding the true effect.

Finally, under this category the authors mention environmental exposures, such as polychlorinated biphenyl or dioxin. Based on animal studies that suggest that polychlorinated biphenyl or dioxin exposure might relate to endometriosis, possibly through their effects on the immune system [80], there has been considerable interest in this exposure as a risk factor in women. Results of studies completed recently, based on serum levels of dioxin or polychlorinated biphenyl, have been contradictory [81,82]. Most recently, a well-designed case-control and retrospective cohort study has found a nonsignificant elevation in risk as high as fourfold. In both studies, however, sample sizes were small and the confidence intervals about these estimates were wide; thus, a null association cannot be excluded [83,84].

For lifestyle exposures, there is the possibility that confounding factors, such as menstrual symptoms or socioeconomic status, might be operating. Because of the importance of these exposures, however, they should continue to be examined in epidemiologic and experimental studies that take this potential confounding into consideration.

Immune disorder comorbidity

In April 1999, the Endometriosis Family Study distributed a press release that included interesting correlations between endometriosis and immune disorders [44,53]. Similar, more detailed results have been presented at several scientific meetings but have not yet been published [85]. They report that within their study population of 4000 cases—all members of the US-based Endometriosis Association who responded to a self-administered questionnaire—the prevalence of physician-diagnosed rheumatoid arthritis was 2% (compared to 0.8% in the general population), 0.8% with systemic lupus erythematosus (compared to 0.05%), 6.8% with hypothyroidism (compared to 1.9%), 1.5% with hyperthyroidism (compared to 1.1%), and 0.6% with multiple sclerosis (compared to 0.1%). If such relations exist, they would support many of the hypotheses that the cause of endometriosis includes abnormalities of immune system function, that the presence of endometrial tissue outside of the uterus increases the risk of autoimmune response, or that the presence of endometriosis catalyzes a chronic inflammatory response that leads to multisystemic immune dysfunction.

Summary and interpretation

Figure 1 illustrates a model for the pathogenesis of endometriosis that attempts to integrate the diverse risk factors. The model emphasizes the importance of retrograde menstruation as a key factor in disease pathogenesis. In some cases, retrograde menstruation alone might be sufficient to cause disease, such as when it is excessive (eg, because of outflow obstruction, vaginal or cervical stenosis, or a noncommunicating uterine horn). In other cases, a ''normal degree'' of retrograde menstruation might operate in conjunction with immune factors that might affect its ability to be cleared from the pelvis or with hormonal stimuli that might affect its growth. One also must consider the possibility that there are other pathways, such as coelomic metaplasia, that are not yet fully understood or we do not know what the epidemiologic correlates might be.

How can one distinguish whether some of the risk factors described are causes or consequences of the disease? Because endometriosis could be asymptomatic for many years, it is arguable that many of the risk factors discussed could be either causes or consequences of the disease. The authors believe that three factors are of particular interest: dysmenorrhea, infertility or subfertility, and immune function.

Dysmenorrhea is a strong risk factor for endometriosis but generally has been considered to represent a symptom of existing disease because it is easy to imagine that monthly bleeding from pelvic lesions is painful. Because dysmenorrhea may correlate with stronger uterine contractility [86], however, an alternate interpretation is that dysmenorrhea is associated with some degree of outflow obstruction, stronger uterine cramping, and increased propensity to retrograde menstruation. Taking a careful menstrual history and recording changes in dysmenorrhea over time with events such as childbearing or operative dilation of the cervix might help clarify the sequence.

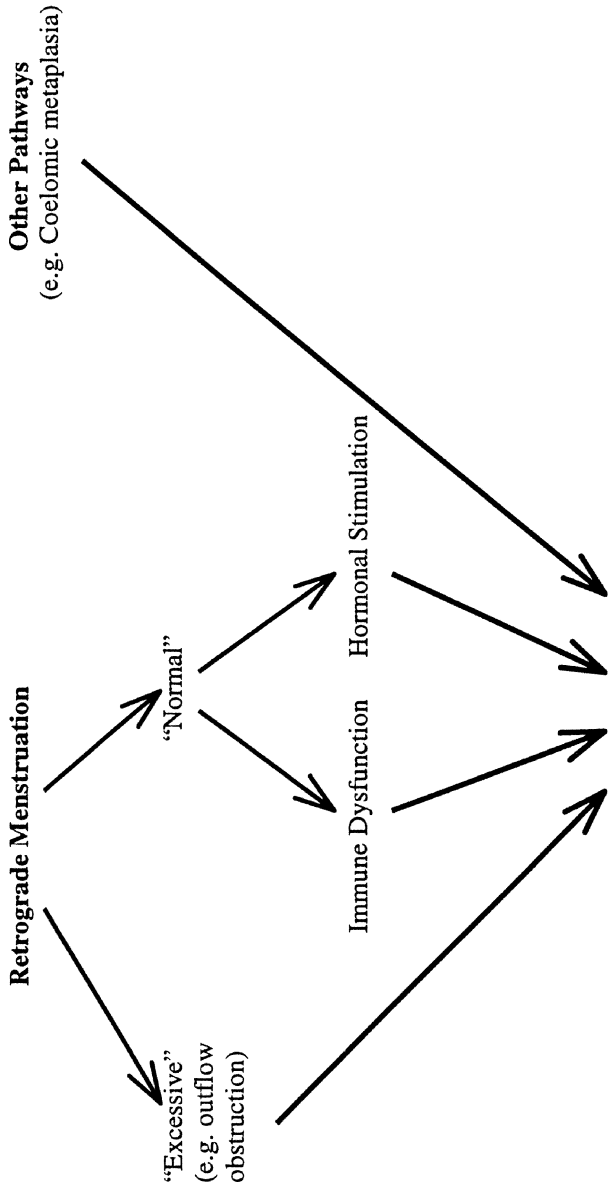

Fig. 1. A summary of the pathogenic hypotheses for endometriosis.

There is similar difficulty in clarifying the relation among childbearing, lactation, and endometriosis. Although disruption of pelvic anatomy and ovarian function that leads to infertility or decreased sexual activity as the result of severe chronic pelvic pain and dyspareunia may be consequences of endometriosis, it is also reasonable to propose that delayed childbearing also could be a cause of endometriosis. Although avoided menstruation is one explanation for a protective effect of childbearing, it should be appreciated that permanent cervical dilation occurs with labor and delivery, possibly reducing the resistance to menstrual flow and decreasing the likelihood of retrograde menstruation. The decidual reaction that occurs on the ovarian and pelvic surface during pregnancy that is attributed to high hormone levels might make this tissue less susceptible to endometriosis implantation or growth. One cannot examine the association between childbearing and endometriosis in studies in which cases are defined on the basis of a presenting complaint of infertility; however, the question can be addressed in cases in which pelvic pain or mass is the complaint.

It has been proposed that retrograde menstruation occurs to some degree in all women; however, only women who are unable to clear the menstrual debris because of immune dysfunction develop the disease [87]. This mechanism has been suggested to explain the possible link with polychlorinated biphenyl or dioxin exposure—substances that might damage the immune system. Alternatively, immune dysfunction also might be a consequence of the disease. Women with endometriosis might develop antibodies against their endometrium and develop autoimmune disorders. The latter issue can be addressed by careful follow-up studies of women diagnosed with endometriosis.

Conclusion

As estimated by the Endometriosis Association, millions of women are severely affected by endometriosis; millions more likely have asymptomatic disease [2]. Limited study suggests that menstrual and reproductive history, anthropometrics, lifestyle, and environmental exposures may play a role in disease etiology. Valid epidemiologic studies of endometriosis can be conducted, the results of which will aid in understanding the pathogenesis and early signs and symptoms of the disease. Early diagnosis and perhaps even prevention of endometriosis may be possible, but researchers must place importance on the epidemiologic characteristics of women who have been diagnosed.

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Genetics of endometriosis

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Endometriosis long has been recognized as showing heritable tendencies, with recurrence risks of 5% to 7% for first-degree relatives. This risk indicates that polygenic and multifactorial etiology is far more likely to be the cause than mendelian inheritance. This conclusion parallels the genetic basis of most adult-onset conditions, including many in reproductive medicine (eg, polycystic ovarian disease, leiomyomata, endometrial or serous ovarian epithelial cancer). The current task is to determine the number and location of genes responsible for endometriosis. Previously only a hypothetical goal, molecular advances of the past decade make identification and elucidation of these genes a reality.

In this article the authors review the basis for concluding that endometriosis is a genetic disorder of polygenic/multifactorial inheritance. Genome-wide strategies for identifying causative genes are considered and available data on association or linkage to putative candidate genes systematically reviewed.

Endometriosis

Familial aggregates

Endometriosis long has been observed as having familial tendencies [1,2]. Case reports of familial aggregates date from the 1940s, and in 1971, Ranney [3] reported a questionnaire survey. In 1984, Simpson et al [4] conducted the first formal genetic study. That study was based on 123 probands with histologically verified endometriosis. Nine of 153 (5.9%) female siblings older than 18 years had endometriosis; 10 of the 123 (8.1%) mothers were affected. Of the patients'

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husband's first-degree relatives (controls), only 1% had endometriosis. Women with an affected sibling or parent were more likely to have severe than mild or moderate endometriosis [5]. Severe endometriosis was present in 11 of the 18 probands (61%) who had an affected first-degree relative. Severe endometriosis was present in only 25 of 105 (23%) of the affected probands who had no affected first-degree relative.

Later studies have been consistent with these initial observations (Table 1). Lamb et al [6] obtained questionnaires from 491 members of the Endometriosis Association, a US-based organization (Table 1). A positive family history was reported by 18% of respondents and was evaluated in more detail in 43 women who returned a detailed questionnaire that also was completed by a friend (control). Endometriosis was present in 6.2% of mothers of probands and 3.8% of sisters; endometriosis was reported in less than 1% in first-degree relatives of friends. The frequency in second-degree relatives was 0.4% in grandmothers and 3.1% in aunts.

The Norwegian study by Moen and Magnus [7] was similar in design to that of Simpson et al [4]. Among 522 informative cases, 3.9% of mothers and 4.8% of sisters had endometriosis; only 0.6% of sisters of women who did not have endometriosis (controls undergoing laparoscopy for other reasons) were affected. In this study, either endometriosis or adenomyosis constituted the basis for diagnosis. Mothers were far more likely to have adenomyosis than affected sisters. As previously observed by Simpson et al [4] and Malinak et al [5], familial cases in Norway also were more likely to show severe endometriosis than were nonfamilial cases. In another report from the same Norwegian center, eight monozygotic twins were observed among 515 endometriosis cases [8]. Six of the eight sets were concordant, and three mothers also were affected.

In the United Kingdom, Coxhead and Thomas [9] reported a sixfold increased frequency of endometriosis among first-degree relatives. In Brazil, dos Reis et al [10] reported 8.6% of first-degree relatives of 81 probands as affected compared with no relatives of 43 controls. It is also evident from studies underway in Taiwan [11] and Puerto Rico [12] that endometriosis will prove to be familial in those venues. An attractive model exists in Rhesus monkeys, with genetic (familial) factors almost certainly responsible [13].

Kennedy et al [14,15] collect familial cases for linkage studies and perform sibling pair analysis using DNA polymorphic variants. They have had little difficulty recruiting familial aggregates worldwide [14,15]. They recommend

Table 1
Frequency of endometriosis among first-degree relatives of index cases to controls

	Mothers(%)	Sisters(%)	All first-degree relatives (%)	Controls (%)
Simpson et al [4]	5.9	8.1	6.9 (19/276)	0.9 (2/211)[a]
Lamb et al [6]	6.2	3.8	4.9	2.0
Moen and Magnus [7]	3.9	4.8	4.3 (45/1038)	0.6 (2/318)[a]
Coxhead and Thomas [9]	—	—	5.5 (7/127)	0.8 (2/258)[a]

[a] Statistically significant.

MRI for diagnosis of endometriosis [16]. Endometriosis was found on MRI in 5 of 14 (14%) first-degree relatives and 1 of 12 (8%) other relatives; equivocal findings were found more often.

Comparable to the studies cited previously, most population-based studies have generated similar recurrence risks. Stefansson et al [17] compared 750 Icelandic women with endometriosis to matched controls. The former were descended from a smaller number of ancestors (minimum number of ancestors) and showed an increased average kinship coefficient, consistent with complex genetic basis. The risk ratio for sisters was 5.2 and 1.56 for cousins.

Data from Utah indicate higher recurrence risks in the Mormon population. Hull et al [18] identified 419 women from Utah with endometriosis. Of these women, 326 had at least one sister of reproductive age; 17.8% of the total of 719 sisters had a surgically diagnosed endometriosis, a twofold to threefold increase over other studies. Of the proband's mothers, 11.2% had a surgical diagnosis, and 25% had "suggestive symptoms" of endometriosis (eg, pelvic pain). Approximately 14% reported an affected cousin. Affected relatives were more often of maternal (10.3%) than paternal (5.6%) lineage. Overall, 33% of probands had at least one affected relative in these large, extended families.

Higher concordance exists for monozygotic than dizygotic twins [8,14,19,20]. Endometriosis as a cause of surgical menopause also is more highly correlated in monozygotic twins than in dizygotic twins (r = 0.52 versus 0.19) [19]. Finally, menstrual pneumothorax was reported in two sisters with pelvic endometriosis [21]. Pneumothoraxes occurred on the right, the usual side.

Probable mode of inheritance: polygenic/multifactorial

The 5% to 8% risk for first-degree relatives is more reminiscent of polygenic/multifactorial tendencies than of a single mutant gene (25% or 50%). One assumes either that more than a single gene is involved or, in theory, that multiple alleles are at a single locus. A rarer mendelian form could still coexist, but polygenic inheritance is far most likely if one assumes that endometriosis is a single entity. In polygenic inheritance, increased severity in familial cases is expected because one predicts that the greater the severity of a polygenic disorder, the greater the underlying genetic liability. The proportion of affected relatives should be increased when the proband has severe endometriosis. The fact that endometriosis is more severe in familial than sporadic cases also lessens the likelihood that presence of an affected family member led to the identification of an affected relative merely because of a higher index of clinical suspicion [5].

One or more forms of endometriosis might still be mendelian, despite the larger proportion being nongenetic or polygenic. This heterogeneity exists in peptic ulcers and other adult-onset disorders. The fact that no human leukocyte antigen associations traditionally have been observed in endometriosis [22–24] is more consistent, however, with polygenic/multifactorial inheritance as the major—if not exclusive—genetic explanation. Recently, Ishii et al [25] found an association for the HLA-DRB1 1403 allele but not for other alleles.

Assuming that more than one gene is involved, how many genes are likely to be involved? There need not be many. The basis for this statement can be discerned by the following rationalization. Suppose that not one but two or more genes influence a given trait, and suppose further that at each there are two alleles (ie, A,a;B,b). Nine genotypes are possible: AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb, and aabb. Assume heuristically that A, B, a, and b each exert dissimilar phenotypic influences. As the number of genes controlling a trait increases, the number of genotypes in the population increases rapidly. For three genes, each with two alleles, 27 classes exist (3^n) (Table 2). Table 3 shows that if there are three alleles per locus and two loci, 36 genotypes exist (6^n). As the number of genotypic classes increases, a histograph showing the distribution of genotypes (phenotypes) in the population more closely approximates a normal distribution. Continuous variation can be approximated in the population by only a few genes.The significance of this information is that finding the genes that cause endometriosis is not necessarily daunting. No more than three or four genes need be pivotally involved; or perhaps only two with multiple alleles.

General strategies for finding the genes responsible for endometriosis

The task of determining the number of genes responsible and their chromosomal location(s) can be undertaken by genome-wide approaches, which include (1) comparative genome hybridization or other cytogenetic-based approaches designed to identify chromosomal regions of interest; (2) quantitative linkage analysis, which is designed to compare DNA of endometriosis cases and normal individuals in a given family; and (3) expression profile patterns, which are designed to compare mRNA (cDNA) between individuals with endometriosis and controls or between endometriosis tissue and eutopic endometrium or nonreproductive tissue from a single individual.

Any of these approaches cumulates in a candidate gene for subsequent analysis. Alternatively, one also can hypothesize a given candidate gene without prior genome-wide analysis. A caveat in the latter is that most studies that purport

Table 2
Relationship between numbers of genes controlling a trait and numbers of genotypes (classes of individuals) in a population, assuming two alleles per locus, each of which exerts a differential phenotypic effect

No. of loci	Alleles	Genotypes (classes of individuals)	No. of genotypes (formula)
1	(M,N)	MM MN NN	3 (3^1)
2	(M, N;R,S)	MMRR MMRS MMSS MNRR MNRS MNSS NNRR NNRS NNSS	9 (3^2)
N	(2 alleles per locus)		3^n

Table 3
Relationship between numbers of genes controlling a trait and number of genotypes in a population assuming three alleles per locus

Loci	Alleles	Genotypes	No. of genotypes
1	(M,N,O)	MM MN MO NN NO OO	6 (6^1)
2	(M,N,O;P,Q,R)		36 (6^2)
N	(3 alleles per locus)		6^n

to study a candidate gene are more accurately termed "association studies," which carry their own pitfalls. General gene mutation screening approaches can be undertaken or, ideally, the entire gene can be sequenced.

Cytogenetic attempts at gene localization: metaphase chromosomal analysis, fluorescent in situ hybridization, and comparative genome hybridization

Chromosomal rearrangements in affected endometriotic tissue may uncover candidate chromosomal loci and, ultimately, causative genes. Initially, cytogenetic studies were unrevealing in endometriosis. Dangel et al [26] found no evidence of abnormalities in any of 42 implants. Given the potential for outgrowth of the selectively advantaged normal cells in heterogeneous tissue specimens, endometriotic tissue may not have been studied. Use of newer techniques has proved more informative. The authors' group used fresh tissue touch preparations of endometriotic tissues to permit direct placement of cells from select tissue areas onto slides and then applied chromosome-specific probes (multicolor fluorescent in situ hybridization) to examine single cells. Nonrandom chromosome alterations included trisomy 11 and monosomy 16 and monosomy 17 in late-stage disease [27]. The authors observed a significantly greater frequency of chromosome 17 aneuploidy in the endometriotic specimens ($n = 8$, mean of 65%) compared to matched normal endometrial cells (mean of 25%) [28]. A significant ($P < 0.0001$) difference between the distribution of fluorescent in situ hybridization signals among the endometriosis samples also was found, which implied a high degree of heterogeneity involving chromosome 17 aneuploidy. These findings provided evidence that acquired chromosome-specific alterations may be involved in endometriosis and support a multistep pathway (see later discussion) that suggests clonal expansion of chromosomally abnormal cells. That is consistent with etiology that involves candidate tumor suppressor genes or oncogenes. Chromosomal loss or gain would, similar to cancer, be expected to play a role in the development or progression of endometriosis.

An alternative approach to in situ analysis of fresh endometriotic tissue is to perform conventional cytogenetic analysis on established cells lines. A human endometriosis-derived permanent cell line (FbEM-l) has been established [29]. Cytogenetic R-banding showed numerous chromosomal aberrations, including monosomy X, 4q+, 5q+, trisomy 7, 8, and 10, and tetrasomy of chromosomes 17,

18, 19, and 20. A caveat for these studies is that cultured cell lines may be unstable, which reflects growth of selectively advantaged cells and is no longer representative of the original tissue. Comparative genomic hybridization also has been used to identify somatic chromosomal alterations from the FbEM-l cell-line [30–32]. Comparative genomic hybridization revealed overrepresentation of chromosomes 1, 2, 3, 5, 6p, 7, 16, 17q, 20, 21q, and 22q, whereas chromosomes 6q, 9, 11p, 12, 13q, 18, and X were underrepresented. Subsequent fluorescent in situ hybridization analysis confirmed that the gain at 17q involved amplification of the protooncogene HER-2/neu in 16% of the nuclei of the cultured cells. Analysis of the original tissue showed loss of 1p, 22q, and chromosome X and gain of 6p and 17q. Gogusev et al [30] also evaluated primary endometriotic lesions (n = 18) and found abnormalities for 1p and 22q in 50% of the samples. Loss was observed for 5p (33%), 6q (27%), 7p (22%), 9q (22%), lq (22%), and 17q in one case. Chromosomal gain was detected for 6q, 7q, and 17q.

Quantitative genetic analysis

Principles

Quantitative linkage analysis is based on the principle that any region in the genome could encode a gene(s) of importance to the disease in question. To find these genes from among the 40,000 total human genes, the most commonly used method of quantitative linkage analysis is sibling-pair analysis. Presumably affected relatives (siblings) inherit identical copies of any given allele (identified by descent from their common ancestors [parents for siblings]) more often than expected by chance alone. Sibling-pair analysis obligatorily requires that informative DNA polymorphic markers exist every few centimorgams (10cM). The polymorphisms usually used are DNA variants, such as dinucleotide, trinucleotide, or tetranucleotide repeats. At any given locus, alleles of variable length (ie, CA^n, CAG^n) exist, easily assayed by molecular techniques. Another option is single nucleotide polymorphisms, spaced 1:1000 nucleotides apart. Regardless, a polymorphic marker near a disease (mutation) locus is more likely to be the same in two affected siblings than in a pair of siblings in which one is affected and the other is not. The further the causative gene from the polymorphism, the less informative it is, which reflects the likelihood of crossing over (recombination) that occurs during meiosis, which, if present, results in polymorphism being found on the opposite (homologous) chromosome. Using DNA obtained from patients and their family members, DNA genotyping can identify a region of interest. Plausible candidate genes—known or novel—emanate from searching preexisting genome databases using computer-based methodologies. Candidate genes of interest can be analyzed by direct (sequencing) or indirect methods (denaturing gradient gel electrophoresis and single-stranded conformational polymorphism) to identify mutations in affected individuals. Distinguishing trivial DNA alterations (polymorphisms) from disease-causing mutations depends on presence or absence of the change in

unaffected relatives or existence of a change known to be disruptive (eg, nucleotide alteration predicted to produce a stop codon that results in premature termination of the message) (mRNA) and thus a truncated gene product.

One strength of sibling-pair analyses for quantitative linkage analysis is that no specific mode of inheritance need be hypothesized because Identity by Descent (IBD) sharing at a given locus is simply being compared with the random expectation of 0.5 for first-degree relatives. Excess IBD sharing can be detected in affected relatives regardless of (incomplete) penetrance, phenocopy, or genetic heterogeneity.

Several pitfalls exist in quantitative linkage analysis. One pitfall is the necessity for large numbers of multigenerational families with accurate diagnosis of affected and unaffected individuals. Another pitfall is difficulty in excluding disease in ostensibly unaffected individuals. Misclassification is a particular problem in disorders such as endometriosis that show clinical mimicry and are not always easily diagnosed clinically. Another final pitfall is gene interaction, which impedes detecting linkage. Simpson and Bischoff [33] provide further discussion of this topic.

Quantitative linkage in endometriosis

The first quantitative linkage search in endometriosis was begun in Oxford, United Kingdom, using sibling-pair analysis [14,16,34]. Over several years, various regions of exclusion were noted, but no specific linkage has been published. A second center pursuing this approach is in Australia. Initially 289 families with 374 sibling-pairs were studied and possible linkages claimed [35]. More recently, these Oxford and Australian groups have combined, along with their current respective commercial partners (Oxagen Ltd, United Kingdom, and Cerylid Biosciences, Australia). A total of 557 families and 683 sibling-pairs have been studied. ''Significant linkage'' was reported for ''one locus'' and ''possible linkage'' for four other loci [36]. A third group that is pursuing quantitative linkage is in Iceland [17]. At one time a suggestive locus was found on 9q [37], but more recently the same group failed to confirm these preliminary results [38].

Gene expression profiling and microarrays

Histochemical studies

Another approach for finding the genes pivotal for endometriosis involves searching for differences between mRNA (or derivative cDNA) or the actual gene products expressed in endometriosis and control tissue. This approach typically uses microarrays (mRNA) but began with immunocytochemical approaches (gene products). The basic principle is not disparate. In earlier studies, high protein (gene product) levels were found for various protooncogenes, compared to levels in normal endometrium: c-*myc,* c-*fms,* c-*erbB*-1/2, and *ras* [39,40].

Using the established endometriosis cell line alluded to previously, overexpression of oncogenes c-*myc* and c-*erbB*-1 and c-*erb*-2 was observed by Gogusev et al [31]. These data originally suggested that altered protooncogene expression may be involved in disregulated growth and differentiation of endometriotic cells. That is, endometriosis involved multi-hit gene mutations, like neoplasia.

In other studies, *bcl*-2 was overexpressed [41]. This gene was interesting because in the normal endometrial cycle, *bcl*-2 regulates cellular homeostasis and apoptosis; increased expression is observed in the proliferative endometrial phase but not in the secretory phase [41]. Overexpression could lead to a decreased rate of cell death [42]. Watanabe et al [43] reported *bcl*-2 to be overexpressed in ectopic endometrial lesions by immunohistochemical staining, which indicates that endometriotic cells fail to undergo apoptosis. Using a cell death detection enzyme-linked immunosorbent assay, Dmowski et al [44] found that apoptosis was significantly decreased in eutopic endometrium of women with endometriosis, compared with fertile controls.

Microarrays and expression profiling in endometriosis

Histochemical studies are fundamentally limited by the inability to study more than one gene (or more than only a few) at a given time. Microarrays overcome this obstacle, making it possible to study simultaneously the presence of thousands of genes or, more specifically, the expression of thousands of genes. High-density oligonucleotide or cDNA arrays are constructed through several thousand oligonucleotides of defined sequence synthesized directly onto derivatized glass slides through photolithography and oligonucleotide chemistry [45,46]. These surface-bound oligonucleotides (probes) subsequently can bind or hybridize labeled mRNA and detect RNAs at frequencies of only 1:300,000. Expression studies require not only that a given DNA sequence be present but also that it must be transcribed into mRNA (expressed). Because mRNA is unstable, it is typically transcribed by reverse transcriptase into stable, single-stranded, cDNA for ease of experimentation. The cDNA is used to challenge the microarrays, none of which has yet been made specific for plausible gene likely to be involved in endometriosis.

Eyster et al [47] illustrated the use of cDNA microarray technology by studying eutopic endometrium and endometriotic implants from three patients. The DNA microarray (Human Genes Gene Filter, Research Genetics, Huntsville, AL) had 4133 genes, actually not a large number for microarrays. Total RNA was extracted and reverse-transcribed to cDNA before being denatured and added to the DNA microarray. Eight genes were overexpressed in endometriotic implants as compared to eutopic endometrium, several of which have roles in the cytoskeleton (Fig. 1).

Giudice et al [48] also presented preliminary data that compared expression profiles of eutopic endometrium from women with and without endometriosis. Eight samples from endometriosis subjects and ten samples from controls were hybridized on the Affymetrix Hu95A microarrays (Affymetrix Inc., Santa Clara, CA), whose probe set contains more than 12,000 full-length human genes or

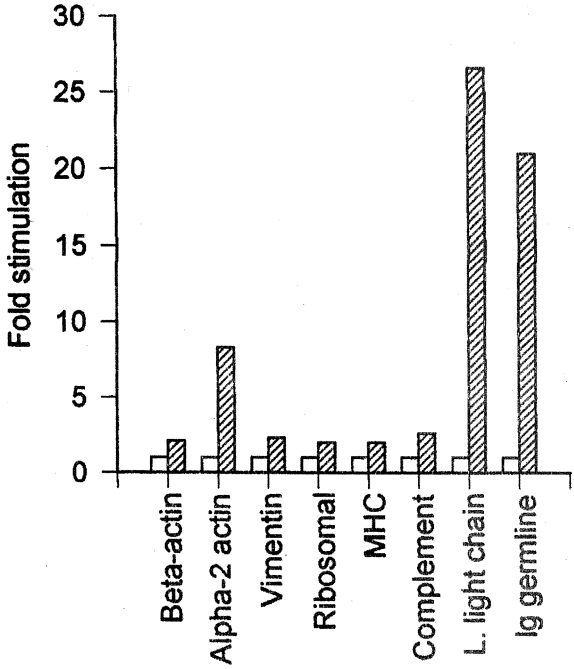


Fig. 1. Illustration of the differential expression between uterine endometrium (*open bars*) and ectopic endometriosis implants (*crosshatched bars*) in relative densitometric units normalized to the expression of cyclophilin C (n = 3). Ribosomal = 40S ribosomal S23 protein; MHC = major histocompatibility complex class 1,C; Complement = complement component 1 S subcomponent; L light chain = lg-λ light chain; lg germline = lg germline H chain G-E-A region B γ-2 constant region. (*From* Eyster KM, Boles Al, Brannian JD, Hansen KA. DNA microarray analysis of gene expression markers of endometriosis. Fertil Steril 2002;77:38–42; with permission.)

expressed sequence tags, which need not connote an entire gene. Of these more than 12,000 probes, 91 were overexpressed and 115 were underexpressed.

Work on cDNA microarrays is also underway in Taiwan [49]. In Singapore, Hu and Tay [50] are performing substraction hybridization to distinguish cDNAs differentially expressed in endometriosis versus normal tissue.

A complementary approach to cDNA microarrays—one favored by the authors' group—is tissue microarrays. Minute tissue cylinders (diameter 0.6–2mm) are removed from hundreds of primary tissue blocks and subsequently juxtaposed in one "recipient" paraffin block (Fig. 2) [51]. A single immunostaining or in situ hybridization reaction provides information on all specimens on the slide; subsequent sections can be analyzed with other probes. Genes that are upregulated or downregulated are specifically targeted for further analysis. One pitfall of tissue microarray technology is the uncertainty concerning the extent to which tissue heterogeneity affects the validity of the approach. Several studies, however, have confirmed the validity and representativity of tissue microarray data [52] compared with conventional techniques. Through microarrays, differ-

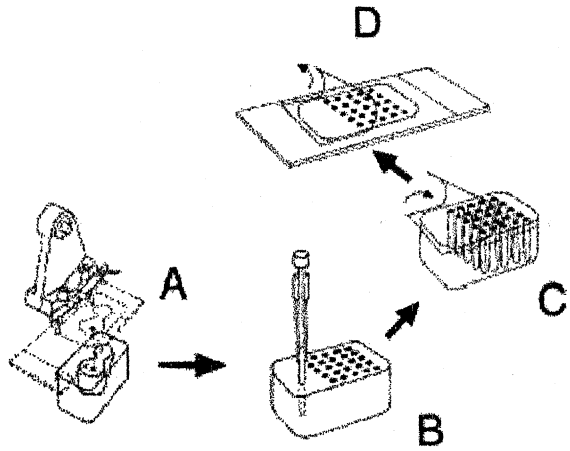


Fig. 2. Tissue microarray construction. (A) A tissue core biopsy of 0.6 mm in diameter is punched from a preselected region of a donor block using a thin-wall stainless steel tube. A hematoxylin and eosin (H&E) stained section overlaid on the surface of the donor bloc is used to guide sampling from representative sites in the tissue. (B) The tissue core is transferred into a premade hole at defined array coordinates in the recipient block. (C, D) An adhesive-coated tape sectioning system assists in cutting the tissue microarray block.

ential genetic perturbations among endometriotic tissue from different patients or at different stages of the disease can be studied in a comprehensive manner.

Proteomics

A new and fashionable technology related to DNA microarrays involves monitoring gene expression by proteomics. This omnibus term encompasses global patterns of gene expression (translated gene product) at the protein level [53]. (It harkens back to histochemical studies, except it currently involves concurrent analysis of many proteins). Proteomics has progressed from analysis by two-dimensional gels to simultaneous high-throughput analysis of many proteins. Technology is evolving in three general areas: biochips that use mass spectrometry, such as surface-enhanced laser desorption/ionization systems; protein arrays; and most recently, lysate (reverse phase protein) arrays [54]. Protein arrays consist of robotically immobilized recombinant proteins or antibodies placed on discrete surfaces. These arrays can hold from hundreds to thousands of ''bait'' molecules (spots). The array is then incubated with the protein sample or lysate of interest, which has been tagged with a detectable enzyme or dye. Concentration of the target protein in the original sample is directly proportional to the level of signal of each capture spot [55]. Proteins are then eluted from the array and their mass determined by time-of-flight mass spectrometry. Identification of peak on the mass spectrometer requires further characterization to identify the protein detected. No studies regarding endometriosis have been published, but these techniques seem promising.

Survey of candidate genes: association, linkage, and molecular perturbation studies

Once one has a specific hypothesis (candidate gene), he or she can search systematically for DNA perturbations at this locus or chromosomal regions. This approach may follow quantitative linkage analysis or gene expression profiling or it may involve a de novo hypothesis derived from nongenetically derived data (or sheer plausibility).

Although many published reports claim to be searches for a specific gene or linkage to purported candidate genes, what is often sought is actually an association. An association is a statistical relationship between a given disease and a locus, such as human leukocyte antigen or other polymorphic allele. Dozens of associations between histocompatibility antigens and various disease states have been identified, but the disease is only rarely on chromosome 6, which encodes human leukocyte antigen. An association may or may not indicate a causative gene and certainly needs not connote linkage.

Three general explanations have been proposed to explain association between a polymorphic allele and a nonlinked disease: (1) ethnic stratification, the disorder that coincidentally (spuriously) occurs in a population subset in which a given marker is also unusually common; (2) direct causal relationship, the disease that occurs as result of presence or absence of the marker itself or the gene coding the marker (ie, pleiotrophy); and by contrast, there could genuinely exist (3) linkage between the marker allele and another gene that truly causes the disease. That is, the marker is not itself etiologically related but would rather indirectly identify other loci integrally related to the pathogenesis.

Differences between association and linkage are underscored by realizing that if two genes are linked, one would not expect a particular allele at one locus to be associated in the population with a particular allele at the second locus. If the two alleles are found together more often than chance, another phenomenon can be deduced to exist. Suppose, for example, that gene A is linked to gene B, and suppose further that each gene has at least two alleles (A,a;B,b). In the general population the following combinations of alleles could exist: AB, Ab, aB, ab. If "linkage equilibrium" exists, the relative frequency of the combinations can be calculated given frequencies of the individual alleles. In a given family, certain combinations would be expected to be preferentially observed. For example, Ab or aB may be more frequent than AB or ab in a given family; in other families, AB or ab may preferentially be observed, Ab or aB arising only after recombination. Recombination frequencies decrease as closeness of linkage increases.

On the other hand, certain pairs of alleles may be found more often than expected in the population. In this linkage case disequilibrium is said to exist. For example, AB and ab might occur more often than predicted on the basis of frequencies in the population; Ab and aB might occur less often. There are various explanations for linkage disequilibrium, but usually none proves uncertain.

Galactose-1-phosphate uridyl transferase

The first candidate gene specifically studied for association or linkage was galactose-1-phosphate uridyl transferase, located on chromosome (9p13). The specific alteration sought was an adenine to guanine transition (polymorphism) in codon 314 of exon 10. This results in substitution of aspartate (D) for asparagine—N314D. This polymorphism was reported to be associated with endometriosis by Cramer et al [56]. Such an association, however, was not confirmed by Morland et al [57], Hadfield et al [58], or Stefansson et al [38,59]. Linkage (as opposed to association) to galactose-1-phosphate uridyl transferase was formally disproved by the latter group.

Phase I and II detoxification genes

Drug metabolism is carried out by phase I (functionalization) and phase II (conjugation) reactions. Phase I drug metabolizing enzymes (eg, CYP1A1) act by introducing a functional group into their endogenous and exogenous substrates. A procarcinogenic compound is metabolically activated by a (phase II) conjugating enzymes to render the compound inactive and no longer carcinogenic or procarcinogenic.

Phase I

Several phase I detoxification enzymes have been studied in endometriosis. Aryl hydrocarbon receptor is present in the cytoplasm and has high affinity for dioxin and dioxin-like compounds. Receptor activation follows stereospecific ligand binding and movement to the nucleus to form a complex that acts as a transcriptional activator of drug-metabolizing enzymes. In aggregate, this complex is a potent inducer of the phase I gene CYP1A1, as it is for phase II Glutathione S Transferase (GSTs). The human aryl hydrocarbon receptor gene is localized to 7p 15 [60]. Approximately 10% of the human population exhibits "high" CYP1A1 induciblity, which suggests germline polymorphisms that may give rise to variations in aryl hydrocarbon receptor affinity. This group is predicted to be at increased risk for cancer or, by analogs, endometriosis. Although an intriguing hypothesis, no association between either the aryl hydrocarbon receptor or CYP1A1 gene polymorphisms was found in Japanese women who had endometriosis [61]. Hadfield et al [62] also found no significant difference in frequencies for CYP1A1 wildtype and MspI mutant alleles between endometriosis (n = 129) and control (n = 147) subjects.

Phase II

Among phase II enzymes, two have been studied extensively: GSTs and N-acetyltransferase 2 (NAT2). GSTM1 and GSTT1 are critical in the detoxification of the products of oxidative stress produced during the repair of the ovarian epithelium [63]. GSTM1 and GSTT1 are polymorphic and have null alleles that can be readily detected by polymerase chain reaction-based methods [64,65].

Homozygous null alleles in the two genes may function synergistically, causing inefficient detoxification of the intermediary compounds produced during stress that if not rapidly metabolized increase damage to various genes in the host cell. Initial studies were by Baranova et al [66], who reported the homozygous GSTM1 null allele to be present in 86% of 50 endometriosis cases compared to 45.8% 72 of controls unaffected. Hadfield et al [62] and Baxter et al [67] failed to confirm this association, finding 45% (n = 132) and 48% (n = 84), respectively, homozygous null genotype frequencies. The authors' group reported only 27% (n = 63) of endometriosis cases to have the homozygous null genotype [68].

Baranova et al [69] also reported increased frequency (60%, n = 65) of the slow acetylation genotypes of aryl amine NAT2, another phase II inactivation enzyme. Nakago et al [70] found contradictory results, with increased frequency of fast acetylation genotypes (57%, n = 54). The authors' group failed to find a difference in the frequency between fast and slow acetylation NAT2 genotypes between endometriosis subjects (59% fast versus 41% slow; n = 111) and controls (63% and 37%; n = 37), respectively. They did observe a significant ($P < 0.001$) difference between allele active and null GSTM1 frequencies (active 79% endometriosis versus 44% controls) [68].

Steroid-related genes (estrogen receptor, aromatase, P21)

Estrogen and aromatase are highly plausible candidate genes [71]. A relationship between endometriosis and increased local production of estrogen has long been a popular thesis. 17 β-hydroxysteroid dehydrogenase type II expression has been shown to be deficient in endometriosis, further leading to impaired conversion of estradiol to estrone. Collectively, these endocrinologic aberrations result in accumulation of increased estradiol and Prostaglandin E2 (PGE_2) [71]. This accumulation could be mediated by estrogen or estrogen receptor gene polymorphisms that yield a more than typical hormonal milieu.

Georgiou et al [72] were the first to study Estrogen Receptor (ER) polymorphisms. Detectable by a PvuII restriction endonuclease, the Estrogen Receptor 1 (ESR1) polymorphism studied was found in 72% (82/114) of patients with endometriosis versus 49% (56/114) of controls. Kitawaki et al [73] studied 109 cases and 179 controls and found the Pallele to be associated with disease free status more so than p in this biallelic polymorphism. In a study of 50 Chinese women, Fu and Wei [74] reported no significant differences between endometriosis subjects and controls for the Pvu polymorphism at the ER locus. Hsieh et al [75] compared 102 surgically diagnosed cases with 119 controls with respect to a p21 polymorphism (Ser31Arg). No association was found.

Cell adhesion genes, matrix metalloproteinase, angiogenic factors

The plausibility for a role by one or more of these gene families is solid. The first formal study that sought an association was that of Vigano et al

[76]. Their study of 117 Italian women found increased frequency of the rare allele P241. Matrix metalloproteinase [77,78], integrins [79], and angiogenic factors are other attractive candidates supported by considerable in vitro and even in vivo experiments. Despite many in vitro and microarray data [47,48], genetic studies seem not to have been reported into specific molecular perturbations in endometriosis.

DNA mismatch genes

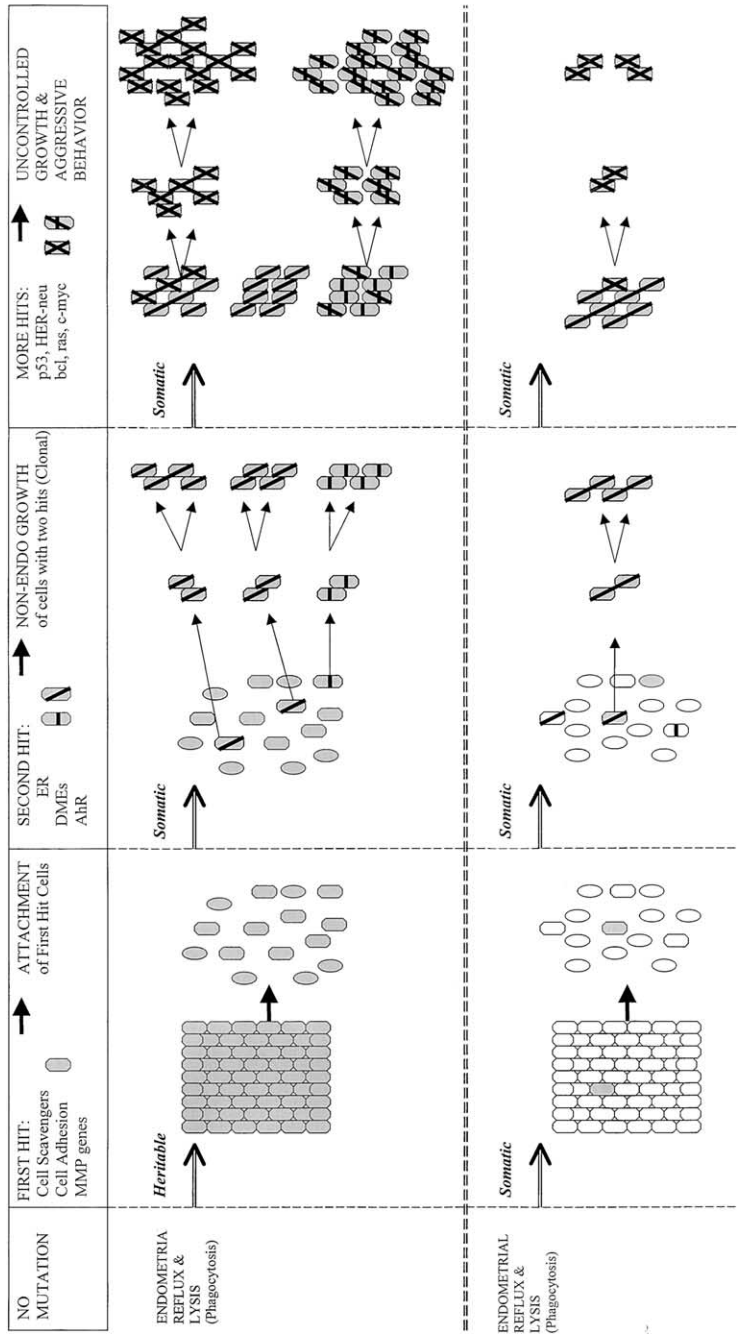
Goumenou et al [80] sought loss of heterozygosity (allelic imbalance) for several other candidate genes, finding negative results for the DNA mismatch repair genes MSH2, MSH6, MLH1, and PMS1. This same study failed to detect loss of heterozygosity for APOA2, a high-density lipoprotein.

Tumor-suppressor genes (p53 and Phosphatase and Tensin nomolog deleted on chromosome Ten (PTEN))

Genes of interest to the authors' group include oncogenes and tumor-suppressor genes. They derive their interest from the belief that endometriosis arises like neoplasia through multi-hit gene mutations [1,33,81]. Monoclonal cell expression has been observed in endometriosis [82,83], with overexpression of certain oncogenes (c-*myc,* c-*erg* B1.

The pathogenesis of neoplasia involves clonal origin of a single progenitor cell that has been subjected to at least two "hits" (sequential mutational events). One would expect more than one gene (polygenic) to be involved. Both mutations could be somatic (arising after birth), or one could be germline (present at birth) and the other somatic. In the former case, heritability is low (sporadic cases), whereas in the latter it is higher (familial). Although this simplistic (two-hit) model should be amended to a more complicated multi-hit hypothesis, the

Fig. 3. Multistep pathogenesis of endometriosis with retrograde menstruation. Endometriosis arises as a result of an initial mutation or hit (heritable or somatic) followed by a series of subsequent somatic hits. Two models are proposed. In one model, the first hit (mutation) is inherited (germline), with all of the cells having the same mutation since birth. In the second model, the initial hit is random and acquired (somatic) after birth in one or few cells. For either model, the initial hit might involve genes that regulate cellular attachment (eg, matrix metalloproteinases and integrins) or unscheduled persistence (eg, macrophage receptor). As a result, refluxed endometrial cells adhere more readily to cellular surfaces within the peritoneal cavity. Additional mutations (all somatic) may arise as a second hit. These genes could involve inefficient metabolism of chemicals and/or toxins, with subsequent buildup of toxic intermediate byproducts that results in oxidative stress. In the heritable model, every cell is susceptible to further mutation; therefore, the likelihood of a second hit occurring increases. In the nonheritable model, the likelihood of two hits is lower on purely stochastic grounds. Still further mutational events could involve tumor-suppressor genes and/or oncogenes (eg, *bcl*-2, p53, *ras*) to confer upon cells the invasive features that are capable of spreading to other areas or organs and causing more severe disease. *Abbreviations:* ER, estrogen receptor; DMEs, drug metabolizing enzymes; MMP, matrix metalloproteinases; AhR, aryl hydrocarbon receptor.


NO MUTATION
FIRST HIT: Cell Scavengers Cell Adhesion MMP genes
ATTACHMENT of First Hit Cells
SECOND HIT: ER DMEs AhR
NON-ENDO GROWTH of cells with two hits (Clonal)
MORE HITS: p53, HER-neu bcl, ras, c-myc
UNCONTROLLED GROWTH & AGGRESSIVE BEHAVIOR
ENDOMETRIA REFLUX & LYSIS (Phagocytosis)
Heritable
Somatic
Somatic
Somatic
ENDOMETRIAL REFLUX & LYSIS (Phagocytosis)
Somatic
Somatic
Somatic

concept still remains valid [84] that two or more genes are necessary to initiate neoplasia and, by analogy, endometriosis. In endometriosis both genes need not involve oncogenes or tumor suppressor genes. In fact, the authors suspect that the first "hit" involves a gene that increases predisposition to implantation of refluxed menstrual endometrial tissue. This gene could involve the cytoskeleton, cell adhesion, or macrophage scavenging. The second "hit" is more likely to involve an oncogene and lead to cellular proliferation (Fig. 3).

If endometriosis mimics neoplasia and involves a tumor-suppressor gene, one would predict loss of heterozygosity. Loss of heterozygosity in endometriosis was found by Jiang et al [85] in fixed archival tissue: chromosomal regions 9p, 11q, and 22q. In another study, chromosomal alterations were also observed in 9 of 11 cases in which ovarian carcinoma had arisen within or adjacent to endometriosis [86]. Alterations in chromosomal regions 5q, 6q, 9p, 11q, and 22q were observed in 25% to 30% of endometriosis with associated carcinoma.

At Baylor College of Medicine, the authors first found alterations involving trisomy 11, monosomy 16, and monosomy 17 in late-stage endometriosis [27] based on studies using chromosome-specific probes. Chromosomal loss or gain seemed to play a role in the development or progression of endometriosis and specifically involved chromosome 17. Tumor-suppressor genes and oncogenes mapped to this chromosome naturally became the authors' focused candidate genes: BRCA1 and p53. A two-color fluorescent in situ hybridization approach for probes specific to the p53 locus (17p13) and 17-centromere [28] was then employed. A significantly greater frequency of chromosome 17 (monosomy) aneuploidy was observed in endometriosis specimens, compared with matched normal endometrial cells. That different tissues showed different extents of aneuploidy is consistent with somatic origin [1,28,33].

Does the perturbation involve the loss of all of chromosome 17 or only a given locus, namely p53? In 12 cases, fluorescent in situ hybridization was lacking signals for p53 and 17-centromere (monosomy 17) [28]. In four other cases, two signals for the 17-centromere probe were observed but only one was observed for the p53 probes. Loss of only the p53 tumor-suppressor gene, rather than loss (monosomy) of chromosome 17 per se, seemed to be the pivotal event. Sequencing the entire p53 gene in endometriosis is currently underway, but the authors have not yet found mutations [87]. In one endometriosis sample studied by Jiang et al [86], a point mutation in p53 gene was found (Tyr 220 Cys). Failure to find molecular perturbations could indicate that any causative roles played by p53 originate through chromosomal rearrangement, not point mutations, and could involve other genes in the region. A second tumor-suppressor gene that has been studied in endometriosis is PTEN, located on chromosome 10q23. PTEN mutations have been reported in endometrioid ovarian epithelial inversion tumors [88], which have a relationship to endometriosis, but in neither serous nor mucinous epithelial ovarian tumors [81]. Somatic mutations involving PTEN have been observed only in endometrioid tumors, albeit only 21% of cases [81]. PTEN mutations could be an early event in transforming benign endometriotic cells to malignancy progenitors.

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Pathogenesis of endometriosis

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Origin of endometriotic implants

Endometriosis is a common gynecologic disorder characterized by the presence of endometrial tissue outside the uterine cavity. Various theories have been put forth to explain the mechanisms for the development of this disease. The authors summarize the existing theories on the origins of endometriotic tissue. They also review the factors that affect the survival and growth of these implants.

Retrograde menstruation (implantation) theory

The retrograde menstruation theory, also known as implantation or Sampson's theory, proposes that viable endometrial tissue is refluxed through the fallopian tubes during menstruation and implants on peritoneal surface or pelvic organs [1]. This theory is based on three assumptions. First, there is retrograde menstruation through the fallopian tubes. Second, refluxed endometrial cells are viable in the peritoneal cavity. Third, the refluxed endometrial cells are able to adhere to peritoneum with subsequent invasion, implantation, and proliferation.

The implantation theory was neglected for a long time because of the presumption that retrograde menstruation was rare and endometrial tissue was not present in menstrual effluent [2–5]. Later, several studies confirmed the high incidence of retrograde menstruation. In 1938, Watkins observed blood dripping from fallopian tubes in women who underwent laparotomy during menstruation [6]. After this observation, Goodall reported that retrograde menstruation occurred in 50% of women who underwent laparotomy during menstruation [7]. The presence of blood in the peritoneal fluid was also observed in women who underwent peritoneal dialysis [8]. Recent studies using laparoscopy have shown

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that retrograde menstruation is a common phenomenon that occurs in 76% to 90% of women with patent fallopian tubes [9, 10].

Later came the demonstration of the viability of sloughed endometrial cells and their capacity to implant at ectopic sites. In 1951, Keettel and Stein cultured endometrial cells obtained from menstrual discharge of seven women who wore diaphragms [11]. Endometrial cells obtained from peritoneal fluid after uterine lavage also were cultured successfully [12, 13]. Endometrial cells collected from the peritoneal cavity after uterine lavage stayed viable in culture for up to 2 months [14]. Finally, endometrial cells obtained from peritoneal fluid also were cultured successfully [15]. These findings proved the viability of menstruated endometrial cells.

Once in the peritoneal cavity, retrogradely menstruated endometrial cells should be able to implant to cause endometriosis. In 1950, Scott and TeLinde reported that shed endometrial cells were able to implant [16]. In monkeys they inverted the uterus and diverted menstrual flow into the peritoneal cavity and showed that 50% of the monkeys developed endometriosis [17]. Similarly, it was demonstrated that endometriosis developed in four baboons after injection of menstrual endometrium into their retroperitoneal space [18]. Ridley and Edwards collected menstrual effluent from women during the second day of menstruation and injected it into the subcutaneous abdominal fat of patients who subsequently underwent laparotomy for other gynecologic indications 90 to 180 days after implantation. The site of injection was excised for histologic study, and viable endometrial glands and stroma were present at the site of implantation in these women [19]. These findings demonstrated that viable endometrial cells in menstrual effluent are able to implant and develop into endometriotic lesions.

Substantial clinical data also exist to support the implantation model of peritoneal endometriosis. There is an increased risk of endometriosis in patients with müllerian anomalies and obstructed flow [20, 21]. There is an increased frequency of endometriotic implants in the dependent areas of the pelvis [22]. This anatomic distribution of endometriosis also supports the concept of retrograde menstruation.

Coelomic metaplasia theory

The theory of coelomic metaplasia initially was introduced at the turn of the twentieth century by Meyer. This theory proposed that endometriosis develops from metaplasia of cells that line the pelvic endometrium [23–25]. Meyer suggested that infectious, hormonal, or other inductive stimuli may result in metaplasia, which in turn could result in endometriosis [26, 27].

Embryologic studies demonstrated that pelvic peritoneum, germinal epithelium of ovary, and müllerian ducts are derived from epithelium of the coelomic wall [28]. This type of transformation may cause ovarian surface endometriosis. Clinical evidence that supports the theory of coelomic metaplasia lies in case reports of endometriosis that occurs in men [29, 30], in prepubertal [31] and adolescent girls [32], in women who never menstruated [33], and in unusual sites, including pleural cavity [34–36].

The occurrence of endometriosis in men is generally thought of as proof of the theory of coelomic metaplasia. The men with endometriosis were undergoing estrogen therapy, however, and the possibility of estrogen stimulation of müllerian rests cannot be excluded. Similarly, although pleural endometriosis could result from local metaplasia of pleural mesothelium, it also might result from transdiaphragmatic passage of endometrial fragments. If coelomic metaplasia is similar to metaplasia elsewhere, an increase in its frequency would be expected with aging. Proofs for the theory of coelomic metaplasia are far from being conclusive.

Induction theory

The induction theory is an extension of the coelomic metaplasia theory and proposes that endogenous biochemical or immunologic factors can induce undifferentiated cells to differentiate into endometrial tissue. This theory is supported by observations in female rabbits. Initial evidence to support this theory came from Levander and Normann, who implanted sections of uterine wall obtained from pregnant rabbits into subcutaneous tissue of 2-month-old female rabbits stimulated with gonadotropins immediately before transfer. In 7 days, they observed cells characteristic of endometrium and cyst formation in the surrounding tissue [37].

Similar experiments were later performed in rabbits by Merill using Millipore filters that contained myometrium, fat, or endometrium [38, 39]. Implants were later excised with the surrounding tissue and examined histologically. Cysts lined with cells that resembled endometrial epithelium and occasional gland-like structures developed in tissues adjacent to filters that contained endometrium but not in tissues adjacent to filters that contained myometrium or fat. Endometrial stroma, an important component of endometriotic implants, was not present in the induced tissue.

More recently, Matsuura et al demonstrated in vitro coelomic metaplasia in vitro in ovarian surface epithelium co-cultured with endometrial stromal cells and treated with 17β-estradiol [40]. The used estradiol concentration was nearly ten times higher than that in the peritoneal fluid. The high concentration could be found in the vicinity of the ovary and may explain ovarian endometriosis. These findings suggest that induction of coelomic metaplasia may be responsible for some cases of endometriosis.

Embryonic rest theory

In the 1890s, Von Recklinghausen [41] and Russell [42] introduced the embryonic rest theory. This theory proposed that cell rests of müllerian origin could be activated to differentiate into endometrium in the presence of a specific stimulus. Transformation of embryonic rests is a plausible explanation for rare cases of endometriosis reported in men.

Lymphatic and vascular metastasis theories

In the 1920s, Halban [5] and Sampson [43] suggested that endometriosis also could result from lymphatic and hematogenous dissemination of endometrial cells. Considerable evidence suggests that endometrial cells can metastasize via lymphatic and hematogenous routes. Metastasis of endometrial cells through the lymphatic system to distant areas, such as pleura, umbilicus, retroperitoneal space, lower extremity, vagina, and cervix, is anatomically possible because of communication of lymphatics among these structures [24, 44–46].

Sampson demonstrated the presence of endometrial tissue in uterine veins in women with adenomyosis [47]. Hobbs and Borthnick induced pulmonary endometriosis by injecting endometrial tissue intravenously in rabbits [34]. Lymph node endometriosis was found to be present in 6.7% of 178 autopsy cases and in 6.5% of 153 women who underwent lymphadenectomy [48].

Lymphatic or vascular metastasis could explain rare cases of endometriosis that have been reported in bone, muscle, brain, nerve, lung parenchyma, vertebral space, and extremities [49, 50].

How do the endometriotic implants survive and grow?

Retrograde menstruation is a universal phenomenon, and of all the theories, implantation of exfoliated endometrial cells is the most widely accepted theory for the development of endometriosis. On the other hand, why endometriosis develops in some women but not others is unknown. Five critical steps have been postulated in the development of endometriotic lesions. The two initial steps are attachment of endometrial cells to the peritoneal surface and invasion of these cells into the mesothelium. After these steps, recruitment of inflammatory cells subservient to the implant, angiogenesis around the nascent implant, and endometrial cellular proliferation occur. Although the endometriotic tissue with its local hormonal environment influences each of these steps, immune cells and inflammatory cytokines and environmental factors also play a role.

Attachment of endometrial cells to mesothelial cells

According to retrograde menstruation theory, fragments of endometrium are refluxed through the fallopian tubes into the peritoneal cavity. Then they attach to and grow on peritoneal surfaces. The mechanisms involved in cell attachment to the peritoneum have been studied in vitro, using extracts of intact amniotic and peritoneal membranes.

First, van der Linden et al [51] evaluated the ability of endometrial fragments from early proliferative phase to adhere to amniotic membrane in vitro. They reported that amniotic membrane was similar to peritoneum with respect to expression of cytokeratins in epithelial lining and of extracellular matrix components. The endometrial fragments did not adhere to the epithelial side of the amniotic membrane, whereas adhesion did occur on the nonepithelial side.

These authors suggested that intact epithelial lining may prevent initial adhesion of retrogradely shed endometrium fragments to peritoneum [51]. After this report, Groothuis et al [52] evaluated the ability of endometrial fragments isolated in the proliferative and secretory phase of the menstrual cycle to adhere to amnion. Endometrial fragments obtained in either phase of the cycle were able to adhere to the epithelial side of the amnion, but only at locations where the amniotic epithelium was damaged or absent [52]. They produced similar results using proliferative endometrium and cultured peritoneal explants. Endometrial cells adhered to peritoneal explants only at locations where the mesothelium was absent or damaged and the basement membrane was exposed [53]. The same authors also evaluated the adherence of shed menstrual tissue to amnion and peritoneum in vitro. Results were similar [54]. They concluded that intact mesothelium constitutes a defense barrier that prevents adhesion of endometrial fragments. They hypothesized that trauma to the mesothelial lining is a prerequisite for endometrial cell adhesion [53].

Using similar techniques, another group of investigators reported contradicting findings. Witz et al [55] cultured whole fragments of mechanically dispersed endometrium obtained during the proliferative and secretory phase with whole explants of peritoneum for 24 to 48 hours. They found that endometrial fragments attached to the mesothelial side and the nonepithelial side of the mesothelium, and the menstrual cycle phase during which endometrial tissue was collected did not make a difference. Approximately 90% of attached endometrial fragments did not have an intact underlying mesothelium, although most had an intact mesothelium running up to the point of attachment. Contrary to the findings of Groothuis et al, however, they identified an intact mesothelium at the site of attachment in 10% of the endometrial implants [55]. When they repeated the experiment using a 1-hour incubation period, they demonstrated the rapid adhesion of endometrium to the peritoneum and confirmed their finding that endometrial cells can attach to intact mesothelium [56]. In most sites of attachment, endometrium adhered to mesothelium via endometrial stroma, although many sites of endometrial epithelium-mesothelium attachment also were detected [56].

These findings led to the investigation of molecular mediators of endometrial cell attachment to mesothelium. Several cell adhesion molecules, including integrins, intracellular adhesion molecule-1, vascular cell adhesion molecule-1, have been implicated. The $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrins are expressed at the mesothelial cell surface and could mediate endometrial-mesothelial adhesion [57]. Integrin-blocking antibodies do not interfere with endometrial stromal or epithelial cell adherence to mesothelium, however [58].

Recently, hyaluronic acid and CD44 have been implicated in the interaction of peritoneal mesothelium with endometrial cells. Peritoneal mesothelium produces hyaluronic acid. Hyaluronic acid is expressed along the cell membrane of peritoneal mesothelial cells, contributes to the pericellular matrix, and is a major component of the extracellular matrix ground substance. CD44 is the principal receptor for hyaluronic acid. It is involved in binding of gastric cancer and

ovarian cancer cells to mesothelium. Endometrial stromal end epithelial cells express CD44. Hyaluronidase pretreatment of mesothelial cells decreases the binding of endometrial stromal and epithelial cells to mesothelium by 40% [59]. These findings suggest that the hyaluronic acid/CD44 binding may be involved in the initial adherence of endometrium to peritoneal mesothelium.

Invasion of endometrial cells into the mesothelium: matrix metalloproteinases and endometriosis

Invasion of endometrial cells into the mesothelium follows their initial adhesion to the peritoneal wall. Matrix metalloproteinase (MMP) enzymes have been implicated in this invasion. MMPs and their inhibitors, the tissue inhibitors of matrix metalloproteinases (TIMPs), play a significant role in normal endometrial remodeling that accompanies menses [60–62]. The MMP family contains several structurally related Zn^{2+}-dependent endopeptidases, which collectively are responsible for the degradation of various extracellular matrix components, including several types of collagen, gelatins, proteoglycans, laminin, fibronectin, and elastin [60–64]. The TIMPs are the natural inhibitors of MMPs [60, 64]. In eutopic endometrium, the expression of MMPs and TIMPs is tightly regulated by steroid hormones and cytokines during each phase of the menstrual cycle [61, 63]. Coincident with the tissue breakdown and remodeling that occurs at menses and during the early proliferative phase of the cycle, a significant upregulation of MMP expression occurs [61, 63]. Then, MMPs are suppressed during progesterone-driven endometrial differentiation in the luteal phase.

A significant amount of data indicates that MMPs are involved in the pathogenesis of endometriosis. In endometriotic lesions, abnormal expression of specific members of the MMP and TIMP families has been identified [65–69]. For example, MMP-1, MMP-3, and MMP-7 are expressed constitutively in endometriotic lesions, whereas they are highly regulated in eutopic endometrium during the menstrual cycle [63, 65, 69]. Although endometriotic cells synthesize and secrete TIMP-1 protein in vitro [70], in vivo TIMP-1 concentrations are lower in the peritoneal fluid of women with endometriosis [71]. The role of MMPs in the establishment of ectopic lesions by human endometrium was evaluated in an animal model of endometriosis using athymic nude mice. In this model, suppression of MMP activity by pretreatment of human endometrial tissues with progesterone or intraperitoneal TIMP injections suppressed the development of endometriotic implants [72]. These findings suggest that increased MMP activity in and around the endometriotic implants may facilitate invasion and growth of lesions.

Progesterone downregulates endometrial MMP expression [68]. Paracrine factors that mediate progestin action on endometrial MMP expression have been investigated in an attempt to identify targets for treatments that would downregulate endometriotic MMP expression. One such factor is transforming growth factor-beta (TGF-β). TGF-β is produced by endometrial stroma in response to progesterone and can suppress expression of an epithelial MMP-7 independent of

progesterone. An antibody directed against the mammalian isoforms of TGF-β abolishes progesterone suppression of MMP-7 in stromal-epithelial co-cultures, which implicates TGF-β as the principal mediator of MMP-7 suppression in the human endometrium [73]. Similarly, in the nude mice model of endometriosis, blocking the action of TGF-β opposes progesterone-mediated suppression of MMP-3 and MMP-7 and blocks the ability of this steroid to prevent experimental endometriosis [74]. On the other hand, TGF-β alone does not lead to sustained suppression of MMPs, as observed after progesterone treatment, possibly because of resumption of MMP production in the absence of progesterone [74]. This finding is consistent with the fact that peritoneal fluid levels of TGF-β are elevated in endometriosis [75].

Another cytokine that regulates MMP expression is interleukin-1α (IL-1α). IL-1α is a potent stimulator of MMP-3 in proliferative phase endometrium in organ culture; however, progesterone exposure in vivo reduces the IL-1α stimulation of MMP-3 in secretory phase tissue [76]. The loss of sensitivity to IL-1α is duplicated in cultured endometrial stromal cells treated with progesterone in vitro. IL-1α stimulation of MMP-3 is restored in a dose-dependent manner with progesterone withdrawal [77]. Conversely, cultured endometriotic cells obtained from a rat endometriosis model express higher levels of MMP-3 mRNA than eutopic rat endometrial stromal cells when treated with progesterone. The elevated and persistent MMP-3 expression by endometriotic stromal cells cultured in the presence of progesterone correlates with elevated levels of IL-1α mRNA detected in the endometriotic stromal cells and IL-1α protein in their culture medium [69]. The production of IL-1α by the endometriotic lesions seems to be able to overcome the progesterone-induced suppression of MMP-3 in these cells, a phenomenon that is not observed in the cultured uterine stromal cells. It is plausible that an IL-1α– related mechanism promotes MMP-3 production by endometriotic cells even in the presence of progesterone.

Aberrant MMP and TIMP expression in the endometriotic environment caused by abnormal levels of paracrine regulators may induce a more aggressive behavior and facilitate invasion of endometriotic implants. The exact mechanisms that lead to the aberrant expression of MMPs and TIMPs have yet to be defined.

Survival and proliferation of ectopic endometrial cells

Immune factors

Impaired immune response that results in inadequate removal of refluxed menstrual debris has been proposed as a possible causative factor in the development of endometriosis. Endometriosis is associated with changes in cell-mediated and humoral component of innate and acquired immunity. Although the peritoneal fluid of women with endometriosis contains increased numbers of immune cells, they seem to facilitate rather than inhibit the development of endometriosis. Leukocytes that would be expected to clear endometrial cells from the peritoneal cavity seem to enhance their proliferation by secreting growth factors and cytokines. Although it is unclear whether these

immunologic alterations induce endometriosis or are a consequence of its presence, they seem to play an important role in allowing endometriosis implants to persist and progress.

Pelvic inflammation in women with endometriosis also seems to contribute to the development of their most common complaints: pain and infertility. Secretory products of immune cells in the peritoneal fluid, such as cytokines and prostaglandins, contribute to dysmenorrhea that may progress to dyspareunia and chronic pelvic pain. Pelvic inflammation also may lead to adhesion formation and scarring and disrupt fallopian tube patency. Similarly, the inflammatory environment may impair folliculogenesis, fertilization, and embryo implantation and result in infertility.

In this section the authors summarize the alterations in the immune parameters of women with endometriosis and discuss how they may play a role in the pathogenesis of endometriosis.

Macrophages. Macrophages are the most abundant nucleated cells found in peritoneal fluid [78]. Their number and activity is increased in the peritoneal fluid of women with endometriosis [79–83]. Although the increased number and activity of peritoneal fluid macrophages in women with endometriosis would be expected to facilitate the clearance of ectopic endometrial cells and slow down or inhibit the development of endometriosis, it seems to promote growth of ectopic endometrium. This effect may be caused by an increase in the release of growth-promoting cytokines and growth factors [84] combined with an impaired scavenger function. Abnormal levels of cytokines and hormones present in the peritoneal fluid [85] and the lack of interaction between macrophages and extracellular matrix components that results in a decreased expression of scavenger receptors [86] are believed to cause the decrease in scavenger function in women with endometriosis.

Secretory products of peritoneal macrophages and circulating monocytes of women with endometriosis seem to mediate growth and maintenance of ectopic endometrium [84]. Peritoneal fluid from women with endometriosis stimulates proliferation of cultured endometrial stromal cells [87]. Peripheral blood monocytes obtained from women with endometriosis enhance proliferation of co-cultured autologous endometrial cells, whereas monocytes from fertile women show the opposite effect and suppress endometrial cell proliferation [88]. In addition to their growth-stimulatory effect on endometriotic implants, macrophage products are also implicated in the pathophysiology of endometriosis-associated pain and infertility.

Natural killer cells. Natural killer (NK) cells are an important component of the innate immune system. Researchers have suggested that a decrease in NK cell activity may lead to impaired clearance of regurgitated endometrial cells from the peritoneal cavity and facilitate development of endometriosis. Initial studies that investigated NK cell numbers in peritoneal fluid of women with endometriosis reported conflicting results. Whereas some studies reported a decrease in peritoneal

NK cells [89], others reported no change [90] or an increase [83]. On the other hand, studies that investigated NK cell activity in women with endometriosis consistently showed a decrease in cytotoxic activity. NK cells from the peritoneal fluid and the peripheral blood of women with endometriosis were found to have decreased cytotoxic activity against autologous and heterologous endometrium [90, 91]. The decrease in NK cell cytotoxicity in the peritoneal fluid was more pronounced in the moderate and severe stages of endometriosis [92]. These findings suggest that the alteration in NK cell activity in women with endometriosis is caused by qualitative rather than quantitative changes.

Multiple mechanisms seem to be involved in the suppression of NK cell activity in women with endometriosis. Sera [93] and peritoneal fluid [94, 95] from women with endometriosis suppress NK cell cytotoxicity [93, 94], which suggests that soluble factors are also involved. Recently, Wu et al found that peritoneal NK cells of women with endometriosis have higher killer-inhibitory receptors expression [96]. When stimulated, killer-inhibitory receptors send inhibitory signals that override the kill signal and suppress cytotoxic activity.

Lymphocytes. More than 20 years ago, Dmowski et al showed that T-cell–mediated immunity to autologous endometrium is suppressed in Rhesus monkeys with spontaneous endometriosis [97]. Similarly, cytotoxic activity of peripheral blood lymphocytes against autologous endometrial cells is decreased in women with endometriosis [98]. These observations led to the speculation that endometriosis develops as a result of impaired cell-mediated immune response that is believed to be critical in clearing ectopic endometrial cells from the peritoneal cavity [99].

The functional alteration observed in T cells of women with endometriosis is not accompanied by a quantitative downregulation. Total lymphocyte numbers and the helper/suppressor ratio in the peripheral blood are not affected markedly in women with endometriosis [83, 100]. Similarly, there is no change in total lymphocyte content or helper/suppressor ratios in the eutopic endometrium of women with endometriosis compared to eutopic endometrium from normal controls [101]. On the other hand, T lymphocyte concentration is increased in the peritoneal fluid [83, 99] and endometriotic implants [102] of women with endometriosis. An increase in helper and suppressor subtypes contributes to this observed increase, although their relative ratio seems to be unchanged [83, 99, 102].

Autoimmunity. Endometriosis is associated with polyclonal B-cell activation and an increased incidence of autoantibodies [103, 104]. Although it seems that autoantibodies may be associated in certain cases of endometriosis-associated infertility, the relative importance of autoimmunity in the pathogenesis and pathophysiology of this disease is still controversial.

Cytokines and growth factors. Cytokines are a large family of low-molecular-weight soluble proteins involved in regulating cellular activity. They act as paracrine and autocrine messengers within the immune system and between the

immune system and other systems of the body. Their action is mediated by specific cytokine receptors. Cytokines and growth factors play an important role in regulating chemotaxis, mitosis, angiogenesis, and differentiation. Although impaired cellular immune response has been implicated as a permissive factor in survival of endometrial cells in the peritoneal cavity, cytokines and growth factors seem to promote implantation and growth of ectopic endometrium by inducing proliferation and angiogenesis.

Several cytokines and growth factors have elevated levels in the peritoneal fluid of women with endometriosis, including IL-1 [105–107], IL-8 [108, 109], monocyte chemotactic protein-1 [110, 111], Regulated upon activation, Normal T cell Expressed and Secreted (RANTES) [112], tumor necrosis factor-α [113], and vascular endothelial growth factor [114]. The growth factors and cytokines found in the peritoneal fluid of women with endometriosis have a multitude of effects that promote survival and growth of endometriotic implants. They induce chemotaxis of mononuclear cells into the peritoneal cavity, which causes a further increase in secretion of growth factors and cytokines. They stimulate adhesion of endometrial stromal cells to fibronectin, which facilitates the initial attachment of endometrial cells to the peritoneal surface [115]. They upregulate metalloproteinase activity that degrades extracellular matrix and facilitate invasion [116], they induce endometrial stromal cell proliferation [117], and they are involved in angiogenesis. They also seem to have adverse effects on fertilization [118] and early embryonal development [106]. In summary, many cytokines and growth factors are elevated in the peritoneal environment of women with endometriosis, and they seem to play an important role in the pathogenesis and pathophysiology of endometriosis.

Endocrine factors

Endometriosis is an estrogen-dependent disorder. Aberrant estrogen synthesis and metabolism have been implicated in the pathogenesis of endometriosis. Aromatase catalyzes the synthesis of estrone and estradiol from androstenedione and testosterone, respectively. It is expressed by many human cell types, including ovarian granulosa cells, placental syncytiotrophoblasts, adipose cells, and skin fibroblasts.

Estrogen action is classically believed to occur via an endocrine mechanism. In other words, circulating estradiol is believed to exert an estrogenic effect after delivery to target tissues via the bloodstream. Studies on aromatase expression in breast cancer demonstrated that paracrine mechanisms play an important role in estrogen action in this tissue [119]. Estrogen also displays an ''intracrine'' effect. Estrogen produced by aromatase activity in the cytoplasm of leiomyoma smooth muscle cells or endometriotic stromal cells can exert its effects by readily binding to its nuclear receptor within the same cell. Disease-free endometrium and myometrium, on the other hand, lack aromatase expression [120, 121].

In the ovary, the most important site of estrogen biosynthesis in a woman of reproductive age, binding of follicle stimulating hormone to its receptor in the granulosa cell membrane induces a rise in intracellular cAMP levels. This in turn

enhances the binding of transcription factors to the promoter region of the aromatase gene [122, 123]. As a result, there is an increase in aromatase expression and, consequently, in estrogen secretion from the preovulatory follicle [122, 124]. In postmenopausal women, estrogen production takes place in extraglandular tissues, such as the adipose tissue and the skin [125, 126]. This action is controlled primarily by cytokines and glucocorticoids [124].

Endometriomas and extraovarian endometriotic implants express high levels of aromatase. Cultured stromal cells derived from endometriotic implants and incubated with a cAMP analog display extraordinarily high levels of aromatase [121]. Growth factors, cytokines, and other factors have been investigated as possible inducers of aromatase activity via cAMP-dependent pathway in endometriosis. Prostaglandin E_2 was identified as the most potent inducer of aromatase activity in the endometriotic stromal cells [121]. Estrogen was found to upregulate prostaglandin E_2 formation by stimulating cyclo-oxygenase type 2 enzyme in endometrial stromal cells in culture [127]. There is a positive feedback loop for continuous local estrogen and prostaglandin E_2 production, possibly favoring the proliferative and inflammatory characteristic of endometriosis. Low levels of aromatase mRNA also are detected in the eutopic endometrial samples of women with moderate to severe endometriosis, whereas it is absent in eutopic endometrium of disease-free women [128]. This finding suggests that a genetic defect in aromatase expression may exist in women with endometriosis. When endometrial tissue with low levels of aberrant aromatase expression reaches the pelvic peritoneum by retrograde menstruation and induces an inflammatory reaction, this would exponentially increase local aromatase activity and local estrogen formation [121].

Although prostaglandin E_2 was identified as the most potent known inducer of aromatase activity by increasing cAMP levels in endometriotic stromal cells, neither cAMP analogs nor prostaglandin E_2 stimulates aromatase activity in cultured eutopic endometrial stromal cells. The mechanisms that mediate the differential regulation of aromatase activity in endometriotic cells and normal eutopic endometrium have been investigated. The cAMP-inducible promoter II seems to be responsible for in vivo aromatase expression in endometriotic tissue [129]. Two transcription factors, the stimulatory transcription factor (SF-1) and an inhibitory factor, chicken ovalbumin upstream promoter transcription factor (COUP-TF), compete for the same binding site in aromatase promoter II. COUP-TF is ubiquitously expressed in eutopic endometrium and endometriosis, whereas SF-1 is expressed specifically in endometriosis but not in eutopic endometrium and binds to aromatase promoter more avidly than COUP-TF [129]. SF-1 and other transcription factors (eg, Cyclic-AMP Response Element Binding Protein (CREB)) activate aromatase gene transcription in endometriosis, whereas COUP-TF, which occupies the same DNA site in eutopic endometrium, inhibits this process [129]. In summary, one of the molecular alterations that leads to local aromatase expression in endometrial cells but not in normal endometrium is the aberrant production of SF-1 in endometriotic cells, which overcomes the protective inhibition maintained normally by COUP-TF in the eutopic endometrium.

The primary substrate for aromatase activity in endometriosis is androstenedione of adrenal and ovarian origins in premenopausal women and adrenal androstenedione in postmenopausal women. The major product of aromatase activity in endometriosis, namely estrone, is only weakly estrogenic and must be converted to estradiol to exert a full estrogenic effect. The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1, which catalyzes the conversion of estrone to estradiol, is expressed in endometriosis [129, 130]. In contrast, 17β-HSD type 2 inactivates estradiol by catalyzing its conversion to estrone in eutopic endometrial glandular cells during the luteal phase [130]. Progesterone induces the activity of this enzyme in endometrial glandular cells in culture, which makes inactivation of estradiol to estrone one of the antiestrogenic properties of progesterone [129]. The expression of 17β-HSD type 2 is absent from endometriotic glandular cells [129]. Consequently, this protective mechanism that lowers estradiol levels is lost in endometriotic tissue [129].

In summary, aberrant expression of aromatase, the presence of 17β-HSD type 1, and the absence of 17β-HSD type 2 from endometriosis collectively give rise to elevated local levels of estradiol compared with eutopic endometrium and may promote survival and growth of endometriotic implants.

Genetic factors

The presence of familial tendencies in endometriosis has long been suspected. In 1980, Simpson et al [131] evaluated 123 women with histologically confirmed endometriosis. 8.1% of their mothers and 5.9% of their female siblings older than age 18 were affected. Their husbands' families were used as controls. Only 1% of the patients' husbands' first-degree relatives had endometriosis. Subsequent studies have been consistent with these initial observations. In a similarly designed study conducted in Norway, 3.9% of mothers and 4.8% of sisters of 522 women with endometriosis had endometriosis [132]. Only 0.6% of sisters of women who did not have endometriosis were affected. Lamb et al [133] used questionnaires received from 491 members of the Endometriosis Association, based in the United States. In sisters and mothers of women with endometriosis, they detected a 6.2% and 3.8% incidence of endometriosis, respectively. In Brazil, dos Reis et al reported that 8.6% of first-degree relatives of 81 women with endometriosis were affected, compared to no relatives of controls [134].

Consistent with these studies, higher concordance for monozygotic than dizygotic twins is observed [135, 136]. Monozygotic concordance does not reach 100% expected for a mendelian trait, however. Investigation of familial cases for linkage studies revealed familial aggregates [136].

Endometriosis seems to be heritable, but the precise mechanism is unclear. The increased risk of 5% to 8% for first-degree relatives suggests polygenic/multifactorial inheritance if one assumes that all endometriosis is a single disorder. The other possible explanation is that endometriosis is not a single disorder but several different disorders of distinct etiologies. That is, genetic heterogeneity may exist. One or more forms of endometriosis might be mendelian, despite the larger proportion being nongenetic or polygenic.

Environmental factors

Exposure to environmental toxins recently has been added to the list of factors that contribute to the pathogenesis of endometriosis. Among environmental toxins implicated in the development of endometriosis, 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) is the best studied [137, 138] and is reviewed in this section. TCDD belongs to the family of polychlorinated diaromatic hydrocarbons and is usually used as a reference compound for the effects of all other polychlorinated diaromatic hydrocarbons. Because of their lipophilic property, these chemicals degrade slowly and tend to accumulate in the food chain. It is believed that the exposure of TCDD and other polychlorinated diaromatic hydrocarbons is mostly through ingestion of contaminated foods, although various industrial accidents also may contribute [139–141]. TCDD and other dioxin-like compounds can exert their effects via aryl hydrocarbon receptor, an orphan nuclear receptor whose natural ligand is not known. Aryl hydrocarbon receptor can bind other compounds, including glucosinolates and constituents of cigarette smoke. This receptor is present in many tissues, including eutopic and ectopic endometrium [142, 143].

2,3,7,8-tetrachlorodibenzo-ρ-dioxin can inhibit ovarian progesterone synthesis [137]. It also inhibits progesterone-induced expression of TGF-β2 [144], a growth factor that suppresses endometrial MMPs. Although both of these effects may promote the development of endometriosis, TCDD also has an antiestrogenic action [145–147]. The exact mechanism by which TCDD, an antiestrogen, promotes the development of endometriosis remains to be elucidated.

The effect of TCDD on the development of endometriosis has been studied in animal models. Rier et al showed that endometriosis spontaneously developed in monkeys exposed to dietary TCDD for 5 years [148]. They performed laparoscopy and found that 71% and 86% of monkeys given 5- and 25-ppt doses of TCDD, respectively, developed moderate to severe endometriosis, although only 33% of control animals had minimal endometriosis. Yang et al studied the effects of TCDD on monkeys with surgically induced endometriosis [148]. They observed a bimodal effect of TCDD on implant sizes. The size of the implants was found to be significantly increased in 25-ppt dose group and decreased in the 1-ppt dose group compared to controls. The implants also were observed to regress in all groups over time.

Rodent studies for the effect of TCDD on endometriosis also have been conducted. In most of these studies TCDD was shown to enhance the growth of endometrial implants in mice [149–151]. In contrast to these findings, Yang and Foster demonstrated that TCDD resulted in regression of previously established implants in mice [152]. The dose of dioxin and the length of exposure may determine its effects on endometrial implants.

Few case-control studies investigated the association of environmental toxins with endometriosis in humans. Gerhand and Runnebaum showed a positive association between endometriosis and exposure to polychlorinated biphenyls ([153]. Similarly, Koninckx et al noted that in Belgium the level of dioxins in breast milk is among the highest in the world and that the incidence of endometriosis is also higher than other countries [154]. Mayani et al reported

that women with endometriosis compared to women with tubal infertility are more likely to have a history of TCDD exposure [155]. On the other hand, other studies found no association between endometriosis and dioxins or polychlorinated biphenyls [156]. Because of inadequacies in the design and sample size, it is not possible to establish a cause-and-effect relationship between these compounds and the development of endometriosis based on these trials.

Although their mechanism of action remains unclear, dioxin and related compounds seem to have potential adverse effects on the development of endometriosis. For a better understanding of the basic mechanisms underlying this disease, further studies are needed.

Summary

Endometriosis is a common gynecologic disorder characterized by the presence of endometrial tissue outside the uterine cavity. Various theories have been put forth to explain the mechanisms for the development of this disease. Although no single theory can explain all cases of endometriosis, the retrograde menstruation theory has gained the widest acceptance. This theory proposes that viable endometrial tissue is refluxed through the fallopian tubes during menstruation and implants on peritoneal surface or pelvic organs. Retrograde menstruation occurs in 76% to 90% of women. The much lower prevalence of endometriosis suggests that additional factors determine susceptibility to endometriosis. Once in the peritoneal cavity, the survival and implantation of endometrial cells seem to be mediated by abnormal MMP and TIMP expression, altered immune milieu, aberrant local aromatase activity, and genetic and environmental factors.

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Obstet Gynecol Clin N Am
30 (2003) 63–82

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Adenomyosis

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Historical background

In the mid-nineteenth century, Rokitansky [1] described a condition in which elongated endometrial glands were found embedded in a hyperplastic endometrial stroma. He noted two variants to this condition: one in which the glands grew into the uterine musculature and another in which they grew downward into the endometrial cavity, forming a polyp. Schatz [2] later interpreted Rokitansky's finding to be a variant of uterine leiomyomata. He qualified this condition as "fibroadenoma cysticus et polyposum."

Subsequently, Chiari [3] described an abnormal growth of endometrial glands into the uterine musculature in the areas of uterine cornu and proximal fallopian tube. This was the first mention of *salpingitis isthmica nodosum*, which he believed to be a variant of adenomyosis.

In the 1880s and 1890s, some investigators claimed that adenomyosis reflects an embryonic error in müllerian cell distribution. They also claimed that this was caused by invasion of the myometrium by the hyperplastic basal endometrium [4–6]. In 1893, Hauser proposed that idiopathic stromal hyperplasia is the cause for adenomyosis. Subsequently, Von Recklinghausen [7] proposed that adenomyosis was the result of displacement of mesonephric (Wolffian) elements. He noted that these ectopic glandular elements were found mainly on the posterior uterine wall in the area of the uterine cornu, and he believed that these regions were more likely sites for Wolffian rather than müllerian vestiges.

In the late 1890s and early 1900s, Meyer [8,9] suggested that chronic endometritis may be responsible for invasive endometrial hyperplasia and referred to this condition as "adenomyometritis." Combining two earlier proposals, Cullen [10] distinguished between adenomyoma, an intramyometrial tumor-like condition constituted by endometrial glands and stroma, and diffuse adenomyoma, in which

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both elements were distributed throughout the myometrium. He claimed that basal endometrial invasion is an explanation for most cases of adenomyosis, but he left open the possibility of müllerian rests that could be implicated for the encapsulated form of adenomyosis (adenomyomas). Later, Taussig [11] described lymphatic transmission of endometrial components. Although this theory was used to describe pelvic endometriosis, it also put forward another possible explanation for adenomyosis. Marcus [12] also suggested that some müllerian totipotential cells could exist within the myometrium that could differentiate into endometrial cells, which gave another explanation for the development of adenomyosis.

Currently, we are back to square one, with most investigators believing that adenomyosis results from basal endometrial hyperplasia invading a hyperplastic myometrial stroma. It should be noted that all organs in the human body that contain cavities also possess a submucosal region, except the uterus. It is believed that one of the main functions of this submucosa is to prevent the inward growth of glands that line these cavities [13].

Definition

The term "adenomyosis uteri" was first used by Frankl [14]. In 1972, Bird et al [15] defined adenomyosis as "the benign invasion of endometrium into the myometrium, producing a diffusely enlarged uterus which microscopically exhibits ectopic, non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium." This definition is still good; however, some investigators qualified it further to include the "presence of endometrial glands and stroma located haphazardly and deep within the endometrium" [16]. Depth is important because the normal endomyometrial junction is often irregular, and adenomyosis must be distinguished from minimally invaginated basalis surrounded by myometrium. There are two ways to get around this problem. The first is to determine the existence of myometrial hypertrophy around foci of adenomyosis. Such differentiation is not seen at the endomyometrial junction. The second is the measurement of the distance between the endomyometrial junction and the closest adenomyotic foci. This should be approximately 25% of the total thickness of the myometrium. The latter approach has a particular significance in the postmenopausal and gravid uterus because periadenomyotic muscular hypertrophy in this type of uterus is basically absent [17]. Although adenomyosis is generally considered a variant of endometriosis, widely referred to as internal endometriosis [18,19], it is preferable to define endometriosis as endometrial glands and stroma located outside the myometrium.

Epidemiology

The reported incidence of adenomyosis has varied widely over the years, ranging from 5.7% to 69.6% [10,20]. Although some of this disparity can be

explained by the use of different histologic definitions for adenomyosis, most of the variation is likely caused by the degree of zeal with which pathologists pursue the diagnosis. Because of the focal nature of this condition, the diagnosis of adenomyosis can be difficult to make. In a prospective study [15], 200 consecutive hysterectomy specimens were examined histologically. When three routine sections of myometrium were examined, adenomyosis was found in 62 women (31%). Six additional tissue blocks were then examined—three each from the anterior and posterior uterine walls—and an additional 61 cases were discovered, which raised the incidence from 31% to 61.5%.

The main difficulty in establishing the true incidence of adenomyosis originates from the fact that although published reports may cite the number of cases of adenomyosis found in relation to patient age, they uniformly fail to report the total number of hysterectomies performed in each age group. The relevant incidence of adenomyosis as a function of age never has been defined [13].

Another source of difficulty in establishing the true incidence of adenomyosis is the fact that most studies are biased (ie, they evaluate only women who undergo hysterectomies), which creates a selection bias. Two necropsy studies have been performed, reporting an incidence of adenomyosis in 50% and 53.7% of specimens [21,22]. Although these studies involve a different type of selection bias (ie, women with hysterectomy have been excluded), they do illustrate the fact that the true incidence of adenomyosis is probably nearer the upper end of the published range.

Superficially, parity seems to correlate with adenomyosis, because up to 93% of treated patients are parous [23,24]. These figures tend to mimic those of the general population, however, so their significance is under consideration. If true, this would confirm an interesting paradox, because parity may protect against endometriosis yet be a risk factor for the development of adenomyosis. There does not seem to be any significant correlation between adenomyosis and race or obesity [25,26]. Likewise, there does not seem to be any significant preference for adenomyosis to coexist with other gynecologic problems. In a retrospective study of 134 patients who underwent hysterectomy, Vercillini et al [27] reported coexistence of adenomyosis with fibroids (23%), genital prolapse (26%), cervical cancer (19%), endometrial cancer (28%), ovarian cancer (28%), and ovarian cysts (21%).

Pathogenesis

The precise etiology and the developmental events that lead to adenomyosis still remain a mystery. Several theories have been proposed in the past 50 years, and these have been reviewed in detail by Ridley [28]. Currently, it is generally believed that adenomyosis develops as a result of downgrowth and invagination of the basalis endometrium into the myometrium. One often can see direct continuity between the basalis endometrium and the underlying adenomyosis in the myome-

trium. In extrauterine regions such as the rectovaginal septum, adenomyosis may develop de novo from embryologically misplaced müllerian remains.

The triggering mechanism of endometrial "invasion" of the myometrium in humans has yet to be determined. Proliferative changes, such as mitotic activity, increased nuclear DNA synthesis, and ciliogenesis, are significantly more manifest in the functionalis than the basalis layer of the endometrium [29]. The biologic rationale for the geographic variation in proliferative indices may be located in the difference in physiologic functions of the functionalis compared to the basalis layer. The former is the seat of blastocyst implantation, whereas the latter provides the origin for the regenerative endometrium after menstrual degeneration of the functionalis [30]. During periods of regeneration, epithelial cells from the stump of basalis glands are in direct contact with the spindle-shaped cells of the endometrial stroma, and ultrastructurally they contain intracellular microfilamentous/trabecular systems and pseudopodial cytoplasmic projections. These features are consistent with migration by amoeboid contraction and expansion [30]. Such morphologic changes have not been described yet in adenomyotic endometrial glandular epithelium.

In vitro studies underlined the invasive potential for endometriotic cells with their invasion index being similar to that of metastatic bladder cell lines [31]. Such invasive potential may facilitate extension of the basalis endometrium into the myometrium. In MCF-7 breast cancer cells, production of tenascin is stimulated by the hormonally regulated epidermal growth factors [32]. The fact that endometrial stromal fibroblasts produce tenascin, a fibronectin inhibitor that facilitates epithelial migration, suggests a complex physicochemical interrelationship during the endometrial ugrowth processes. Tenascin has been immunolocalized around proliferative phase endometrial glands but not in postovulatory phase endometrial glands [33]. It is likely that tenascin mediates epithelial-mesenchymal interactions by inhibiting cell attachment to fibronectin in adenomyotic type endometrium, as it does in its endometrial counterpart.

A study using in situ hybridization and immunohistochemistry revealed that endometrial glands in adenomyosis selectively express more human chorionic gonadotropin/luteinizing hormone receptor mRNA and immunoreactive receptor protein than the noninvaginating glandular epithelium [34]. In normal endometrium, the glands fail to demonstrate geographic variation (as a function of their depth) in human chorionic gonadotropin/luteinizing hormone receptor expression. It is possible that the increased receptor expression of invaginating endometrial epithelium may be related to the possibility of invaginating into the myometrium and forming adenomyotic foci. Increased human chorionic gonadotropin/luteinizing hormone receptor expression was found in endometrial carcinomas compared to normal endometrial glands [35] and in invasive versus noninvasive trophoblasts in choriocarcinomas [36].

Studies on steroid receptor using cytosol preparations yielded inconsistent results; some found no progesterone receptors in 40% of the adenomyotic cases studied [37], whereas others found higher progesterone than estrogen receptor concentrations [38]. Using immunohistochemical tracing techniques, relatively

high concentrations of estrogen and progesterone receptors were found in the basalis and adenomyotic endometrium [16]. Estrogen receptors are prerequisites for estrogen-mediated endometrial growth. Despite the lack of apparent evidence of impaired hormonal environment in most women with adenomyosis, high estrogen levels may be implicated in the invagination process because high frequency of endometrial hyperplasia is found in women with adenomyosis. Some researchers claim that a relatively high estrogen concentration is necessary for the development and maintenance of adenomyosis and endometriosis [39]. This claim is supported by the clinical observation that suppression of estrogenic environment by danazol induces involution of the ectopic endometrium and associated symptoms, such as menorrhagia and dysmenorrhea [39].

As is the case in uterine leiomyomata, estrogen is synthesized and secreted in adenomyotic tissues [39]. Aromatase and estrogen sulphatase activities have been demonstrated by steroidobiochemical analysis in the supernatant faction of myometrium-containing foci of adenomyosis. Estrogen sulphatase, and particularly aromatase, activity was higher (1 mg protein of tissue) than that observed in the normal adjacent myometrium and leiomyomata and overlying endometrium ($P < 0.01–0.001$). Endometrial enzymatic activity was suppressed in vitro by up to 50% after addition of 10^6 M danazol [39]. Aromatase also was demonstrated by immunohistochemistry in the cytoplasmic substance of gland lining cells but not stromal cells in foci of adenomyosis in human uteri. The production of estrogens by adenomyotic tissue is also supported by finding more women with adenomyosis who have high estradiol concentration (>30 pg/mL) in the menstrual blood than women without adenomyosis who have normal ovulatory cycles [40]. The secretory response of adenomyotic tissue to hormonal stimulation is consistent with effective progestogenic influence on the ectopic endometrium. Progestogens promote aromatase activity in eutopic and adenomyotic tissues [41], which contributes to estrogen biosynthesis in adenomyotic foci.

It is likely that bioavailability of sex steroids is not sufficient by itself to produce adenomyosis. It could be that in cases of adenomyosis, the myometrium is predisposed to invasion by the basalis endometrium. The myometrial susceptibility could be primary or secondary. Disruption of the mesenchymal layers surrounding the endometrium in the neonatal period can trigger disordered development of uterine stroma, smooth muscle, blood vessels, and, possibly, innervation. This alteration in the development of normal functional fibromuscular anatomy of the uterine body forms the basis for the abnormal and aberrant growth of endometrial tissue [42]. The ‘‘benign invasion’’ of the endometrium also could occur secondary to acquired ‘‘weakness’’ of the myometrium caused by trauma, such as curettage, myomectomy, and cesarean section. From this point of view, adenomyosis can be induced in pregnant rabbits by curetting one horn and tube while maintaining pregnancy in the opposite horn [21]. It is likely that myometrial invasion by the endometrial basalis is favored by increased intrauterine pressure, which, according to Cullen [10], could be induced by high circulating progesterone levels.

Increased expression of the major histocompatibility complex class II antigen (HLA-DR) in the gland cells of normal (eutopic) and ectopic endometrium (18 patients) and adenomyosis (50 patients) was observed by immunohistochemistry [43, 44]. Macrophages in the myometrium of adenomyosis also seem to increase, which may activate helper T cells and B cells to produce antibodies [45]. Autoantibodies against phospholipids in endometriosis and adenomyosis and marked deposition of immunoglobulin (Ig)S or complement components have been observed [46,47]. The exact significance of these aberrant immune phenomena in adenomyosis or endometriosis remains to be elucidated.

Experiments in vitro showed that activated CD3+ T cells in the endometrium and their secretory product interferon-γ induce expression of HLA-DR immunoreactivity in endometrial gland cells and inhibition of their proliferation [48]. The closer the endometrial cells are to activated T cells, the greater is their growth inhibition. It seems likely that lymphoid follicle-like structures, mainly located in the endomyometrial junction, are rich in activated T helper cells. Their location coincides with maximal inhibition of endometrial growth observed morphologically [49] and by proliferation marker studies [48]. Conversely, endometrial proliferation is at its maximum near the endometrial surface far away from basalis-containing lymphoid aggregates [48,49]. It is likely that adenomyotic uteri are poor in activated T cells, with the basalis endometrium having growth advantage over nonadenomyotic, lymphoid-rich basalis endometria. It remains to be determined whether such anomaly is necessarily associated with acquired myometrial weakness or whether it is an independent prerequisite for the development of adenomyosis.

A series of immune responses is activated in adenomyosis. These responses include a strong expression of cell surface antigens, an increase in the number of macrophages or immune cycles, and the deposition of immunoglobulins and complement components. Endometrial cells are under immunologic stress and protect themselves by synthesizing heat shock proteins. Activated immune cells secrete different cytokines or growth factors that stimulate expression of cell surface antigens, which results in an immunologic ''vicious cycle''[50].

The exact reason for myometrial hyperplasia/hypertrophy, located around deep foci of endometrium, is not known but may indicate an attempt at controlling endometrial invagination of the myometrium or simply may represent smooth muscle bundles displaced by the ingrowing endometrium. By immunohistochemistry, the myometrium that surrounds the ectopic endometrium, whether diffuse (adenomyosis) or focal (adenomyoma), contains no abnormalities. Smooth muscle cells in adenomyotic foci, normal myometrium, and leiomyomata (coexistent or not with adenomyosis) are all rich in actin and desmin [16].

Several animal models are available for the study of the pathogenesis of adenomyosis. In one, intrauterine isografts of anterior pituitary mice lead to the development of adenomyosis [51]. Prolactin may amplify this event, for the isograft-free horn contained comparatively less developed adenomyosis. Whereas ovariectomy prevented adenomyosis, estradiol benzoate stimulated the development of adenomyosis in this animal model. In another model, mice

treated prenatally with high doses of diethylstilboestrol developed adenomyosis. It seems likely that certain strains of mice are prone to develop adenomyosis in response to high concentrations of prolactin, estrogens, and progestogens. More recently, Ficicioglu et al [52] induced adenomyosis in noncastrated rats with hyperprolactinemia. The authors suggested that high prolactin concentrations cause myometrial degeneration in the presence of ovarian steroids, which may result in myometrial weakness and subsequent myometrial invasion by the endometrial basalis.

Most interesting were the observations of Mori and Nagasawa [53] in mice, in which myometrial invasion by stromal fibroblasts along the branches of blood vessels preceded invagination of endometrial glands. Sakamoto et al [54] induced a high rate of uterine adenomyosis in mice by ectopic pituitary isografts. DNA-synthesizing activities and related enzymes (ie, thymidilate synthetase and thymidine kinase) were markedly increased in adenomyosis compared to control uteri. In the same experimental animal model, small molecular weight matrix metalloproteinases were probably involved in the development of adenomyosis at the level of gene transcription, activation, and inhibition [55,56]. Certain experimental observations suggested that some hereditary factors may be involved in the pathogenesis of adenomyosis. For example, the uteri of recombinant inbred SMXA mice develop spontaneously histologic changes similar to adenomyosis and contain tenascin around adenomyotic glands [57]. These observations together with the biologic property of tenascin are consistent with the endometrial origin of adenomyosis and the intramyometrial imagination concept of a genetically predisposed myometrium. Compared to SMXA mice, the uteri of F1 mice, a strain between SMXA and NJL strains, contain even more prominent spontaneous changes that resemble human adenomyosis. Whether heredity plays an important role in adenomyosis in humans remains to be determined [58,59].

The observation of adenomyosis in the rectovaginal septum supports the de novo origin of adenomyosis from müllerian remains in extrauterine sites [60,61]. Endometrial glands and stroma associated with smooth muscle cell hypertrophy may be found in this location forming adenomyotic nodules [62]. Although these nodules may develop as a result of invaginating peritoneal endometriosis, the müllerian remains origin theory is favored by some. According to Nisolle and Donnez, in most cases adenomyotic nodules are located deep in the septum and occasionally in the muscularis propria of the rectum far from the pelvic peritoneum. Coexpression of vimentin and cytokeratin in endometrium, whether lining the endometrial cavity or located within adenomyotic foci, is typical of müllerian-derived tissue. Morphologically and receptor content-wise, rectovaginal adenomyosis is identical to its intramyometrial counterpart, including poor or no response to postovulatory progestational stimuli. Despite large doses of exogenous progestational agents given to women with rectovaginal adenomyosis to produce secretory transformation, hormonal therapy is poor. Definite cure of rectovaginal lesion by surgery also suggests ametaplastic de novo process from müllerian remains in that location rather than implantation/invagination of peritoneal endometriosis.

We are still left in the dark concerning the precise origin and pathogenic mechanism(s) of adenomyosis. Experimental and human studies are required to clarify the pathophysiology of this condition of the female genital tract.

Histopathology

At the time of hysterectomy, the adenomyotic uterus usually has been described as globular or boggy. It appears enlarged in at least 60% of cases but rarely exceeds 12 weeks' gestation in size; it weighs between 80 and 200 g [15,63]. In his classical article in which he found parity to be the primary determinant of uterine weight, Langlois [64] defined the upper limits of normal uterine weight as 130 g for nulliparous women, 210 g for parity of one to three, and 250 g for parity of four or more. With these criteria, discounting cases with associated leiomyomata, uterine weight is not appreciably elevated by adenomyosis.

Generally, these uteri are usually hyperemic with thickened walls. Although most investigators have reported that the posterior wall is more frequently involved than the anterior wall, Bird et al [15] found adenomyotic foci to be equally located when they took an additional six sections for histopathologic examination. The foci are frequently scattered diffusely throughout the myometrium but sometimes can be large and localized, forming structures called adenomyomas.

The characteristic gross appearance of adenomyosis is caused by myometrial hypertrophy that surrounds endometrial mucosa. When the whole myometrium or one of the myometrial walls is diffusely involved, the uterus is enlarged and globular. On cross-section, the haphazardly distributed hypertrophied muscular trabeculae that surround foci of adenomyosis are apparent. The latter sometimes may contain brown-staining ''old blood'' that corresponds to hemolysed blood and hemosiderin pigment deposits [65]. The focally involved uterus with adenomyosis resembles a leiomyoma; the term ''adenomyoma'' is applied to this frequent presentation of adenomyosis. Because the process is not neoplastic, the term ''focal adenomyosis'' is preferred by Hendrickson and Kempson [66]. Because adenomyoma is often confused clinically with leiomyoma, a benign but neoplastic condition, it is believed that the use of the term ''adenomyoma'' is acceptable. Typically, adenomyoma does not have definite margins because they are mixed with the surrounding normal myometrium. In contrast, leiomyomata compress the surrounding myometrium and have clear-cut, well-circumscribed margins. The latter can be enucleated, whereas the former cannot [16].

Histologically and by immunohistochemistry, the endometrial glands and stroma in foci of adenomyosis look much alike the basalis endometrium. It seldom responds to hormonal stimuli, a phenomenon that partially explains the occasional hemorrhagic or reparative morphologic events in foci of adenomyosis. The reason for an increased tendency for focal hemorrhage in deeply located adenomyotic foci is not clear [67]. In contrast, ectopic endometrium in foci of endometriosis often undergoes cyclic changes, including degeneration, bleeding, and regeneration, in

all respects similar to the functionalis layer of the endometrium. The different frequency in menstrual-type changes between the two endometria is probable caused by the relatively poor vascularization of the basalis-type adenomyotic endometrium compared to the richly vascularized functionalis-type endometrium in endometriosis. Adenomyotic endometrium seems to retain its proliferative potential to be the place of endometrial growth and to be responsible for failure of the amenorrhea or hypomenorrhea after endometrial ablation [68].

Secretory transformation, including stromal decidualization in foci of adenomyosis, is observed mainly during gestation and exogenous progestational therapy, the changes being mediated by estrogen and progesterone receptors. Progestational effect in the nongravid uterus occurs in 30% to 50% of adenomyotic foci [63, 69]. During intrauterine pregnancy, 57% of the articles reviewed by Azziz [70] described decidualization. Others observed decidualization during pregnancy only in deeply located foci (at depth of two low power fields), whereas decidualization was absent or inconspicuous in foci located less than two low power fields from the basalis-myometrium junction [67]. It is not unusual to find hyperplastic changes with or without atypia in adenomyosis associated with similar conditions in the overlying endometrium. Hyperplasia in adenomyotic foci may present metaplastic changes of the glandular epithelium, such as tubal metaplasia, squamous metaplasia (squamous morules), and mucinous metaplasia. Adenocarcinoma also may involve foci of adenomyosis. When carcinoma is limited to adenomyotic foci, it should be referred to as intramucosal, because it does not make the prognosis worse than the carcinoma for which the patient has had surgery. It is not possible to determine by histology whether adenocarcinomas located in the overlying endometrium and foci of adenomyosis represent simultaneous primaries or extension of the former in adenomyotic foci. The latter hypothesis is more viable because adenocarcinoma in foci of adenomyosis without surface component is a rare event [71,72].

Clinical presentation

Approximately 35% of adenomyotic cases are asymptomatic [73]. In the remaining cases, the most frequently cited profile comprises the triad of abnormal uterine bleeding (50%), secondary dysmenorrhea (30%), and enlarged, tender uterus. Other symptoms, such as dyspareunia and chronic pelvic pain, present less commonly. Unfortunately, however, none of these symptoms (or even the triad itself) is pathognomonic for adenomyosis. The frequency and severity of symptoms correlate with the extent [73] and depth [74] of adenomyosis.

The exact cause of menorrhagia of adenomyotic cases is not known. It may be caused by poor contractibility of the adenomyotic uterus and compression of the endometrium by submucous adenomyomata or leiomyomata. Mefenamic acid administration can reduce blood loss, which suggests that prostaglandins ($F_{2\alpha}$) also may play a role in a greater degree of blood loss in women with adenomyosis

[65]. Other factors may be anovulation, hyperplasia, and, rarely, adenocarcinoma. Finally, upregulation of the basic fibroblast growth factor receptor/ligand system and increased cellular proliferation in adenomyosis may contribute to the pathogenesis of abnormal uterine bleeding associated with adenomyosis [75]. Dysmenorrhea is caused by uterine irritability, which in turn is secondary to increased amounts of blood loss.

Adenomyosis-related symptoms are hard to determine conclusively. For example, in a study of 136 patients with histologically verified adenomyosis, symptoms were variable, nonspecific, and, according to the investigators, related to the associated pathologic conditions such as leiomyomata, endometriosis, and polyps rather than adenomyosis [76]. In another study, there were no differences in either the frequency or severity of dysmenorrhea and pelvic pain between 28 women with adenomyosis and 157 "controls" [77]. In a study of 23 women with uterine adenomyosis no qualitative differences were found in the spontaneous mobility of isolated myometrial tissue throughout the menstrual cycle from normal regenerative and leiomyomatous uteri [78]. The mobility pattern was of low amplitude and high frequency of spontaneous contractions during the proliferative phase; both changes were amplified in the secretory phase. Histamin-produced myometrial contractions were similar in all myometrial tissues investigated [78].

The fact that adenomyosis is not always diagnosed correctly preoperatively is the result of the nonspecificity of those symptoms. Most investigators have reported a correct preoperative diagnosis in less than 10% of cases [12,79–81]. Because of selection bias, incomplete pathologic examination of surgical specimens, and a limited number of well-designed studies, however, the true ability to diagnose adenomyosis prospectively is impossible to ascertain.

Adenomyosis and infertility

Until recently, little attention has been paid to the possible relationship between adenomyosis and infertility [82,83], and only case reports are available [84–87]. There are several reasons for this lack of information. The incidence of adenomyosis begins to rise from the age of the mid-thirties. It is difficult to diagnose adenomyosis before surgery because there are no pathognomonic signs, symptoms, or physical findings. Recent developments in methods of evaluation, including the measurement of CA125, hysteroscopy [88], and MRI, have shown the importance of adenomyosis in infertile patients [89–91].

Miscarriage rates in patients with endometriosis are reported to be high, ranging from 11% to 63% [92]. Treatment with danazol of patients with endometriosis decreases the rate to an average of 11%. Another report has suggested the possible involvement of adenomyosis in infertility and early miscarriage [93–95].

The mechanisms that cause infertility or induce early miscarriage in adenomyosis are not clear. It is possible that nitric oxide, a potential vasodilator, might

be involved in the mechanisms. Recent reports indicated that endothelial nitric oxide synthase, originally identified in vascular endothelial cells, is present in glandular epithelial cells in the endometrium [96,97]. The expression of endothelial nitric oxide synthase in the endometrium varies with the menstrual cycle and is most marked in the midsecretory phase in fertile women. In contrast, the expression of the enzyme in adenomyosis is constantly high compared with controls throughout the menstrual cycle [98]. Several studies in vitro indicate that nitric oxide affects human spermatozoa [99,100] and rat embryos [101] and that optimal levels of nitric oxide are critical for normal sperm function and embryonic development. The endometrial environment in patients with adenomyosis has different immune parameters from those in normal fertile women. These abnormal immune responses eventually might stimulate macrophages or endometrial cells to produce persistently large amounts of nitric oxide and impede fertilization and implantation. Even after successful implantation, the embryo may be attacked by activated macrophages or T cells or be exposed to an excess of nitric oxide, which results in early miscarriage [50].

Adenomyosis in pregnancy

A large study on adenomyosis in pregnancy conducted approximately 50 years ago that involved analysis of uteri obtained at cesarean hysterectomy noted that the incidence of this condition is 17.2% [67]. The study claimed that adenomyosis in pregnancy markedly increased the risk of obstetric complications, specifically postpartum hemorrhage, uterine atony, and uterine rupture, but has not been proved [102]. In his outstanding review, Azziz [70] noted only 29 cases of complications in more than 80 years' worth of literature, a surprisingly low figure in light of the incidence of this entity.

Associated gynecologic pathology

Adenomyosis is rarely an isolated finding. Up to 80% of adenomyotic uteri are associated with such conditions as leiomyomata, endometrial hyperplasia, peritoneal endometriosis, and uterine cancer. The fact that all of these conditions, except endometriosis, are associated with prolonged estrogen exposure has been considered frequently as evidence that adenomyosis results from hyperestrogenemia. Adenomyosis occurs most frequently in association with leiomyomata (up to 57% of the time), and the similarity of symptomatology in these two conditions serves to make accurate preoperative diagnosis difficult [73]. Despite their obvious similarities, adenomyosis and pelvic endometriosis coexist in only 28% of women or less [15,63,69,79,103].

Salpingitis isthmica nodosum, an inflammatory process of uncertain etiology that affects the proximal fallopian tube, also occurs in association with adenomyosis. Its observed coexistent frequency was 1.4% in one study and 19.8% in

another [63,73]. Abnormalities of the endometrial lining that range from hyperplasia to adenocarcinoma frequently are associated with adenomyosis. The reported incidence of coexistent hyperplasia also demonstrated hyperplasia in the adenomyotic foci [63]. Most of these cases have shown simple endometrial hyperplasia; however, atypical hyperplasia can occur. Molitor [63] reported an incidence of 3.5% for atypical hyperplasia in a series of 281 adenomyotic uteri.

Adenomyosis frequently occurs in association with endometrial adenocarcinoma. In one study, 60% of 100 patients with adenocarcinoma also had adenomyosis [12]. Other reported incidences are much lower, at 10% to 33% [104]. In addition to arising within the same uterus as adenomyosis, adenocarcinoma may arise from within adenomyotic foci. It seems likely that the coexistence of adenomyosis does not have an impact on the prognosis for patients with endometrial adenocarcinoma [105]. Isolated reports have described other types of uterine cancer that have been reported in association with adenomyosis. Specifically, müllerian adenosarcoma, endometrial stromal sarcoma, and leiomyosarcoma, all of which were believed to have developed within adenomyotic foci, have been reported [106,107]. Although no one specifically has reported on the incidence of adenocarcinoma within adenomyotic uteri, it is believed to be relatively rare.

Diagnosis

The clinical diagnosis of adenomyosis is only suggestive at best (50%) [108] and most often is either not made (75%) [23,65] or overdiagnosed (35%) [109]. Menorrhagia and dysmenorrhea in a multiparous woman in her late 40s early 50s are suggestive symptoms but not diagnostic of adenomyosis. The uterus may be diffusely enlarged (12 weeks' gestational size) and soft and tender on palpation. The presence of endometrial hyperplasia at the time of hysterectomy was the only variable significantly associated with adenomyosis [110].

Several investigators have examined the use of various radiologic modalities to aid in the prospective diagnosis of adenomyosis. In the largest study of hysterosalpingography to date, Marshak and Eliasoph [111] were able to diagnose adenomyosis correctly in only 38 of 150 patients with "proven" adenomyosis (25%). They did not note either the total number of patients examined or the incidence of false-positive diagnosis. The most commonly described findings on hysterosalpingography include endometrial diverticuli and honeycomb defects that protrude into the myometrium [112,113]. This test is fraught with inaccuracy, however, because the myometrial speculations frequently ascribed to adenomyosis resemble those of lymphatic or vascular dye intravasation.

Unfortunately, abdominal ultrasonography cannot diagnose the pathology. In the late 1970s, one group proposed 5- to 7-mm irregular myometrial sonolucencies as ultrasonographic findings characteristic of generalized adenomyosis [114]. This was subsequently disputed by Siedler et al [115], who noted generalized

uterine enlargement, normal myometrial echogenicity, and preservation of uterine contour in most of their patients with documented adenomyosis. Several more recent studies have failed to clarify this issue.

Transvaginal ultrasonography has been evaluated only as a diagnostic modality since the early 1990s. Fedele [116] evaluated 43 women who underwent hysterectomy for menorrhagia with preoperative transvaginal ultrasound. He described numerous small myometrial anechoic areas with irregular hyperechogenic outlines in 22 women. The sensitivity of this technique was reported to be 80% with a specificity of 74%. Other investigators have reported lower sensitivities of 48% [117] and 53% [118]. Further studies are needed in this area.

Magnetic resonance imaging has been applied to pelvic pathology, and preliminary results in adenomyosis patients are encouraging [119–121]. Mark et al correctly predicted adenomyosis in 8 of 20 patients studied using T2-weighted images. Ten of the remaining 12 patients were correctly diagnosed not having adenomyosis; in the other 2 patients, radiologic diagnosis was uncertain. The investigators described a unique-appearing, wide low-signal-intensity band surrounding the normal high-signal-intensity endometrium in patients with diffuse adenomyosis. Microscopic adenomyotic foci, however, were not demonstrated. T2-weighted imaging seems to offer significant advantages over unenhanced and contrast-enhanced T1-weighted imaging. MRI also has been evaluated as a technique for differentiating adenomyosis from leiomyomata [122]. 93 patients were evaluated preoperatively, and the results were correlated with surgical pathology. All 16 cases of adenomyosis were diagnosed correctly preoperatively. This new technology, however, needs further study. Cost also may prohibit development of MRI as a widespread screening test.

CA-125 is an antigen produced by ovarian epithelial cells. It is secreted into the blood, and its use has been advocated in various gynecologic conditions. Although some researchers have used it to predict recurrences of nonmucinous ovarian carcinomas, others have attempted to assess nonoperatively the status of recurrent endometriosis by determining serial CA-125 levels [123,124]. In 1985, Takahasi et al [125] reported elevated preoperative serum levels of CA-125 in six of seven study patients. Although these levels were elevated, they were significantly lower than those commonly found in patients with ovarian carcinomas. One month after hysterectomy, all patients had normal levels of CA-125. Using immunohistochemistry, these same investigators localized the CA-125 antigen to glandular epithelium present in the adenomyotic foci of eight hysterectomy specimens [126]. Another study, however, failed to confirm these findings [127]. In their report of 22 women, 11 of whom had adenomyosis, Halila et al [127] noted normal preoperative CA-125 levels in all adenomyosis patients. These levels did not significantly change when tests were repeated 1 and 5 weeks postoperatively. The reason for the discrepancy in these studies is not clear, but it is hoped that further work will be conducted in this field.

Serum cystine aminopeptidase and leucine aminopeptidase levels also have been used as potential markers for adenomyosis. Levels of these enzymes have been reported to be elevated in several benign and malignant conditions

involving the uterus and ovary [128]. No controlled trials have been performed to evaluate the clinical use of these measurements.

Although adenomyosis can be diagnosed by myometrial needle biopsies, the overall sensitivity of this technique is low and depends on the number of biopsies and the depth of penetration of adenomyosis. This technique is of little or no value in detecting minimal or moderate disease, but it may provide histologic confirmation in cases in which there is extensive invasion of the myometrium. If biopsy is contemplated, patients should be selected on the basis of the clinical presentation and after assessment of the myometrial echotexture by endovaginal ultrasonography or MRI. These investigations also may help to determine the site for biopsy. Routine biopsy of the myometrium in patients with pelvic pain cannot be performed, however [129–131].

Treatment

The mainstay of the diagnosis and treatment of adenomyosis remains hysterectomy. Until a safe and consistently effective method exists for directed myometrial biopsy, one can diagnose adenomyosis accurately only by surgical removal of the uterus, thus effectively treating this condition simultaneously [13].

Concerning medical management, in the mouse model, bromocriptine has a suppressive effect on adenomyosis [132]. Conversely, prolactin, progesterone, and possibly even growth hormone seem to accelerate the development of the disease [133,134]. RU-486, an antiprogestational agent that inhibits the effects of progesterone at uterine receptor sites, has been shown to suppress the development of adenomyosis markedly when given for up to 30 days. This finding may have some implication for future human studies [135].

Anecdotal evidence exists that progesterone may exacerbate the development of adenomyosis in humans as in mice [136]. Danazol, an antigonadotropic derivative of 17a-ethinyl testosterone used effectively in the treatment of endometriosis, has not been studied extensively in this condition [88, 137–139]. Tamaoka et al treated adenomyotic women with a danazol-containing intrauterine device from June 1993 to August 2000, and a significant decrease in dysmenorrhea and serum CA-125 levels were observed. In the endometrial hyperplastic patients, histopathologic findings of endometrial hyperplasia disappeared after treatment with the danazol-containing intrauterine device. The mechanisms of direct effect of danazol on endometrial hyperplasia must be clarified [140].

Hormonal administration of progestins or gonadotropin-releasing hormone analogs may be effective, as in endometriosis [141,142]. Enlargement of the uterus and recurrence of symptoms usually reappear within 6 months after the cessation of therapy. Conservative surgery using endomyometrial ablation, laparoscopic myometrial electrocoagulation, or excision of adenomyosis has been helpful in some patients, although follow-up has been restricted to 3 years [143,144]. Recently, the inhibitory effects of a novel, orally active matrix metal-

loproteinase inhibitor, ONO-4817, on the development of uterine adenomyosis induced experimentally by pituitary grafting were examined in mice. The results indicate that ONO-4817 may be an effective inhibitor of the development of adenomyosis [145].

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Typical and subtle atypical presentations of endometriosis

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The diagnosis of peritoneal endometriosis at the time of laparoscopy is often made by the observation of typically puckered black or bluish lesions. There are also numerous subtle appearances of peritoneal endometriosis; these lesions, frequently nonpigmented, were diagnosed as endometriosis after biopsy confirmation by Jansen and Russell in 1986 [1]. The greatest change has been in the case of "subtle" lesions, the diagnosis of which increased from 15% in 1986 to 65% in 1988 [1–6].

Black or bluish lesions: so-called typical lesions

The macroscopic appearance of ectopic endometrium probably depends on the longevity of the process. Viable cells may implant and the initial appearance may be an irregularity or discoloration of the peritoneal surface—the earliest sign being hemosiderin staining of the peritoneal surfaces. Initially, these lesions may appear hemorrhagic, but menstrual shedding from a viable endometrial implant initiates an inflammatory reaction that provokes a scarification process. This process, in turn, encloses the implants. The presence of entrapped menstrual debris is responsible for the typical black or bluish appearance. If the inflammatory process obliterates or devascularizes the endometrial cells, eventually this discoloration disappears. A white plaque of old collagen is all that remains of the ectopic implant. Scarring of the peritoneum around endometrial implants is a

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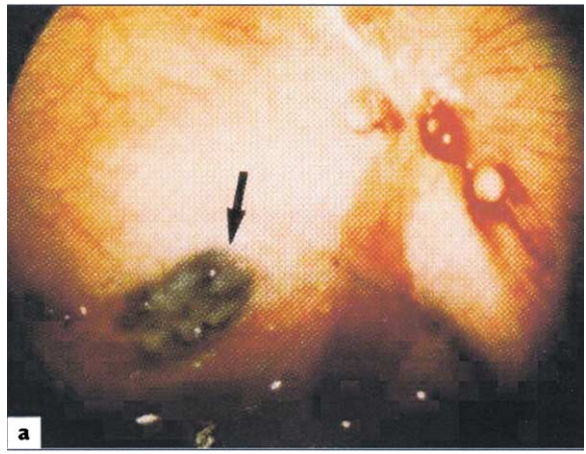

Fig. 1. Black lesion. The black color is caused by the presence of intraluminal debris.

typical finding. In addition to encapsulating an isolated implant, the scar may deform the surrounding peritoneum or result in the development of adhesions.

The typical black peritoneal endometriotic lesion (Fig. 1) results from tissue bleeding and retention of blood pigment, which produce brown discoloration of tissue. Puckered black lesions are a combination of glands, stroma, and intraluminal debris.

Other appearances: red and subtle lesions

Confirmation of endometriosis in subtle lesions was made by Jansen and Russell [1]. Endometriosis was confirmed in 81% of white opacified lesions, 81% of red flame-like lesions, 67% of glandular lesions, 50% of subovarian adhesions, 47% of yellow-brown patches, and 45% of circular peritoneal defects (Table 1). Later, Stripling et al [4] confirmed endometriosis in 91% of white lesions, 75% of red lesions, 33% of hemosiderin lesions, and 85% of other lesions. In the authors'

Table 1
Different appearances of peritoneal lesions

Color	Description
Black	Typical puckered black lesions
Red	Red flame-like lesions
	Glandular excrescences
	Petechial peritoneum
	Areas of hypervascularization
White	White opacification
	Subovarian adhesions
	Yellow-brown peritoneal patches
	Circular peritoneal defects

study, they confirmed the presence of endometriosis in nonpigmented lesions of the peritoneum in more than 50% of cases.

Red lesions

Red flame-like lesions, glandular excrescences, and subovarian adhesions must be considered as the most active lesions [7].

- Red flame-like lesions of the peritoneum (Fig. 2) or red vesicular excrescences more commonly affect the broad ligament and the uterosacral ligaments. Histologically, red flame-like lesions and vesicular excrescences are caused by the presence of active endometriosis surrounded by stroma.
- In color, translucency, and consistency, glandular excrescences on the peritoneal surface closely resemble the mucosal surface of the endometrium seen at hysteroscopy. Biopsy reveals the presence of numerous endometrial glands.
- Subovarian adhesions, or adherence between the ovary and the peritoneum of the ovarian fossa, are distinctive from adhesions characteristic of previous salpingitis or peritonitis. They are caused by a consequence of an inflammatory reaction induced by active lesions.

Subtle lesions

Sometimes subtle endometriotic lesions can be the only lesions seen at laparoscopy.

- White opacification of the peritoneum (Fig. 3) appears as peritoneal scarring or as circumscribed patches, often thickened and sometimes raised. Histologically, white opacified peritoneum is caused by the presence of an

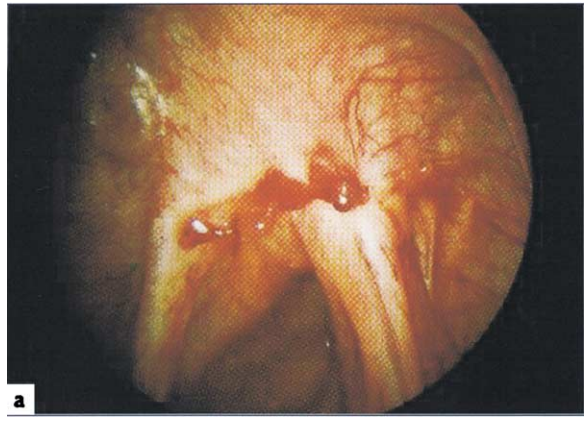

Fig. 2. Red flame-like lesion.

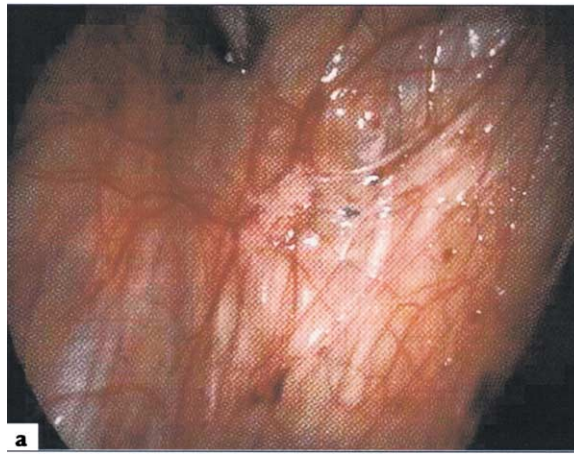

Fig. 3. White lesion.

occasional retroperitoneal glandular structure and scanty stroma surrounded by fibrotic tissue or connective tissue.

- Yellow-brown peritoneal patches resemble café au lait patches. The histologic characteristics are similar to those observed in white opacification, but in the yellow-brown patches, the presence of the blood pigment hemosiderin among the stromal cells produces the café au lait color.
- Circular peritoneal defects, as described by Chatman [2], are present. Serial section demonstrates the presence of endometrial glands in more than 50% of cases.
- Areas of petechial peritoneum or areas with hypervascularization, which were diagnosed as endometriosis in the authors' recent study [6,8], are present. These lesions resemble the petechial lesions that result from manipulation of the peritoneum or hypervascularization of the peritoneum. They generally affect the bladder and the broad ligament. Histologically, red blood cells are numerous, and endometrial glands are rare.

Unsuspected peritoneal endometriosis: nonvisible lesions

In one of the authors' studies [6] (Table 2), biopsies were taken from visually normal peritoneum of 32 women who underwent laparoscopy for infertility, in whom neither typical nor subtle appearances of endometriosis were found (group II). In another group of 52 women with apparent endometriosis, biopsies also were taken from visually normal peritoneum (group I). The peritoneum was considered normal if no lesions, as described previously, were seen. A biopsy was taken from the normal peritoneum of the uterosacral ligaments. Histologic study revealed the presence of endometriotic tissue in two cases (6%) in the group of

Table 2
Peritoneal endometriosis and infertility

	Group I (n = 52)	Group II (n = 32)
Number of biopsies		
From visible endometriotic lesions[a]	86	—
From normal-appearing peritoneum[a]	52	32
Histologic proof of endometriosis		
In visible lesions[a]	80/86 (93%)	—
In normal-appearing peritoneum[a]	7/52 (13%)	2/32 (6%)

Biopsies were taken from the peritoneum of women with (group I) and without (group II) apparent endometriosis. All the women were undergoing laparoscopy for infertility.

[a] Refers to the macroscopic appearance.

32 infertile women without endometriosis. This rate was less than one half the rate (13%) observed in normal peritoneum taken from women with visible endometriosis. Identification of endometriosis in biopsy specimens from areas of normal peritoneum in patients with known endometriosis was reported by Murphy et al [9]. By scanning electron microscopy, 25% of their specimens, which appeared normal by gross inspection, were found to contain evidence of endometriosis.

In the authors' study, by light microscopy, they reported a rate of 13% [6]. Histologic study of biopsies from visually normal peritoneum in infertile women without any typical or ''subtle'' endometriotic lesions revealed the presence of endometriosis in 6% of cases [6]. Unsuspected peritoneal endometriosis can be found in the visually normal peritoneum of infertile women, with or without known associated endometriosis. Although the rate (13%) in women with visible endometriosis was twice the rate observed in women without endometriosis, the difference was not significant. The size of the endometriotic lesions in visually normal peritoneum (313 $\pm$ 185 μm) probably explains why the peritoneum had a normal aspect and why the lesion was not visible, although a meticulous inspection was made to identify small and nonhemorrhagic lesions [6]. As recently demonstrated in infertile women, the diagnosis of endometriosis at laparoscopy has increased. The authors' data confirm that the operating surgeon did not make the diagnosis in at least 6% of cases, however, despite the significant increase in the diagnosis and documentation of endometriosis.

Hormonal independence

Using qualitative histochemistry, the microscopic changes [10] present in endometrium have been observed in ectopic implants, but endometrial implants do not demonstrate the characteristic ultrastructural changes of normal endometrium [11]. The fact that endometrial implants can undergo cyclical histologic changes similar to those found in normal endometrium demonstrates that ectopic endometrium responds to gonadal hormones. Most implants, however, do not

demonstrate histologic changes synchronous with the comparable uterine endometrium [12]. Some of the reasons [13] may be as follows:

- a deficiency in steroid receptors
- the influence of the surrounding scarification process
- the pressure atrophy
- the hormonal independence of ectopic endometrial glands

The evaluation of steroid receptors in ectopic endometrial implants could be difficult because of the small number of glandular and stromal cells within the implant and the heterogeneity of the tissue. Whereas most implants can be demonstrated to possess progesterone receptors [14], only 30% have estrogen receptors. In the ovary, implants have far fewer estrogen and progesterone receptors than does normal epithelium [15,16]. Castration, menopause, pregnancy, or therapeutic suppression of gonadal function can alter the pattern of the disease dramatically. The authors have shown [17] that hormonal treatment is unable to eradicate endometriosis. In peritoneal endometriosis and ovarian endometriosis, microscopic examination of specimens (taken after 6 months of therapy) revealed a high incidence of active endometriosis without signs of degeneration. Mitotic activity was found, which suggested the presence of hormonally independent glands in endometriotic foci.

Is vascularization the key growth factor?

Vascularization of endometriotic implants is probably one of the most important factors in the growth and invasion of other tissue by endometrial glands. A stereometric analysis was applied to study, as precisely as possible, the vascularization in peritoneal endometriotic foci [18,19]. The authors histologically evaluated the vascularization of typical peritoneal endometriosis and its modifications, according to the macroscopic appearance of peritoneal endometriosis.

The method of descriptive and computerized interactive morphometry for different tissue was applied to the study of endometriotic foci to evaluate the stromal vascularization [19] (Table 3). The study demonstrated significant

Table 3
Morphometric study of the stromal vascularization

	Typical lesions (black) group Ia (n = 135)	Red lesions group Ib (n = 35)	White lesions group Ic (n = 50)
Number of capillaries/mm^2 stroma	243	147[a,b]	206[b]
Capillary mean surface area (μm^2)	118 ± 84	234 ± 192[a,b]	78 ± 43[b]
Capillaries/stroma relative surface (%)	2.4	3.2[a,b]	1.5[b]

[a] Significantly different from groups Ia and Ic ($P < 0.05$).
[b] Significantly different from group Ia ($P < 0.001$).

differences between typical (black or bluish) lesions, red lesions, and "subtle" lesions. When compared to typical lesion data, the vascularization was found to be significantly higher in red lesions and significantly lower in white lesions. This change was caused by an increase (red) or a decrease (white) in the volume occupied by the vessels, as proved by the mean capillary surface area and the ratio of capillaries:stroma surface area. This change was more evident in the group of red lesions, in which the number of capillaries/mm^2 was significantly lower than in the other subgroups. In red lesions, the increased level of vascularization was caused by a larger number of larger vessels than in the other groups. In white lesions, there was a greater number of smaller vessels; the number of capillaries was higher than in red lesions. The mitotic index also was significantly different in the three groups. Mitotic processes permit the maintenance and growth of peritoneal endometriosis. The absence of mitosis in white lesions proves their low "activity" [6,18,19].

According to the authors' data, they suggest that there are different types of peritoneal endometriotic lesions in different stages of development. Red flame-like lesions and glandular excrescences are probably the first stage of early implantation of endometrial glands and stroma. The growth and aggressiveness of endometrial glands in the stroma have been demonstrated by a three-dimensional evaluation [18]. In this group, a higher incidence of glands with ramifications was observed when compared to typical and white lesions. The significantly higher stromal vascularization and epithelial mitotic index could be responsible for the invasion of ectopic sites by glands and stroma. Thereafter, menstrual shedding from viable endometrial implants could initiate an inflammatory reaction that provokes a scarification process, which encloses the implant. The presence of intraluminal debris is responsible for the typical black coloration of the same lesion. This scarification process is probably responsible for the reduction in vascularization, as proved by the significant decrease in the capillaries/stroma relative surface area. Thereafter, the inflammatory process devascularizes the endometriotic foci, and white plaques of old collagen are all that remain of the ectopic implant. Concerning white lesions, the authors' study demonstrated the absence of mitosis and poor vascularization, although a similar number of capillaries were found when compared to typical lesions. Their hypothesis is that white opacification and yellow-brown lesions are latent stages of endometriosis. They are probably nonactive lesions that could be quiescent for a long time [18,19].

Influence of GnRH agonist

Some morphologic changes in endometriotic foci after GnRH agonist therapy have been described previously [17]. The mitotic index has been found to be significantly reduced. One of the authors' hypotheses concerning the mechanism of action was the reduction in the vascularization of glandular epithelium after GnRH agonist therapy. Macroscopically, preoperative hormonal therapy resulted in the reduction of pelvic vascularity and inflammation, diagnosed at the time of

second-look laparoscopy. Their results demonstrated that there was a significant decrease in the vascularization of the endometriotic foci after GnRH agonist therapy [19]. This change was caused not by a reduction in the number of capillaries in the lesion but by a decrease in the area of the vessels. In the treated patients, a predominance of smaller vessels was observed when compared with the untreated patients. The vascularization decrease, observed histologically, was in accordance with the observations made by laparoscopy after hormonal therapy. Vascular effects of GnRH agonist on the uterine arteries also have been demonstrated by Doppler [20]. The hypoestradiol state induced by GnRH agonist therapy also could have an effect on the vascularization of the endometriotic stroma. The reduction in vascularization after hormonal therapy could account for the decrease in the inflammatory reaction observed around the endometriotic foci.

In conclusion, evaluation of the stromal vascularization permitted the differentiation and classification of the different appearances of peritoneal endometriosis, according to their vascularization level. The authors' study proved that the "activity" of peritoneal endometriosis is related to the vascularity. This concept must be taken into account in further discussions of the American Fertility Society Endometriosis Classification. Typical, red, and white lesions are three different stages of peritoneal disease, and their relative relation to infertility probably also differs.

Hypothesis

Morphologic and morphometric data allow us to suggest that eutopic endometrium and red peritoneal lesions are similar tissues, with red lesions being recently implanted and regurgitated endometrial cells [21,22]. These data constitute an argument in favor of the transplantation theory for peritoneal endometriosis (Fig. 4). After endometrial tissue entry into the peritoneal cavity, the attachment phase is followed by degradation of the extracellular membranes by matrix metalloproteinases present in the menstrual cavity. Red lesions are consistently located on the surface of the peritoneum, which histologically consists of a thin layer of loose connective tissue covered with a layer of mesothelium. There is a rich supply of subperitoneal blood vessels and lymphatics [23]. Vascularization of endometriotic implants is one of the most important factors of growth and invasion of other tissue by endometrial glands [19,24]. Thereafter, detachment of glands from viable red endometrial implants, explained by the presence of matrix metalloproteinases, could initiate their implantation in other peritoneal sites, as in a "metastatic" process [25].

Data from the authors' group [25] revealed the presence of matrix metalloproteinases in the stroma of red lesions throughout the menstrual cycle, although in eutopic endometrium, matrix metalloproteinases are detected only during the marked decline in progesterone. After this partial shedding, the remaining red lesion always regrows constantly until the next shedding, but menstrual shedding finally induces an inflammatory reaction, which provokes a

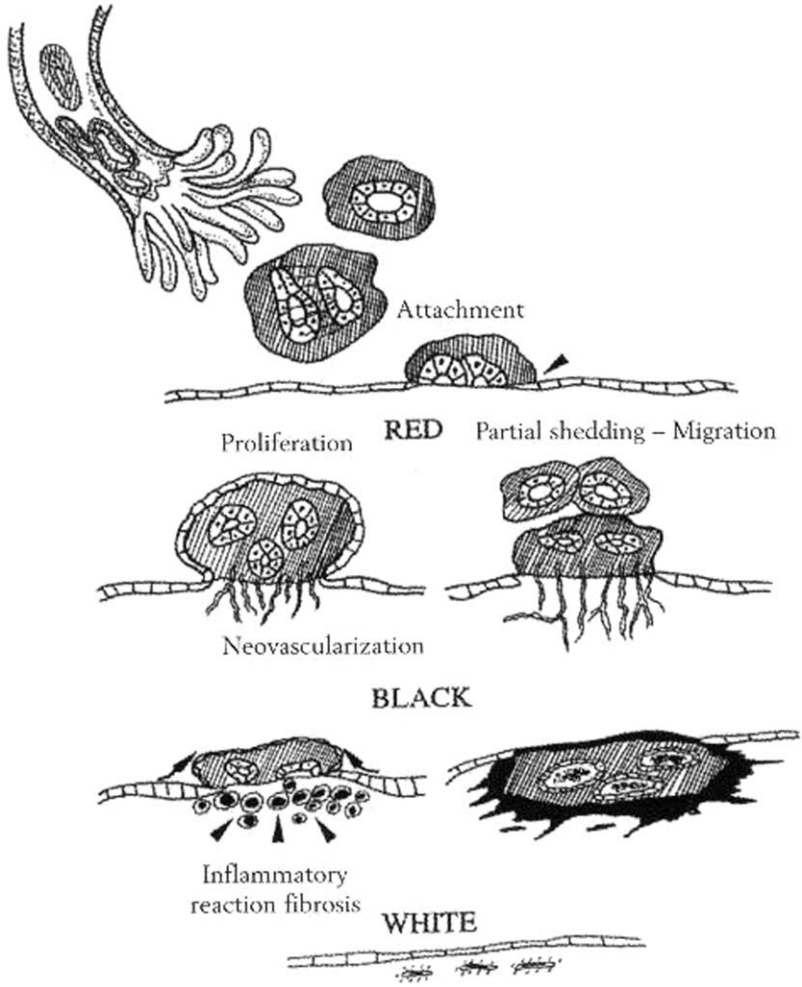

Fig. 4. Hypotheses of evolution.

scarification process that encloses the implant. The enclosed implant becomes a ''black'' lesion because of the presence of intraluminal debris. The scarification process is probably responsible for the reduction in vascularization, as proved by the significant decrease in the relative surface areas of the capillaries and stroma [19]. In some cases, the inflammatory process and subsequent fibrosis totally devascularize the endometriotic foci, and white plaques of old collagen are all that remain of the ectopic implant [21,22]. White opacification and yellow-brown lesions are latent stages of endometriosis [19]. They are probably inactive lesions that could be quiescent for a long time. In agreement with Brosens [26], the authors regard red lesions as early endometriosis and black lesions as advanced

endometriosis [7,19,27–29]. White lesions are believed to be healed endometriosis or quiescent or latent lesions [7,19].

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Noninvasive diagnosis of endometriosis: the role of imaging and markers

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Endometriosis is defined by the presence of endometrial tissue outside the uterus. This definition is based on Sampson's concept that the disease is caused by peritoneal regurgitation and implantation of viable endometrial cells in menstrual debris [1]. Consequently, the diagnosis of endometriosis is based on histologic identification of ectopic endometrial glands and stroma. Inordinate smooth muscle proliferation is also a typical component of endometriotic lesion, however [2,3]. Deep endometriosis, which is found along the outside of the müllerian tract, is characterized predominantly by fibromuscular hyperplasia and the formation of an adenomyotic nodule and microendometriomas [4,5]. On the other hand, peritoneal and ovarian endometriosis is characterized by chronic bleeding that results in the formation of hemorrhagic blisters, fibrosis, adhesions, and ovarian endometriomas. Endometriosis is further characterized by altered immune cell responses, inflammation, neoangiogenesis, and ovarian and uterine dysfunction. These observations indicate that the disease is not merely the sum of all ectopic implants but represents a fundamental disorder that affects the entire reproductive tract [6].

Clinical and basic research in endometriosis has been hampered severely by the lack of accurate noninvasive diagnostic techniques. Transvaginal ultrasonography

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(TVU), Magnetic Resonance Imaging (MRI), and endometrial and serum markers have the potential to facilitate the diagnosis and can be useful in the follow-up of patients. Endometriosis research has entered the postgenomic era, and powerful genomic and proteomic technology is being applied in the search for novel diagnostic and therapeutic approaches. This article explores the recent advances in imaging techniques and the development of diagnostic molecular markers of endometriosis.

Diagnostic imaging of endometriotic lesions

Superficial endometriosis and endometriotic adhesions

Superficial peritoneal endometriosis and ovarian surface implants are not detectable by TVU. MRI also fails to detect subtle endometriotic lesions, although fat-saturated MRI improves the detection rate of small hemorrhagic lesions that measure less than 5 mm from 4% at conventional MRI to 50% [7]. Current imaging technology does not permit reliable assessment or classification of endometriotic adhesions.

Ovarian endometrioma

Gross and microscopic features

The ovarian endometrioma is caused by recurrent menstrual shedding of endometrial-like tissue that lines the wall of the cyst [8]. The macroscopic and microscopic features of the endometrioma have been detailed by analysis of in situ lesions in ovarian specimens [8,9] and by ovarioscopy combined with targeted biopsies [10]. More than 90% of endometriomas are pseudocysts formed by invagination of the ovarian cortex, which is sealed off by adhesions. The inside of the cyst is characterized by fibrosis and retraction of the cortex, the presence of islands of glandular endometrial tissue, and organized blood clots. The remainder of the cyst wall is smooth and lined by a thin endometrial-like tissue that consists of surface epithelium and highly microvascularized stroma. Recent endometriomas have a marble-like cortical surface, whereas the cyst lining in older lesions is pigmented, fibrotic, and poorly vascularized. The preferential site for endometriomas is the left ovary [11], which is readily explained by the anatomic position that favors the formation of adhesions between the ovary and the opposing pelvic structures. The typical macroscopic features are not applicable in recurrent endometriomas after surgery.

There is no evidence that endometriotic tissue invades the ovarian stroma; however, large multilocular cysts frequently combine endometriomas with a hemorrhagic corpus luteum or lutein cyst. These associated cysts sometimes can become colonized by surface epithelium and stroma that originate from the endometrioma [8]. The chocolate-like content represents old and chronic bleed-

ing but is not a specific feature of the endometrioma, because it is also found in other hemorrhagic cysts of the ovary.

Ultrasound diagnosis

Transvaginal ultrasonography is a useful tool to detect and monitor ovarian endometriomas that are larger than 10 mm in diameter. Several authors have evaluated the diagnostic accuracy of TVU (Table 1). The characteristic features are mainly based on the presence of diffuse, low-level internal echoes and hyperechoic foci in the wall (Fig. 1). A major limitation of these studies is that the sonographic findings have not yet been correlated with histologic examination of in situ specimens or targeted biopsies. The pathologic significance of increased wall thickness, nodularity, and hyperechoic foci remains speculative. Other possible discriminatory factors, such as location, lesion shape, and position, also have not yet been assessed.

Sonographic features of endometriomas can be present in hemorrhagic cysts, dermoid cysts, and occasionally in epithelial ovarian tumors. A repeat ultrasound is highly recommended for unilocular cysts with low-level internal echoes but without wall nodularity or hyperechoic foci [12]. If papillary structures that protrude from the internal cyst wall are visualized, then ovarian malignancy, such as endometroid adenocarcinoma, must be excluded (Fig. 2) [13].

Transvaginal ultrasonography combined with Doppler ultrasound

Whether the addition of color Doppler studies adds to the diagnostic efficiency of TVU remains uncertain. Alcazar et al [14] found no improvement in the performance of ultrasound in the diagnosis of endometriomas by including color Doppler. Aleem et al [15], however, concluded that scattered vascularity is typical of ovarian endometriomas and distinct from the dense vascularization associated with corpus luteum cysts and ovarian neoplasms. Similarly, observations were made by Guerriero et al [16], who reported that endometriomas are associated with "poor" blood supply, whereas nonendometriomas are characterized by

Table 1
Efficiency of transvaginal ultrasound for the diagnosis of ovarian endometriomas

Reference	No. of patients	Diagnostic feature	Sensitivity (%)	Specificity (%)
B-mode				
Mais et al [70]	21	Cyst content	84	90
Volpi et al [71]	57	Cyst content and wall	82	98
Dogan et al [72]	107	Cyst content and wall	86	99
Patel et al [12]	40	Cyst content and wall	45–60	98–100
Color doppler				
Alcazar et al [14]	27	Without CD	89	91
		With CD	76	89
Guerriero et al [16]	58	Without CD	81	91
		With CD	90	97

CD, color Doppler.

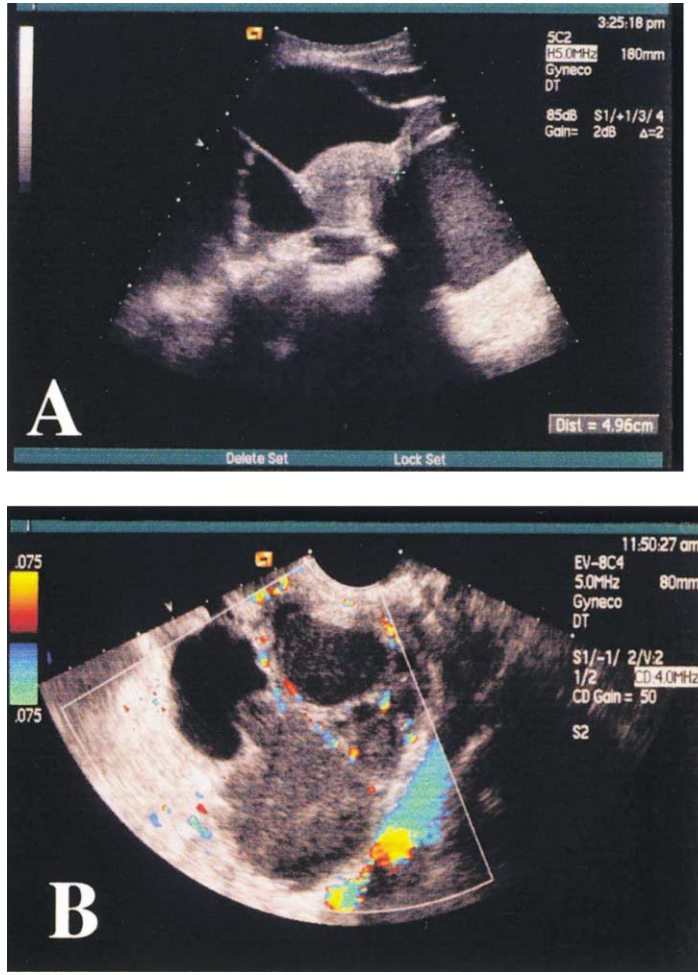

Fig.1. (A) Transabdominal sonography (*transverse view*) of a large endometrioma with "ground glass" appearance (*right*) and anechogenic pseudocysts formed by adhesions (*center* and *left*). The uterus is centrally located (*between crosses*). (B) Transvaginal sonography of a multilocular endometrioma with "ground glass" appearance of cyst contents. Color Doppler imaging enables visualization of limited vascularity in the cyst wall and blood flow in the external iliac vein (*blue color*). The anechogenic cyst represents parts of a hydrosalpinx (*left*).

"rich" vascularization or the presence of arterial flow in the papillary structures or echogenic areas of the cyst.

Transvaginal ultrasonography-guided aspiration

Transvaginal ultrasonography can be used for transvaginal aspiration of endometriomas. Aspiration is not an effective treatment for ovarian endome-

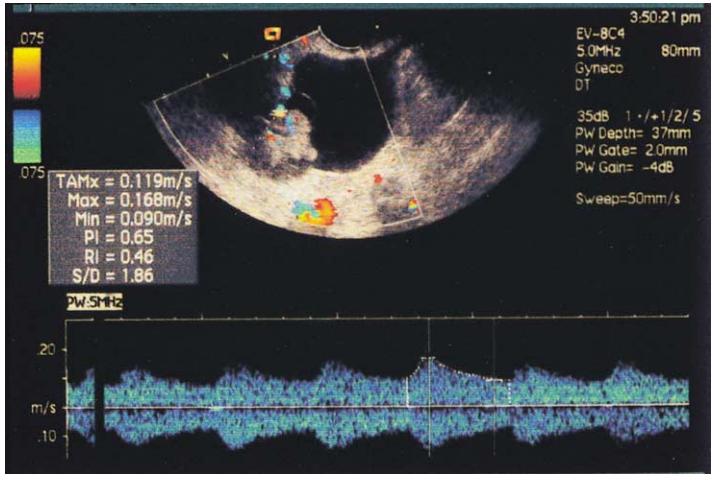

Fig. 2. Transvaginal sonography of an endometroid adenocarcinoma with solid papillary projection within an endometrioma. Color Doppler imaging and spectral flow analysis reveal low-resistance flow within this solid projection.

trioma but may be helpful in patients who had prior surgery and present with a recurrence. It has been reported that up to 73% of recurrent hemorrhagic cysts after surgery are actually dysfunctional cysts [10]. Measurement of CA-125 levels in the aspirate may be helpful in differentiating endometriomas from dysfunctional hemorrhagic cysts [17].

Scoring systems

Several scoring systems have been proposed to improve the diagnosis and differentiate between malignant and benign adnexal masses. Complex scoring systems are unlikely to be clinically useful, however. Most ultrasonographers subjectively interpret the sonographic features of adnexal tumors and, depending on prior experience, subjective assessment can be accurate in distinguishing between malignant and benign adnexal masses and among endometriomas, cystic teratomas, and other common adnexal masses in young women [13]. Okaro et al [18] reported that the combination of "soft" markers, such as site-specific tenderness, the presence or absence of free fluid in the pelvis, and the degree of ovarian mobility, with the conventional "hard" ultrasound markers improves the detection of endometriosis and adhesions in women with chronic pelvic pain. The authors reported that in 83% of symptomatic patients with a normal ultrasound examination no discernible pelvic pathology was found at laparoscopy. Conversely, pelvic pathology was detected in 78% of patients with abnormal scan findings using a combination of "soft" and "hard" markers.

Table 2
Efficiency of MRI for the diagnosis of ovarian endometriomas

Reference	No. of patients	Imaging modality	Sensitivity (%)	Specificity (%)
Zawin et al [73]	31	No fat suppression	71	82
Arrive et al [74]	8	No fat suppression	88	—
Togashi et al [75]	86	With fat suppression	90	98

Magnetic resonance imaging

The role of MRI for the diagnosis of ovarian endometrioma has been evaluated by several investigators (Table 2). The reported MRI features are almost exclusively based on the detection of chronic or recurrent bleeding in the endometrioma. The larger endometrioma (> 1 cm) appears as a homogeneously high-signal intensity mass on T1-weighted images and as a low-signal intensity mass with focal high-signal intensity areas on T2-weighted images [19]. In the presence of recent bleeding, the cyst content has high-signal intensity in both types of sequences. Some authors have described the thickened hyposignal wall in T2-weighted images with a retracted part that produces the typical appearance of a "grain de café" [20]. This retraction may divide the pseudocavity in two parts, each filled with blood of different age.

Deep retroperitoneal endometriosis

Histologic appearance of deep endometriosis

Deep endometriosis represents a nodular, myoproliferative lesion characterized by the presence of microendometriomas and a sparse amount of glandular and stromal tissue. Similar to uterine adenomyosis, deep endometriotic lesions have no capsule and are in continuity with the surrounding fibromuscular or muscular structures. Not all deep lesions are proliferative. In a series of 28 deep sacrouterine lesions and 11 rectovaginal lesions, only fibrotic tissue was found in 6 (21%) and 1 (9%) of the specimens, respectively [21].

Deep retroperitoneal endometriosis occurs preferentially in the rectovaginal and vesicouterine spaces and uterine ligaments. Rectovaginal septum endometriosis is a misnomer, because involvement of this septum rarely occurs. Endometriotic nodules in the rectovaginal space eventually can reach the upper extremity of the rectovaginal septum, but on MRI the septum invariably appears distinct and regular [22]. The lesions may extend laterally into the parametrium and even involve the ureters [23]. Pelvic endometriosis also can affect the rectosigmoid colon, the appendix, and ileum, where it can cause marked overgrowth of the external muscular coat. These lesions may be constricting or may produce an eccentric intraluminal filling defect that resembles colon carcinoma [19]. Unlike colon carcinoma, however, endometriosis does not breach the bowel mucosa or causes mucosal ulceration.

Ultrasonography and magnetic resonance imaging

To date, few ultrasound studies have focused specifically on the detection of deep retroperitoneal endometriosis. On ultrasonography, these endometriotic nodules can appear as solid hypoechogenic lesions that range from 0.5 to 4 cm and adhere to the anterior rectal wall (Fig. 3). Characteristically, these lesions are more painful when examined during menstruation. Rectal endoscopic sonography has been used in evaluating the thickness of the uterosacral ligaments and the presence of rectal infiltration in patients with deep endometriosis [24–27].

Chapron et al [22] recently described in detail the MRI appearances of rectovaginal endometriotic nodules that varied between 2 and 2.5 cm in eight affected patients. On T1-weighted images, the signal intensity of rectovaginal

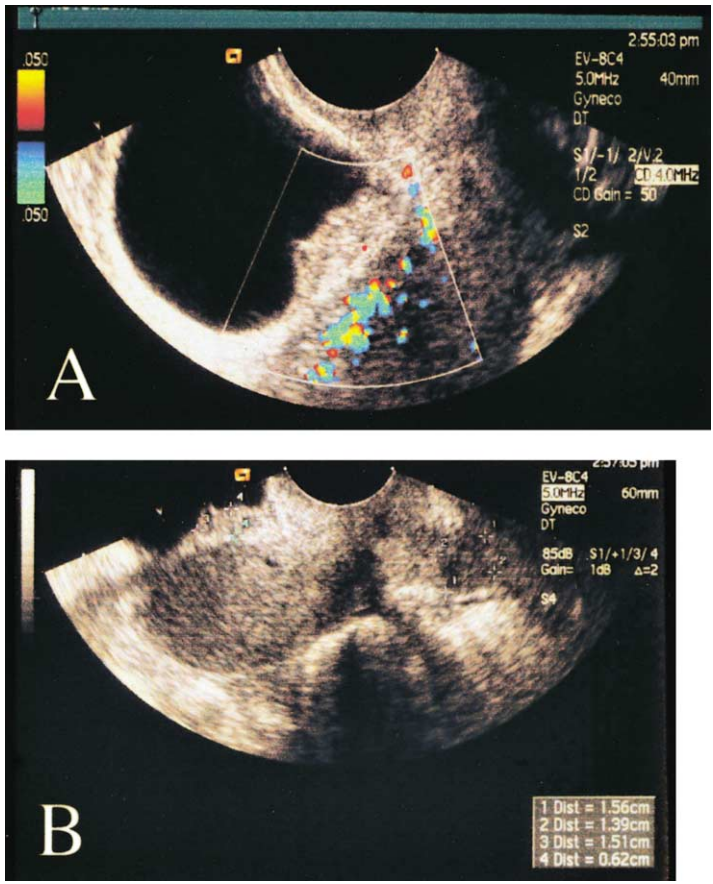

Fig. 3. (A) Transvaginal sonographic image of deep endometriosis within the posterior bladder wall. At color Doppler imaging, normal myometrial flow is seen, whereas the endometriosis lesion has limited vascularity. (B) Transvaginal sonography shows a sagittal view of the uterus, bladder endometriosis (*left*), and deep rectovaginal endometriosis (*right*).

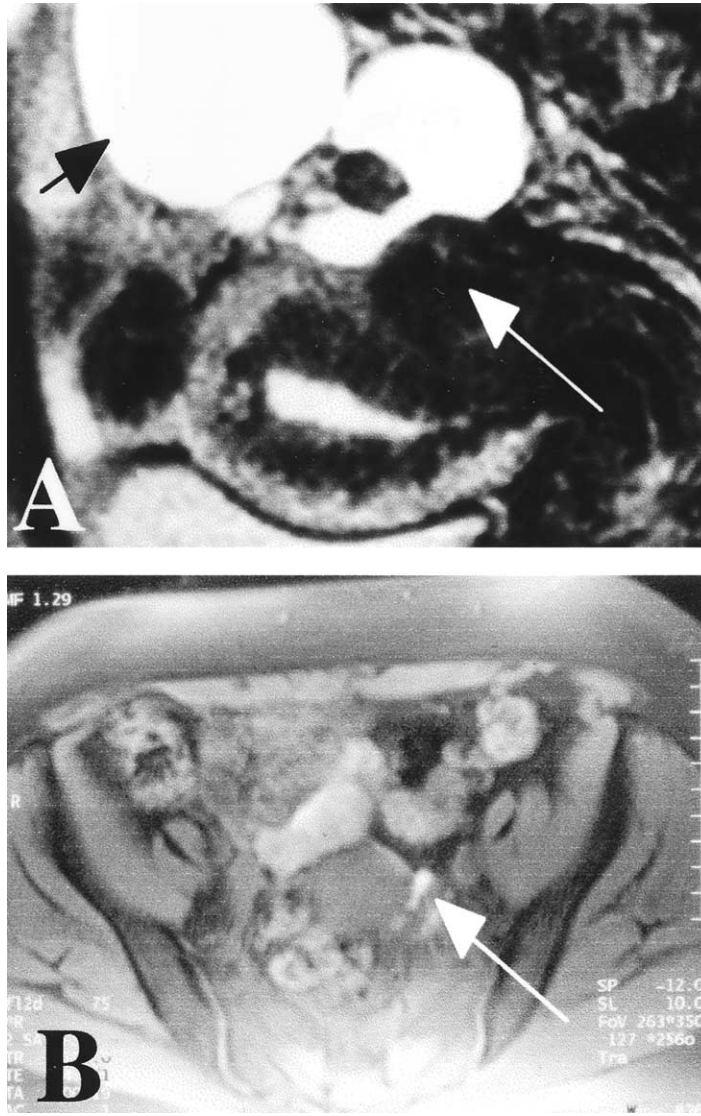

Fig. 4. (A) T2-weighted uterine MR scan demonstrates that multiple pelvic endometriotic lesions are visible, such as large ovarian endometriomatas (*black arrow*) and deep rectovaginal endometriosis (*long white arrow*). (B) Axial T1-weighted image obtained with a fat-saturation technique shows a small, hyperintense lesion surrounding the left ureter at the site of obstruction (*arrow*). The hyperintense lesion represents a blood clot, which corresponds to a small endometrioma.

nodule is isointense to the myometrium with hyperintensive spots that remain visible in the fat-suppressed sequences, indicating the presence of microendometriomas. On T2-weighted images, the signal intensity of the nodules is isointense or hypointense to the myometrium with hyperintensive spots (Fig. 4). The nodules have an irregular contour and are indistinguishable from the uterovaginal structures. In some cases a hyper-signal intensity transition zone can be identified between the rectum and the nodule, which has been termed the "safety margin." In other cases, this "safety margin" is not seen, and thickening of the rectum wall is noticed. The "safety margin" is likely to represent interposing fat tissue. The retraction among the torus uterinum, the endometriotic nodule, and the rectum results in obliteration of the pouch of Douglas. This occurrence can give a false impression of the lesion being located below the pouch and of radiated infiltration of the perirectal space with thickening and stiffness of the rectum wall.

Detection of parametrial disease with MRI is difficult but should be suspected if there is asymmetric signal intensity on T2-weighted or contrast fat-saturated images [19]. False-positive detection of endometriosis on MRI may be caused by misinterpretation of normal anatomic structures, MRI-related artifact, or previous surgery. It has been suggested that the diagnostic efficiency of MRI in endometriosis could be improved by the routine use of phased-array coils and negative signal-reducing bowel contrast agents [19].

Bladder endometriosis

Nodular bladder endometriosis is not easily palpable at vaginal examination. Typically it is found in patients with dysmenorrhea with associated urinary symptoms, such as micturition frequency. TVU may reveal a solid nodule within the posterior bladder wall if the bladder is slightly filled. Color Doppler studies may detect low to moderate vascularity (Fig. 3), and mild pressure with the vaginal probe often elicits focal pain. In a series of 12 patients with nodular bladder endometriosis that varied between 10 and 31 mm in diameter, TVU was normal in 4 patients. In contrast, MRI using a body coil enabled visualization of the lesions in all patients [28]. The use of an endocavitary coil was found to be superior to imaging with a body coil in determining the extent of infiltration of the bladder wall [28].

Obstructive uropathy secondary to endometriosis

Ureteral obstruction is an infrequent but serious complication of deep peritoneal endometriosis. In a large retrospective study on ureteral endometriosis, the proportion of lesions located on the left side was found to be significantly higher than on the right side [29], although this was not confirmed in two recent studies [23,30]. External ureteral endometriosis is more frequent than internal and can be treated by laparoscopic ureterolysis [31]. Obstructive uropathy caused by a hemorrhagic endometrioma has been described in several patients who received unopposed estrogen replacement therapy after hysterectomy and bilateral salpingo-

oophorectomy [32]. MRI has been shown to be useful for diagnosing periureteric endometriomas and monitoring the response to medical therapy (Fig. 4) [33].

Diaphragmatic endometriosis

One case study reported on the use of CT and MRI in visualizing diaphragmatic endometriosis, although the patient was already known to have implants on the diaphragm [34]. Other researchers have found imaging scans to be of no help, and a negative scan cannot exclude the presence of diaphragmatic endometriosis [35,36].

Imaging of endometriosis: conclusion and perspective

Current imaging techniques do not allow accurate staging of endometriosis because they lack the resolution necessary to visualize small superficial peritoneal and ovarian implants and cannot detect the presence or extent of adhesions. A major role of MRI, however, is to help visualize laparoscopic blind spots, such as the retroperitoneal space or lesions obscured by dense adhesions. TVU and MRI are useful in evaluating recurrence and response to treatment in patients with known disease.

Early detection and staging of disease are crucial in cancer. It is questionable, however, if a similar approach is required for a benign disease such as endometriosis. No evidence exists that all small endometriotic lesions necessarily progress or acquire a destructive invasive phenotype. Although there is unequivocal evidence that endometriotic cells have invasive potential in invasion assays in vivo [37,38], endometriosis does not invade the ovarian stroma or the fat tissue in the retroperitoneal space. Nondestructive invasion is seen in structures with a fibromuscular or muscular wall, however, and it seems possible that the extent of invasion is determined primarily by changes in the local microenvironment, interstitial bleeding, inflammation, and subsequent colonization by endometriotic cells. Clinically, there is poor correlation between the size of the lesion and symptoms such as infertility and pelvic pain. Finally, a surgical paradox shows that surgery is more effective in pelvic pain and infertility in severe rather than in mild disease. These observations question the assumption that the management of suspected cases of endometriosis necessarily requires an invasive procedure for meticulous staging and ablation of visible lesions, as is the case for cancer. Instead, it seems reasonable to initiate medical treatment in symptomatic patients with ultrasound or MRI evidence of endometriosis if this is the preferred treatment option.

Endometrial and serum markers of endometriosis

Normal endometrial responses to ovarian hormones

During the menstrual cycle, ovarian estradiol and progesterone stimulate the ordered growth and differentiation of endometrial tissue compartments. In

humans, this action includes synchronous growth and coiling of the spiral arteries, secretory transformation of glandular epithelium, migration of bone marrow-derived cells, and decidualization of the stroma, which is believed to be essential for blastocyst implantation and subsequent formation of a hemochorial placenta. At a molecular level these morphologic events are controlled by highly coordinated activation of certain gene sets [39–41]. The sequential expression of these progesterone-dependent genes controls the influx of uterine natural killer cells, defines a limited period of uterine receptivity, controls trophoblast invasion, and, in the absence of pregnancy, maintains vascular integrity before menstruation.

Strong evidence suggests that the response to ovarian hormones in the various endometrial cellular compartments is affected through complex interactions among activated steroid hormone receptors, estrogen and progesterone receptors, and signaling pathways activated by locally released factors [41–44]. Cytokines and growth factors released by uterine immune cells, including T cells, uterine natural killer cells, polymorphonuclear neutrophils, macrophages, and monocytes, are believed to play a pivotal role in establishing the specific microenvironments of the basal and superficial endometrial layers [40,44]. For instance, it is believed that interferon-γ secreted by lymphoid aggregates in the basal endometrial layer contributes to the low apoptotic and proliferative activities in this layer and could account for the higher local expression of interferon-γ–dependent genes, such as class II major histocompatibility complex antigens and heat shock protein-70.

It is important to note that the spatio-temporal distribution of uterine immune cells is in turn tightly controlled by ovarian hormones. For instance, the lymphoid aggregates in the basal layer are small during the early proliferative phase but significantly increase in size during the second half of the cycle [45]. During the proliferative phase, the superficial endometrial layer contains only a few uterine natural killer cells, macrophages, and T cells dispersed throughout the stroma and glands. After ovulation, the number of uterine natural killer cells, but not T cells or macrophages, increases dramatically until a few days before menstruation [46].

Aberrant gene expression in endometrium from women with endometriosis

The uterine endometrium in women with endometriosis is histologically normal but biochemically profoundly abnormal. Alterations have been documented in the immune cell compartment and in the responses of the stromal and glandular cells to ovarian steroid hormones. These observations reinforce the view that immune cell function and steroid hormone response are intricately linked in the endometrium. Increased numbers of CD45+, CD43+, and CD3+ intraepithelial leukocytes have been documented in the endometrium of women with endometriosis during the proliferative phase of the cycle [47]. Klentzeris et al [49] reported that the endometrium of affected women is also characterized by fewer T-suppressor/cytotoxic (CD8+) cells and CD56+ uterine natural killer cells but more T-helper/inducer (CD4+) cells and macrophages (CD68+). Although in the latter study the observed differences in the various immune cell populations

Table 3
Nonexhaustive list of genes that are aberrantly regulated in the endometrium of women with endometriosis

Gene	Function	Reference
αvβ3 integrin	Extracellular matrix/cell adhesion molecule	[76, 77]
β1-integrin	Extracellular matrix/cell adhesion molecule	[78]
E-cadherin	Extracellular matrix/cell adhesion molecule	[78]
P450 aromatase	Estrogen biosynthesis	[50–52]
17b-hydroxysteroid dehydrogenase type-1	Estrogen metabolism	[79]
Interleukin-6	Proinflammatory cytokine	[80]
Monocyte chemotactic protein-1	Chemotactic cytokine	[81, 82]
Interleukin-1 receptor type II	Cell signaling	[83]
Cyclooxygenase-2	Prostaglandin synthesis	[84]
Endoglin	Cell surface receptor	[85]
C3 complement	Immune response	
Heat shock protein 27	Signaling modulator	[86]
Xanthine oxidase	Detoxification	[87]
Superoxidase dismutase	Detoxification	[88]
Endometrial bleeding-associated factor	Growth factor	[89]
HOX gene	Transcription factors	[90]

were found not to be statistically significant, they may reflect differences in lymphocyte activation status and cytokine expression profiles [48,49].

The temporal and spatial expression of a growing number of genes has been shown to be aberrant in the endometrium of women with endometriosis. These genes are involved in diverse cellular functions, including steroid hormone biosynthesis and metabolism, prostaglandin synthesis, cell signaling and signal transduction, free radical scavenging, angiogenesis, cell adhesion, and extracellular matrix remodeling (Table 3).

Despite the considerable progress in characterizing the endometrium of women with endometriosis, few attempts have been made to assess the diagnostic value of endometrial markers of disease. There are several reasons for this. First, the level of expression of a given gene may vary considerably among individuals and among biopsy samples. Second, abnormal expression pattern may be confined to a certain phase of the cycle. Third, the altered expression pattern may be too subtle to be used as a discriminatory marker. Finally, the expression profiles of many endometrial factors have been determined only by immunostaining. This approach is not only time consuming but also the assessment of immunoreactivity is, to a certain degree, subjective and observer dependent. The lack of easy, reliable, and quantitative techniques to assess expression levels in biopsy material restricts the use of endometrial markers.

Endometrial aromatase expression as a marker for endometriosis

Several studies reported that aromatase P450, the enzyme that catalyses the conversion of C_{19} steroids (androstenedione and testosterone) to estrone (E_1), is expressed in the eutopic endometrium of women with endometriosis but not in

endometria of disease-free controls [50,51]. Aromatase P450 mRNA expression seems to be independent of the phase of the cycle, which renders it a potential "ideal" marker that does not require quantitation or timed biopsy samples. In a retrospective study, Kitawaki et al reported that detection of aromatase P450 protein in endometrial biopsy samples strongly correlates with the presence of endometriosis and adenomyosis. The authors suggested that this approach could be used as an outpatient screening test for endometriosis, with sensitivity and specificity rates of 91% and 100%, respectively [52].

In a prospective study, Dheenadayalu et al reported that endometrial aromatase P450 mRNA expression, detected by Reverse Transcriptase-PCR (RT-PCR) and Southern blot analysis, is not confined to women with endometriosis but is also associated with most hormone-dependent proliferative disorders of the uterus, including leiomyomata, adenomyosis, and proximal tubal disease [53]. As a diagnostic marker for endometriosis, aromatase P450 mRNA expression yielded a sensitivity of 82%, a specificity of 59%, a positive predictive value of 76%, and a negative predictive value of 67%. If additional uterine pathology was taken in account, the sensitivity increased to 84%, the specificity to 72%, and the positive predictive value to 87%, but the negative predictive value remained unchanged (67%). The authors concluded that although endometrial aromatase P450 gene expression is predictive of the presence of pelvic disease, the relative high incidence of false-negative results and lack of specificity are likely to impair clinical application [53].

MetrioTest

To date, there is only one commercially available test for endometriosis based on simultaneous analysis of an endometrial biopsy and peripheral blood sample. The MetrioTest (PROCREA BioSciences, Inc., Montreal, Quebec, Canada) has been developed through a clinical study that compared the proportion of several subsets of T and B lymphocytes, natural killer cells, and macrophages in the endometrium of 173 patients with endometriosis and 195 normal controls (P. Hugo, MD, personal communication, 2002). It is based on the assessment of eight proprietary leukocyte subsets by flow cytometry analysis combined with a blood biochemical marker analyzed by ELISA. Using a decisional algorithm, the test provides a specificity rate of 95% and a sensitivity rate of 61%. Given a prevalence of 45%, these values can be further converted into positive and negative predictive values of 91% and 75%, respectively. MetrioTest has been approved by Health Canada.

Serum markers of endometriosis

There is considerable interest in the development of serum markers for endometriosis. Ideally, such markers should exhibit the following features: high sensitivity and specificity, excellent prognostic value, and good correlation between the serum levels and severity of disease. Such markers could be used

Table 4
Reported accuracy of serum markers for the diagnosis of endometriosis

Marker	Sensititvity (%)	Specificity (%)	Correlation with stage?
CA-125	27–94	83–93	Yes
Placental protein-14 (glycodelin)	50–73	—	Yes
Endometrial antibodies	74–83	79–100	No
Carbonic anhydrase antibodies	35–66	85–90	?
Interleukin-6	90	67	?

Modified from Pittaway DE. Serum markers of endometrium and endometriosis. In: Diamond MP, Osteen KG, editors. Endometrium and endometriosis. London: Blackwell Science; 1997; p. 31–41.

not only for diagnosing endometriosis but also for monitoring disease progression and responding to medical or surgical treatment.

Peripheral blood levels of CA-125, placental protein-14 (glycodelin), and anti-endometrial and anti-carbonic anhydrase antibodies have been investigated for their diagnostic potential in women with endometriosis. Table 4 summarizes the diagnostic performance of these candidate markers. CA-125, a high molecular weight membrane glycoprotein, is clinically the most widely used serum marker of endometriosis. This glycoprotein is expressed in all tissues derived from embryonic coelomic epithelium, including endometrium, endocervix, fallopian tubes, peritoneum, pleura, and pericardium [54]. In patients with advanced endometriosis, CA-125 is elevated predominantly during the first days of the menstrual cycle [55,56]. Elevated serum concentrations of CA-125 are not specific for endometriosis, however, and have been associated with many epithelial cancers and benign gynecologic and nongynecologic disorders, such as adnexitis, pancreatitis, peridontitis, pregnancy, and ovarian hyperstimulation syndrome. Combining CA-125 plasma levels with TVU does not result in a better predictive capacity than TVU alone [57].

A recent report suggested that serum levels of interleukin-6, with a cut-off level of 2 pg/mL, could discriminate between patients with and without endometriosis [58]. Similarly, Matarese et al [59] recently reported marked increased leptin levels in serum and peritoneal fluid of patients with pelvic endometriosis. The authors suggested that the proinflammatory and neoangiogenic actions of this adipocyte-derived helical cytokine may contribute to the pathogenesis of endometriosis. Follow-up studies have failed to confirm that the presence of pelvic endometriosis is associated with elevated serum leptin concentrations [60]. Larger prospective studies are required to determine the diagnostic potential of measuring circulating inflammatory cytokine levels in endometriosis.

Novel strategies in the search for markers of endometriosis

Functional genomics and proteomics

Increasing evidence suggests that endometriosis is a polygenic and multifactorial disease, which indicates that multiple distinct pathways could be involved in its pathogenesis. Many cardinal features of endometriosis, such as inflammation

and neoangiogenesis, are shared with a plethora of other diseases, which renders it unlikely that a single biochemical marker will yield sufficient sensitivity and specificity to be used in clinical practice. In recent years, the human genome sequencing project has been the driving force in the development of functional genomics. Different methodologies, including suppression subtractive hybridization, differential display reverse transcriptase PCR, and microarrays, have proved to be powerful in detecting and characterizing differentially expressed genes. Microarray technology allows simultaneous analysis of the expression of large numbers of genes and has been used to characterize the expression of genes, gene families, and signal transduction pathways during the implantation window in human endometrium [61]. A similar approach has been used to characterize gene expression upon decidualization of human endometrial stromal cells in culture [62,63]. Recently, Eyster et al [64] used cDNA microarrays to identify differentially expressed genes between eutopic and ectopic endometrium and reported that the expression of eight genes from a total of 4133 genes on the microarray was increased in endometriotic implants.

Comparative expression analysis of mRNAs and proteins has shown that expression levels of mRNA do not necessarily correlate with those of the encoded protein. A given gene can encode for many different protein species through the use of alternative promoters, pre-mRNA splicing, alternative translation, or postranslational modifications. The term "proteomics" refers to the currently emerging technology that allows large-scale and high-throughput identification of proteins in cells, tissue samples, or body fluid. Classically, two-dimensional electrophoresis, based on a combination of isoelectric focusing and sodium dodecyl sulpfate polyacrylamide gel electrophoresis, was the only method to analyze the protein complement in a sample with high resolution. The introduction of protein chips and mass spectrometry has facilitated protein identification greatly [65]. In particular, matrix-assisted laser desorption and ionization time-of-flight and surface-enhanced laser desorption and ionization time-of-flight are increasingly used for rapid protein profiling. As is the case for microarrays, these protein profiles can contain thousands of data points, which necessitates sophisticated bioanalysis.

The power of proteomic pattern technology as a diagnostic tool recently has been demonstrated for ovarian cancer, however. Petricoin et al [66] first identified an optimum discriminatory proteomic pattern from analysis of serum from 50 unaffected women and 50 women with ovarian cancer. Subsequently, the discovered pattern was used to classify an independent set of 116 serum samples from women with and without ovarian cancer. The discriminatory protein pattern yielded sensitivity and specificity rates for ovarian cancer of 100% and 95%, respectively. Currently, functional genomics and proteomics are being applied in all areas of medicine, including in the search for novel diagnostic modalities of endometriosis [66].

Immunotargeting of endometriotic lesions

The possibility of raising antibodies specific to endometriotic implants is currently under investigation. If successful, such antibodies could prove to be

powerful tools for the diagnosis and treatment of endometriosis. For instance, antibody-conjugated paramagnetic liposomes would allow visualization of specific molecules expressed on endometriotic cells by MRI. Conceivably, coupling of an endometriosis-specific antibody to a phototoxic molecule might enable the surgeon to carry out "targeted in situ killing" of microscopic implants.

Immunotargeting of endometriotic implants requires the development of monoclonal antibodies against strong immunogens specific to endometriotic cells. Antigens specific to endometriotic cells have not yet been identified. Immunization of animals with cells or cell membrane fractions has the potential of generating cell type-specific antibodies, however. The source of such cells could be tissue fragments, although fragments invariably contain a mixture of cells. Alternatively, laser microdissection could be used to target certain cell types, thereby reducing the complexity of the immunogens. Alternatively, selected cell populations that are cultured as primary or secondary cells or even as immortalized cell lines could be prepared for immunization. Subsequently, the harvested monoclonal antibodies require immunohistologic testing to confirm cell specificity, and the targeted antigen requires characterization at molecular level.

Endometrial and serum markers of endometriosis: summary and perspective

An accurate test for endometriosis based on biochemical analysis of eutopic endometrium or peripheral blood sample potentially could reduce the number of uninformative laparoscopic procedures and provide a rational basis for initiating medical treatment. Any attempt at evaluating the diagnostic accuracy of a biochemical marker of endometriosis must overcome two intrinsic biases: (1) the inevitable selection of patients who require laparoscopy, which inevitably results in a high disease prevalence and (2) the intrinsic limitations of the visual diagnosis of endometriosis. Peritoneal endometriosis can be microscopic and can change in appearance and location [67,68]. Spontaneous disappearance of minimal peritoneal endometriosis, determined by second-look laparoscopy, has been shown to occur in 42% of affected patients [69], which indicates that there will be inevitable discrepancies between biochemical markers of disease and the visual assessment of pelvic lesions. Conversely, severe lesions can represent a regressive or inactive stage of endometriosis. These observations have prompted some researchers to argue that biochemical tests should focus on predicting clinical correlates of endometriosis, such as infertility and pain, rather then the presence or absence of ectopic implants per se [53].

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The current staging system for endometriosis: does it help?

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Sampson began to classify endometriosis in graded stages as early as 1921 [1]. Despite its long recognition as a continued and progressive disease process, the etiology, pathophysiology, and natural history of endometriosis remain unpredictable. Ideally, a classification system should correlate outcomes with an observed stage of disease. Predictable treatment responses also should be related to stage, allowing a prognosis to be given based on the observed stage and the potential response to treatment. Given these parameters, most staging systems for endometriosis have been modeled after those for malignant disease. Unfortunately, treatment outcome for the symptoms of endometriosis may not depend on volume of disease or even morphologic types of lesions. There is current recognition that the existing system for endometriosis classification does not meet these criteria well in terms of predicting treatment response for either infertility or chronic pelvic pain. Potential sources of error include observational error, incomplete knowledge of the pathophysiology of the disease with a failure to consider morphologic lesion types, limited reproducibility, and the arbitrariness of the scoring system [2–5].

Historical background

In 1921, Sampson classified endometriosis by modifying a previously used category of hemorrhagic cysts of the ovary. Because of the histologic appearance of endometrial-like glands and stroma in several ovarian hemorrhagic cysts, he added the endometriotic cysts category to the other types (follicular, corpus luteal, and stromal cysts). He noted that these endometriotic cysts were often adjacent to adhesions formed, he thought, by escaped contents of the cysts into the peritoneal cavity. During a later publication, Sampson noted that retrograde menstruation was another pathogenesis for peritoneal endometriotic implants and adhesions.

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In 1949, Wicks and Larson [6] proposed histologic criteria to evaluate endometriosis based on the histology of resected lesions and not on the anatomic location or clinical findings. They proposed a grading system similar to Broder's system used for malignancy staging. Patients were staged at laparotomy into four groups based on the amount and spread of disease. Grade 1 lesions were relatively inactive and composed primarily of phagocytic cells, blood pigment, and debris. Grade 4 lesions demonstrated glands and stroma typical for active endometrial tissue responsive to ovarian hormonal stimulation. This system was suggested as a guide for therapeutic intervention, and frozen section biopsy at the time of surgery could then aid in the surgical decision. Because of its perceived similarity to malignant disease, however, hysterectomy with bilateral salpingo-oophorectomy was almost uniformly performed in the early part of the twentieth century. This procedure was supported by the recurrent and progressive nature of endometriosis and its ability to invade adjacent organs. The presence of endometriosis was believed to be justification for castration because it often resulted in sterility. Clinicians began to follow pregnancy rates in patients with endometriosis, however, and an interest in conservative management was cultivated [7].

Huffman presented anatomic staging in 1951 [8], which argued in favor of treatment based on the extent of disease, similar to contemporary malignancy staging. Superficial disease was differentiated from invasive disease, but no attempt was made to classify adhesions. Although he reported a subsequent pregnancy rate of 47% in women with stages I and II, routine exploration of pelvic viscera was not performed in this era. Intuitively, he recommended that preservation of childbearing was reasonable for patients with stage I or II disease and possibly even with stage III.

In 1962, Riva et al [9] studied medical treatment of endometriosis with the progestational agent norethynodrel. They studied patients by culdoscopy, colpotomy, or laparotomy and noted the number of pelvic structures involved and the surrounding adhesions. Patients then were meticulously divided into categories according to the number of pelvic structures involved. They were the first to attempt a scale to define who might benefit from medical treatment. Unfortunately, this classification method was unable to correlate with clinical outcomes.

In 1966, Beecham stated that a tedious effort to detail endometriotic location and lesion "would serve no purpose." He developed a simple classification scheme of four stages that used physical and operative findings. He believed that this scheme would be appropriate to follow patients being managed by medical or surgical therapies [10].

No attempt at classification before 1973 received widespread acceptance, which made reports of pregnancy rates in response to conservative operations difficult to interpret [11]. It was difficult for a physician to counsel an infertile couple adequately with regard to their clinical prognosis because there was little means to group patients accurately and compare clinical outcomes. Before endoscopic surgical technique, severe disease was necessary to receive surgical treatment. Milder disease was often an incidental finding at surgery for unrelated pelvic disease.

In a collaborative effort, Acosta et al [12] proposed a classification of endometriosis (see Box 1) that divided the disease into mild, moderate, and severe categories based on surgical findings. These categories included the site of lesions, presence of adhesions, and presence of scarring or retraction to distinguish these stages with the presence of small endometriomas (< 2 cm) or the presence of minimal peritubal or periovarian adhesions that distinguished moderate from mild disease. Using this staging system with retrospective data, a direct relationship was established with initial stage of disease and pregnancy rates that were confirmed by other investigators [13]. Disease also was automatically classified as severe in the presence of an endometrioma larger than 2 cm in size. Peritubular and periovarian adhesions separated mild from moderate disease because ovarian adhesions were recognized as having a damaging effect on fertility.

Box 1. Acosta's classification of pelvic endometriosis [12]

Mild	1. Scattered, fresh lesions (ie, implants not associated with scarring or retraction of the peritoneum) in the anterior or posterior cul-de-sac or pelvic peritoneum. 2. Rare surface implant on ovary, with no endometrioma, without surface scarring and retraction, or small endometrioma. 3. No peritubular adhesions.
Moderate	1. Endometriosis involving one or both ovaries, with several surface lesions, with scarring and retraction, or small endometriomata. 2. Minimal periovarian adhesions associated with ovarian lesions described. 3. Minimal peritubular adhesions associated with ovarian lesions described. 4. Superficial implants in the anterior and/or posterior cul-de-sac with scarring and retraction. Some adhesions, but not sigmoid invasion.
Severe	1. Endometriosis involving one or both ovaries with endometrioma $> 2 \times 2$ cm (usually both). 2. One or both ovaries bound down by adhesions associated with endometriosis, with or without tubal adhesions to ovaries. 3. One or both tubes bound down or obstructed by endometriosis; associated adhesions or lesions. 4. Obliteration of the cul-de-sac from adhesions or lesions associated with endometriosis. 5. Thickening of the uterosacral ligaments and cul-de-sac lesions from invasive endometriosis with obliteration of the cul-de-sac. 6. Significant bowel or urinary tract involvement.

Many physicians used the appearance of adnexal adhesions as the basis for conservative therapy rather than medical treatment. Pertersohn [14] reported that patients with endometriotic lesions alone had subsequent 80% pregnancy rates, whereas if adnexal adhesions were present the pregnancy rate was only 40%. This finding led Acosta et al to treat the extent of ovarian involvement as a major factor because of the potential risk of adhesion formation after the resection of an endometrioma. Many physicians believed that this classification system had several disadvantages, however, because of the arbitrariness of the staging and inability to distinguish unilateral or bilateral disease. Kistner et al [15] believed that one such detractor was the natural progression of the disease, which should be weighted heavily in the staging process, and a different staging system was developed that moved from early peritoneal implants to ovarian involvement to tubo-ovarian involvement to dissemination throughout the pelvis (see Box 2). This group was strongly emphasized that tubo-ovarian mobility, once impaired, was the major cause of infertility.

In 1974, Mitchell and Farber [16] proposed a staging system similar to that used in gynecologic malignancies, including a stage V for malignant transformation. Buttram [13] proposed an expanded classification (see Box 3) based on the Acosta scheme that allowed for more flexibility and less ambiguity than the Acosta classification. Each patient was graded by peritoneal, ovarian, tubal, and cul-de-sac involvement. Stages II and III provided for laterality, and all stages were divided into graduated severity levels. Although detailed and precise, most physicians believed that this grading system was too cumbersome and another system was proposed. Cohen developed a scheme that used ten states according to severity based on laparoscopic findings. Distant organ involvement, adenomyosis, and pelvic inflammatory disease were included [17].

American Fertility Society classification schemes

Despite modifications, none of the classifications before 1978 received widespread acceptance and use, which prompted the American Fertility Society (AFS) to form a panel to design a classification system for endometriosis; its recommendations were published in 1979 (Fig. 1) [18]. This unique and innovative classification scheme stratified endometriosis into mild, moderate, severe, and extensive disease and for the first time used a weighted point score that included assessment of the extent of endometriosis (two-dimensional) and presence of adhesions in the peritoneum, ovaries, and tubes. It allowed for assessment of unilateral versus bilateral disease. The sizes of endometriomas were weighted differently, as was the presence of filmy versus dense adhesions. An anatomic drawing was included to aid in surgical finding documentation, and a cumulative score was attained. At the outset, the point scores were recognized as arbitrarily assigned, and it was anticipated that changes in the assignment would be based on clinical studies and disease progression or response to treatment.

Box 2. Kistner's classification of endometriosis [15]

Stage I	Areas of endometriosis are present on the posterior pelvic peritoneum (cul-de-sac, uterosacral ligaments) or on the surface of the broad ligaments but do not exceed 5 mm in diameter. Avascular adhesions may involve the tubes, but the fimbriae are free. The ovaries may show a few avascular adhesions, but there is no ovarian fixation. The surfaces of the bowel and the appendix are normal.
Stage IIA	Areas of endometriosis are present on the posterior pelvic peritoneum (cul-de-sac, uterosacral ligaments) and the broad ligaments but do not exceed 5 mm in diameter. Avascular adhesions may involve the tubes, but the fimbriae are free. Ovarian involvement by endometriosis has been subclassified as follow: IIA-1: Endometrial cyst or surface is 5 cm or less IIA-2: Endometrial cyst or surface is over 5 cm. IIA-3: Ruptured endometrioma; the bowel and the appendix are normal.
Stage IIB	The posterior leaf of the broad ligament is covered by adherent ovarian tissue. The tubes present adhesions not removable by endoscopic procedures. The fimbriae are free. The ovaries are fixed to the broad ligament and show areas of endometriosis over 5 mm in diameter. The cul-de-sac presents multiple implants, but there is no adherent bowel nor is the uterus in fixed position. The bowel and the appendix are normal.
Stage III	The posterior leaf of the broad ligament may be covered by adherent tube or ovary. The tubal fimbriae are covered by adhesions. The ovaries are adherent to the broad ligament, and tube may or may not show surface endometriosis or endometriomas. The cul-de-sac shows multiple areas of endometriosis, but there is no evidence of adherent bowel or uterine fixation. The bowel and the appendix are normal.
Stage IV	Endometriosis involves the bladder serosa, and the uterus is in fixed, third-degree retroversion. The cul-de-sac is covered by adherent bowel or is obliterated by the fixed uterus. The bowel is adherent to the cul-de-sac, uterosacral ligaments, or uterine corpus. The appendix may be involved by the endometriotic process.

Box 3. Buttram's expanded classification of endometriosis

Stage I (Peritoneum)
- A. No peritoneal involvement.
- B. Scattered superficial surface endometrial implants on the pelvic peritoneum (anterior or posterior cul-de-sac, uterosacral ligaments, or the broad ligaments), which do not exceed 5 mm in diameter. Neither tubal nor ovarian involvement.
- C. Same as for B, but invasive endometriosis or plaques or endometrial implants > 5 mm in diameter. Fine, filmy adhesion may be present that may be lysed without great danger of resultant adhesions.

Stage II (Ovarian): 1, Right; 2, Left; 3, Bilateral
- A. No ovarian involvement.
- B. Superficial surface endometrial implants of ovary of < 5 mm in diameter, which can be removed by scraping or fulgaration without great danger of resultant adhesions. Fine, filmy adhesions may be present and lysed without great danger of resultant adhesions.
- C. Invasive endometriosis (plaques or endometrioma) > 5 mm but < 2 cm that requires surgical removal. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.
- D. Invasive endometriosis > 2 cm that requires surgical removal or a ruptured endometrioma of any size. Fine, filmy adhesion may be pres- ent, which may be lysed without great danger of resultant adhesions.
- E. B, C, or D with sufficient dense adhesions to fix ovary to adjacent tissue (usually posterior leaf of broad ligament).

Stage III (Tubal): 1, Right; 2,Left; 3, Bilateral
- A. No tubal involvement.
- B. Superficial endometrial implants on tube that do not exceed 5 mm in diameter and can be removed by scraping or fulgaration without great danger of resultant adhesions. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.
- C. Invasive endometriosis (plaques or endometrioma) > 5 mm but < 2 cm that require surgical removal. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.
- D. Tube involved with adhesions that distort tubal anatomy and/or limit tubal movement. Fimbriae are free and tube is patent. C may be present.
- E. Fimbriae are covered by adhesions or distal end of tube is occluded. B, C, or D may be present.

Stage IV (Cul-de-sac)
- A. Neither B nor C is present.
- B. Invasive endometriosis of bladder or colon.
- C. Posterior cul-de-sac obliterated and/or uterus fixed and retroverted. Bowel or adnexa may be adherent to cul-de-sac area. B is usually present.

From Buttram VC. An expanded classification of endometriosis. Fertil Steril 1978;30:240 – 2; with permission.

The American Fertility Society*†

Birmingham, Alabama

Patient's Name _____ Date _____

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 _____

PERITONEUM	ENDOMETRIOSIS	<1cm	1-3cm	>3cm
	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
OVARY	ADHESIONS	<1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
	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.

Fig. 1. The American Fertility Society classification of endometriosis. (*From* American Fertility Society. Classification of endometriosis. Fertil Steril 1979;32: 633–4; with permission.)

Several advantages were initially evident. Although the AFS staging system was based on a presumed natural history of disease progression, it allowed for significant flexibility in point assignment, which allowed any case to be categorized, including those that were recognized to not follow the usual chronology of disease progression. It included the need for a comprehensive pelvic evaluation at the time of staging. Because the AFS classification provided a standardized reporting system, it also met a main objective of any classification scheme: easy and clear communication among practitioners. Critics pointed out the shortcomings. Hassan [19] stated that the features of infertility were emphasized but not the features necessarily related to pelvic pain. He offered a modification that increased the point scoring of uterosacral ligament involvement and deep retroperitoneal lesions. Rock et al [20] evaluated the AFS system against the classification of Kistner and Buttram by retrospectively classifying 214 patients

who previously underwent conservative surgery for endometriosis. The schemes by Kistner and Buttram revealed significantly different monthly fecundity rates for the different stages; however, the AFS scheme showed a significant difference only if the mild and moderate stages were combined and compared to severe and extensive stages.

Guzick et al [5] compared the point-scoring system by dose-response methodology. They noted that the arbitrary point scores and the arbitrary cutoff points to divide the patients into the various groupings failed to show a correlation to the severity of endometriosis with pregnancy rates after surgery. Guzick et al [5] delineated a method to redefine the optimal breakpoint among groups empirically and were able to demonstrate an improvement in the predictability of the AFS scale. In the revised cutoff scheme, the pregnancy rate for patients with mild disease was significantly better than for patients with severe disease but not significantly different than for patients with moderate disease. Adamson et al [21] proposed a clustering technique to analyze the combinations of variables and ultimately develop a better method for identifying factors that would predict pregnancy rates in patients with endometriosis. The results of this study suggested several flaws in the AFS system. The arbitrary point scores may not reflect the actual relative likelihood of fertility. Once again, empirically derived point scores and breakpoints were recommended to define disease categories and better predict pregnancy rates [21].

In 1985, in response to the earlier identified problems with the AFS classification, a revised classification scheme was presented (R-AFS) (Fig. 2) [22]. The more detailed system created a separate category for minimal disease and eliminated the extensive disease category. A three-dimensional assessment of disease was included that differentiated superficial from invasive disease. A quantification of the number of adhesions around the tubes and ovaries was included, as was a distinction between filmy and dense adhesions. A distinction also was made for complete enclosure of the fimbria, with a point score that automatically placed it in the moderate category. As before, the system did not include extragenital sites but included space to record additional pathology and less frequently involved sites. Cul-de-sac obliteration was heavily weighted, with complete obliteration automatically qualifying as a severe stage. This classification was strengthened by Buttram, who reported that in his series only 36% of patients with complete obliteration of the posterior cul-de-sac were able to conceive compared to 68% of patients with only partial posterior cul-de-sac obliteration [11].

The R-AFS classification still has similar flaws noted in its predecessors, because the point assignments and breakpoints are still arbitrarily assigned and largely unsupported by data. The relative weights of the point system, however, were shifted with better understanding of the disease. Although there were few clinical data to substantiate the system, the designers hoped that the new scheme would be a useful clinical tool in the documentation and individualized study of the disease. It was the intention that the system again would be subject to revision as clinical data became available.

Patient's Name _____ Date _____

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 _____

PERITONEUM	ENDOMETRIOSIS	<1cm	1-3cm	>3cm
	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
OVARY	ADHESIONS	<1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
	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

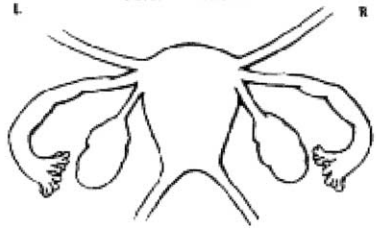
*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.

Density appearance of superficial implants types as red (RB), red, red-pink, flamelike vascular blobs; clear vesicles); white (W opacifications, peritoneal defects; yellow-brown), or black (BB) black, hemosiderin deposits, blue). Density percent of total described as R ___%, W ___% and B ___%. Total should equal 100%.

Additional Endometriosis: _____

Associated Pathology: _____

To Be Used with Normal Tubes and Ovaries



To Be Used with Abnormal Tubes and/or Ovaries

Fig. 2. The American Fertility Society revised classification of endometriosis. (*From* American Fertility Society. Revised classification of endometriosis. Fertil Steril 1985;43:351–2; with permission.)

Limitations of the revised American Fertility Society classification of endometriosis

Arbitrariness of the scoring system

The use of the scalar system with its arbitrarily weighted grouping has limited the overall effectiveness of the R-AFS classification. Several reports have failed to demonstrate a significant difference in cumulative pregnancy rate as a function of the AFS score or R-AFS stage [5,20]. Two main problems have been identified. First, the points associated with the individual categories do not reflect the empirically derived weights. For example, the point score is 20 for an unruptured endometrioma of 4 cm, whereas the point score is 4 for widely scattered deep peritoneal implants that total 3 cm. Is the former lesion five times worse than the latter in terms of pain or fertility impairment? The second problem is that the demarcations between stages are equally arbitrary. Empirically derived scores and stage demarcations might add discriminatory power to the R-AFS classification system.

Potential for observational error

Endometriosis has many appearances, which increases the likelihood of observational error. These differences in interpretation may affect staging. Nonpigmented lesions are often subtle and variable in appearance. Stripling et al noted that surgeon expertise increased the documentation of these nonpigmented lesions [23]. Martin et al [24] also reported an increase in the overall diagnosis of endometriosis attributed largely to the increased awareness of subtle lesions.

Microscopic endometriosis is also well documented [25,26]. Murphy et al [26] detected endometriosis by scanning electron microscopy in 25% of visually normal peritoneal biopsies in patients with endometriosis. By definition, these lesions are not detectable by current endoscopic modalities. The clinical significance of this observational deficit is not yet known. There is also observational error in the assessment of small deep ovarian endometriomas. Candiani et al [27] reported that laparoscopic ovarian puncture greatly facilitated the appropriate diagnosis and staging of 52 infertile patients who demonstrated enlarged ovaries (3.5–5 cm maximum diameter) but lacked distinguishable endometriomas [27]. The debate also continues as to the need for histologic confirmation of endometriotic cysts for staging purposes [28,29].

Limited reproducibility

The reproducibility of the R-AFS has been questioned. Hornstein et al [30] assessed the degree of intraobserver and interobserver variability of this staging system. Five subspecialty-certified reproductive endocrinologists viewed the tapes of the diagnostic portions of 20 laparoscopies on patients with endometriosis and scored each twice in random order. The variability in assigned scores was then measured for each of the five components of the AFS system and the

total scores and stage of endometriosis. Among the individual component of the scoring system, they noted that the greatest variability occurred in the endometriosis of the ovary and cul-de-sac obliteration, with less variability for peritoneal endometriosis and ovarian and tubal adhesions. Interobserver variability for endometriosis staging was notable, which resulted in differences between two observers that caused a change of endometriosis stage in 52% of cases. Intraobserver variability also was common, with the same observer restaging endometriosis in 38% of cases.

Rock et al [31] reported better reproducibility for the R-AFS system. Visual documentation of laparoscopies of 315 women with endometriosis before and after gonadotropin releasing hormone (GnRH) agonist therapy was scored by the various investigators and a blinded reviewer. All visual documentation that was defined as unreadable or readable with difficulty was excluded from analysis. This exclusion resulted in readable documentation for 88 patients (43%) at the pretherapy evaluation and for 83 patients (41%) at posttherapy evaluation. The kappa statistic, which measures the association between two raters when data are on a categorical scale, was used to compare the assignment of the R-AFS classification between the group of investigators and the blinded reviewer. The kappa statistic was 0.44, which indicated fair to good agreement.

Failure to consider lesion morphologic type

Age-related evolution and color changes have been suggested by Redwine [32]. He reported that nonhemorrhagic lesions were usually seen in younger women, whereas dark pigmented lesions primarily were seen in older women. Vernon et al [33] related the gross and histologic appearance of endometriotic implants with the capacity to produce prostaglandin F. As judged by these criteria, younger, reddish petechial implants were more biochemically active than intermediate, brownish implants, which in turn were more active than older, powder burn black implants. This factor likely reflects the amount of functional endometrial glands in each type of lesion.

Other investigators have noticed a correlation with the type of lesion and pelvic pain symptoms. Vercellini et al [34] analyzed the prevalence and severity of dysmenorrhea, intermenstrual pain, and deep dyspareunia in relationship to different morphologic features of peritoneal lesions. They classified the lesions as typical (black nodules, yellow-brown patches, and stellate scars) and atypical (clear vesicles, clear or red papules, and red polypoid lesions) or mixed. There was a higher prevalence of deep dyspareunia in patients with typical or mixed lesions versus atypical lesions. The authors speculated that fresh, papular atypical lesions exposed to peritoneal fluid may cause functional pain, whereas "old," darkly pigmented nodules immersed in infiltrating scar may cause organic pain.

Donnez et al [35] recently applied advanced stereographic computer technology to analyze the three-dimensional architecture of peritoneal endometriotic lesions. They identified two main types of peritoneal endometriotic lesions

according to the presence or absence of glandular ramifications. They also observed through stereometric study that the effect of GnRH agonist therapy exerts a stronger effect on the stroma rather than on the epithelium. It remains to be seen whether this latter method has overall practicality in the determination of lesion architectural type and correlation with either pain symptomatology or pregnancy prognosis.

Pelvic pain and the classification of endometriosis

The ability of the current R-AFS classification of endometriosis to aid in the evaluation and management of endometriosis in the setting of pelvic pain, such as the assessment of infertility, seems limited. The poor understanding of the cause of pelvic pain and the well-recognized multiple causes of chronic pelvic pain make the evaluation of treatment in clinical trials difficult. The current classification scheme was designed primarily to address endometriosis in the setting of infertility. Multiple attempts have been made to correlate stage of disease and severity of pain. Using the first AFS classification, Buttram [13] studied the incidence of dysmenorrhea by disease stage and found that disease severity by the staging system was a poor predictor of dysmenorrhea. Fedele et al [36], using the R-AFS classification, also found no correlation between type of pain and disease stage. In a second study, these investigators did find severe dysmenorrhea in a higher proportion of stage III–IV patients than in stage I–II patients or in controls [37]. These patients were recruited from an infertility population, and results may reflect selection bias.

As in the case of infertility assessment, investigators have examined specific lesion characteristics to explain the poor correlations with stage. During the evaluation of 53 patients with CO_2 laser excisional techniques at laparoscopy, Cornillie et al [38] noted a strong correlation between depth of invasion and pelvic pain with all patients with implants larger than 10 mm deep having severe pain. They also found lesions more than 5 mm deep to be histologically more active than shallower lesions. Koninckx et al [39] similarly found significant correlation between depth of invasion and degree of pain. They found no relationship, however, among lesion type, total surface area of endometriosis, and amount of pain. The parameters associated with pelvic pain might be different from those that assess and classify infertility causing endometriosis; the current staging system does not adequately address the former.

In recognition of some of the shortcomings of the R-AFS classification in the evaluation of pelvic pain, the American Society for Reproductive Medicine (then AFS) formed a subcommittee to evaluate the evidence and develop an instrument to aid in the assessment of endometriosis in the setting of pelvic pain. The committee developed a form that included preoperative assessment that documents pain quality and location on examination and adjunct investigations [40]. Operative assessment included detailed assessment of adhesion type, description of peritoneal lesion type by morphologic appearance, and the mean diameter and depth of invasion, encouraging histologic correlation. Using these guidelines, it is

believed that the accumulation of such data will be useful in future evaluation and revisions of the classification scheme in the presence of pelvic pain.

Suggestions regarding endometriosis pathophysiology and staging

Peritoneal lesions

Peritoneal lesions have multiple appearances, including microscopic, early active (red, glandular, or vesicular), advanced (black, puckered), and healed (white, fibrotic) lesions. Wiegerinck et al [41] suggested that early lesions appear and disappear "like mushrooms on the peritoneal surface." During laparoscopic evaluation of 14 women before and 6 months after a 3-month medical trial, the authors noted that the R-AFS score remained unchanged or decreased in 13 of 14 patients. On the other hand, the presence of early active lesions was variable and seemed to be independent of the overall R-AFS score. Red papular or vesicular lesions were present in both laparoscopies in 5 patients and absent in 4 patients. Three patients had disappearance of such lesions from the first laparoscopy to the second, although 2 patients had these types of lesions appear de novo. The authors suggested that the staging of endometriosis include the type of active lesion in addition to the R-AFS score.

Ovarian endometriomas

Several authors recently have offered suggestions regarding possible modifications to the current system of grading ovarian endometriosis. Based on the histopathologic findings of Hughesdon [42], who reported that more than 90% of endometriomas present as a proliferation of endometrial tissue on the surface of an invaginated ovarian capsule, Brosens [43] distinguished between two types of endometriomas: red and black. He promoted the endoscopic technique of ovarioscopy to help the surgeon distinguish between the two types. Red cysts had red, vascularized areas on a white surface; black cysts had a dark, pigmented, and fibrotic wall. The distinction lies in the fact that precise coagulation or vaporization of the red implants and their associated vascularization was all that was necessary for adequate treatment, whereas the entire walls of the black, fibrotic cysts should be excised. Brosens et al [44] further suggested a scoring of the endometriotic cysts according to their internal diameter: small (< 1 cm); medium (1–5 cm); large (>5 cm).

Nezhat et al [45] proposed another classification system for ovarian endometriomas based on clinical and histologic study of 187 patients. Presumed endometriomas were classified into three types according to size, cyst contents, ease of capsule removal, adhesion of the cyst to other structures, and location of the superficial endometriotic implants relative to the cyst wall. Histologically small (< 2 cm), superficial ovarian cysts were invariably endometriomas, and their cyst walls often were difficult to remove (type I). Large cysts with easily

removed walls were usually luteal cysts (type II). Large cysts with walls adherent in multiple areas adjacent to superficial endometriosis were generally endometriomas, although some had histologic characteristics of functional (luteal or follicular) cysts (types IIIa and IIIb). During a later publication, these authors modified their classification system [46]. Type I cysts (primary endometriomas) were small superficial cysts of "true endometrioma" origin. Type II cysts (secondary endometrioma) were follicular or luteal cysts that have been invaded by superficial endometriotic lesions or adjacent primary endometriomas. Type II cysts were further divided into three subtypes (IIa, IIb, IIc) depending on the degree of penetration of endometriotic lesions with the cyst walls.

Severe disease: stage V

A severe disease subcategory for endometriosis has been suggested repeatedly. Canis et al [47] observed an intrauterine pregnancy rate of 37.5% in a study of laparoscopic treatment in patients with severe endometriosis. There were no pregnancies achieved, however, in women with an R-AFS score of more than 70. 52.9% of patients with an R-AFS score of less than 70 achieved pregnancy. These investigators reported that bilateral adnexal disease was significantly more frequent in women with an R-AFS score of more than 70. The differences in postoperative fertility were attributed to a higher rate of bilateral dense adhesions in patients with increased scores. A stage V was proposed for patients with extensive disease, especially with bilateral dense adhesions, because poor fertility results are consistently obtained with conservative therapy alone in this group of patients. Using a revised classification scheme, a plan to proceed quickly toward in vitro fertilization would be uniformly recommended for all infertile, stage V patients.

Recently, Pal et al [48] examined 61 patients with a primary diagnosis of endometriosis undergoing 85 cycles of in vitro fertilization. The patients were divided into groups based on their R-AFS stage. Group A included patients with stages I and II (minimal and mild), and Group B included patients with stages III and IV (moderate and severe). Patient age of more than 40 years, basal day 3 follicle-stimulating hormone level of more than 20 IU/L, male factor infertility, assisted hatching, and gamete intrafallopian transfer cases all were excluded. The stimulation was similar for all cycles that used pituitary downregulation with GnRH agonist in a midluteal protocol, and ovarian stimulation was achieved with a combination of follicle-stimulating hormone and human menopausal gonadotropin. The response to controlled ovarian hyperstimulation and the number, maturity, and quality of the oocytes was comparable between patients with varying severity of disease. Fertilization rates in oocytes from patients with moderate to severe endometriosis were significantly impaired when compared to rates of women with minimal or mild disease. The patients in Group B required significantly more ampules of gonadotropin to attain a serum estradiol and follicular size and number comparable to Group A. The rates for implantation, clinical pregnancy, and miscarriage were comparable between the two groups. The authors deduced that the reduced fertilization potential of preovulatory oocytes obtained from patients

with severe endometriosis in the absence of male factor infertility suggests an adverse biologic impact on the oocytes in women with advanced disease. The outcome of in vitro fertilization-embryo transfer (ET) is unaffected by the increasing severity of disease and suggests that in vitro fertilization may compensate for or overcome this reduction in the biologic potential of the oocytes associated with severe disease.

Other investigations suggest that patients with severe endometriosis have defects in endometrial receptivity, including an embryotoxic intrauterine environment, the presence of autoantibodies [49,50], and aberrant integrin expression in the endometrium, which suggest a defect in intrauterine receptivity [51].

The endometriosis pain instrument

A panel of international experts recently addressed the limitations of the R-AFS with respect to pelvic pain [40]. The committee recommended a visual instrument that documented the extent of endometriosis and pelvic pain. The importance of mapping the pain location accurately was considered essential. Anatomic diagrams were provided to document the distribution of pain and the area of most severe pain. The quality and intensity of the pain were determined, and abnormal physical findings could be documented on the same diagram. The distribution of tenderness (diffuse or local), extent of nodularity, and the presence of other findings (pelvic organ fixation) were noted. Operative diagrams are provided for the documentation of pelvic adhesions and endometriotic lesions. The gross appearance of pelvic adhesions is classified according to the following scheme: A, vascular or thin; T, thick or dense; B, band-like or string-like; S, sheet-like. The appearance of other peritoneal endometriotic lesions should be documented after intraoperative mobilization of the pelvic viscera. Implants are classified according to the following designations: C, clear; V, vesicular; P, pink; R, red or flame-like; B, black or blue; Y, yellow-brown; W, white; F, fibrotic. Mean diameter and an estimated depth of infiltration of all implants are recorded. To obtain accuracy, the committee recommended using a scaled instrument, such as a calibrated endoscopic probe. Finally, correlation with histology was encouraged with individual implants.

The committee believed that the use of anatomic diagrams to document posttreatment symptoms and physical findings would be helpful. Operative diagrams also could be used in patients at subsequent surgeries who failed to respond to treatment. The committee also believed that the accumulation of data from this diagrammatic system would help to develop more appropriate classification schemes that correlate to endometriosis-associated pelvic pain.

Summary

A multicenter collaboration for data collection and statistical analysis may be necessary to establish and validate a classification system based on empirically derived scores for specific pathologic observations. The endometriosis pain instrument may be a tool for some of those variables with regard to pelvic pain.

A similar strategy for uniform collection of data for analysis of important factors also is necessary for infertility.

The challenge of creating a satisfactory classification of endometriosis remains. The ability of the current classification schemes to predict pregnancy outcome or aid in the management of pelvic pain is recognized to be inadequate. Further revisions of the current classification scheme are anticipated as the understanding of how endometriosis contributes to infertility and pelvic pain evolves. In any revision of the classification system, use of empirically derived weights and breakpoints to define disease stages based on outcome data in larger clinical trials should be attempted. It is also possible that additional factors, such as CA-125 level or lesion characteristics, may be shown to play an important role in prognosis. If so, these must be accounted for in the classification scheme. Careful and consistent use of the recommendations of the American Society for Reproductive Medicine classification of endometriosis subcommittee should allow for collection of data for use in further revisions.

It is possible that a classification scheme that is designed to predict outcome with respect to pregnancy may be totally inadequate in assessing patients who have endometriosis and pelvic pain. Factors found to be important in the assessment of pelvic pain may be different from those involved with the pathophysiology of endometriosis and infertility. The AFS form suggested for use in the management of endometriosis in the presence of pelvic pain allows for recording of variables such as depth of invasion, histology, and documenting adjunct investigations and preoperative physical findings. Such prospective data collection and review in large centers may provide a large clinical base from which to derive empirical point scores and breakpoints in a classification scheme.

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Medical management of endometriosis-associated pain

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Endometriosis-associated pain is among the most challenging conditions in gynecologic practice. It may be found in up to 60% of women with dysmenorrhea and 40% to 50% of women with pelvic pain or dyspareunia [1]. Despite this high prevalence, diagnosis is often delayed because of the need for surgical confirmation. When treatment is initiated it often achieves only partial success. Frequently, various treatments are attempted, tolerated to varying degrees, and rejected. This cycle may result in increasing frustration felt by the patient and the physician. It is not unusual for the resulting sense of despair to sever physician-patient relationships.

This need not be the case. In recent years, various new insights into the medical treatment of endometriosis-associated pain have been gained. These developments should allow most women with endometriosis to find suitable relief. It is incumbent on physicians who provide gynecologic services to women of reproductive age to be intimately familiar with these treatments and the nuances of medication delivery and combination therapy.

How does one assess improvement?

Objective measures of response to treatment include second-look laparoscopy to assess changes in endometriotic lesions. A reduction in the endometriosis staging score [2] is the classic benchmark. Although often used in scientific trials [3–10], this method is almost never appropriate in clinical practice. It also may be misleading. It is well established that there is no correlation between pain symptomatology and the number of endometriotic lesions (Table 1) [11,12]. There may be differences in lesion size and number based on observer variation

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Table 1
No correlation between staging of endometriosis and pain symptomatology

Stage	N	Dysmenorrhea (%)	Pelvic pain (%)	Dyspareunia (%)
I	40	73	38	30
II	28	86	46	25
III	58	72	36	41
IV	34	85	41	29

Modified from Fedele L, Parazzini F, Bianchi S, et al. Stage and localization of pelvic endometriosis and pain. Fertil Steril 1990;53:156; with permission.

and timing in relation to the menstrual cycle. Finally, some treatments (eg, nonsteroidal antiinflammatory drugs [NSAIDs]) may provide substantial patient benefit without any reduction in the size or number of endometriosis lesions. Although surgery has a necessary role in the diagnosis of endometriosis, it is not a practical method for monitoring the success of treatment.

Ultimately, the patient's point of view is more important. This may be measured in myriad ways and must be comprehensive. In clinical trials, visual-analog pain scores are frequently used. Of equal importance, however, are improvements in health-related quality of life (as may be quantified by the short form-36) [13–15] and overall individual impressions of satisfaction [16].

The main problem with most medical therapies for endometriosis is the frequency and severity of side effects. Focusing exclusively on reductions in pain may prove myopic if the side effects of treatment are more intolerable than the disease. A treatment that relieves pain but simultaneously causes severe headaches, mood changes, or hot flashes may not be perceived as beneficial from a patient perspective. It is important to keep the well-being of the whole patient in mind when evaluating clinical trials and assessing individual treatment options.

Keys to selecting appropriate therapy

Careful evaluation of the patient is paramount to minimizing the possibility of misdiagnosis and maximizing the chance of therapeutic success. Attention should be given to the onset, location, and temporal distribution of the pain. Even more importantly, precise documentation of past surgical and medical treatments is critical in planning future management. If the diagnosis of endometriosis is not well established, consideration should be given to laparoscopic evaluation. Although hormonal suppressive therapies are effective for endometriosis, they generally have no effect on peritoneal adhesions, adnexal cysts, interstitial cystitis, inflammatory bowel disease, or other causes of pelvic pain.

Once the diagnosis of endometriosis-associated pain is confirmed, a wide armamentarium of medical treatments exists. The choice of which medication to use depends primarily on side effects and cost. Considerable effort should be made to anticipate potential side effects and, whenever possible, to mitigate them. This effort may imply selecting the levonorgestrel intrauterine device (IUD) over

oral or intramuscular progestins or initiating a gonadotropin-releasing hormone (GnRH) agonist with add-back therapy as opposed to without.

The hormonal therapies currently used in the treatment of endometriosis are equipotent in terms of reducing pain scores and inducing lesion regression (Table 2) [17–19]. In general, their ability to relieve pain is directly related to their capacity to induce amenorrhea.

Progestins (eg, Provera, DepoProvera) are the least expensive options, and GnRH agonists with immediate add-back therapy may offer the most benign side effect profile.

In this article all of these treatments are evaluated in depth. The use of oral contraceptives and NSAIDs also is examined. Special attention is given to the question of routinely initiating medical therapy immediately after conservative surgery for endometriosis-associated pain. Finally, with an eye to the future, some exciting recent advances that may spawn entirely new therapies for endometriosis are discussed.

Nonsteroidal antiinflammatory agents

Although NSAIDs do not directly treat endometriosis lesions, they long have been a mainstay in the treatment of endometriosis-associated pain. NSAIDs are particularly well suited for dysmenorrhea, because the symptom is mediated by prostaglandin synthesis [20]. By inhibiting cyclooxygenase, NSAIDs reduce prostaglandin production and alleviate pain.

A wide variety of NSAIDs are available. The most commonly used NSAIDs in gynecology include ibuprofen and naproxen. They are of similar efficacy in the

Table 2
Equipotent treatments for endometriosis-associated pain

Medical therapy	Common side effects (frequency $\geq 50\%$)	Monthly cost[a]($)
Danazol 600–800 mg qd	Acne, hot flashes, weight gain	460
Medroxyprogesterone acetate 50–100 mg orally every day	Breakthrough bleeding, weight gain	135
DepoProvera 150 mg intramuscularly every 3 mo	Breakthrough bleeding, weight gain	23
GnRH agonists	Hot flashes, sleep disturbances	600
Depot leuprolide 3.75 mg intramuscularly every mo, or 11.25 mg intramuscularly every 3 mo		
Goserelin 3.6 mg sc every mo, or 10.8 mg sc every 3 mo		
Nafarelin 200 μg intranasally twice daily		
GnRH agonists with add-back		650
Gestrinone 2.5 mg orally twice daily	Acne, hirsutism	NA

Abbreviations: NA, not applicable; sc, subcutaneously.

[a] Prices taken from the Yale Physician Building Pharmacy, August 2002.

relief of pelvic pain and dysmenorrhea, but they may differ slightly in cost and dosing schedule. A common problem with NSAIDs is gastric irritation. It usually relates to the frequency and duration of use and may result in peptic ulcers. Another serious, but rare, complication of long-term NSAID use is kidney damage, including papillary necrosis and renal failure.

Recently, a new generation of NSAIDs has been introduced that specifically inhibits cyclooxygenase-2 (COX-2). These medications (Celebrex, Vioxx) are no more effective at treating dysmenorrhea than naproxen or ibuprofen, but they have a much lower risk of gastric ulceration [21–23]. The main disadvantage of COX-2 inhibitors is high cost. The average price of a 30-day supply of ibuprofen or naproxen is US$5 to 15 US dollars, whereas a similar quantity of a COX-2 inhibitor is $60 to 80.

Oral contraceptives

Oral contraceptives are also widely used in the initial management of endometriosis-associated pain. Oral contraceptives regulate and reduce menstrual flow. They also are generally well tolerated and inexpensive. There is a paucity of data regarding their use in women with endometriosis, however [24]. In the only prospective, randomized trial published to date that compares them to a GnRH agonist, oral contraceptives were shown to be effective in the treatment of dysmenorrhea, dyspareunia, and pelvic pain [25]. The relief of dysmenorrhea and dyspareunia after 6 months of therapy was less pronounced than in women who received the GnRH agonist, however.

Low-dose monophasic oral contraceptives may be particularly well suited for endometriosis when given continuously, starting a new pack every 21 days. Such a method provides constant progestin-mediated suppression of endometrial growth and is more likely to induce amenorrhea. Satisfaction rates of 60% to 70% have been reported with this approach [26]. Side effects experienced by more than 10% of women who use this method include breakthrough bleeding, bloating, nausea, weight gain, and headache.

If a woman with endometriosis fails to respond to a 3-month trial of continuous oral contraceptives and NSAIDs, then more aggressive hormonal therapy or surgery or both are warranted. There is no evidence that switching from one oral contraceptive or one NSAID to another is beneficial in this setting.

Danazol

Danazol has been used in the treatment of endometriosis since the early 1970s. Soon after its introduction, it became the worldwide gold standard. Over the last 15 years, however, its use in North America has been curtailed greatly, primarily because of its high cost and significant side effect profile.

Danazol is a synthetic derivative of 17-ethynyltestosterone. Its efficacy in the treatment of endometriosis derives from its capacity to produce a high androgen/low estrogen environment [27–30]. The hormonal profile induces endometrial atrophy within the uterus and at ectopic sites [31,32]. Danazol also suppresses the midcycle surge of luteinizing hormone and follicle stimulating hormone [33]. As a result, most women who use high-dose danazol experience amenorrhea.

Danazol, 600 mg/day, has been compared to placebo in two small prospective, randomized trials [4,34]. In the first study, women had only laparoscopic confirmation of endometriosis (Table 3), whereas in the second study endometriosis implants were treated surgically and the impact of postoperative therapy on disease recurrence was evaluated. Both studies found that danazol significantly alleviated endometriosis-associated pain. This finding was in keeping with earlier cohort studies that reported symptomatic improvements in 80% to 90% of women who used danazol, 600 to 800 mg/day [35–37]. Not surprisingly, the highest response rates were achieved in women who developed amenorrhea [36].

Unfortunately, up to 80% of women who take danazol, 600 to 800 mg/d, experience major side effects (Box 1) [38]. These side effects are usually androgenic. In one placebo-controlled trial that used danazol, 600 mg/day, 60% of women developed acne, edema, or breakthrough bleeding, whereas 30% noted muscle cramps [4]. Adverse lipid changes (37% increase in Low Density Lipoprotein (LDL), 53% decrease in High Density Lipoprotein (HDL)) also occur during therapy with danazol [39]. Liver transaminases may become elevated, and a rare potential complication of danazol is liver failure [40].

Table 3
Placebo-controlled trials evaluating medical treatments of endometriosis-associated pain

Medication	Sample size[a]	Duration of therapy	Results
Danazol [4]	$n = 18$	6 mo	Significant reductions in pain scores
600 mg/d			Decrease in number and size of endometriotic lesions
Provera [4]	$n = 16$	6 mo	Significant reductions in pain scores
100 mg/d			Decrease in number and size of endometriotic lesions
GnRH agonists			
Lupron Depot [55]	$n = 32$	6 mo	90% complete relief of dysmenorrhea
• 3.75 mg intramuscularly every 28 d			Significant reductions in pelvic pain, tenderness, and nodularity
Triptorelin [56]	$n = 24$	6 mo	Significant reductions in pain scores
• 3.75 mg intramuscularly every 28 d			Decrease in number and size of endometriotic lesions

[a] Number of participants who received the active study medication.

Box 1. Side effects of danazol[a]

Androgenic
- Hot flashes (50%)
- Acne, oily skin (30% – 60%)
- Weight gain, fluid retention (30% – 50%)
- Muscle cramps (30%)
- Adverse lipid changes(↓HDL, ↑LDL)
- Decreased breast size (25%)
- Hirsutism (15%)
- Irreversible deepening of the voice (8%)

Breakthrough bleeding (40%)
Mood changes (20%)
Liver damage

[a] Estimates of prevalence are a composite from published clinical trials [4,34,38].

Because of the side effects, some investigators have evaluated lower doses of danazol (in the range of 50–200 mg daily) [36,41–44]. Although the total number of patients studied is small, the studies consistently have demonstrated a reduction in the frequency and severity of side effects. Most women continue to menstruate on these dosages, however, and clinical efficacy rates between 50% and 75% have been reported. Finally, it is important to stress the need for a barrier form of contraception with this therapy, because danazol is a teratogen.

Progestins

An effective alternative to danazol in the treatment of endometriosis-associated pain is progestins [45,46]. The best described medication is oral medroxyprogesterone acetate (Provera) at doses of 50 to 100 mg/day. Approximately 80% to 90% of women who use such high-dose progestins have reported symptomatic improvement [45,47]. With high-dose progestins, eutopic and ectopic endometrial tissue undergo atrophic changes and pseudodecidualized reaction. Progestins offer significant cost savings over danazol, and in two prospective, randomized placebo-controlled trials the efficacy of medroxyprogesterone acetate, 100 mg/day, was equivalent to danazol, 600 mg/day, with fewer side effects [4,34].

An alternate route to oral progestins is intramuscular depot medroxyprogesterone acetate (Depoprovera). Depoprovera is inexpensive and in a prospective randomized trial it was shown to be as effective as low-dose danazol combined with an oral contraceptive [48]. Within 6 to 12 months, most women who use Depoprovera develop amenorrhea. A disadvantage of Depoprovera, however, is

a 6- to 9-month delay before the resumption of ovulatory cycles when treatment is discontinued.

Use of high-dose systemic progestins may be limited by side effects (Box 2). The most common side effect is breakthrough bleeding. Fluid retention, weight gain, breast tenderness, and mood changes are also reported frequently [4,45].

The levonorgestrel-releasing IUD has been available for many years in Europe and recently received approval in the United States (Mirena). It releases approximately 20 mg of levonorgestrel per day and is effective for at least 7 years. Unlike copper IUDs, the levonorgestrel IUD results in hypomenorrhea or amenorrhea [49]. Recently, two small studies have investigated its potential in women with endometriosis-associated pain [50,51]. Both studies found excellent patient satisfaction (85%–95%) and significant reductions in pain scores. Because progestin levels are concentrated locally within the pelvis, therapeutic efficacy can be maximized while minimizing side effects. The levonorgestrel IUD may be particularly appropriate for women with endometriosis of the rectovaginal septum [51].

Gonadotropin-releasing hormone agonists

Depot GnRH agonists are widely used in the treatment of endometriosis-associated pain. After an initial gonadotropin flare, they induce downregulation of the pituitary and a hypoestrogenic state [52]. Like danazol and high-dose progestins, they also induce amenorrhea.

There is no therapeutic advantage of one GnRH agonist over another [53,54]. Choice of an agonist depends largely on the preferred route of administration: depot leuprolide (Lupron) is intramuscular, goserelin acetate (Zoladex) is subcutaneous, and naferelin (Synarel) is via nasal spray.

Two prospective, randomized, placebo-controlled, double-blind studies have evaluated the use of GnRH agonists for endometriosis-associated pain (see Table 3)

Box 2. Side effects of progestins

- Breakthrough bleeding (40%–80%)
- Weight gain, fluid retention (40%–50%)
- Acne (20%)
- Breast tenderness (10%)
- Headaches (10%)
- Mood changes (10%)
- Muscle cramps
- Adverse lipid changes (↑ LDL, ↓ HDL)

Estimates of prevalence are a composite from published clinical trials [34,36,48].

[55,56]. The studies reported highly significant improvements in pain compared to placebo. In one of the studies, 77% of women on placebo withdrew early because of worsening pain, whereas 94% of the women who used the GnRH agonist successfully completed 6 months of therapy [55]. Numerous randomized trials have compared GnRH agonists to danazol [3,5–10,57–61]. In every case, the clinical efficacy of danazol and the GnRH agonist was equivalent.

The timing of the first dose of the GnRH agonist is a matter of some debate. In a 1-month, randomized, double-blind, placebo-controlled trial, Miller demonstrated a significant increase in endometriosis-associated pain 2 and 4 weeks after initiating a GnRH agonist [62]. In this study, depot leuprolide, 3.75 mg, was administered in the early follicular phase. Previous investigators demonstrated that the ovarian flare effect of GnRH agonists is greatest at this time of the cycle [63] and that pituitary suppression is more rapidly achieved when GnRH agonists are initiated in the midluteal phase [64]. For these reasons, it may be advisable to initiate GnRH agonists in the midluteal phase rather than the early follicular phase. One also should note that neither of the GnRH agonist placebo-controlled trials mentioned previously [55,56] documented an increase in pain after the first month of GnRH agonist use.

Side effects of GnRH agonists are well documented (Box 3), nearly all of which relate to the rapid induction of a hypoestrogenic state, similar to surgical menopause. Most patients (80%–90%) experience hot flashes and other common menopausal symptoms [55,65]. GnRH agonists also have adverse effects on bone density and lipid profiles. The average loss of bone density after a 6-month course of a GnRH agonist is 4% to 6%. Although most patients slowly regain any losses in bone density as estrogens return to premenopausal levels, the use of a GnRH agonist without add-back therapy is generally limited to a maximum of 6 months.

Box 3. Side effects of GnRH agonists

- Hot flashes (80% – 90%)
- Sleep disturbances (60% – 90%)
- Vaginal dryness (30%)
- Joint pain (30%)
- Breakthrough bleeding (20% – 30%)
- Headaches (20% – 30%)
- Mood change (10%)
- Bone loss (↓ bone density 5% – 6%)
- Adverse lipid changes (↑ LDL, ↓ HDL)

Estimates of prevalence are a composite from published clinical trials [19,55,56,65].

Gonadotropin-releasing hormone agonist add-back therapy

The hypoestrogenic effects of GnRH agonists have stimulated interest in so-called "add-back therapy." Add-back therapy relies on the hierarchy of end-organ responses to estrogen, the estrogen threshold hypothesis [66]. The concept is that the threshold estrogen level for hot flashes and bone loss may be lower than the threshold estrogen level that stimulates growth of endometriosis implants. One might be able to add-back a sufficient amount of estrogen to alleviate hypoestrogenic side effects without compromising the efficacy of the GnRH agonist.

Add-back therapy rests on the assumptions that the endometriosis estrogen threshold is fairly constant among different groups of women and that the threshold for endometriosis growth is higher than that for hot flashes and bone loss. In protocols that add-back estradiol, serum levels typically rise to the 30- to 50-pg/mL range, whereas levels on GnRH agonists without add-back are usually less than 30 pg/mL [67,68].

Numerous add-back regimens have been investigated. Simple add-back regimens include norethindrone acetate, 5 mg/day, with or without 0.625 mg of conjugated equine estrogen (Premarin). These regimens have been proven to preserve bone density for up to 1 year of continuous GnRH agonist use (Box 4) [69,70]. Other regimens, including those that use bisphophonates, have been described [71,72]. These regimens are often more complicated, however, and do not necessarily alleviate vasomotor symptoms.

There is no reason to reserve add-back therapy only for women who may use a GnRH agonist for longer than 6 months. Numerous placebo-controlled studies have demonstrated equivalent clinical efficacy between GnRH agonists used alone versus GnRH agonists combined with immediate add-back therapy [67,70,73–75]. In all of these studies the immediate introduction of add-back therapy did not compromise pain relief but significantly relieved vasomotor symptoms and loss of bone density.

Several caveats apply to add-back regimens. If the dose of estrogen is too high, then clinical efficacy may be compromised. In the large, multicenter lupron add-back study, a significant number of women randomized to 1.25 mg of daily Premarin dropped out because of recurrent pain [70]. A second caveat is that if the dose of progestin is too high (eg, 10 mg norethindrone acetate/d), adverse

Box 4. Add-back regimens proven to preserve bone density for 1 year

- Norethindrone acetate 5–10 mg orally every day
- Premarin 0.625–1.25 mg + norethindrone acetate 5 mg orally every day
- Cyclic etidronate 400 mg + Os Cal 500 mg + norethindrone acetate 2.5 mg orally every day

lipid changes (↓HDL, ↑LDL) may occur [71]. Third, any woman who receives add-back therapy to prevent GnRH agonist-induced osteoporosis also should receive supplemental calcium. Finally, when long-term GnRH agonist therapy is used, the treating physician should consider performing periodic assessments of bone mineral density and a lipid profile.

Gonadotropin-releasing hormone antagonists

Gonadotropin-releasing hormone antagonists are rapidly gaining acceptance in controlled ovarian hyperstimulation protocols used in the treatment of infertility. Unlike GnRH agonist side effects, there is no initial flare of follicle-stimulating hormone and luteinizing hormone. GnRH antagonists block pituitary GnRH receptors, which causes an immediate, dose-dependent decline in gonadotropin (follicle-stimulating hormone, luteinizing hormone) secretion.

Because the end result is the same as with GnRH agonists (ie, decreased estrogen production) there is every reason to expect that they can be used to treat endometriosis. Although currently no human studies have been published, the efficacy of GnRH antagonists in endometriosis already has been shown in animal models. Current formulations are less than ideal for long-term use, however. They are expensive and require frequent subcutaneous injections. If they are to be used in women with endometriosis-associated pain, then longer acting, more affordable depot preparations are needed. Finally, physicians who may be tempted to combine agonists with antagonists should be aware that starting with an antagonist does not suppress the gonadotropin flare when a GnRH agonist is introduced.

Antiprogestins

The first clinically available antiprogestin was RU-486 (mifepristone). This medication generated considerable controversy because of its capacity to induce miscarriage. Because it inhibits ovulation and disrupts endometrial integrity, it has potential applications for women with endometriosis. In small, open-label, cohort studies, doses of mifepristone, 50 to 100 mg/day, have been shown to induce amenorrhea (without hypoestrogenism) and lower pain scores [76,77].

Long-term administration leads to mixed proliferative/secretory endometrium associated with cystic dilation, increased stromal density, and frequent mitotic figures without cytologic atypia [78]. Mifepristone also may alter glucocorticoid levels [79]. It is generally well tolerated, however, and does not result in a loss of bone density [76,80]. To date, mifepristone has not been used widely for the treatment of endometriosis-associated pain.

Gestrinone

Gestrinone bears many similarities to danazol. Although used in Europe, it is not available in the United States. Gestrinone is derived from a 19-nortestoster-

one steroid nucleus and has antiestrogen, antiprogesterone effects on the endometrium. Like danazol, it results in endometrial atrophy or amenorrhea [81].

Several large, prospective, randomized trials have demonstrated equivalent efficacy for gestrinone (2.5 mg twice a week) compared to either danazol [82,83] or a GnRH agonist [84,85]. The side effects of gestrinone are also similar to danazol (acne, hirsutism) and GnRH agonists (hot flashes). Gestrinone significantly reduces serum progesterone and sex hormone-binding globulin levels, with negligible impact on gonadotropins and estradiol [86]. Gestrinone does not negatively impact bone density but has an androgenic effect on serum lipids (↓HDL, ↑LDL) [84,87].

Longevity of medical treatment effects

Most (80%–90%) women with endometriosis-associated pain experience a reduction in pain, lower endometriosis staging scores, and decreases in the size and extent of endometriotic implants with medical treatments. There is a widely held misconception that medical treatments destroy endometriosis implants, however. Just as uterine endometrial lining resumes functioning after cessation of medical therapy, so too may endometriotic lesions [56,88].

Bulletti et al performed a prospective study comparing danazol to GnRH agonists, each given for 6 months, in two groups of 110 women with endometriosis [10]. Laparoscopy was performed twice: before initiating treatment and at 6 months. With both treatments endometriosis staging scores declined, but in only 4% to 18% of cases were biopsy specimens of previously identified endometriotic lesions consistent with complete, permanent resolution of endometriosis.

The length of time to recurrence of pain after stopping medical therapy varies based on how long patients are followed. Recurrence rates between 30% and 70% have been reported [35,36,41,42,55,58,65,89]. The mean length of time before recurrence is generally between 6 and 18 months [41,55,58,89]. In short, medical treatment is suppressive therapy, not extirpative therapy [90].

The role of medical therapy after conservative surgery for endometriosis

Whether medical treatment routinely should be commenced after conservative surgery for endometriosis-associated pain is controversial. Two points are clear from the literature, however. First, recurrence of pain after conservative surgery for endometriosis is common. The average time to pain recurrence after conservative first-line surgery for endometriosis is approximately 1 year [91]. Second, a short (3-month) course of postoperative treatment (eg, danazol, GnRH agonist) does not delay the recurrence of endometriosis-associated pain [92–94].

Longer courses of treatment (eg, at least 6 months) have been shown in at least three studies to extend pain relief or reduce the need for future surgeries (Box 5) [34,91,95]. Another study first treated women postoperatively for

Box 5. Postoperative therapies proven to delay the recurrence of endometriosis if given for at least 6 months

- Medroxyprogesterone acetate 100 mg orally every day [34]
- Danazol 600 mg orally every day [34]
- Nafarelin 200 g intranasally twice daily [91]
- Goserelin 3.6 mg sc every month [95]

6 months with a GnRH agonist then randomized them to a low dose of danazol (100 mg/day) for an additional 6 months or no further therapy [44]. The danazol group had significantly lower pain scores after 12 months without any significant androgenic or metabolic side effects. It seems that the longer the course of postoperative medical treatment the greater the potential benefit, assuming that the medication is well tolerated.

Future treatments

Basic research in endometriosis implant development is beginning to yield novel strategies for treatment. Among these developments, the most promising are aromatase inhibitors and immune system modulators.

Aromatase inhibitors

Aromatase converts C19 steroids (ie, testosterone and androstenedione) to estrogens (ie, estradiol and estrone). Although normal endometrium does not express aromatase, endometriosis implants have this capacity. They may generate the fuel that sustains and potentiates their existence. Estrogen also stimulates prostaglandin E2, which in turn stimulates aromatase activity. A local positive feedback loop is created within endometriosis implants, which augments local levels of estrogens, prostaglandin E2, and aromatase [96]. Complicating matters, another abnormality in endometriosis implants (deficient 17beta-hydroxysteroid dehydrogenase) impairs inactivation of estradiol to estrone [97].

Clinical trials are currently in progress to assess if aromatase inhibitors will benefit premenopausal women with endometriosis. Studies in animal models have shown promise, with near total resolution of endometriotic nodules [98]. Already, there is reason to believe that aromatase inhibitors may be the treatment of choice for postmenopausal women with endometriosis [99]. If the etiology of persistent lesions is the conversion of adrenal androgens to estrogens, then an aromatase inhibitor is the logical response.

Immune system modulators

Endometriosis is known to induce an inflammatory environment characterized by alterations in the immune system, cytokine secretion, and growth factors

[100,101]. One cytokine in particular, tumor necrosis factor-α, a major product of activate macrophages, seems to play a pivotal role in modulation of immune system responses, endometrial cell turnover, and cell adhesion. Studies of pentoxifylline (an anti–tumor necrosis factor therapy) in animals have shown regression of implants [102] and reversal of surgically induced endometriosis-associated infertility [103]. A small pilot study of pentoxifylline in humans with endometriosis-associated infertility also showed benefit [104].

More studies are needed to assess fully the safety, efficacy, and side effects of immune system modulators for women with endometriosis.

Summary

In the coming years, basic science research into the mechanisms of endometriosis development and persistence almost certainly will open new avenues for treatment. A wide armamentarium of medical therapies already exists, however. The efficacy of most of these methods in reducing endometriosis-associated pain is well established. The choice of which to use depends largely on patient preference after an appropriate discussion of risks, side effects, and cost.

Typically, oral contraceptives and NSAIDs are first-line therapy because of their low cost and mild side effects (Box 6). Because of its greater potential for suppressing endometrial development, consideration should be given to prescribing a low-dose monophasic oral contraceptive continuously. If adequate relief is not obtained or if side effects prove intolerable, consideration should be given to the use of progestins (oral, intramuscular, or IUD) or a GnRH agonist with immediate add-back therapy. Progestins are less expensive, but GnRH agonists with add-back may be better tolerated. If none of these medications proves beneficial or if side effects are too pronounced, then repeat surgery is warranted. The surgery may have analgesic value and serves to reconfirm the diagnosis. Finally, if endometriosis is identified at the time of surgery, then consideration should be given to prescribing medical therapy postoperatively.

Box 6. Suggested approach to endometriosis-associated pain

1st line: continuous low-dose monophasic oral contraceptive with NSAIDs as needed

2nd line: progestins (start with oral dosing, consider switching to levonorgestrel intrauterine device or depo if well tolerated)

3rd line: GnRH agonist with immediate add-back therapy

4th line: repeat surgery, followed by 1, 2, or 3[a]

[a] May consider low-dose (100–200 mg every day) danazol if other therapies poorly tolerated.

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Surgical management of endometriosis-associated pain

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Pain may be caused by endometriosis, endometriosis and other factors, or other factors with endometriosis as a coincidental finding. Although pain may be difficult or impossible to cure in some patients, other patients have coincidental and asymptomatic endometriosis that requires no treatment. Surgical guidelines are reasonable, but treatment frequently must be individualized. Distinguishing patients who need no treatment from patients who need intermediate or extensive treatment can be difficult.

Endometriosis and pain

Endometriosis is often an unpredictable disease and pain is hard to quantitate. Measurements of pain and correlation of this with endometriosis are affected by a patient's personality type [1]. Pain may be caused by endometriosis, endometriosis and other factors, or only other factors with endometriosis as a coincidental finding [2,3]. Although pain may be difficult or impossible to cure in some patients, other patients have coincidental and asymptomatic endometriosis that requires no treatment. Care is needed to attempt to ensure that patients are neither overtreated nor undertreated.

Is there a "gold standard" for diagnosis?

Recognition of lesions may be more important than the surgical technique used. A ''gold standard'' would be useful in research and in clinical care. This

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standard has not been established, however [4,5]. Near contact laparoscopy [6] has been used to identify lesions as small as 180 to 200 μ [7,8]. All lesions are not recognized at laparoscopy or laparotomy, however. Small lesions can be missed because of their size [9–12], whereas larger lesions may be missed because of their position [13]. Deep lesions may be more palpable than visual [14–17]. One study was published with none of 66 endometriomas confirmed by histology [18]. Although there is no "gold standard" for diagnosis, histologic examination is attempted in all cases, when it can be performed safely [19]. Histologic confirmation has been suggested as a standard for research studies [5].

Medical management

Surgery is avoided with no palpable mass until after a trial of birth control pill suppression and nonsteroidal antiinflammatory agents (NSAIDs) is attempted. This is particularly true in teenagers. Pain increases the chance that patients with infertility may benefit from laparoscopy.

Medical management may be useful in preoperative preparation to decrease rectovaginal mass size, ovarian size, and functional cysts [20,21]. The mass generally returns to the original volume by 6 months [20].

Expectant management

Expectant management is based on data that some patients with endometriosis have spontaneous regression [22–24]. Expectant management seems useful for bowel, periureteral, and rectovaginal endometriosis when pain can be controlled with medical suppression. This approach can avoid the morbidity and expense of extensive pelvic surgery in some of these patients.

As a specific note, biopsy-proven, nontender, rectovaginal endometriosis must be observed for proximity to the anal verge. A distance of 1.5 cm or more may be necessary for placement of the circular gastrointestinal anastomotic device. Although the need for diverting colostomy increases with a distance of less than 6 cm [25], successful primary anastomosis may be reasonable [26,27]. If a rectovaginal mass is growing and extending down the posterior vagina, surgery may be indicated to decrease the chance of a colostomy.

Laparotomy

Laparotomy is a standard for surgical therapy. Palpation, examination of retroperitoneal spaces, examination of bowel, and delicate handling of deep lesions are enhanced at laparotomy when compared with laparoscopy. Laparoscopic excision of deep bowel lesions has been associated with a high persistence of pelvic pain [16]. Open surgery is used when circumstances indicate a need for laparotomy [28,29]. Laparotomy is most useful in patients with persistent pelvic

pain after initial laparoscopic approach and in those with bulk tumor that appears more than appropriate for laparoscopy.

Laparoscopy

Laparoscopy can be used to identify, remove, and confirm lesions as small as 180 μ [7]. Video monitoring provides increased magnification and resolution [30]. This monitoring technique increases the ability of the assistants and other personnel to assist at surgery; however, video can decrease detection, resolution, depth of field, and field of vision.

Although coagulation and vaporization are adequate for most cases, excision has been used to resect lesions as deep as 14 mm and dissect the ureter and bowel away from endometriosis and adhesions [16,31–33]. The CO_2 laser can be used for deep and delicate dissections with excellent visualization. Scissors, bipolar or thermal coagulation, and unipolar knives are more commonly available and have adequate accuracy [34–37].

Although laparoscopy is generally equal to or better than laparotomy [30, 38–42], the learning curve for extensive disease is long. Complications of deep dissection at laparoscopy have been significant [43]. Physicians may decide to proceed with laparotomy rather than work through the complications associated with demanding laparoscopic techniques.

Extent of surgery

The extent of surgery that is planned depends on the patient's history, physical findings, and goals of surgery. A primary approach is generally used on patients with infertility or focal pelvic tenderness but no palpable nodules who are having their first operation. A secondary approach is generally used when nodularity is present or when pain and focal tenderness are the main problems. The tertiary approach is one in which the surgeon and patient are prepared for bowel surgery, ureteral anastomosis, ureteral implantation and laparotomy. These patients generally have received a diagnosis during previous laparoscopy or laparotomy.

Six or more approaches to the surgical treatment of endometriosis have been reviewed, with much overlap in the terms used by different investigators. Minimalistic surgery is often used in infertility or in young patients with diffuse scattered lesions. Conservative surgery may include deep incision and resection of all identifiable lesions. Semi-conservative surgery denotes hysterectomy with preservation of the ovaries. Radical surgery involves abdominal hysterectomy with bilateral salpingo-oophorectomy but leaves certain lesions alone, particularly on the bowel, ureter, or other vital systems. This type of radical surgery anticipates that surgical menopause will cure endometriosis. Definitive surgery includes not only abdominal hysterectomy with bilateral salpingo-oophorectomy but also resection of all palpable and visual lesions, including those on the bowel,

ureter, and other vital organs. As a last and rare approach, bilateral salpingo-oophorectomy with preservation of the uterus may be occasionally useful in patients who are preparing for in vitro fertilization [44].

The authors' primary approach to all patients undergoing laparoscopy is to be prepared to coagulate any recognized lesions and lyse adhesions. This can be performed with bipolar or thermal coagulation and mechanical scissors. Monopolar equipment is not necessary for this level of care.

A secondary level of care is used in those patients who previously underwent laparoscopy or who have clinical history or physical findings that suggest the need for deep tissue techniques. Deep tissue techniques include vaporization with laser or monopolar electrosurgery or dissection and excisional techniques using any form of dissecting and excisional equipment.

A tertiary approach is for patients who have large palpable nodules or who have failed to respond to other forms of therapy. These patients frequently undergo intravenous pyelograms, barium enemas, colonoscopies, sonographies, and other diagnostic testing. Their bowels are prepared and they frequently self-bank their blood. Preoperative permission for exploratory laparotomy, bowel resection, bowel anastomosis, and the possibility of ureteral reimplantation is discussed and clarified. This type of approach is generally used for pain and is uncommonly used for infertility.

Peritoneum and soft tissue

Small implants (≤ 2 mm) are treated with many energy types. They are sampled by biopsy or excision before treatment. Coagulation can distort tissue because of the thermal transfer and heating, which may interfere with recognition and dissection. Vaporization or excision is more useful for larger lesions. These techniques are carried down to the level of healthy tissue.

Deep lesions [16,45] are more accurately excised than vaporized [14,31–33]. Coagulation is inadequate for lesions larger than 2 mm. This is also true for bipolar electrosurgery, thermal cautery, argon laser, and Potassium-Titanyl-Phosphate (KTP) laser. Neodymium: Yttrium Aluminum Garnet (Nd:YAG) lasers are adequate to 5 mm. For lesions larger than 5 mm, deep vaporization or excision is needed [21,36,46]. Excision is started by cutting through the peritoneum and into the loose connective tissue with scissors, knife, or laser. A probe, irrigating solution, knife, or laser is then used to dissect these layers. Once the tissue is excised, it is removed. Specimens that are too large to be removed through the trocar can be cut or morcellated into smaller areas or bagged to be removed through the trocar incision, a minilaparotomy, or a colpotomy [32].

If carbon accumulates, the field is obscured at the time of surgery and at subsequent laparoscopy. Carbon can be confused with or can hide endometriosis [15,47]. High-power density electrosurgical or superpulse laser techniques decrease carbonization by facilitating rapid vaporization with a decrease in the amount of lateral tissue desiccated or coagulated [48,49].

Five patients with deep retroperitoneal involvement had little or no peritoneal disease. Four of these patients had a revised American Fertility Society [50] classification of stage 0/score 0, and the fifth patient had classification of stage 1/score 1. Three of these patients had complete ureteral obstruction, hydronephrosis, or full-thickness involvement of the rectum. The disease was severely symptomatic, difficult to diagnose, and challenging at surgery [13]. This deep retroperitoneal disease may be recognized only during menses [14]. Retroperitoneal disease with no peritoneal component (revised American Fertility Society score of 0) can be associated with significant symptoms [7,51].

Endometriosis in the retrocervical area and deep pouch of Douglas is a specific area of concern [26]. Surgical recognition and care can require probes in the vaginal fornix and the rectum. If the posterior fornix can be expanded with the vaginal probe, and if it shows no evidence of involvement, then the cul-de-sac may be intact. In all of the authors' patients, the test showed that the posterior fornix or retrocervix or both were involved when the rectovaginal septum was involved. (See illustrations at www.DanMartinMD.com/rvendo.htm.)

Ovary

Ovarian endometriomas are managed according to their size. Endometriomas that measure less than 5 mm are biopsied and coagulated, vaporized, or excised. When they are between 5 mm and 2 cm, they are handled according to the general characteristics at the time of surgery. The infiltration of these intermediate lesions can be irregular, and the treatment is taken 2 to 4 mm into healthy appearing stroma.

At 2 to 5 cm in size, the ovary is opened and drained and the inner wall inspected. A modification of Semm's stripping technique is used [52]. The opening of the ovary for this stripping can be performed with any type equipment but should be at the dependent portion or on the lateral (broad ligament) side to avoid bowel adhesions. A relaxing incision to facilitate definition of the plane of the pseudo-capsule may be useful in the development of the dissection plane and in determining the histology [15]. Surface endometriosis and endometriosis that infiltrate into a corpus luteum have a different score than an ovarian endometrioma. A differential excision of the surface of the ovary with sectioning to analyze for this possibility is needed [21]. When the capsule adheres to the hilar vessels, coagulation is used instead of stripping to avoid tearing these vessels. Hydrodissection may facilitate removal of the capsule [53].

When an endometrioma exceeds 5 cm, stripping techniques can take 2 to 5 hours of laparoscopy before performing a laparotomy [15]. Removing these large cysts may increase the chance of sacrificing the ovary when compared to performing a staged procedure [29,52,54,55], which involves draining, biopsy, and coagulating the inner lining of large cysts at an initial laparoscopy. Serial sonography with or without medical suppression is used to monitor the ovary. A subsequent laparoscopy is performed if the cyst recurs.

Limiting the amount of surgery [56], using laparoscopy, avoiding sutures [15,29,30,57,58], and encouraging early ambulation [59] have been associated with decreased need for repeat operations.

Bowel

Bowel involvement is suggested by palpable tumor near the bowel, rectovaginal tenderness, a rectovaginal shelf, rectal bleeding at the time of menses, or persistent pain after laparoscopic removal of recognized lesions. Recognition requires careful palpation because lesions smaller than 1 cm are easier to feel than to see.

Bowel lesions may have most of the lesion pushing into the lumen with a superficial area representing the tip of an iceberg [17,29]. Approximately 50% of appendiceal lesions are more readily recognized by palpation than visualization [60]. Few lesions are found by barium enema, colonoscopy, sonography, CT scan, or MRI. The main advantage to colonoscopy in the presence of a bowel mass is in ruling out adenocarcinoma of the bowel.

A laparoscopic initial attempt at partial-thickness resection of infiltration with bowel distortion associated with pain and tenderness was attempted in five patients [16]. Immediate laparotomy was performed for bowel resection in two of the patients. Although the other three patients had apparent resection of their endometriosis, persistent pain and tenderness resulted in delayed laparotomy in all of them. All five had deep muscular involvement. Patients who have persistent pain may require medical suppression or laparotomy [16,17,28,52,61–63].

Tumor in the rectovaginal septum generally requires a gynecologist or general surgeon who is familiar with bowel surgery in this area. Deep rectosigmoid resection and anastomosis is a distinct possibility at this level, and laparotomy may be indicated [17,28,52,62]. A distance of 1.5 cm or more from the anal verge may be needed for the gastrointestinal anastomotic device. Sure line dehiscence has occurred in 25% of patients with a distance of less than 6 cm [25].

The uterosacral ligaments can be infiltrated with extension toward the sacrum or pelvic floor. This level of the uterosacrals or perirectal tissue is more palpable through the rectosigmoid than through the vagina. If it is not noted preoperatively, it is easy to miss at surgery. If the mucosa is fixed, full-thickness penetration is often present. With any concern regarding bowel muscle involvement, a general surgery consultation, barium enema, sigmoidoscopy, and colonoscopy are considered to rule out bowel cancer.

Patients who are preparing for laparotomy generally have bowel preparation because the most common indication for laparotomy is suspected bowel involvement. Self-banking of blood is discussed with these patients because these procedures frequently last 3 to 5 hours and can be associated with significant blood loss and subsequent transfusion. Bowel procedures generally are avoided in infertility patients who do not have symptoms attributable to bowel infiltration.

Bladder

Bladder implants of up to 5 mm are handled in the same way as the peritoneal lesions. Larger lesions approach and may invade the bladder muscle. Deep muscular penetration should be anticipated as the lesions get larger. Lesions of 2 cm and larger require resection of the bladder dome at laparotomy when the indication is pelvic pain or when there is tubal distortion.

Ureter

When endometriosis lies over the ureter, two techniques are used. Solution can be injected to push the ureter away and provide a barrier between the ureter and the surgical destruction [37,64]. An alternate technique is to make an incision in the peritoneum above and away from the ureter. The peritoneum is then grasped and pulled toward the midline. A blunt probe is used to push the ureter away, or the laser is used to incise into loose connective tissue. The laser is not aimed at the ureter. The lesion is resected in its entirety using this technique.

When the ureter does not push away from the peritoneum, the chance of infiltration into the ureter is great. The periureteral vessels also can bleed, and techniques for hemostasis may harm the ureter. If the ureter is transected or damaged in the process of resecting disease, some urologists believe that anastomosis in a diseased area should not be performed and that an implantation is indicated. If one is not prepared for ureteral implantation, avoid cutting near the ureter, especially when it adheres to endometriosis.

Medial deviation of one or both ureters has been seen in 26% of patients with peritoneal pockets [65]. Increased concern for the position of the ureter is needed in these patients.

Staged procedures

Staging of surgery for endometriosis has been described by Semm [55] and Donnez [54]. Staging involves removing as much endometriosis as reasonable at first surgery, placing the patient on 3 to 9 months of hormonal suppression, and observing if the patient is asymptomatic or repeating the pelviscopy to remove any remnant endometriosis and treat the adhesions and tubal disease.

A staged procedure for ovarian endometriomas that involves drainage, irrigation, examination, biopsy, and coagulation of the inner lining of large cysts at an initial laparoscopy may be useful. This approach decreases the extent of removal of healthy ovary [66]. Serial sonography with or without medical suppression is used to monitor persistence or recurrence. A subsequent laparoscopy is performed if the cyst recurs.

Other surgeons suggest that endometriosis, particularly around the ureter, bowel, or major blood vessels, be left behind intentionally [61,63]. Observation may prove that partial treatment provides sufficient relief so that laparotomy or

medical therapy is not necessary. When the pain persists, medical therapy, a second laparoscopy, or laparotomy is considered. When significant endometriosis is present, a second laparoscopy may remove additional lesions or recurrent lesions. Repeat laparoscopy for recurrent endometriosis is particularly useful in younger patients.

Laparotomy may reveal palpable lesions that are not seen at laparoscopy [7,13,14,16,51]. Because of the increased incidence of palpable bowel disease in this group, bowel preparation is routinely performed on all patients who are prepared for laparotomy.

Presacral neurectomy and uterine nerve ablation

Although a study of conservative surgery and laparoscopic uterine nerve ablation (LUNA) suggests that LUNA is helpful [67], other studies show little or no positive effect. There is no evidence that LUNA adds value to conservative surgery for endometriosis-associated pain [68,69].

Presacral neurectomy has been investigated in two randomized trials as an adjunctive procedure to conservative surgery. In both studies, there was a significant and substantial decrease in midline menstrual pain but no effect on other types of pain. Conclusions of reviews have varied. On the positive side, presacral neurectomy may be valuable when endometriosis is associated with midline pain at the time of menses [69]. On the other hand, there is insufficient evidence to recommend the use of surgical pelvic neuroablation in the management of dysmenorrhea, regardless of cause [68].

Malignant transformation

The frequency of malignant transformation of extragenital endometriosis is low. Endometrioid carcinoma has been the most frequent histologic type, and cases of clear cell carcinoma are reported. Malignant transformation that involves the obliterated rectovaginal pouch-posterior vaginal formix was reported not only with use of opposed estrogen therapy but also with the use of Enovid and DepoProvera. Between 15% and 20% of endometrioid adenocarcinomas of the ovary are derived from preexisting endometriosis. In premenopausal patients, ovarian carcinoma also can cause cul-de-sac and uterosacral ligament nodularity and should be investigated [19].

Pain response

Pain relief is hard to quantitate. Pain may be caused by endometriosis, endometriosis and other factors, or only other factors with endometriosis as a coincidental finding [2]. Randomized, controlled trials are uncommon [67], and study design has been challenged. In noncontrolled studies, local tenderness associated with scarred lesions resolves when these lesions are resected [13–16,70].

Pain relief was similar at laparoscopy and laparotomy [36,71–73]. Diffuse pain is much harder to predict, and pain associated with chlamydia is the hardest to relieve [74].

Integrated approach

An integrated approach can show improvement over surgical or medical intervention [75]. The long-term therapeutic approach requires establishment of effective patient/physician relationships. Reasonable goals are set to help the patient assume responsibility. Medication, mental health referral, specific psychological treatment, and multidisciplinary pain centers are used as necessary [3,76].

Available data suggest that measured outcomes, including pain severity, global health status, and somatization, associated with this approach are significantly better than those observed after isolated medical interventions. Because of the chronicity of many of the psychological and social variables that predispose to recurrent symptom formation, care must be continuous and longitudinal if recurrent adverse sequelae, including disability, inappropriate health care use, and depression, are to be prevented [77].

Summary

General surgical guidelines are reasonable, but treatment frequently must be individualized. Laparoscopic coagulation can be used for many cases of superficial endometriosis. Resection seems to be associated with an increased resolution of endometriosis. Resection increases the difficulty of the procedure, the time of the operation, and the cost, however. When endometriosis is found coincidentally, it may need no treatment because many women have endometriosis as a self-limited disease. Distinguishing patients who need no treatment from patients who need intermediate or extensive treatment can be difficult. Care is needed to attempt to ensure that patients are neither overtreated nor undertreated.

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Endometriosis
Preoperative and postoperative medical treatment

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The management of endometriosis recently has undergone major modifications based on several advances in surgical, medical, and alternative treatments. After the introduction of safe and effective endoscopic techniques for debulking or radical conservative surgery of the disease, diagnostic-only laparoscopy has almost disappeared [1,2]. Currently it is standard practice to eliminate or reduce visible lesions on the same occasion as visual confirmation [3] because randomized, double-blind, controlled trials demonstrated the efficacy of endometriosis ablation in moderately increasing the pregnancy rate in infertile women [4] and in reducing pelvic pain in symptomatic patients [5,6]. As a result of the increasing surgical approach to endometriosis, the combination of medical treatment with laparoscopic procedures, either preoperatively or postoperatively, represents a growing field of application of drugs [7–10]. Unfortunately, little information is available on the potential benefits of hormonal treatments in combination with conservative surgery for endometriosis [11–13]. Accordingly, the authors considered it of interest to identify, analyze and, when appropriate, pool published data on combined medical and surgical treatment in the scientific literature of the last 15 years. The main purpose of this article is to assess the effect of administering drugs either before or after the intervention on postoperative pregnancy rate and on endometriosis-associated pelvic pain and compare it with that of surgery alone. This article is partly based on a previously published review [14].

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The literature search

The authors adopted several different strategies to identify all English-language medical articles published on hormonal treatment combined with conservative surgery for endometriosis. They conducted a Medline search from 1987 to 2002 using relevant medical subject heading terms (endometriosis, infertility, pelvic pain, dysmenorrhea, dyspareunia, medical therapy, surgery). The authors personally searched the main specialty journals (*American Journal of Obstetrics and Gynecology*, *British Journal of Obstetrics and Gynaecology*, *Fertility and Sterility*, *Human Reproduction*, *Obstetrics and Gynecology*) from January 1987 to June 2002. They identified additional reports by systematically reviewing all references from retrieved papers and by consulting books and monographs on endometriosis published in the last 15 years.

Because of the absence of randomized, controlled trials on the use of preoperative medical treatment, the authors decided to collect data from the available observational studies on this specific topic. On the other hand, only randomized trials that included a placebo or no-treatment arm were considered for the evaluation of the effects of drugs administered postoperatively. Only studies in which results were presented as a proportion of pregnant/nonpregnant women after surgery and of treatment responders/nonresponders in terms of pain persistence or recurrence were selected. Studies were excluded if it was not possible to identify how many patients desired a pregnancy or were infertile or to categorize the outcome of interest (such as reports that presented results only in terms of reduction in pain scores) or if partial results were reported in advance of an available later full report.

Response to treatment was considered as postoperative conception independently of pregnancy outcome or as absence or amelioration of pain in previously symptomatic subjects. For the analysis of pain, including overall estimates, the authors considered the effect on dysmenorrhea, because this was the main endpoint of most studies, or, when the types of symptoms were not specified, on any pelvic pain. Two of the authors (O.D.G. and G.F.) abstracted data in an unblinded fashion on standardized forms. An initial screening of the title and abstract of all articles was performed to exclude irrelevant citations when both observers agreed. The year of publication, type and design of study, clinical characteristics of subjects, surgical details, drug treatment schedule, and main and secondary outcomes were recorded independently. The number of patients who wanted pregnancy and had pain at baseline, the duration of follow-up, and the conception and symptomatic recurrence rates after surgery were obtained from individual studies. Discrepancies among the evaluators were identified and resolved by consensus.

For noncomparative studies, the authors calculated the postsurgical conception rate with the respective 95% confidence interval (CI) based on binomial distribution. For each comparative trial, a 2×2 table was generated, including the number of pregnant/nonpregnant women and of responders/nonresponders in the study groups at the end of treatment or follow-up. Odds ratios and their 95% CI were calculated using Epi Info 6.0 software [15]. A combined estimate of the odds ratio across studies (the typical odds ratio) was then calculated for the

outcome of interest (pregnancy and/or pain) using the Mantel-Haenszel method [16]. Differences among comparative studies were assessed quantitatively using the Breslow-Day method [17]. Attribution of validity class for randomized, controlled trials was based on the criteria suggested by Chalmers et al [18].

Results of the literature search

Medical treatment before conservative surgery

The initial screening yielded 12 citations for further assessment. Of these, the authors excluded 5 citations because the outcomes of interest were not included in the results, which regarded mainly surgical aspects [19–23], 1 citation because no follow-up data were available for the women allocated in the preoperative medical treatment arm [24], 1 citation because it did not indicate how many of the 70 women who underwent surgery for endometriosis were treated with a progestin before surgery [25], and 1 citation because ad interim analyses were reported [26]. Data on the effect of preoperative medical treatment were extracted from the remaining 4 articles, which were published in full in peer-reviewed journals. Of the studies considered, 2 were noncomparative (1 prospective and 1 retrospective) and 2 were comparative and retrospective. The main characteristics and details of the 4 trials considered are shown in Tables 1 and 2. Endometriosis was always staged according to the American Fertility Society (AFS) classification in its original [27] or revised [28] version.

Effect on fertility

Olive and Martin [29] analyzed data from 129 patients with infertility and endometriosis who underwent CO_2 laser laparoscopy with or without perioperative danazol treatment. Cumulative pregnancy rates at 36 months were 39%, 46%, and 50%, respectively, for AFS stages I, II, and III disease. Monthly fecundity rate in subjects with stage I disease ($n = 59$) was 1.58 in the preoperative danazol group, 3.45 in the postoperative danazol group, and 3.50 in the laparoscopic surgery-only group. Corresponding figures at stages II ($n = 48$) and III ($n = 20$) were 4.38, 2.50, 2.51, and 6.06,1.49, and 5.79, respectively, without significant differences. Treatment allocation was not random, however, and the size of some subgroups was limited. In the same year, Donnez et al [30] described 50 patients with ovarian endometriosis at AFS stages II and III who were treated with diagnostic laparoscopy followed by a 6-month course of oral lynestrenol, 5 mg/day, and then microsurgery at laparotomy. After progestin therapy, the AFS score fell in 36 cases, rose slightly in 2 cases, and remained unchanged in 12 cases. The pregnancy rate after 18 months of follow-up was 56% (28/50; 95% CI, 41%–70%), being 60% in stage II and 47% in stage III patients. The authors claim an advantage of preoperative medical treatment with lynestrenol compared with published results of surgery alone or combined with postoperative medical therapy, but the noncomparative nature of the study precluded conclusions.

Table 1
Main characteristics of studies on medical treatment before conservative surgery for endometriosis

Source	Origin, year	Type of study	Treatment schedule	Control	Surgical modality	Life table analysis
Olive and Martin [29]	United States, 1987	Retrospective, comparative	Oral danazol[a]	Immediate surgery	CO_2 laser laparoscopy	Yes
Donnez et al [30]	Belgium, 1987	Prospective, noncomparative	Oral lynestrenol 5 mg/d for 6 mo	NA	Microsurgery at laparotomy	Yes
Napolitano et al [31]	Italy, 1994	Retrospective, comparative	Oral MPA 20 mg/d for 3 mo or danazol, 600 mg/d for 3 mo	Immediate surgery	Microsurgery at laparotomy	No
Donnez et al [32]	Belgium, 1996	Retrospective, noncomparative	Subcutaneous goserelin depot 3.6 mg/28 d for 3 mo	NA	Co_2 laser laparoscopy	Yes

Abbreviations: MPA, medroxyprogesterone acetate; NA, not applicable.

[a] Dose and duration of therapy not available.

From Vercellini P, et al. Endometriosis: drugs and adjuvant therapy. In: Templeton A, Cooke I, Shaughn O'Brien PM, editors. Evidence-based fertility treatment. London: RCOG Press; 1998; p. 225–45, with permission.

Table 2
Details of patients enrolled and main results of studies on medical treatment before conservative surgery for endometriosis

Source	Sample size	Mean age	Endometriosis stage	Infertility factors other than endometriosis	Months of follow-up	Pregnancy rate	
						Experimental (%)	Control (%)
Olive and Martin [29]	129[a]	31	I – III[b]	Yes	36	43% in the entire series	
Donnez et al [30]	82	NR	II and III[b]	Yes	18	56	NA
Napolitano et al [31]	117	NR	III and IV[c]	No	NR	34	26
Donnez et al [32]	814	NR	III and IV[c]	Yes	12	51	NA

Abbreviations: NA, not applicable; NR, not reported.

[a] Including 11 women who took danazol postoperatively.

[b] According to the original AFS classification [27].

[c] According to the revised AFS classification [28].

From Vercellini P, et al. Endometriosis: drugs and adjuvant therapy. In: Templeton A, Cooke I, Shaughn O'Brien PM, editors. Evidence-based fertility treatment. London: RCOG Press; 1998; p. 225–45, with permission.

Napolitano et al [31] treated 117 infertile women with stage III or IV endometriosis according to the revised AFS classification [28]. Oral medical therapy with medzoxyprogesterone acetate (MPA), 20 mg/day, or danazol, 600 mg/day, was administered perioperatively for 3 months in 90 women, whereas 27 subjects underwent immediate surgery. The crude pregnancy rate at 18-month follow-up was 31% (8/26; 95% CI, 14%–52%) in the MPA group, 36% (23/64; 95% CI, 24%–49%) in the danazol group, and 26% (7/27; 95% CI, 11%–46%) in the surgery-only group, without significant differences. Donnez et al [32] recently published the results obtained in 814 patients with large ovarian endometriomas (>3 cm) treated by drainage, ovarioscopy, and biopsy at first-look laparoscopy, followed by 3.6 mg monthly goserelin depot injections for 12 weeks and, finally, a second-look laparoscopy with CO_2 laser vaporization of the remaining endometriotic cysts. The mean cyst diameter was reduced by 50% of the baseline value (from 47 ± 6 mm to 22 ± 4 mm), and the 12-month postoperative cumulative conception rate was 51%. After a follow-up of 2 to 11 years, the recurrence rate of ovarian endometriomas was 8%.

Effect on pain

Donnez et al [30] reported that the combination of preoperative progestin therapy and surgery at laparotomy for moderate and severe endometriosis in a series of 50 patients resulted in complete relief of pelvic pain in 45% and improvement in 42% of previously symptomatic women. Corresponding figures for dyspareunia were 16% and 58%, respectively. Only percentages—but not absolute numbers—are indicated. When Napolitano et al [31] retrospectively reviewed their series of 117 women operated for moderate or severe endometriosis, they observed that 64% of previously symptomatic women who underwent combined medical and surgical treatment experienced complete pain relief, and 16% experienced partial improvement. Corresponding figures in the surgery-only group were 55% and 18%, without significant differences. Absolute numbers are not indicated in the article. These data are of doubtful value given the noncomparative or nonrandomized study design.

Medical treatment after conservative surgery

The initial screening yielded 17 citations for further assessment. Of these, the authors excluded 4 because the studies were either not comparative or not randomized [33–36], 2 because the outcomes of interest were either not included in the results or not clearly identifiable [37,38], 1 because it is unclear if laparoscopy performed before danazol treatment was ablative or simply diagnostic [39], 1 with a misleading title because the effects of gestrinone were evaluated without prior surgical debulking [40], 1 because no follow-up data are provided [24], and 1 because mean pain score variations but no absolute numbers of nonresponders are reported [41].

Data on the effect of postoperative medical treatment were extracted from the remaining 7 articles. Two studies [42,43] reported exclusively variations of

symptoms and they were included only in the evaluation of the effect on pain. In the study by Parazzini et al [44], it was not possible to extrapolate the absolute numbers of nonresponders after the intervention because the effect of treatments was expressed in terms of median reduction of pain scores. The effect on conception was assessed in evaluating studies by Telimaa et al [45], Parazzini et al [44], Vercellini et al [46], Bianchi et al [47], and Busacca et al [48], which were published in full in peer-reviewed journals.

The main characteristics and details of the seven randomized, controlled trials considered are shown in Tables 3 and 4. Four studies evaluated drug therapy after operative laparoscopy, two evaluated drug therapy after microsurgery at laparotomy, and in one the surgical approach was mixed. Three trials were placebo controlled, and four included a no-treatment arm; the method of randomization was defined for five of them. A preplanned calculation of the sample size based on the expected difference in the main outcome measure was reported in three articles only, and no blinding procedure was adopted in another four. Specific protocol objectives were not identified in the study by Telimaa et al [45]. The mean number of patients included was 107, but more than one third of the subjects were recruited in the multicenter Italian trial [46]. Endometriosis was always staged according to the revised AFS classification [28]. Two studies included subjects with disease at all stages and three only at stages III and IV, whereas in studies by Hornstein et al [42] and Muzii et al [43], mean scores but not stage attribution are indicated. The numbers of patients excluded from each study and the reasons for exclusion are reported in Table 5. These subjects are not included in the calculation of rates.

Effect on fertility

Assessment of effect in terms of postoperative conception rate at the end of follow-up of the randomized, controlled trials identified is shown in Fig. 1. A total of 344 women were evaluated. Medical treatment schedules varied among studies: (1) three studies used a gonadotropin-releasing hormone (GnRH) agonist (given in a subcutaneous depot formulation for 6 months in one study, intramuscularly for 3 months in one study, and intranasally for 3 months in one study); (2) one study used either oral MPA or danazol for 6 months; (3) one study used oral danazol for 3 months. The odds ratio of conception in the trials considered ranged from 0.52 to 1.20, all with 95% CI, including unity. The common odds ratio from these randomized, controlled trial was 0.77 (95% CI, 0.42–1.39), which suggests that the treatment effects of surgery plus postoperative medical therapy or surgery alone were equivalent. Clinical heterogeneity among studies is inevitable because different interventions are used for comparison. Similarity in effect sizes was reflected by a lack of statistical heterogeneity, however (Breslow-Day test for heterogeneity, $\chi^2 = 0.95$, 4 df, $P = 0.91$).

Effect on pain

In the study by Telimaa et al [45], MPA and danazol given postoperatively reduced pelvic pain scores more effectively than placebo, the difference being significant at 6 months of therapy. It is unclear if the difference in symptomatic

Table 3
Main characteristics of randomized, controlled trials on medical treatment after conservative surgery for endometriosis

Source	Origin, year	Validity class	Type of randomization	Surgical modality	Treatment schedule	Control	Life table analysis
Telimaa et al [45]	Finland, 1987	B	NR	Laparotomy	Oral danazol 600 mg/d for 6 mo, oral MPA 100 mg/d for 6 mo	Placebo	No
Parazzini et al [44]	Italy, 1994	A	Computer-generated list centralized allocation by phone	Laparotomy	Nasal nafarelin 400 μg/d for 3 mo	Placebo	No
Hornstein et al [42]	United States and Canada, 1997	A	NR	Laparoscopy	Nasal nafarelin 400 μg/d for 6 mo	Placebo	Yes
Vercellini et al [46]	Italy, 1999	A	Computer-generated list centralized allocation by phone	Laparotomy and laparoscopy	Subcutaneous goserelin depot 3.6 mg/28 d for 6 mo	No postoperative treatment	Yes
Bianchi et al [47]	Italy, 1999	B	Computer-generated list	Laparoscopy	Oral danazol 600 mg/d for 3 mo	No postoperative treatment	No
Muzii et al [43]	Italy, 2000	A	Computer-generated list	Laparoscopy	Cyclic oral contraceptive for 6 mo	No postoperative treatment	Yes
Busacca et al [48]	Italy, 2001	A	Computer-generated list	Laparoscopy	Intramuscular leuprolide acetate depot 3.75 mg/28 d for 3 mo	No postoperative treatment	Yes

Abbreviations: MPA, medroxyprogesterone acetate; NR, not reported.

Table 4
Details of study patients enrolled in randomized, controlled trials on medical treatment after conservative surgery for endometriosis

Source	Sample size	Mean age	Endometriosis stage	No. of women with infertility at entry	Infertility factors other than endometriosis	No. of women with pain at entry	Months of follow-up
Telimaa et al [45]	60	30	AFS[a], all stages	22	NR	NR	6
Parazzini et al [44]	75	<38	Revised AFS stages III and IV	75	No	60	12
Hornstein et al [42]	109	31	Revised AFS[b]	NA	NA	109	18
Vercellini et al [46]	269	30	Revised AFS, all stages	NA	NA	269	24
Bianchi et al [47]	77	NR	Revised AFS stages III and IV	27	No	60	6–36
Muzii et al [43]	70	28	Revised AFS[b]	NA	NA	70	22
Busacca et al [48]	89	29	Revised AFS, stages III and IV	30	No	89	19

Abbreviations: NA, not applicable; NR, not reported.

[a] American Fertility Society classification [27].

[b] Only mean scores are available but not stratification by stage.

Table 5
Number of patients enrolled who were excluded from studies on medical treatment after conservative surgery for endometriosis, with reasons for exclusion

Author	No. of patients excluded	Reason for exclusion after enrolment
Telimaa et al [45]	1	Adverse effects during placebo
Hornstein et al [42]	16	Various reasons unrelated to drug efficacy (n = 15), irregular drug assumption
Vercellini et al [46]	59	Various reasons other than symptoms recurrence or major protocol violations (n = 57); case report forms not completed (n = 2)
Muzii et al [43]	2	Various reasons unrelated to disease recurrence or side effects of drugs

relief persisted also after the withdrawal of medical therapy. Absolute numbers of responders/nonresponders were not indicated, a limit that applies also to the trial by Parazzini et al [44]. The latter study assessed the antalgic effect of nasal nafarelin for 3 months after surgery using a seven-point multidimensional verbal rating scale and a ten-point linear analogue scale. At 12-month follow-up, the mean reduction of the multidimensional score was 3.6 and 4.0, respectively, in women allocated to nafarelin or placebo, and the mean reduction of the ten-point linear scale score was 7.0 and 6.9, respectively. These differences were not statistically significant.

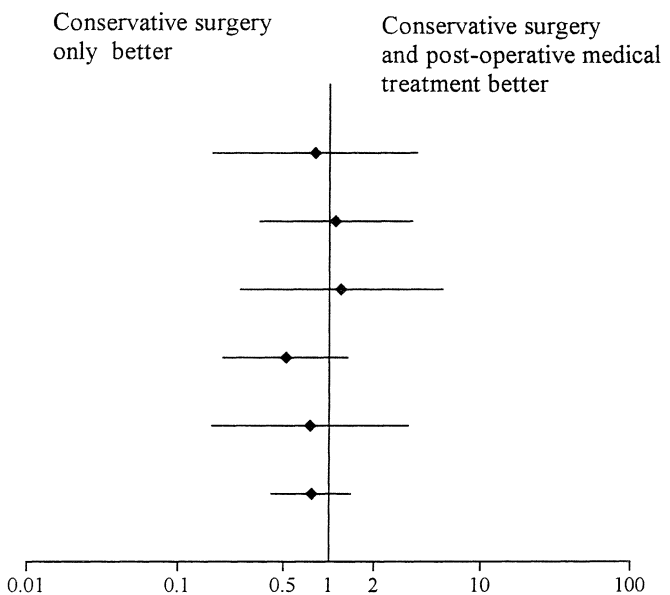
Assessment of effect in terms of pelvic pain relief at the end of follow-up for the remaining five trials is shown in Fig. 2. The authors computed the results of the study by Hornstein et al [42] by considering the number of patients who required alternative treatment during follow-up as the number of nonresponders; this may not match the exact estimate, which was not indicated in the article. Consequently, the results of the combination of data across the trials considered must be interpreted with caution. Given these limitations, the common odds ratio was 0.54 (95% CI, 0.34–0.82), which suggests an effect of postoperative medical treatment in reducing the rate of pain symptoms recurrence.

Comment

A major misunderstanding that undermines the entire concept of medical therapy of endometriosis lies in the conviction that ectopic implants may regress, degenerate, and ultimately disappear because of an unfavorable hormonal milieu.

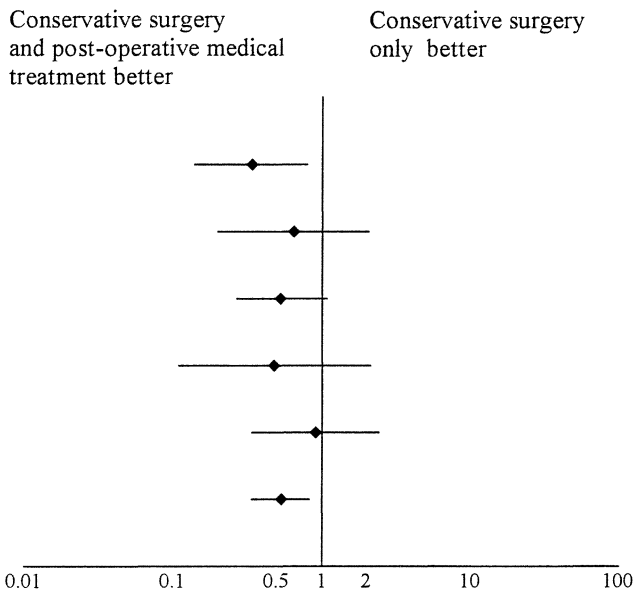
Fig. 1. Overview of randomized, controlled trials that compare conservative surgery for endometriosis with or without postoperative medical treatment. Diamonds represent odds ratio of conception, and horizontal lines are 95% CI. Asterisk represents subjects allocated to danazol and medroxyprogesterone acetate treatment.

Trial Identifier	Pregnancies / Patients	
	Experimental	Control
Telimaa *et al.* (45)	5/40*	3/20
Parazzini *et al.* (44)	7/36	7/39
Bianchi *et al.* (47)	6/11	8/16
Vercellini *et al.* (46)	8/76	14/76
Busacca *et al.* (48)	5/15	6/15
Common odds ratio		



Breslow-Day = 0.95 ($P = 0.91$)

Trial Identifier	Pain Recurrences / Patients	
	Experimental	Control
Hornstein *et al.* (42)	15/49	25/44
Bianchi *et al.* (47)	7/31	9/29
Vercellini *et al.* (46)	19/81	27/74
Muzii *et al.* (43)	3/33	6/35
Busacca *et al.* (48)	10/44	11/45
Common odds ratio		



Breslow-Day = 2.12 ($P = 0.71$)

This erroneous belief has been supported largely by findings at follow-up laparoscopies that were performed with the patients still under the effect of treatment and showed reductions in the AFS scores. The difference in the aspect of lesions before and at the end of treatment is not definitive, however, because implants undergo simple modification of their appearance with just partial and temporary regression but no resorption or healing [49–51]. Ectopic endometrium is still there, ready to regrow with a metabolic activity no different than before treatment and, most importantly, independently of the type and dosage of the drug administered [52–54].

Drugs that suppress ovulation do not constitute a cure for endometriosis in terms of healing processes and resorption, whereas the chance of conception increases moderately when limited ectopic implants are effectively eliminated by means of laparoscopic ablation [4]. In light of this scenario, hormonal drugs no longer should be prescribed in combination with surgery, with the aim of increasing the pregnancy rate in infertile women.

The theoretical advantages of medical treatment before surgery are reduced inflammation and vascularization and shrinkage of implants. According to some authors, these effects may contribute to easier, quicker, and less traumatic surgery, with more chance of complete eradication of the disease and a reduced risk of postoperative adnexal adhesions [7,9–13,21,26]. Practical advantages include avoidance of operating in the secretory phase with the disturbing presence of the corpus luteum and the possibility of hospital admission at any time [8]. This may be important in large, busy, public hospitals. The carry-over effect of most drugs used preoperatively prevents short-term ovulation in a recently traumatized gonad [8]. Finally, with preoperative treatment lasting a few months, the differential diagnosis between endometriotic and luteal cysts can be made easily, avoiding an untimely intervention when a functional formation is present. On the other hand, under medical suppression, small endometriotic foci may temporarily regress and thus escape laparoscopic recognition and ablation. Delaying surgery may be inopportune in some circumstances, especially when the nature of the cysts is not completely defined and serum CA-125 levels are particularly elevated. Indisputable disadvantages include the increase in the overall cost of treatment and drug-related side effects.

Apart from general considerations, only limited data are available to evaluate the effect of preoperative medical treatments on surgical aspects and long-term outcome. According to the extensive evaluations of preoperative medical therapies by Donnez et al [19,20,26], a GnRH agonist in depot formulation proved superior to progestins, danazol, gestrinone, and the same GnRH agonist as nasal spray in terms of reduction of inflammation, vascularization, AFS score, mean endometrioma diameter, and mitotic index. In a randomized trial, Donnez

Fig. 2. Overview of randomized, controlled trials that compare conservative surgery for endometriosis with or without postoperative medical treatment. Diamonds represent odds ratio of symptoms recurrence, and horizontal lines are 95% CI.

et al [21] demonstrated that goserelin administration for 3 months after drainage of endometriomas partially prevented the regrowth to the original dimensions observed in the subjects who were not medically treated between first- and second-look laparoscopy. Whether all of these factors lead to easier, quicker, and more effective surgery remains debatable, however. When Muzii et al [22] compared the intraoperative results of 20 patients who underwent laparoscopy after 3 months of GnRH agonist treatment with results of 21 women who were allocated to immediate surgery for unilateral ovarian endometriomas, no significant difference could be demonstrated in total operative time, cyst wall stripping time, and the time needed to obtain complete hemostasis. Audebert et al [24] did not observe significant differences in surgical feasibility when using a GnRH agonist before laparoscopy. In pretreated women, 6/25 (24%) of the procedures were classified as difficult or very difficult compared with 8/28 (28%) in the subjects who underwent immediate surgery.

In the absence of convincing evidence of a treatment effect in terms of surgical advantages, pregnancy rate, and symptomatic relief, preoperative medical treatment seems unjustified, especially if this modality includes the performance of two surgical procedures some months apart. In these circumstances the increase in morbidity and costs seems to far outweigh the hypothetical benefits.

Enthusiasm for adjuvant drug therapy after conservative interventions for endometriosis increased after publication of the retrospective findings of Wheeler and Malinak [55]. These authors reported a pregnancy rate of 79% (15/19) after combined surgery and postoperative danazol therapy compared with 30% (36/119) after surgery alone. The hypothetical advantages of postoperative medical treatment include resorption of residual visible lesions whose surgical removal was considered inopportune or not possible, ''sterilization'' of microscopic implants, and reduction in the risk of disease dissemination when endometriomas rupture during mobilization. These advantages should increase the postoperative pregnancy rate and reduce the recurrence rate [7,9–13]. Unfortunately, the lesson learned with medical therapies when used alone applies also to postoperative treatments that render these considerations naive. Medical treatment might prevent a pregnancy just when a conception may be more likely (ie, in the immediate postoperative period). This last notion has never been confirmed formally, however.

As far as pelvic pain is concerned, pooling of data from five trials demonstrated a reduced long-term symptoms recurrence rate in women who were allocated to postoperative medical therapy. More information is needed to confirm these findings, however, particularly in view of the discordant results obtained by Parazzini et al [44], Vercellini et al [46], and Busacca et al [48].

The observed differences among various drugs used after surgery are limited in clinical terms and, in the absence of formal randomized comparisons, are difficult to interpret. If and when a postoperative medical treatment is deemed opportune, progestins with or without estrogens should be considered first because of their tolerable side effects, limited cost, and antalgic efficacy similar to GnRH agonists and danazol.

Summary

The quality of the evidence that supports the use of medical treatment before conservative surgery for endometriosis is manifestly poor, and no recommendations can be made based on the results of the published studies. There are practical advantages inherent to this schedule, but whether this translates into better conception rates and reduced pain recurrence rates is unproven. The effect of drug therapy after surgery can be assessed better as data from seven true randomized, controlled trials are available. The results of the current review do not support the notion that suppressing ovarian activity postoperatively increases the long-term pregnancy rate. As far as pelvic pain is concerned, more data are needed to verify the reduced symptoms recurrence rate found in four trials in women who were allocated to postoperative medical therapy, particularly in view of the different results obtained in some of the considered studies. The observed differences among various drugs used before or after surgery are limited in clinical terms and, in the absence of formal randomized comparisons, are difficult to interpret. Because of their tolerable side effects and limited cost, progestins with or without estrogens should be considered strongly as first-line postoperative medical treatment if and when suppression of ovulation after conservative surgery is deemed opportune.

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How does endometriosis affect infertility?

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Endometriosis has been one of the most confusing gynecologic diseases since it was first described approximately a century ago. The rate of endometriosis in infertile women ranges from 4.5% to 33% [1].

The non–in vitro fertilization (IVF) clinical scenario confirmed the intricate relation between endometriosis and infertility by means of three approximations: (1) Prospective studies on the prevalence of endometriosis have shown that mild-to-moderate stages are more frequently found in infertile women and in women with pain or dysfunctional bleeding than in women who request tubal sterilization [2]. (2) When women with endometriosis were treated randomly by surgery or managed expectantly, cumulative pregnancy rates significantly increased in treated patients [3], which demonstrated that mild-to-moderate lesions could interfere with fertility. (3) Artificial insemination with donor sperm and lack of other infertility factor endometriosis were factors against successful outcome [4].

Assisted reproductive technology has provided a diagnostic and therapeutic approach for endometriosis (Fig. 1). Some IVF-based basic and clinical research found alterations in all the steps of the normal reproductive physiology [5], including an altered embryo quality [6–8] and a higher in vitro embryo blockage [9].

Women with endometriosis display lower implantation capacity and further diminished pregnancy rates [5,10]; however, the pathophysiology by which endometriosis affects implantation is an unresolved medical question. We do not know much about the endometrium and the embryo intrinsic mechanisms in relation to the implantation process.

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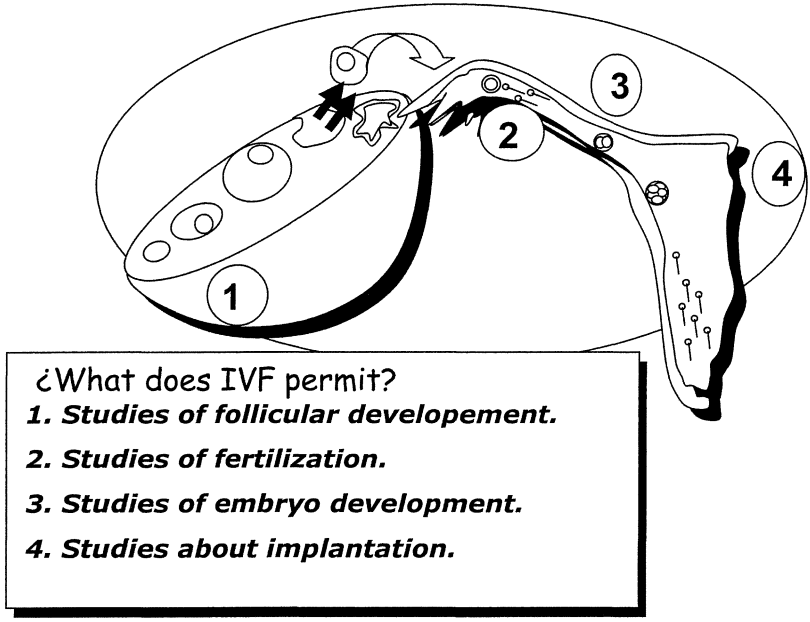

Fig. 1. Strategies in the study of endometriosis.

From experience, the authors can conclude that implantation is drastically altered in women with endometriosis. The likely cause of this is the impaired quality of the embryos obtained [5], which points to the follicular microenvironment and the quality of the oocyte that might be compromised in patients with endometriosis. This suggests that infertility may be related to alterations within the follicle, which result in embryos of lower quality and capacity to implant. Many processes and molecules have been studied in women with endometriosis and nonendometriosis controls, with the aim to describe their implications on fertility in the endometrium and follicles (Fig. 2).

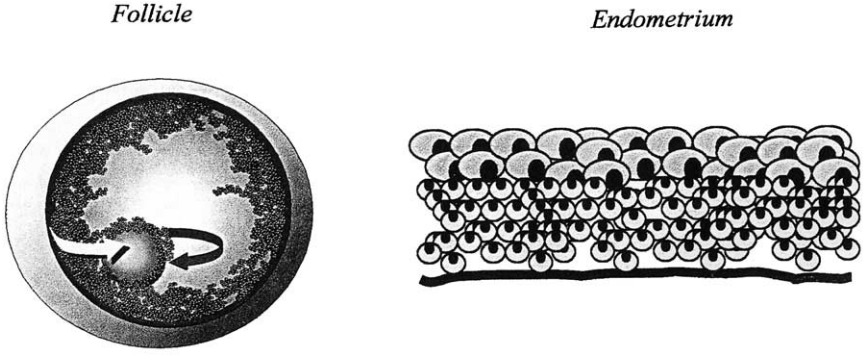

Fig. 2. Schematic representation of a follicle and the endometrium.

Within this article, the authors consider the clinical evidence that supports a causal relationship between endometriosis and fertility and the molecular basis of this relationship based on the available data on the literature.

In vitro fertilization in women with endometriosis

Some authors have reported a poor outcome with IVF in patients with endometriosis compared with other etiologies [11–17]; others have reported favorable results [18–21]. The poor results have been associated to two related mechanisms: a poor oocyte quality that results in decreased fertilization rates [12–16] and a defective implantation capacity of the embryos [7,8,11,17].

The authors' team has focused on the reproductive outcome of women with endometriosis who undergo assisted reproductive technology procedures. Two studies were conducted to investigate the subfertile status of endometriosis patients compared with tubal infertility patients [6] and the issue of embryo quality in women with endometriosis [9,22]. The first study included 96 cycles from patients with different endometriosis stages and a control group of 96 cycles corresponding to patients with tubal factor. Number of oocytes retrieved, fertilization rates, and the number of embryos transferred in each group were similar, but pregnancy rates per cycle, pregnancy per transfer, and implantation rate were halved in the endometriosis group.

A second analysis showed the same trends in the pregnancy and implantation rates, with the added observation of a significant decrease in the number of blastomeres in the endometriosis group [6]. The number of blastomeres and the degree of fragmentation were established after 48 and 72 hours in culture [23]. There was no difference between groups in age, number of oocytes retrieved and fertilized, and mean number of blastomeres after 48 hours. After 72 hours in culture, however, there was a significant decrease in the number of blastomeres and a significant increase in the percentage of arrested embryos in the endometriosis group as compared to the group with tubal infertility.

From those studies the authors concluded that implantation was impaired significantly in women with endometriosis, and the quality of the transferred embryos was probably responsible for such observation.

Similar conclusions have been drawn from two different groups' metaanalyses of the available literature. Recently, to increase knowledge on this topic, the authors further reviewed the situation by means of the metaanalyses of eight relevant studies on this issue [24], including nearly 900 cycles of approximately 700 women with endometriosis and more than 2500 cycles of 1700 women without the disease used as controls. All these studies were selected depending on their design, data presentation, and inclusion criteria. The authors found a statistically considerable decrease in the pregnancy and implantation rates in nonendometriosis controls versus women with endometriosis, respectively, whereas no differences were found in the fertilization rates or the number of oocytes retrieved.

In another recent study [10], patients were classified by level of endometriosis, which classified women according to the indication for IVF. Classical IVF parameters were considered, which were extracted from 22 published studies. Women with endometriosis had a low pregnancy probability when compared with controls with tubal factor. Multivariate analysis also showed a decrease in fertilization and implantation rates and a significant decrease in the number of oocytes retrieved for endometriosis patients. Stage of disease also was influential, because women with severe endometriosis displayed significantly lower rates than women with mild disease.

Oocyte donation in women with endometriosis: the assisted reproductive technology experiment

Ovum donation provides a unique opportunity to investigate the reproductive outcome of women with endometriosis. It is a therapeutic option for patients with endometriosis-associated infertility and repeated IVF failures. Reproductive outcome comparison between patients with severe endometriosis who receive healthy donor oocytes and patients without endometriosis under the same circumstances provides an appropriate set-up to address how this disease affects fertility.

The authors retrospectively analyzed the results of their oocyte donation program [6] in three groups of women with premature ovarian failure (n = 54), low response (n = 77), and women with endometriosis (n = 10) who underwent oocyte donation because of low response. With a similar number of embryos being replaced in each group, there were no differences among groups in the pregnancy rates per patient, per cycle, or implantation.

The authors also analyzed the outcome according to the source of the donated oocytes [6], which provided the opportunity to compare the implantation of embryos from women with tubal factor, endometriosis, or ovulation disorders, fertile women who underwent tubal ligation, and healthy women with partners with male infertility. There was no difference in pregnancy rates per transfer; however, implantation rates were significantly lower in women who received oocytes from women with endometriosis. In a prospective design, three groups were compared: 44 donors and recipients without endometriosis; 14 donors with endometriosis who donated oocytes to recipients without the disease; and 16 donors without endometriosis who donated oocytes to recipients with endometriosis. The second group showed a decrease in the pregnancy rate per transfer and the implantation rate, which confirmed the fact that embryos from women with endometriosis display a reduced capacity to implant.

Other researchers observed that the percentage of aberrant forms was higher in women with endometriosis [25]. Some previous studies have shown that endometriosis is not detrimental to implantation [11,26]; however, these studies were retrospective and were not controlled with respect to the origin of the oocytes.

In the authors' experience, the quality of the oocytes in donors is not a variable that affects the results of oocyte donation. A good prospective comparative study

that focuses on implantation should rule out the possibility of assigning oocytes of different quality to the preestablished groups, however.

At this point, the authors designed a study in which sibling oocytes were donated to recipients with and without severe endometriosis to confirm or reject the hypothesis that the endometrial environment did not affect fertility in women with severe endometriosis [27]. Twenty-five oocyte recipients with endometriosis stage III-IV and 33 recipients without the disease were compared. The donors included ten women who underwent IVF and 15 young fertile women who voluntarily donated oocytes. The number of oocytes donated, fertilization rate, number of embryos available, number of embryos transferred, and the average number of good quality embryos transferred were not significantly different between the two groups. Pregnancy, implantation, and miscarriage rates were not affected by stage III-IV endometriosis when compared with the control group. The live birth rate in the women with endometriosis and the control group was not different.

In conclusion, women with endometriosis have a poor IVF outcome in terms of reduced pregnancy rate per cycle, reduced pregnancy rate per transfer, and reduced implantation rate [6,9,23]. On the contrary, some authors [11,16,26] showed that the severity of the disease does not affect the IVF outcome. In these studies, the oocytes came from different donors and from different cycles; therefore, it could be argued that other factors might have obscured the possible existing differences. The authors' studies obviate this criticism and support the observations of Simón et al [11], because recipients were allocated oocytes provided by the same donor. They have shown that implantation is not affected in patients with advanced stages of endometriosis, which suggests that infertility in these patients is not related to an unsuitable peritoneal or endometrial environment affecting endometrial receptivity.

Women with severe endometriosis who undergo hormonal replacement therapy are as likely to conceive as the controls, which suggests that uterine receptivity is not impaired. The question still to be answered is whether this situation applies for natural cycles or whether the use of gonadotropin-releasing hormone analogs and hormone replacement therapy affects the endometrial milieu of these cycles and does not affect outcome in ovum donation endometriosis.

This research indicates that endometrial alterations previously related in these women must describe the relationship among endometrial characteristics and facilitate endometrial cell attachment and growth instead of a clear interference on the implantation process, which is important to the endometrium of these women [5].

The authors' accumulated experience over the years published elsewhere [6,9], and the results of the current study clearly suggest that severe endometriosis does not affect implantation in ovum donation. The poor IVF outcome in cases with advanced stages of endometriosis may be related to a reduced number of retrieved oocytes, which leads to a reduced number of selected embryos available to be transferred. A strong body of evidence indicates that embryo morphology correlates with implantation rates and IVF success. The better the embryo selection, the better the outcome, despite the presence of endometriosis.

Does endometriosis alter follicular environment?

Reduced oocyte quality can be caused by an altered follicular environment in the ovaries. Different endocrine alterations and a malfunction of the hypothalamic-pituitary axis have been found in women with the disease [28]. To increase knowledge on this topic, many basic studies have focused their efforts on the description of the intrafollicular milieu in women with and without the disease. This microenvironment directly influences the oocyte growth and development via the close relationship with granulosa cells and other ovarian cell types.

Classic works that used in vitro cultures of granulosa cells were able to demonstrate a decrease in the synthesis of Estradiol (E2) and Progesterone (P) in women with endometriosis when compared with women without the disease [28]. Pellicer et al [29], by using a similar design, found contradictory results and demonstrated that an increase in the P production (indicating mature oocytes) was higher in the more severe cases of the disease, whereas E2, androstenedione, and testosterone were not different. This study included 24 patients with different degrees of endometriosis and 26 healthy women as demonstrated by laparoscopy. Other parameters were considered, and there were no differences in terms of follicular volume. The E2/testosterone ratio, which previous works considered to be important in the prognosis of the IVF results, was surprisingly more favorable in patients with endometriosis.

An increase in P accumulation in vitro in the presence of peritoneal fluid from patients with endometriosis [30] indicates that peritoneal fluid may contain factors that stimulate P production and increase the response to human chorionic gonadotropin. The authors believed that three hypotheses could explain the enhanced P accumulation: (1) The enzymatic pathway of ovarian steroids somehow may be altered in women with endometriosis. (2) P may be sequestered within the follicle by its binding proteins, specifically albumin and cortisol-binding globulin [31,32]. (3) The immune cells present within the human ovary throughout the menstrual cycle are involved [33].

Endometriosis is a disease in which the immune system suffers critical alterations. The belief is widely accepted that steroid production can be regulated by many secretory products of the immune cells. The authors have studied granulosa cell function with the previous removal of disturbing white blood cells (a well-known source of cytokines and growth factors) with the help of physical and immunologic treatments [34], focused their studies on the enzyme that catalyzes the production of P (3-β-hydroxysteroid dehydrogenase), and measured its expression and function by competitive polymerase chain reaction and radioimmunoassay, respectively. The measures were made in vivo—in the granulosa cells after purification and Follicular Fluid (FF) to determine basal situations after being influenced by ovarian leukocytes—and in vitro—after purification and 24 hour cell culture—to avoid any interference by the leukocytes [35].

Similar determinations also were made for P main blood carrier protein, the corticosteroid-binding globulin. This molecule is expressed by granulosa cells, is present in FF, and is in charge of the transport of approximately 70% of

blood P. When we compare these parameters (in vivo or in vitro levels of corticosteroid binding globulin and steroid dehydrogenase their gene expression and secretion to the media or follicular fluid) in women with and without endometriosis, no differences were found [35].

This information suggests that previous results were not coherent and that the P ovarian physiology is not impaired in women with endometriosis definitely, at least in the endocrinologic aspects, which discards the two first hypotheses.

The authors completed previous experiments and confirmed some discrepancies with previous results. The reasons for the differences even within the results in the same laboratory are caused by the white blood cell contamination caused by the aspiration procedure, which accounts for 5% to 60% (J. Navarro et al, unpublished data) [36] of the cells present in follicular aspirates.

Some authors have shown an increase in some resident immune cell types, such as monocytes and natural killer cells in endometriosis [37,38], and suggest how FF modulates oocyte development directly or modulating granulosa cells.

By flow cytometry, the authors analyzed the proportion of immune cell subtypes within the follicle to unmask the deficiencies in the adequate oocyte formation [37]. They determined the presence of total CD45+ cells and CD14+ cells, positive indication of CD8, CD3, and CD4, and the quantitative presence of each antigen as a symptom of their activation status. This determination allows the discrimination between granulosa cells and white blood cells. Within white blood cells, different subpopulations can be detected and quantified: monocyte/macrophages, total lymphocytes, T lymphocytes, T-CD3+ lymphocytes, and T-CD3- and 8+ lymphocytes.

No differences in proportion and activity were found in women with endometriosis when flow cytometry experiments were used to determine white blood cell populations in the follicles. This conclusion led to the rejection of the third hypothesis, which explained alterations in the endocrine intrafollicular milieu.

Finally, other non-endocrine factors are able to influence oocyte growth and development. Some autocrine and paracrine factors are able to modulate ovarian function apart from gonadotropins and ovarian steroids. These factors can be secreted by granulosa cells and ovarian leukocytes. Some factors are neoangiogenesis related, such as vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6), which is released in response to IL-1β. They are closely interrelated; IL-6 also has been shown to induce VEGF expression [39]. IL-6 mRNA is expressed during ovarian neovascularization, and detectable levels of IL-6 have been found in human FF [40]. Positive immunostaining for this cytokine has been shown in the thecal compartment of antral follicles and corpora lutea [42]. Neulen et al [41] have shown that VEGF mRNA is expressed in human granulosa-luteal cells.

In the human ovary [42], a gonadotropin-dependent preovulatory induction of IL-lβ transcripts in the theca-interstitial cell layer has been described. The mediation of IL-1β in several ovulation-associated phenomena should be considered.

In a first prospective study, the authors analyzed the IL-1β, IL-6, and VEGF production in women with endometriosis [43]. They studied patients with and without endometriosis in natural cycles and women who underwent IVF. The authors found that endometriosis resulted in a significant increase of IL-6 release in serum, FF, and granulosa cell cultures. There was also a decrease in VEGF accumulation in these patients. All the data agreed with the observation that the immune system may be activated, because the ovary has an increased population of macrophages during the periovulatory period [44]. These results also confirm that ovarian cells produce cytokines [45,46] and function differently in women with endometriosis.

Unfortunately, the authors' research did not permit them to conclude definitely that granulosa cells are the only source of cytokines. Whether an enhanced IL-6 production is a marker of altered follicular function that results in oocytes and embryos of lower quality has yet to be determined.

An increase in the release of several peritoneal fluid vasoactive substances was initially reported. Specifically, peritoneal fluid concentrations of IL-1β [47,48], IL-6 [49,50], and VEGF [51,52] have been shown to be increased in the presence of endometriosis. The authors' observations agree with the findings of Machelon et al [46], who showed an increased P accumulation in vitro by human granulosa cells as increasing concentrations of IL-6 were added to the culture medium. FF VEGF concentrations were proved to be significantly lower in women with endometriosis. The significance of this finding requires further investigation, but elevated VEGF levels have been correlated with a healthy follicular vascular network [53].

The authors undertook another study to confirm their previous results: depleting contaminating white blood cells before they studied intracellular IL-6 and VEGF by means of flow cytometry and the gene expression by means of competitive polymerase chain reaction [54]. They found no differences in the paracrine aspects of women with and without endometriosis, contrary to what they initially described.

Recent studies implicate other paracrine modulators, such as IL-8, IL-1β, and tumor necrosis factor-α in FF [55]. Only tumor necrosis factor-α presents itself differently in women with endometriosis. Apoptosis also has been related to ovarian function and oocyte quality [56]. These same authors correlated the total account of apoptotic bodies with the stage of the endometriosis; significant differences were found in the number of oocytes and the fertilization rate [57].

Currently, no definitive conclusions about follicular microenvironment can be drawn.

Summary

Prospective and retrospective clinical trials suggest a decreased oocyte and embryo quality in women with endometriosis. Based on these observations, the

authors described an altered intrafollicular milieu in endometriosis, which explains the bad quality oocytes and the resulting embryos with lower capacity to implant. Whether these changes affect the oocytes or are the consequence of genomic alterations manifested by biochemical and chromosomal differences in healthy women is an unresolved issue. If the effects of endometriosis on follicular development are nongenomic in origin, modulation of the process of folliculogenesis may be sufficient to treat the disease and cure infertility associated with endometriosis. A genomic defect needs specific genetic therapy, which currently is not available.

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30 (2003) 193–208

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Management of endometriosis-associated infertility

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The origin of the infertility associated with endometriosis has been reviewed in detail elsewhere in this issue (see the article by Pellicer). In patients with more advanced disease, anatomic distortion and pelvic adhesions may play a primary role. The pathogenesis of infertility in patients with minimal or mild endometriosis in the absence of mechanical distortion is more controversial. A host of alterations within the immunologic milieu of the peritoneal cavity creates a ''hostile'' environment for successful gamete interaction and early embryo development [1,2]. Other researchers have proposed that endometrial receptivity may be inhibited in patients with minimal or mild endometriosis and in otherwise unexplained infertility [3,4].

Several approaches have been used to achieve conception in this group of patients, with varying degrees of success (Box 1). In this article the authors critically review the data associated with each.

Expectant management

Before evaluating the benefit of any specific therapies, it is important to assess the likelihood that a patient with endometriosis will conceive in the absence of any intervention. Clearly, any such analysis depends on the extent of mechanical distortion and tubal obstruction. As would be expected, the likelihood of spontaneous pregnancy in patients with severe disease is limited. Olive et al reported no pregnancies in such a group [5].

When other stages of endometriosis are considered, the potential for spontaneous conception is slightly more encouraging. Olive et al reported a monthly

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Box 1. Management options for endometriosis-associated infertility

- Expectant
- Surgical resection/ablation
- Ovarian suppression
- Combination surgical and medical therapy
- Controlled ovarian hyperstimulation ± intrauterine insemination
- Assisted reproductive technologies

fecundity rate (MFR) of 4.7% among patients with endometriosis who did eventually conceive [5]. Bérubé et al evaluated 168 infertile patients with endometriosis who were managed expectantly in a prospective multicenter cohort study [6]. The MFR was 2.52 per 100 person months, which was not significantly different than a similar group of 263 women with unexplained infertility. Hull et al reported cumulative pregnancy rates of 55% in 56 patients with stage I and II endometriosis followed expectantly for at least 18 months [7]. Others have described MFRs ranging from 0.14 to 0.45 in similar groups [8–10].

The data suggest that infertile patients without significant anatomic distortion who have endometriosis are capable of conceiving, albeit at a significantly compromised rate compared to the general population. This finding is important not only regarding patient counseling but also regarding our ability to interpret the benefit of therapeutic interventions. Because patients with endometriosis are able to conceive spontaneously, studies that are not controlled become difficult to interpret and are of somewhat compromised value.

Surgical therapy

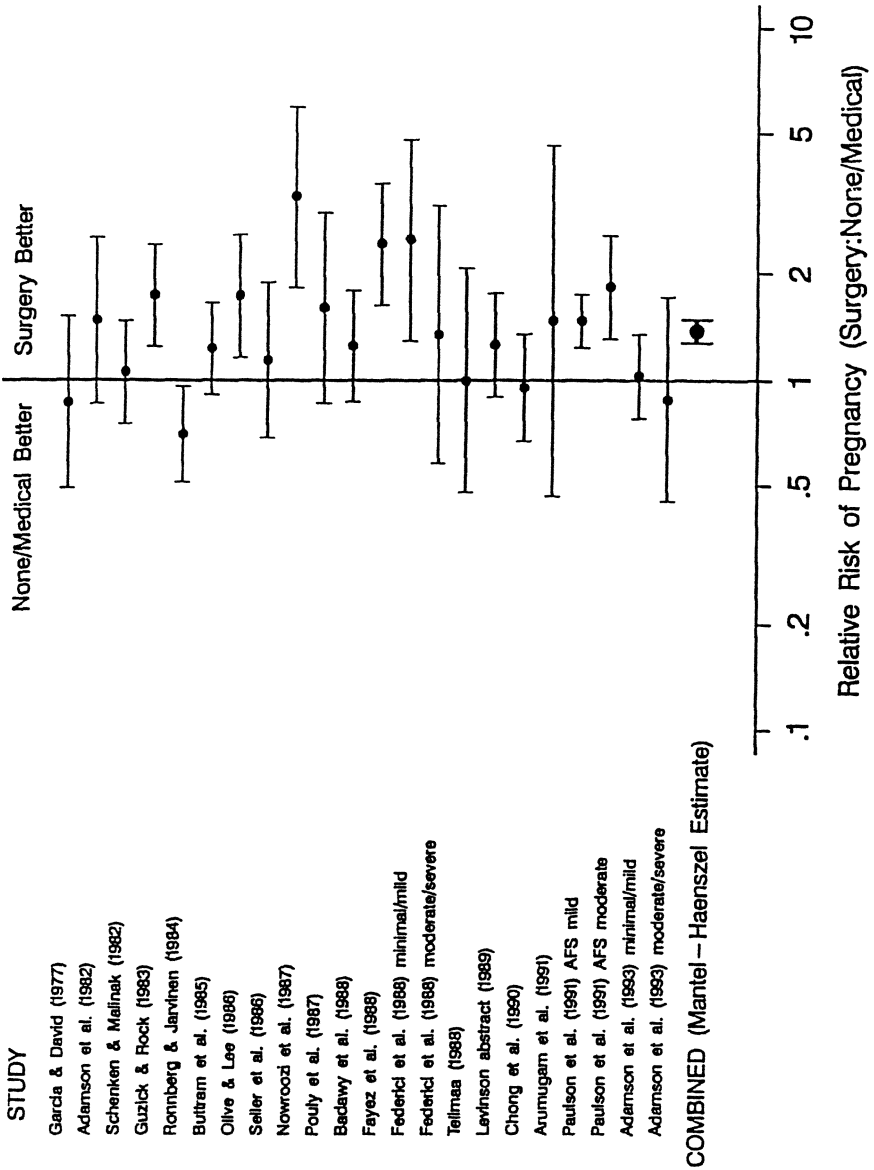
Surgical management represents the standard approach toward overcoming endometriosis-associated infertility. Miller et al have demonstrated in a murine in vitro fertilization (IVF) model that laser laparoscopy reversed the inhibitory effects of serum from infertile women on fertilization and embryo development rates [5]. Closer reading of the literature provokes a series of unanswered questions, however. Few of the clinical studies are controlled. Only a handful of investigators provide more than crude pregnancy rates, with lack of description of the lengths of follow-up and calculation of fecundity rates to allow for meaningful analysis. Technique clearly differs among surgeons. There is little description as to whether lesions were completely or partially resected. More significantly, the recognition of so-called ‘‘atypical’’ lesions has increased dramatically in the last 10 years, which suggests that early investigators may have unknowingly failed to resect all disease. The goal of surgery is not only to

resect all visible endometriotic lesions but also to restore and maintain normal anatomic relationships. Meticulous tissue handling, pin-point hemostasis, and use of appropriate adhesion prevention agents are critical.

The overall likelihood of achieving conception after surgical intervention has been calculated using several mathematical models. In a metaanalysis that did not control for stage, Adamson and Pasta reported a crude pregnancy rate for all surgical interventions to be 38% higher than either medical therapy or non-intervention [11] (Fig. 1). Most pregnancies occurred within 1 to 2 years of surgery. Similarly, Hughes et al evaluated one ''quasi-randomized'' and five cohort trials that compared laparoscopic surgery to no treatment or medical therapy [12]. The common odds ratio was 2.67, which implied a beneficial effect, but the heterogeneous nature of the study designs makes interpretation difficult. Guzick reported an overall pregnancy rate of 56% after laser laparoscopy, which resulted in an MFR of 9.7% [13].

The relative value of laparoscopy in comparison to laparotomy has not been evaluated in prospective randomized trials. In metaanalysis of eight trials, Adamson and Pasta revealed no significant difference between the techniques (Relative Risk (RR) 0.93, 95% confidence index [CI] 0.84–1.02) [11]. Either approach yielded significantly better outcomes than no therapy or medical therapy alone, however. One of the difficulties in interpreting these data is the fact that most of the laparotomies would have been performed for more extensive disease. Using a life-table analysis, Adamson et al previously noted no difference in cumulative pregnancy rates between laparoscopy and laparotomy in patients with minimal or mild disease at 3 years postoperatively (67.8 ± 4.1 vs. 74.3 ± 8.1%, respectively) but significantly higher pregnancy rates when laparoscopy was used in patients with moderate or severe disease (62.2 ± 6.2% vs. 44.4 ± 5.6%, respectively) [14]. Using a cost analysis of 120 patients with moderate and severe disease, Luciano et al noted significantly decreased total cost of medical care for laparoscopy versus laparotomy ($223,260 vs. $424,000; $P < 0.001$), along with significantly lower number of total days of incapacitation (216 vs. 1284; $P < 0.001$) [15].

Management of infertility caused by moderate-to-severe endometriosis has not been evaluated in well-controlled trials. Differences in the technique of the individual surgeon, completeness of resection, and use of adhesion prevention agents represent critical variables that make analysis of data challenging at best. In an evaluation of conservative laparoscopic surgery for severe endometriosis-related infertility, Candiani et al reported a crude pregnancy rate of 47.6% in 206 patients evaluated in 15 studies [16]. The MFR ranged from 2.1% to 3.3% in these trials, however, which was a less impressive but more accurate statistic. Luciano et al described outcomes in 60 patients with stage III and IV endometriosis and noted a 70% cumulative pregnancy rate after surgery, but with an MFR of only 6.7% [15]. More recently, Busacca et al prospectively followed a group of women who underwent laparoscopic surgery for stage III-IV endometriosis using resection or ablation with bipolar cautery for a minimum of 6 months postoperatively [17]. Most patients had stage III disease. The cumulative pregnancy rate at 24 months was 57.5% (MFR 2.4%).


STUDY
None/Medical Better
Surgery Better
Garcia & David (1977)
Adamson et al. (1982)
Schenken & Malinak (1982)
Guzick & Rock (1983)
Ronnberg & Jarvinen (1984)
Buttram et al. (1985)
Olive & Lee (1986)
Seller et al. (1986)
Nowroozi et al. (1987)
Pouly et al. (1987)
Badawy et al. (1988)
Fayez et al. (1988)
Federici et al. (1988) minimal/mild
Federici et al. (1988) moderate/severe
Tellmaa (1988)
Levinson abstract (1989)
Chong et al. (1990)
Arumugam et al. (1991)
Paulson et al. (1991) AFS mild
Paulson et al. (1991) AFS moderate
Adamson et al. (1993) minimal/mild
Adamson et al. (1993) moderate/severe
COMBINED (Mantel–Haenszel Estimate)
.1
.2
.5
1
2
5
10
Relative Risk of Pregnancy (Surgery:None/Medical)

The management of endometriomas is somewhat controversial. Drainage of these cysts is associated with a high rate of recurrence, which approaches 50% [18–20]. One trial that used a 3-month follow-up period and postoperative danazol provided more positive results [21]. In contrast, excision, drainage, and either ablation or resection of the cyst wall are generally associated with lower recurrence and encouraging pregnancy rates. Wood et al studied 52 patients who were treated in this fashion and reported a 9.6% recurrence rate and 50% pregnancy rate in 1 year [22]. Using various laser modalities, Daniell et al reported a 37.5% pregnancy rate in 32 patients [23]. More recently, Jones and Sutton reported outcomes in 39 women—28 of whom had stage IV disease—with endometriomas treated laparoscopically [24]. The cysts were opened and drained, and the capsules were ablated with Potassium-Tridenterium-Phosphate (KTP) laser or bipolar cautery. The cumulative pregnancy rate over 12 months was 39.5% (39.3% in stage IV patients), with all pregnancies occurring within 9 months of surgery. It is important to avoid becoming overly aggressive during ovarian surgery in an effort to prevent adhesion formation and disruption of the ovarian blood supply. Once again, an absence of randomized trials comparing treating modalities hampers data evaluation.

Patients without anatomic distortion who suffer from infertility and minimal-to-mild endometriosis also seem to benefit from surgical therapy. Two recent prospective randomized trials addressed this issue. Marcoux et al reported the results of a large multicenter trial that evaluated 241 infertile women with minimal-to-mild endometriosis (ENDO-CAN) [10]. During diagnostic laparoscopy, patients were assigned randomly to laparoscopy alone or ablation/resection of implants. Patients were subsequently followed for up to 36 weeks postoperatively and through 20 weeks of pregnancy. Pregnancy rates were significantly higher in the surgery group (30.7% vs. 17.7%, respectively). The MFR also was significantly higher (4.7% vs. 2.4%, respectively). These findings have been confirmed by earlier trials [25–30]. Based on further analysis of the ENDO-CAN trial, however, Taylor and Olive calculated that one would need to perform 6.7 laparoscopic surgeries in women with early stage endometriosis to achieve a single pregnancy [31].

One also should note that in a prospective randomized multicenter Italian trial with a similar design to that of the ENDO-CAN study, no differences in birth rates were appreciated between women who underwent resection/ablation (19.6%) or no treatment (22.2%) [32]. It is possible that the differences between the two studies may be the result of a lower number of patients in the Italian trial with a decreased power to detect a difference between the groups. Alternatively,

Fig. 1. Results of a metaanalysis of trials comparing surgical versus nonsurgical (medical suppression or no treatment) therapy in endometriosis-related infertility. (*From* Adamson GD, Pasta D. Surgical treatment of endometriosis-associated infertility: meta-analysis compared with survival analysis. Am J Obstet Gynecol 1994;171:1488–505; with permission.)

this trial evaluated birth rates, whereas the ENDO-CAN trial evaluated fecundity rates alone.

The authors are aware of a single study that compared the effects of laparoscopic excision versus ablation in the management of infertility associated with mild endometriosis. Tulandi and al-Took evaluated 53 women treated with excision and 48 historic controls treated in the same center with electrocoagulation [33]. Pregnancy rates were similar between the groups (53.5% vs. 57.1%, respectively), as were the median intervals between surgery and conception.

Medical therapy

The efficacy of various medications on the suppression of symptomatic endometriosis has been well established. In contrast, the efficacy of progestins, danazol, or GnRH agonists when used as primary therapy to enhance fertility in these patients has not been demonstrated. Hughes et al evaluated data from nine trials that compared ovulation suppression with either danazol, gestrinone, or medroxyprogesterone acetate to no treatment or placebo, which all failed to show any beneficial effect on enhancing pregnancy rates (common odds ratio 0.85; 95% CI 0.95–1.22) [12]. In the same study, an additional six randomized trials that compared a gonadotropin-releasing hormone (GnRH) agonist, gestrinone, or an oral contraceptive to danazol also failed to demonstrate any differences (common odds ratio 1.07; 95% CI 0.71–1.61) [12]. These findings were confirmed by Adamson and Pasta in a separate metaanalysis [11]. The investigators recommended that medical therapies should not be used as a treatment of infertility associated with asymptomatic endometriosis.

There are several possible explanations for these findings. One could propose that minimal-to-mild endometriosis has no impact on fertility given the proven efficacy of these agents in treating the underlying disease but lack of efficacy in improving conception. A second explanation is that the mechanism of infertility associated with endometriosis is different from that associated with pelvic pain and is unaffected by these medications. Neither of these explanations can be supported by data. Several investigators have demonstrated that danazol and GnRH agonists may have a positive impact on peritoneal cytokine levels, natural killer cell activity, metalloproteinase-1 tissue inhibitor concentrations, and endometrial cell apoptosis [34–37].

A third—and perhaps more plausible—explanation may be that by the time a patient resumes normal ovulatory patterns, which may be months after completion of therapy, the deleterious effects of the disease process on fertility that were suppressed initially by medications recur even if the patient remains asymptomatic. If a patient could attempt conception when the disease process is maximally suppressed, pregnancy rates would be heightened. The successful use of prolonged GnRH agonist therapy immediately before IVF would confirm this hypothesis [38].

Combined medical and surgical therapy

The impact on conception of combining surgical resection or ablation with medical therapy administered either preoperatively or postoperatively has been evaluated. Unfortunately, most studies are nonrandomized, which creates a high degree of selection and inclusion bias. Hughes et al evaluated five older cohort studies that compared laparoscopic surgery plus danazol to danazol alone [12]. The common odds ratio for this group was 1.42 (95% CI 0.94–2.14), which suggested no benefit of adjunctive danazol therapy. A similar finding was noted in patients who underwent laparotomy. Telimaa et al reported the results of a placebo-controlled trial that compared postoperative medroxyprogesterone acetate to danazol after conservative surgery [39]. Although only a small subset of patients in each group attempted pregnancy, the conception rates were similar among the three treatment groups.

Donnez et al prospectively evaluated 126 infertile women with ovarian endometriosis that was resected microsurgically at laparotomy who were treated with preoperative danazol, gestrinone, or the GnRH agonist buserelin in a nonrandomized trial [40]. The cumulative pregnancy rates after 18 months of follow-up in patients treated with buserelin (58%) were significantly higher ($P < 0.05$) than those treated with danazol (45%) or gestrinone (47%). Two randomized, placebo-controlled trials evaluated the effect of either 3 or 6 months postoperative GnRH agonist therapy [41,42]. Although pain relief was prolonged with 6 months of postoperative medical therapy, no difference in pregnancy rates was appreciated in either study. It is important to note that the primary endpoints in these studies were symptom recurrence and not fertility, which may have created a degree of selection bias.

The preponderance of data suggests that preoperative or postoperative adjunctive medical therapy adds little to the benefit achieved with surgery alone in overcoming endometriosis-associated infertility in the asymptomatic patient.

Controlled ovarian hyperstimulation

Bérubé et al have reported that the fecundity of women with minimal or mild endometriosis is similar to that of women with unexplained infertility [6]. Several investigators who have attempted to treat these patients in a similar fashion with controlled ovarian hyperstimulation with or without intrauterine insemination have reported varying degrees of success. The caveats for proceeding with this approach are that either the patient with endometriosis has inherently normal pelvic anatomy or that anatomic relationships have been restored to normal. A male factor and decreased ovarian reserve also should be ruled out.

Two studies that primarily address the use of clomiphene citrate have been reported. Simpson et al undertook a prospective nonrandomized trial of clomiphene use alone and described an odds ratio for pregnancy in comparison to untreated controls of 2.9 (95% CI 1.2–7) [43]. Deaton et al published a

prospective randomized crossover trial of clomiphene and intrauterine insemination versus no treatment that combined couples with unexplained infertility and surgically corrected endometriosis [44]. Using life-table analysis, the MFR in the treated group (0.095) was significantly higher than that of the untreated group (0.033). There were no differences in outcome between the 27 patients with endometriosis and the 24 with unexplained infertility.

The benefit of gonadotropin therapy in this patient population also has been explored in two well-designed prospective randomized trials. Fedele et al reported on 49 patients with stage I or II endometriosis randomized to human menopausal gonadotropins and human chorionic gonadotropin for three cycles versus expectant management for six cycles [9]. The cycle fecundity was significantly greater in the treated group (0.15% vs. 0.045%; $P < 0.05$). The

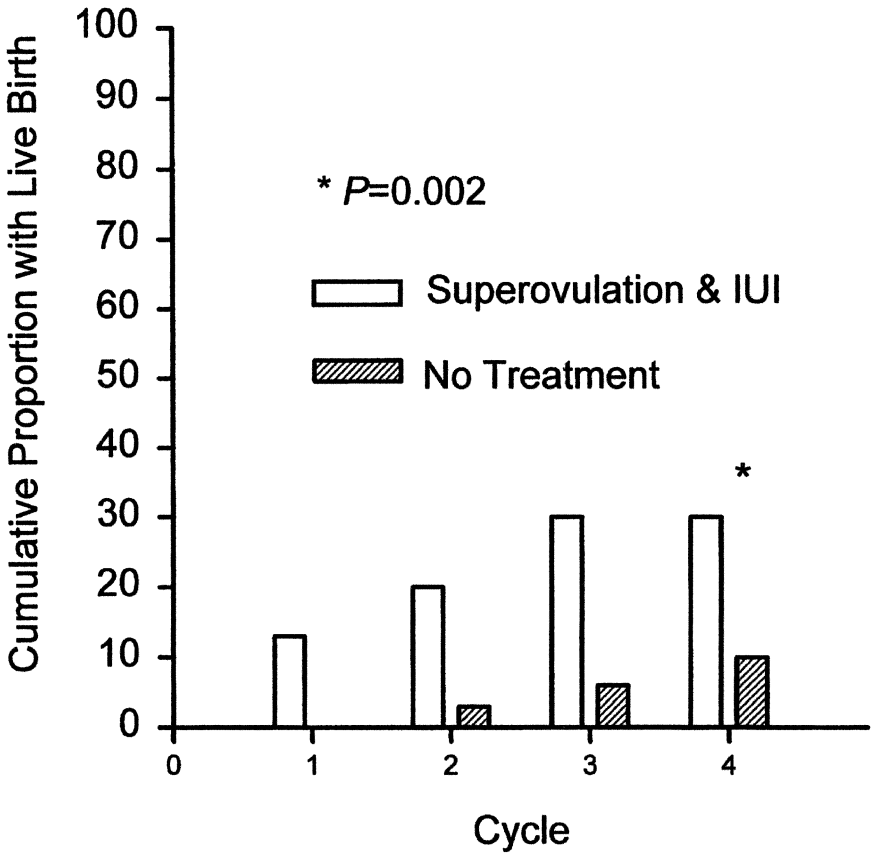


Fig. 2. Cumulative proportion of endometriosis patients with live births after undergoing superovulation and intrauterine insemination versus expectant management. (*From* Tummon IS, Asher LJ, Martin JSB, Tulandi T. Randomized controlled trial of superovulation and insemination for infertility associated with minimal or mild endometriosis. Fertil Steril 1997;68:8–12; reprinted with permission from the American Society for Reproductive Medicine.)

cumulative pregnancy rates were not significantly different, however (37.4% vs. 24%). In a similar study design, Tummon et al randomized 103 couples to urinary follicle-stimulating hormone, human chorionic gonadotropin, and intrauterine insemination or expectant management for four cycles and reported a similarly superior outcome with controlled ovarian hyperstimulation (odds ratio 5.6; 95% CI 1.8–17.4) [45]. All of the pregnancies during therapy occurred within the first three cycles (Fig. 2). In contrast, Serta et al reported that the addition of controlled ovarian hyperstimulation had little additional impact over that achieved with intrauterine insemination alone in a 3-month trial in 50 patients with minimal endometriosis [8]. In a metaanalysis of 962 cycles of controlled ovarian hyperstimulation with intrauterine insemination in patients with a primary diagnosis of endometriosis, Peterson et al reported pregnancy rates per cycle of 15% in patients with stage I and II disease and 8% for stage III and IV disease [46]. These statistics are similar to those reported by Bérubé for untreated patients with minimal disease [6].

The use of controlled ovarian hyperstimulation with or without intrauterine insemination may be beneficial for a short (3-month) course of therapy in patients with endometriosis who have normal pelvic anatomic relationships and in the absence of a significant male factor or decrease in ovarian reserve before considering more aggressive approaches.

Assisted reproductive technologies

The assisted reproductive technologies, in particular IVF, theoretically should maximize fertility rates by removing gametes and zygotes from the "hostile" peritoneal environment and bypassing abnormal pelvic anatomy associated with endometriosis. This hypothesis has been borne out by a host of investigators. Geber et al reported an overall pregnancy rate of 40% after IVF in 140 patients with endometriosis, which was no different than three groups of controls with male factor, tubal factor, or unexplained infertility [47]. Olivennes et al reported delivery rates per embryo transfer of 30% in 360 cycles performed on 214 patients with endometriosis in comparison to 37.5% in 166 cycles performed on 111 controls with tubal factor infertility, a difference that was not statistically significant [48]. These data have been confirmed by other researchers [49]. In contrast, several studies have reported lower fertilization, implantation, or pregnancy rates in patients with endometriosis who underwent IVF in comparison to controls [50–53]. In these latter studies, pregnancy and implantation rates also were somewhat compromised in the control groups. Pregnancy rates all remain significantly higher than those achieved with other forms of therapy.

The impact of the stage of endometriosis on assisted reproductive technology cycle outcome also has been evaluated. Several large investigations have demonstrated that the severity of disease had no effect on the outcome of IVF or on the incidence of pregnancy loss [47–49]. Guzick et al reported that overall pregnancy rates in patients with endometriosis who underwent gamete intrafallopian transfer

procedures were lower than controls with unexplained infertility [54]. No differences were noted between groups of patients with endometriosis based on the stage of the disease. A relatively small group of patients with moderate and severe disease combined were included in this study, however, because of the fact that it is unlikely that patients with significant pelvic adhesions and tubal distortion would be candidates for gamete intrafallopian transfer.

Earlier trials reported significantly lower pregnancy rates after IVF in patients with more advanced disease [55,56]. It is important to note that in these trials, oocytes were obtained laparoscopically. Dense pelvic adhesions and ovarian disease may have limited significantly the ability to aspirate oocytes effectively, which compromised outcome. More recently, Azem et al noted reduced fertilization, pregnancy, and birth rates per cycle in 58 patients with stage III and IV endometriosis in comparison to 60 controls with tubal factor infertility [57]. No comparisons were made to patients with less extensive disease, however. It is important to note that delivery rates were low in both of the groups (6.7% vs. 16.6%, respectively). Pal et al reported that although fertilization rates were significantly lower in patients with stage III and IV in comparison to stage I and II endometriosis, implantation, clinical pregnancy, and miscarriage rates were similar between the groups [58]. Diaz et al essentially ruled out an implantation effect in these patients by using an elegant case-control design [59]. Oocytes derived from a single donor were shared between recipients who had been diagnosed laparoscopically with stage III-IV endometriosis and infertile control patients who were free of disease. Implantation, miscarriage, and live birth rates were similar between the two groups.

The effect of ovarian endometriotic cysts (endometrioma) on IVF outcome also has been addressed. Al-Azemi et al described a decrease in ovarian response that required the use of higher gonadotropin doses in patients with such lesions [60]. Cumulative pregnancy and live birth rates were unaffected, however. An earlier trial reported uniformly poorer outcomes in all parameters, although the results may have been colored by the effects of laparoscopic oocyte retrieval and small numbers of patients evaluated [61]. Yanushpolsky et al reported a higher incidence of pregnancy loss and an adverse effect on number of oocytes retrieved with transvaginal ultrasound-guided techniques and embryo quality in patients with endometriosis [62]. In contrast, Olivennes et al demonstrated no effect of persistent endometriomas on any outcome parameter of either controlled ovarian hyperstimulation or IVF [48]. Unfortunately, none of these investigators has correlated endometrioma size with outcome. Similarly, it is difficult to differentiate the effect of an isolated endometrioma per se on cycle outcome because patients with these lesions may have varying extents of concomitant peritoneal disease that may represent a confounding variable. At least one group of investigators suggests that limited inadvertent exposure of oocytes to endometrioma fluid does not seem to have a significant impact on fertilization rates or early embryo development [63]. It is only logical, however, to make every effort to avoid placing the aspirating needle through an endometrioma during oocyte retrieval procedures to prevent rupture and inadvertent exposure if at all possible.

The effect of surgical resection of endometriomas before IVF also has been evaluated. Canis et al reported the outcome of a series of 41 patients who underwent precycle laparoscopic resection of large (> 3 cm in diameter) ovarian endometriotic cysts (unilateral in 30 patients and bilateral in 11 patients) in comparison to 139 controls with endometriosis but without endometriomata and 59 additional controls with tubal infertility [64]. No differences regarding the resulting number of oocytes or embryos were described despite extensive ovarian surgery. In contrast, Loh et al reported reduced follicular response in natural and clomiphene-stimulated cycles but no effect on ovarian response after gonadotropin stimulation in a retrospective report of 40 patients with ovarian endometriotic cysts of mean diameter 4.23 ± 2.2 cm who underwent precycle resection [65]. More recently, Donnez et al reported on 85 patients (187 cycles) who underwent laparoscopic cyst wall vaporization of ovarian endometriomas before IVF and compared responses to 289 patients (633 cycles) with tubal factor infertility [66]. Response to stimulation and clinical pregnancy rates were similar between the groups. Resection of large lesions clearly enhances access to follicles within underlying normal ovarian tissue and eliminates the potential for rupture during oocyte aspiration. Meticulous surgical technique with an eye toward carefully avoiding compromise of ovarian blood supply and destroying healthy ovarian tissue is mandatory, however.

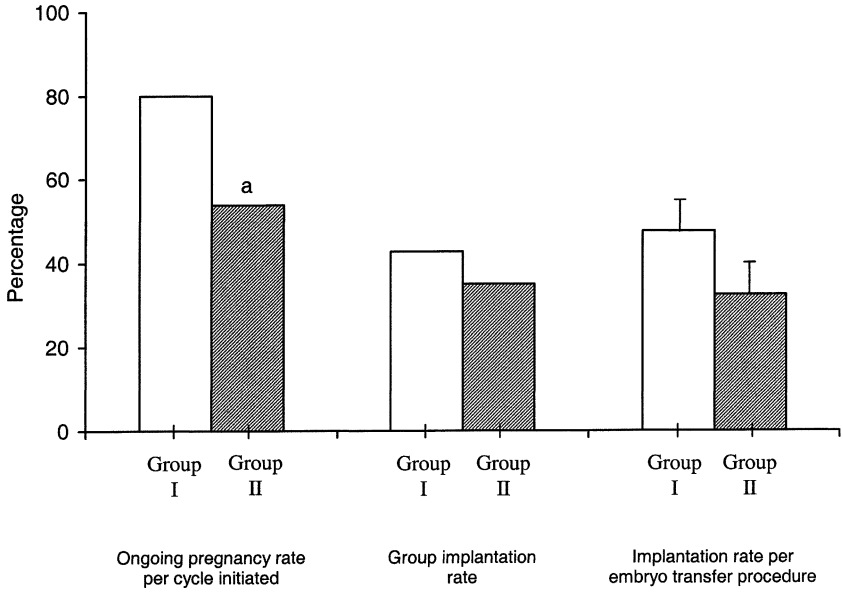


Fig. 3. IVF cycle outcomes for patients with endometriosis who were pretreated with a GnRH agonist for 3 months (group I) immediately before controlled ovarian hyperstimulation or undergoing standard controlled ovarian hyperstimulation (group II). $P < 0.05$ versus group I (*a*). (*From* Surrey ES, Silverberg KM, Surrey MW, Schoolcraft WB. The effect of prolonged GnRH agonist therapy on in vitro fertilization-embryo transfer cycle outcome in endometriosis patients: a multicenter randomized trial. Fertil Steril 2002;78:699–704; reprinted with permission from the American Society for Reproductive

Given the benefit of surgical management of endometriosis on achieving spontaneous conception, the question of whether such intervention in the absence of ovarian endometriomata would enhance IVF cycle outcome has been addressed. In a prospective randomized trial, Surrey and Hill reported that although laparoscopic CO_2 laser ablation of endometriosis at the time of gamete intrafallopian transfer had no effect on cycle outcome, pregnancy rates in subsequent cycles of patients who failed to conceive from gamete intrafallopian transfer were significantly higher than in controls with endometriosis who underwent gamete intrafallopian transfer alone [67]. The authors recently reported that controlled ovarian hyperstimulation and IVF cycle outcomes were similar between groups of patients with endometriosis who underwent surgical resection within 6 months or longer than 6 months to 5 years of oocyte aspiration (ongoing pregnancy rates 63.6% vs. 60.53%, respectively) [68]. Regression analyses demonstrated no effect of the time interval between surgery and oocyte aspiration on implantation rates. It seems that the described benefit derived from such surgery in enhancing spontaneous conception may be masked by the greater impact on implantation and pregnancy achieved with the assisted reproductive technologies.

The authors previously discussed the fact that medical therapy for endometriosis has little impact on enhancing spontaneous pregnancy rates despite beneficial effects on symptomatic disease and the peritoneal environment. If the negative effect of this disease process on fertility returns rapidly after discontinuation of medication, however, then one could hypothesize that any benefits of medical suppression on enhancing fertility would be most evident if pregnancy could be achieved during a time of maximal suppression. This could occur only with the use of assisted reproductive technologies.

In a prospective randomized trial, Surrey et al recently evaluated the effect of a 3-month course of a GnRH agonist administered immediately before IVF in patients with surgically confirmed endometriosis [39]. Significantly higher ongoing pregnancy rates with a trend toward higher implantation rates were appreciated in this group of 25 patients in comparison to 26 controls with endometriosis treated with standard controlled ovarian hyperstimulation techniques before oocyte aspiration in the absence of prolonged GnRH agonist (Fig. 3). These findings have been confirmed by other researchers [69–72]. This may be a result of a beneficial effect of these agents on either peritoneal cytokine levels or endometrial markers of implantation [35,38,73].

Summary

Management of infertility associated with endometriosis remains challenging. The clinician must rule out all other causes of infertility before creating a treatment plan. It is important to remember that women with infertility and endometriosis with tubal patency can conceive spontaneously, albeit at lower rates than in the fertile population. Surgical ablation or resection seems to provide benefit even in the absence of correctable anatomic defects. One should note,

however, that the goal of surgery is not only to eliminate disease effectively but also to restore pelvic anatomy to normal. After reconstruction or in patients with less extensive disease, controlled ovarian hyperstimulation techniques potentially in conjunction with intrauterine inseminations can be effective. It is important to monitor patients carefully given the risk of high order multiple gestation reported with these techniques. IVF represents an effective means of bypassing the hostile peritoneal environment and anatomic distortion associated with this disease state. Although medical suppression of endometriosis alone has virtually no benefit in the asymptomatic patient, there seems to be significant benefit of pretreatment with GnRH agonists immediately before IVF cycle initiation. Whether only a specific subset or all patients with endometriosis would benefit from this approach has not yet been determined. The use of endometrial implantation markers may be helpful in this regard.

The selection of the most effective approach to overcome infertility must be individualized and based on extent of disease, additional infertility factors, patient comfort, and a frank discussion of success rates and risks with patients.

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Relieving endometriosis pain: why is it so tough?

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Chronic pain is a major problem in the United States [1–3]. According to the American Pain Society, pain is the most common reason that individuals seek medical attention, and it is widely undertreated. Women with chronic pain from endometriosis or adhesions secondary to endometriosis face additional hurdles on their way to relief, however. Surveys conducted by the Endometriosis Association about pain from endometriosis and adhesions and the stories of women in pain shed some light on the nature of endometriosis pain and the problems with treatment.

Treating pain is a pain: strike one

Attitudes—such as the fear of treating and being treated aggressively for pain because of perceptions of dangers of pain medications (legal, social, and physical)—and a skewed sense of machismo or courage that causes us to believe that only weak people experience pain or that tough people do not need relief but ''tough it out'' combine to perpetuate undertreatment. In response to the fear of addiction, many physicians are understandably wary of aggressively treating pain with narcotics, and patients may be overly wary of taking prescription medication out of fear of becoming addicted [2]. In much of their literature, the American Pain Foundation proposes that it is unwise to allow the behavior of deliberate drug abusers to dictate medical treatment of pain.

Viva la difference: strike two

Why is it that 72% of chronic pain sufferers are women? A mix of biologic, psychological, and attitudinal factors probably explains the higher incidence of

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chronic pain and its undertreatment in women [3]. A report in the *Journal of Law, Medicine and Ethics* in 2001 entitled "The Girl Who Cried Pain: A Bias Against Women in the Treatment of Pain" stated that women's reports of pain are taken less seriously than men's, and they receive less aggressive treatment. It is also likely that women experience pain differently than men. Women's pain actually may be more intense. Research presented to the US National Institutes of Health, Gender and Pain meeting in 1998 suggested that hormones likely play a role. Women are more sensitized to some pain during the premenstrual period rather than the postmenstrual period, and higher estrogen levels were associated with heightened sensitivity to temperature [3].

Women also experience certain diseases that produce chronic pain more frequently than men. Women experience fibromyalgia nine times more frequently than men (many of these women may have endometriosis) and experience migraines more than twice as often. Women are also more vulnerable to arthritis and temporomandibular disorders. Of the leading causes of chronic pain, only back pain affects men as often as it does women. A collaborative study between the National Institutes of Health and the Endometriosis Association showed a higher incidence of fibromyalgia, rheumatoid arthritis, multiple sclerosis, chronic fatigue immune dysfunction syndrome, and other autoimmune diseases in women with endometriosis than women in general, so women with endometriosis are more likely to have multiple sources of chronic pain [4]. If a woman says to her doctor, "My pelvic pain is terrible, my hands hurt, and my legs and shoulders are killing me," the doctor may be tempted to believe that the woman is exaggerating, although she may be suffering from arthritis, fibromyalgia, and endometriosis.

Attitudes that women cannot handle "normal" pain, that they are hysterical, and that their pain is exaggerated abound and play a role in the treatment of their pain [3]. Perhaps because physicians encounter more women complaining of chronic pain, they may assume that many of them must be exaggerating. Another factor may be that women are more expressive of their pain or express pain in ways that physicians interpret as overly dramatic and find suspect. In our culture, it is more acceptable for women to talk about pain, but this might not be to their advantage when it comes to convincing a physician to treat it [3]. The American Chronic Pain Association, Partners Against Pain, the Endometriosis Association, and most patient organizations encourage patients to discuss symptoms openly with physicians and be active partners in health care. It only makes sense. There seems to be a fine line, however, between adequately describing pain and having it discounted as emotional, psychogenic, or not real. Exactly where that line is located differs from day to day, from physician to physician, from patient to patient, and from pain to pain.

> "Physicians often think that pain is either in your body or in your mind and that's not true. It's *always* in the body and it's *always* in the mind, and we need to integrate our treatment approaches." — Deborah Metzger, MD, PhD [5].

To muddy the waters even more, there are gender differences in the efficacy of pain treatments. Research from the University of California, San Francisco,

suggests that pain medications are metabolized differently by men and women and some medications work on some types of pain better than others [3, 6]. This difference becomes an even more complex issue in the instance of endometriosis, because the pain is experienced in a wide variety of locations and times (Fig. 1).

> "Women with the same amount, the same lesions, of the same size of endometriosis have different experiences of pain." — Paolo Vercellini, MD [5].

Just some "female trouble": strike three

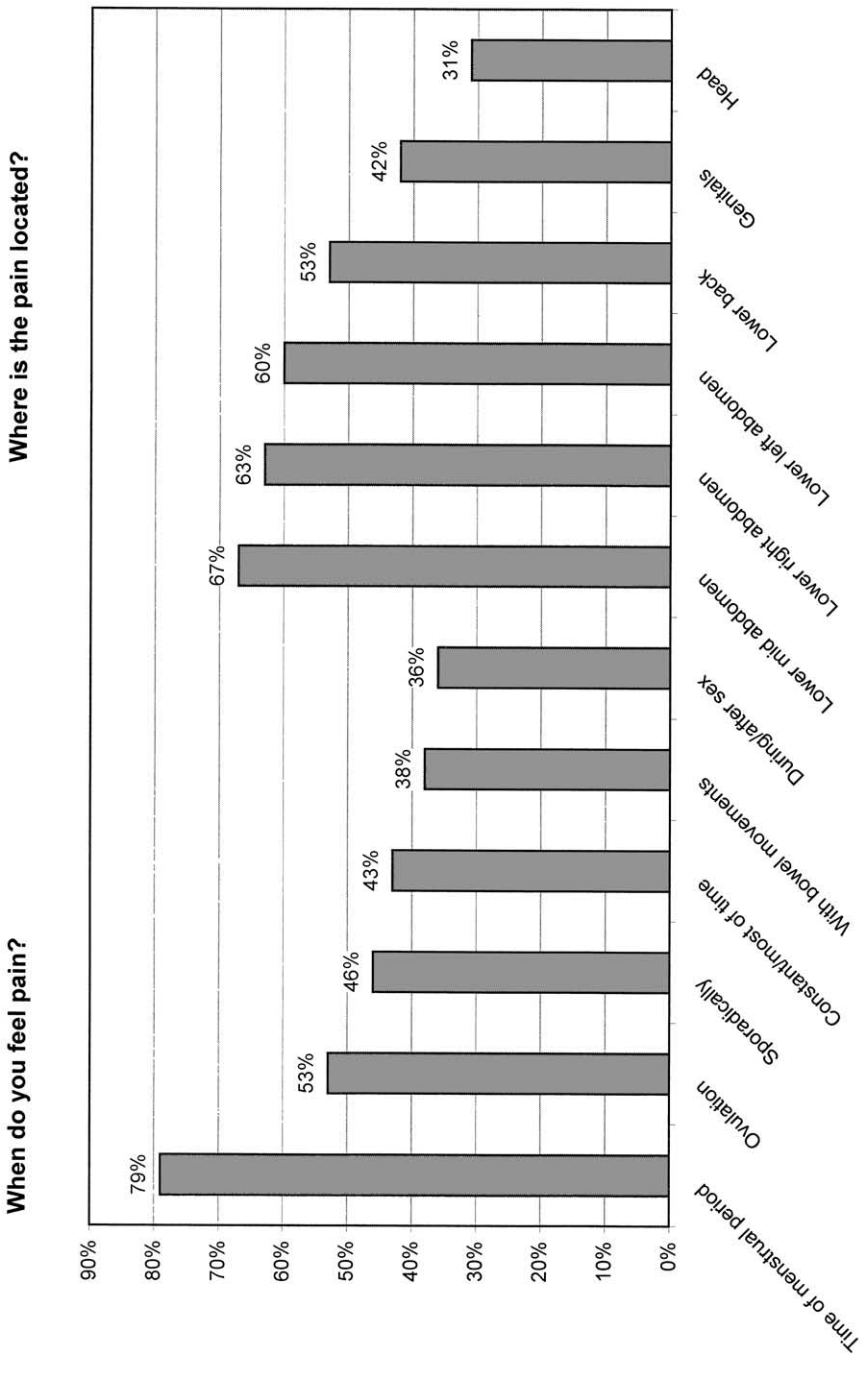
When chronic pain is caused by endometriosis or adhesions secondary to endometriosis or its treatment, it may be even more undertreated. Misinformation and stigma associated with the symptoms of endometriosis and with chronic pelvic pain seem to be major factors in this common, potentially disabling, painful disease.

The Endometriosis Association maintains a database of more than 7000 North American women with endometriosis. The data from 4000 of these women surveyed in 1998 show that it takes an average of 9 years between onset of symptoms and correct diagnosis of endometriosis. Approximately half of that time is lost because women and girls do not report the symptoms to their physicians [7]. Friends and family, media, and society in general often tell women that "female pain," including painful menstruation and pain with sex, is normal. According to feminist theory of psychotherapy, a myth in our culture states that the more a woman suffers, the better wife, mother, and woman she is. The impression seems to be that if pain is not a sign of superior femininity, at least it is a sign of normal femininity and must be accepted. Perhaps it goes back as far as the Garden of Eden, when Eve was punished for eating that apple, with painful childbirth as her punishment, which was transferred to women for all time.

> "My grandmother told me that to be a woman was to hurt, and that I should forget about complaining so much and just get on with my work." — Nancy, South Carolina

The concept of justified pain leads to additional misunderstanding about the nature of endometriosis. According to a chronic pain survey mailed to 4000 randomly selected members of the Endometriosis Association in 2002, 80% of respondents experience 1 to 3 days each month when they are unable to carry on regular activities, including work. Women reported anecdotally that no one took their endometriosis seriously, however, even after endometriosis was diagnosed, unless and until they had trouble conceiving.

Ironically, one persistent myth that involves endometriosis is that it is cured by pregnancy. Some physicians continue to prescribe pregnancy as a treatment for endometriosis, even when the patient is a young teen! The implied message seems to be that if women would get pregnant as they should, they would not have these troubles [8]. Although endometriosis was once considered a "white


When do you feel pain?
Where is the pain located?
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
79%
53%
46%
43%
38%
36%
67%
63%
60%
53%
42%
31%
Time of menstrual period
Ovulation
Sporadically
Constant/most of time
With bowel movements
During/after sex
Lower mid abdomen
Lower right abdomen
Lower left abdomen
Lower back
Genitals
Head

career woman's disease," blaming women who postponed marriage and children for career, we have known for a long time that endometriosis occurs across races, education levels, and socioeconomic backgrounds. Endometriosis also occurs across a wider age range than once believed, from age 9 and up. Medical myths, even among physicians, are sometimes hard to kill.

Despite research showing otherwise, many physicians seem to believe that the only real problem with endometriosis is the possibility of infertility. For whatever reasons, they dismiss the pain involved as insignificant or believe it does not matter until and unless a woman cannot perform her "natural function." The truth is, endometriosis patients present to the general gynecologist three times more often with pain than with infertility [2]. A patient may present with pain in a wide variety of locations, of differing intensities, and at various times [3]. Some physicians have their own set of criteria to support their suspicion or lack of suspicion of endometriosis.

> "The gynecologist basically told me I did not have endometriosis after he did a pelvic exam because I did not have painful sex. But I had very severe pain mid cycle. All he said was if I was not pregnant in a year, to come back to see him." — Tylene, Ohio
>
> "My general practitioner and fertility doctors say that my endo has no impact on my fertility or ability to conceive and that my endo has nothing to do with my overall general health and the fact that I seem to be ill a lot with low grade fevers, recurring infections, etc. My general practitioner suggested counseling to cope with my obvious depression. I'm extremely frustrated with my treatment!" — Cathy, Wisconsin

Women with endometriosis are often told that their pain is exaggerated, imagined, or normal. Unfortunately, the person most likely to tell them these things is their obstetrician/gynecologist (Fig. 2). The opinions of family and friends add to that pressure, and women with endometriosis are likely to begin to doubt their bodies and believe that their pain is psychogenic, somehow their own fault, or that they should just buck up and shut up, although the pain is often disabling. 43% of women surveyed responded that they had sometimes wondered if their pain was "all in their head."

Not only is there stigma involved with chronic pain [8] but also there is misunderstanding about the nature of pain in women and additional stigma attached to menstruation or sex-related pain. The stigma and misunderstanding are fed by physicians and others who continue to proclaim that to feel some "womanly pain," even when that pain is disabling, is normal.

> "I have lower abdominal pain usually with my period and around ovulation. This usually triggers GI problems. No one has ever thought to treat my 'cramps' beyond ibuprofen, because it's 'normal'." — Kathy, Michigan

Fig. 1. In the case of endometriosis, pain is experienced in a wide variety of locations and during various times.

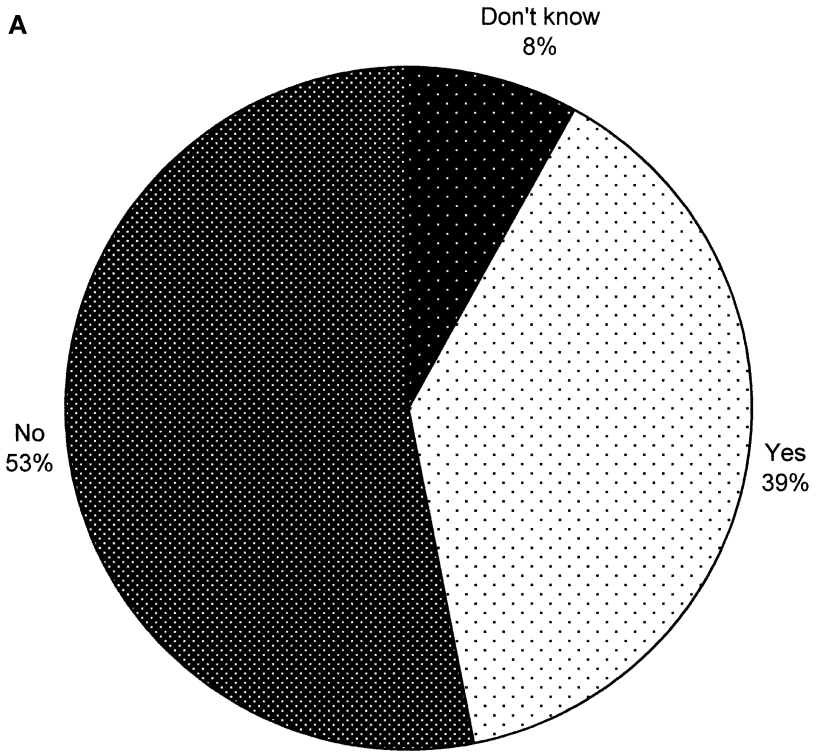


Fig. 2. (A) Answers to a survey question asking whether the woman with endometriosis ever has been told that she exaggerates her pain. (B) Answers to the question asking by whom the woman was told she was exaggerating her pain.

> "For eighteen years my mother, then my doctors, told me my pain was normal and to take some acetaminophen. At age 38, I was treated for infertility and found out I had endometriosis and adhesions. Guess what! The pain is not normal!" — Arlina, Colorado
>
> "My gynecologist told me that I was too nervous and it was making my pain worse. Of course I'm nervous! I wonder how nervous he'd be if it felt like his nuts were in a vice a week each month!" — Ann, Wyoming

Now what?

It is typically a long road from onset of pain from endometriosis to having it identified, justified, and verified by a woman and her physician. Unfortunately, arriving at that point is just the beginning of an even longer journey. Finding an effective treatment for the pain of endometriosis is not a straightforward proposition, and a woman with endometriosis may feel as if she has struck out.

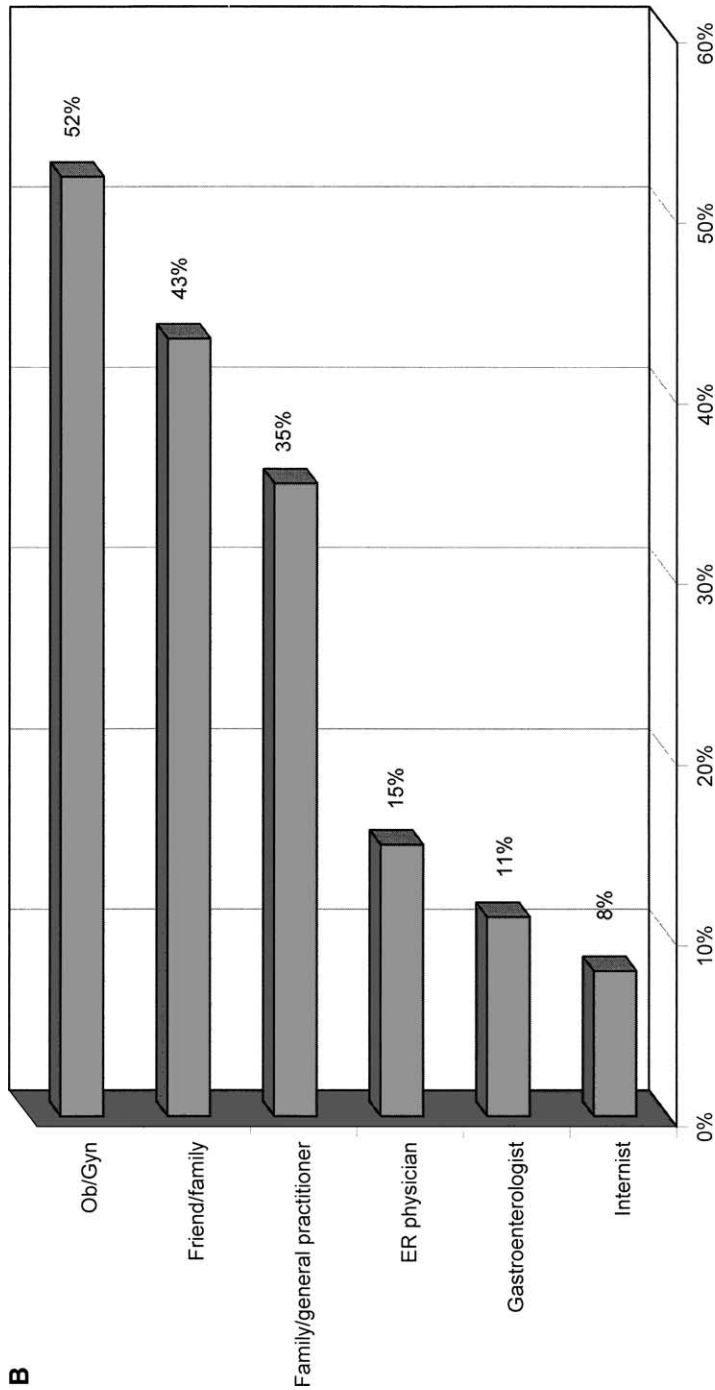


Fig. 2 (*continued*).

> "I suffer from chronic pain, which has left me depressed at times. I was always a strong athletic person, but endometriosis has taken that away."
> — Gloria, Connecticut

There are probably as many diverse treatments for pain as there are types of pain that can be associated with endometriosis, and the Endometriosis Association survey reinforces what we hear from women with endometriosis: what works for one pain or one person at one time may not work for a slightly different pain, a different person, or the same person at a different time.

Survey

The Endometriosis Association surveyed women with pain from endometriosis and/or adhesions to determine the extent, location, suggested treatments, treatment professionals consulted, results of treatment, and attitudes toward their pain and treatments. Surveys were sent to 4000 randomly chosen members of the Endometriosis Association, and data were tabulated on the first 1000 surveys returned.

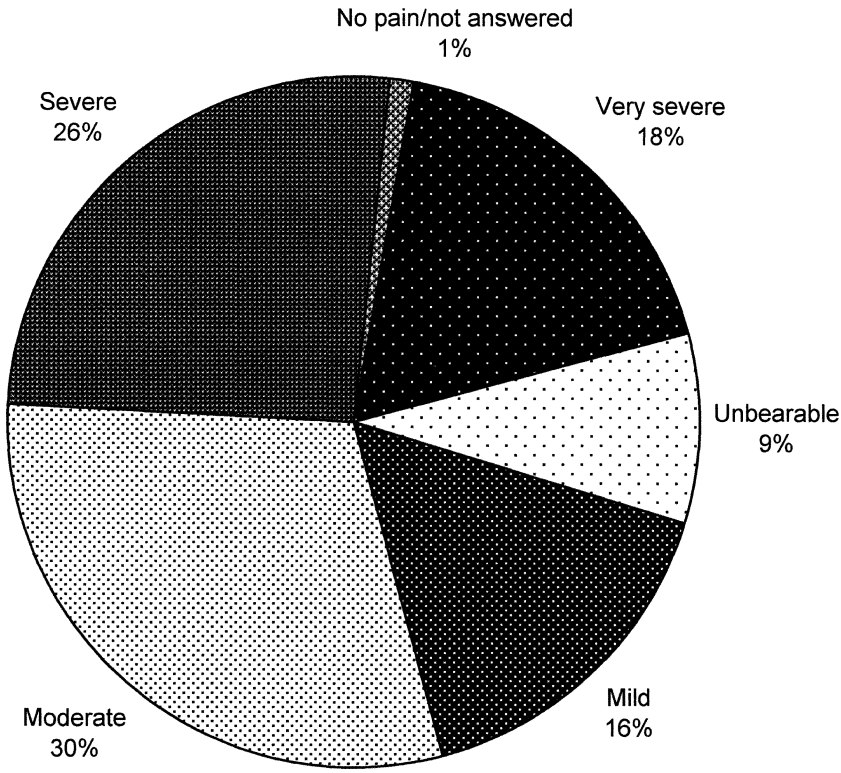


Fig. 3. Rating of intensity of pain of endometriosis.

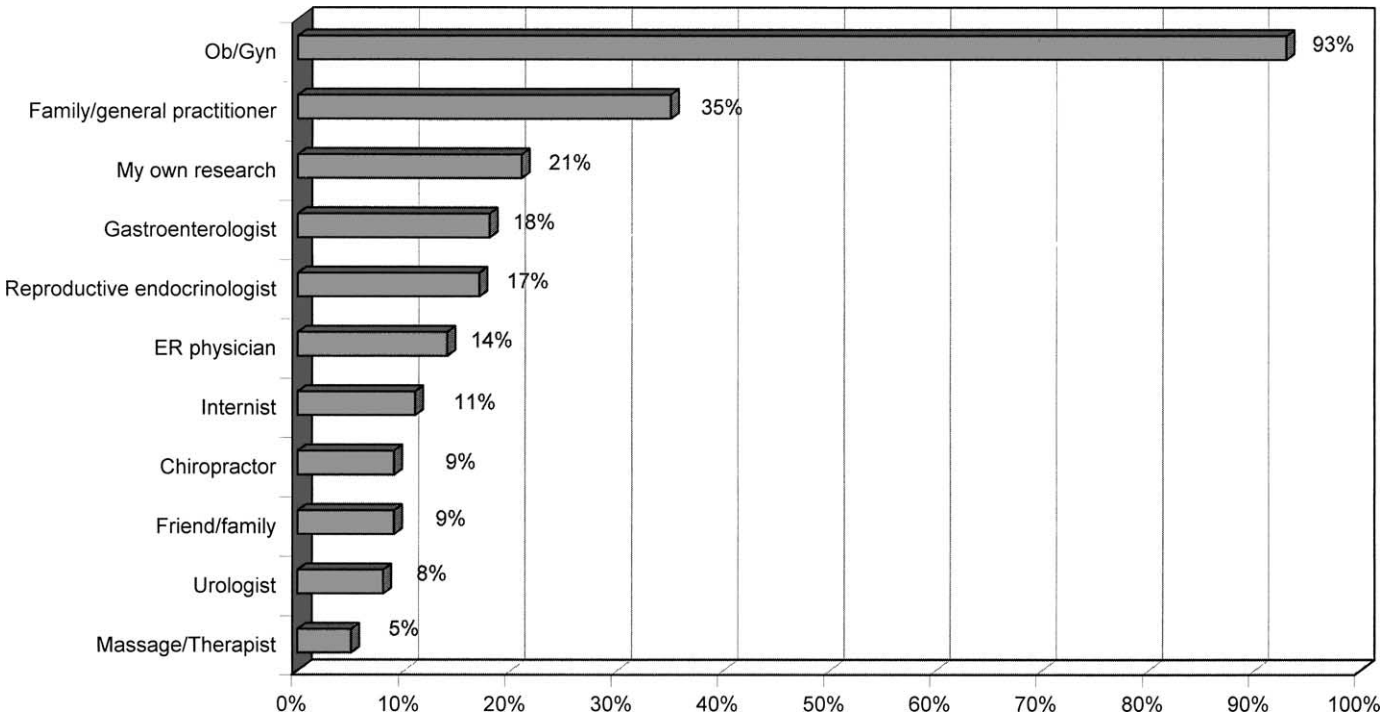


Fig. 4. A graph indicating whom the patient with endometriosis consulted about her pain. Most women see more than one physician for treatment of endometriosis in search for adequate treatment.

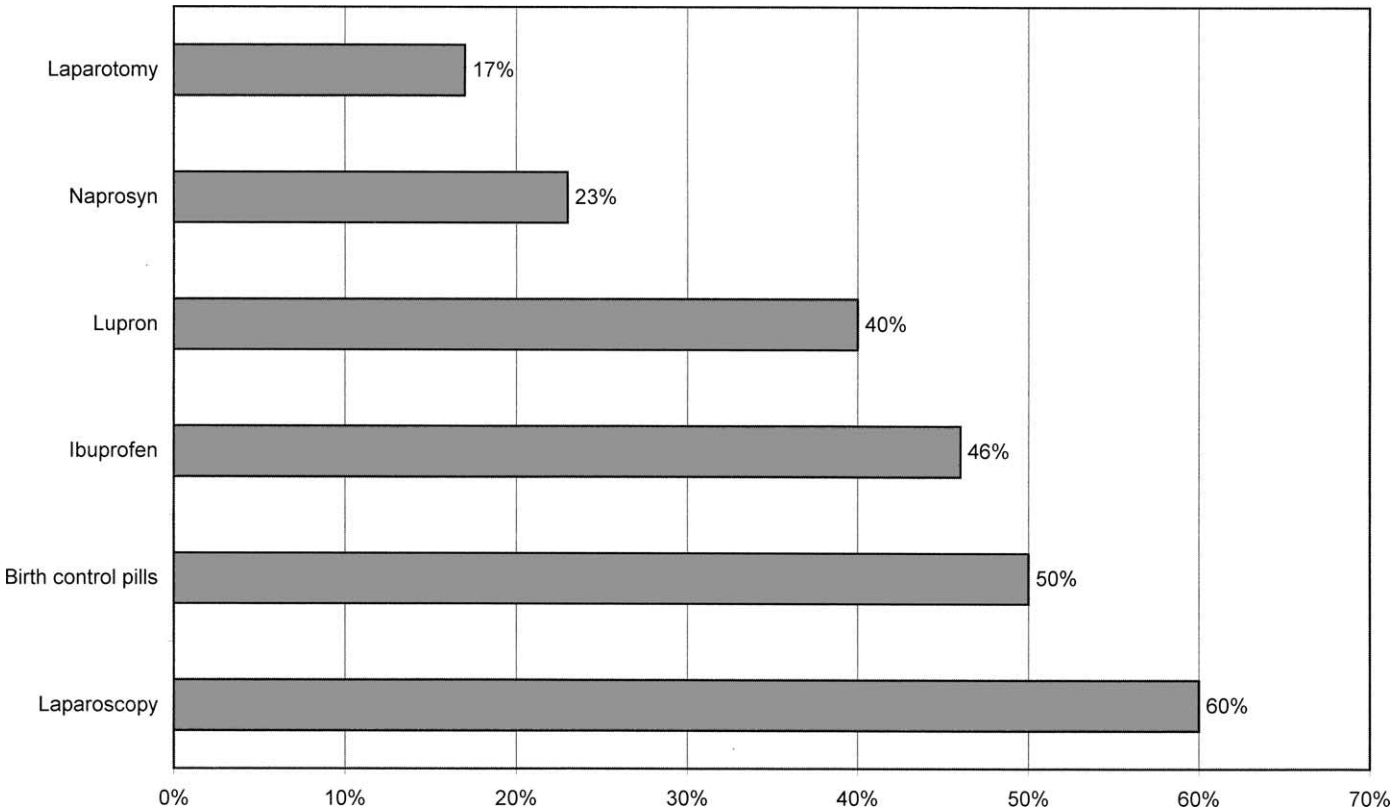


Fig. 5. An indication of some of the treatments suggested for endometriosis-associated pain.

Type of pain reported

Sixty-seven percent of the women reported that they felt pain in their lower mid-abdomen; 63% said lower right abdomen; 60% percent said lower left abdomen. The most common reported time for pain was at the time of menstruation, followed by constant pain and pain around ovulation. 53% of the responders reported that their pain was severe, very severe, or unbearable (Fig. 3).

Women with endometriosis consult various sources to seek relief from their pain. Thirteen different medical specialists were mentioned on the survey; family and friends, "my own research," and "other" also were mentioned. Most women see more than one physician for treatment of endometriosis in search for adequate treatment (Fig. 4). In Endometriosis Association data from 1998, 47% of sufferers indicated that they had to see a doctor five times or more before they received a diagnosis or referral [4].

A seemingly endless variety of treatments is suggested, many of which work well for some and not at all for others. It is hard for someone to be patient when experiencing chronic pain, but that is often what it takes to find a treatment that works well (Fig. 5). It is also frustrating for physicians when patients do not respond to treatments; however, it can be devastating when a patient believes that her physician has given up on her.

> "The doctors have told us that there is nothing more they can do for our daughter. Can you imagine that? She's seventeen years old and there's nothing they can do for this nearly constant pain she's in. That's just not good enough!"
> — Marie, Colorado

Summary

Finding the solution for the pain of endometriosis is likely to be a time-consuming, often frustrating task. But it is a task that can begin in earnest only once the pain is identified and believed. If a girl or woman with endometriosis is ashamed to discuss her pain or her symptoms are dismissed or minimized by her physician, it is inevitable that her pain will continue untreated. The first step in treating the pain of endometriosis is to encourage patients to discuss their pain frankly. A pain map, diary, and descriptors may be helpful, but listening and believing the patient are essential.

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Future directions in endometriosis research

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Lack of progress in endometriosis research

Endometriosis is an important benign gynecologic disease that is pathologically defined by the ectopic presence of endometrial glands and stroma and is clinically associated with pelvic pain and infertility. The current knowledge of pathogenesis, pathophysiology of related infertility, and spontaneous evolution is still limited, although endometriosis has been described for many years. The diagnosis still can be made only by invasive tests (laparoscopy), and treatment either temporarily suppresses the disease (medical approach) or temporarily removes the disease (surgical excision). Recurrences of endometriosis after the stop of medical treatment or after surgery are common, especially in cases of moderate to severe endometriosis. Several reasons contribute to this state.

First, at the time of diagnosis most patients have had endometriosis for an unknown period of time. It is impossible to undertake clinical research that would definitely determine the onset, etiology, or progression of the disease [1].

Second, an important reason for the lack of progress in endometriosis research is study design [2]: few studies have been carried out so far using adequate control groups. When symptomatic patients with endometriosis are compared to women with a normal pelvis, adenomyosis, leiomyomata, adhesions, or other pelvic pathologic conditions, two factors are usually studied in a combined way:

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the pelvic condition (presence of endometriosis or other pathology) and symptoms (none, infertility, pain, other symptoms). To study the effect of endometriosis itself, it is necessary to exclude patients with possible other causes of infertility or pain and compare patients with endometriosis and infertility to women with a normal pelvis and unexplained infertility or compare patients with endometriosis and pain to women with a normal pelvis and pain. To study the effect of endometriosis on infertility, the study group should include infertile patients with endometriosis and women with unexplained infertility, whereas the control group should include fertile women with endometriosis and a normal pelvis (population available at interval tubal sterilization). Similarly, to study the effect of endometriosis on pain, the study group should include patients who experience pain with endometriosis and women with unexplained pain, whereas the control group should include asymptomatic and pain-free women with endometriosis and with a normal pelvis (population available at interval tubal sterilization). It is hard to conduct these adequately controlled studies with sufficient numbers of patients, and multicenter research is needed.

Third, endometriosis long has been considered a surgical gynecologic disease. Currently, there is a need for clinical management of endometriosis by multidisciplinary teams that address medical, surgical, and psychological issues associated with endometriosis. Multidisciplinary research teams also are needed to address the heterogeneous clinical, histologic, immunologic, endocrinologic, toxicologic, genetic, epidemiologic, and psychosocial aspects of endometriosis.

Finally, endometriosis occurs naturally in humans and nonhuman primates only. Because of ethical and practical considerations, properly controlled studies are difficult, and invasive experiments cannot be performed in humans. It follows from these considerations that there is a need for the development of a good animal model with spontaneous and induced endometriosis.

The need for primate models for the study of endometriosis

The main advantage of rodent (rat and rabbit) models is the low cost relative to the primate models, but the disadvantages are numerous. Rodents lack a menstrual cycle and do not have spontaneous endometriosis. Although the rat is a spontaneous ovulator, it has a shorter luteal phase humans. The reproductive pattern of the rabbit even lacks a luteal phase. There is also a wide phylogenetic gap between these two species and humans. In the rodent models, induction is performed through the autotransplantation of endometrial fragments or uterine squares [3], which is not physiologic, damages the uterus, and causes adhesions that interfere with fertility. The resulting ''endometriotic lesions'' consist of cysts that contain clear serous fluid in the rat, and vascularized hemorrhagic solid masses can be found in the rabbit. This type of lesion in both species seems to be different from the various pigmented and nonpigmented lesions found in humans [4–6]. Recently, the use of nude mice [7] or Severe Combined Immunodeficiency (SCID) mice [8] has offered the advantage that these immunodeficient rodents do

not reject xenographic human endometrial tissue, which can be introduced subcutaneously or into the peritoneal cavity. This advantage enables researchers to study human endometrial-murine peritoneal interaction. The question remains how data from these rodent models can be extrapolated to the human situation, given the enormous species difference between mice and humans.

Monkeys, although difficult and expensive to maintain in captivity, offer unique advantages in endometriosis research when compared to rodents. First, they are phylogenetically much closer to humans and have a comparable menstrual cycle. Second, nonhuman primates—rhesus monkeys [9], pigtailed macaques [10], cynomolgus monkeys, De Brazza monkeys [11], and baboons [12,13]—are known to be afflicted with spontaneous endometriosis. It has been reported that irradiation is associated with an increased incidence of spontaneous endometriosis in rhesus monkeys, but only after at least 6 years [14]. In the same species, a positive correlation was found between dioxin dose and severity of endometriosis [15]. Third, induced endometriosis resulted in macroscopic lesions, which showed similarity to the human disease [16–21].

The great apes (eg, chimpanzee, gorilla, orangutan) are closest to humans in many anatomic and physiologic aspects of reproduction. Because all of them are protected, endangered species in the wild, however, they are not practical models for most studies.

Baboons are intelligent animals with a well-studied and interesting social life. Hypotheses about the early evolution of human social behavior have been developed by carefully studying the behavior of baboon troops living on the grassy plains of Africa [22]. The baboon may offer clear advantages for the study of endometriosis when compared to rhesus and cynomolgus monkeys [2]. First, the baboon is phylogenetically close because human and baboon karyotypes (46 and 42 chromosomes, respectively), evolving slowly, share many ancestral characters [23]. Second, detailed accounts of baboon reproductive anatomy and physiology, similar to human, are available, including menstrual cycle characteristics, embryo implantation, and fetal development [24]. Perineal skin inflation and deflation correspond with relative precision to follicular and luteal phases, which offers external follow-up of the menstrual cycle without the need for serial blood samples for determination of estradiol and progesterone levels. Third, the baboon is a proven model for research in cardiovascular and endoscopic surgery [25], endocrinology, teratology, toxicology, testing of contraceptive agents [26], and placental development [27]. Fourth, the baboon is a continuous breeder, with menstrual cycles throughout the year, also in captivity. Fifth, the baboon is a larger and stronger primate than rhesus or cynomolgus monkeys, which allows repetitive blood sampling and complex experimental surgery [26]. Sixth, specific advantages of the baboon model in gynecologic research include the spontaneous presence of peritoneal fluid and the accessibility of the uterine cavity via the cervix, which allows endometrial sampling without hysterotomy [28]. For these reasons, the baboon is considered to be a good model for research in reproduction [26]. Finally, spontaneous endometriosis in the baboon has been found to be minimal [13] and disseminated [12], similar to the different disease stages in

women. More advanced stages of endometriosis can be induced after intrapelvic seeding of menstrual endometrium inside the pelvic cavity [28]. Experimental induction of endometriosis offers the opportunity to make serial observations in the same animal before and after induction, which enables investigators to identify factors in peripheral blood and peritoneal fluid as the consequence of endometriosis.

Over the last 10 years, the baboon has been developed at the Institute of Primate Research as a model for the study of endometriosis, and its clinical relevance has been reviewed extensively [2]. Briefly, spontaneous endometriosis was found in approximately 25% of baboons [29], and prevalence increased with the duration of captivity [30]. The laparoscopic appearance, pelvic localization, and microscopic aspect were similar to endometriosis in women [29,31]. Microscopic endometriosis in macroscopically normal peritoneum was rare [32]. Sampson's hypothesis (retrograde menstruation causes endometriosis) was supported by the increased incidence of retrograde menstruation in baboons with spontaneous endometriosis [33], the observation that cervical occlusion could cause retrograde menstruation and endometriosis [34], and the finding that intrapelvic injection of menstrual endometrium caused experimental moderate to severe endometriosis similar to the spontaneous disease [28]. During follow-up of more than 2 years, endometriosis in baboons seemed to be a progressive disease, with active remodeling among several types of lesions [35]. Progression also was stimulated by high-dose immunosuppression [36]. Fertility was normal in baboons with minimal disease but was reduced in baboons with mild, moderate, or severe endometriosis [37], possibly related to an increased incidence and recurrence of the luteinized ruptured follicle syndrome [38].

In the future, the baboon model for endometriosis should be used to test new drugs in the prevention or treatment of endometriosis and endometriosis-associated subfertility. Because induction of endometriosis is followed by moderate to severe endometriosis in most baboons [28,39], it is possible to conduct either prevention studies (prevent attachment of menstrual endometrium on the uterine peritoneum) or treatment studies (reduce extent of induced endometriosis after medical or surgical therapy). Treatment studies also can be conducted in baboons with spontaneous endometriosis, but it is difficult to have sufficient numbers of them. Placebo-controlled randomized trials can be conducted to evaluate the effect of new anti-endometriosis drugs on endometriosis-associated subfertility with the possibility of complete standardization for the degree of endometriosis (after intrapelvic injection of menstrual endometrium), the presence of ovulation (can be interpreted based on the perineal cycle), and male factors (timed intercourse with male baboon of proven fertility, controlled by behavioral observation and postcoital test) [37].

Intrapelvic injection of menstrual endometrium also allows the possibility to study early endometrial-peritoneal interaction at short-term intervals during in vivo culture and could give important insight into the early development of endometriotic lesions. This observation would be important to assess the validity of the Sampson hypothesis [40].

Etiology: how right or wrong was Sampson?

Although endometriosis has been described since the 1800s, its widespread occurrence was acknowledged only during this century. Endometriosis is an estrogen-dependent disease. Three theories have been proposed to explain the histogenesis of endometriosis: (1) ectopic transplantation of endometrial tissue, (2) coelomic metaplasia, and (3) the induction theory. No single theory can account for the location of endometriosis in all cases.

Transplantation theory

The transplantation theory, originally proposed by Sampson in the mid-1920s, is based on the assumption that endometriosis is caused by the seeding or implantation of endometrial cells by transtubal regurgitation during menstruation [40]. Substantial clinical and experimental data support this hypothesis [1,41]. Retrograde menstruation occurs in 70% to 90% of women [42,43], and it may be more common in women with endometriosis than in women without the disease [43]. The presence of endometrial cells in the peritoneal fluid, which indicates retrograde menstruation, has been reported in 59% to 79% of women during menses or in the early follicular phase [44,45], and these cells can be cultured in vitro [45]. Evidence that supports retrograde menstruation is the presence of endometrial cells in the dialysate of women who undergo peritoneal dialysis during menses [46]. Endometriosis also is most often found in dependent portions of the pelvis, on the ovaries, the anterior and posterior cul-de-sac, the uterosacral ligaments, the posterior uterus, and the posterior broad ligaments [47]. Endometrium obtained during menses can grow when injected beneath abdominal skin or into the pelvic cavity of animals [28,48]. Endometriosis has been found in 50% of rhesus monkeys after surgical transposition of the cervix to allow intra-abdominal menstruation [17]. Increased retrograde menstruation by obstruction of the outflow of menstrual fluid from the uterus is associated with a higher incidence of endometriosis in women [49] and in baboons [34]. Women with shorter intervals between menstruation and longer duration of menses are more likely to have retrograde menstruation and have a higher risk of developing endometriosis [50].

Ovarian endometriosis may be caused by either retrograde menstruation or lymphatic flow from the uterus to the ovary [51]. Extrapelvic endometriosis, although rare (1%–2%), potentially may result from vascular or lymphatic dissemination of endometrial cells to many gynecologic (vulva, vagina, cervix) and nongynecologic sites. The latter sites include bowel (appendix, rectum, sigmoid colon, small intestine, hernia sacs), lungs and pleural cavity, skin (episiotomy or other surgical scars, inguinal region, extremities, umbilicus), lymph glands, nerves, and brain [52]. No solid scientific data are available to support the hypothesis that extrapelvic endometriosis is caused by vascular or lymphatic dissemination of endometrial cells to extrapelvic sites. It is not understood why the presentation of this disease is so variable and why endome-

triosis is progressive in many women. This lack of understanding can be explained at least partly because the phenomenon of retrograde menstruation has not been studied in depth. For instance, it is widely accepted that retrograde menstruation occurs in all women, but detailed studies concerning the mechanisms, quantity, and quality of retrograde menstruation are not available. The presence of endometrial cells in peritoneal fluid during menstruation and nonmenstrual phases of the cycle remains an underinvestigated area of research in the pathogenesis of endometriosis [53].

In a classic article published approximately 20 years ago [54], only a weak correlation was reported between red colored peritoneal fluid and the presence peritoneal fluid endometrial cells at the time of menstruation. Overall, only 8 of 33 aspirates (24%) of red colored peritoneal fluid contained endometrial cells [54], which questioned whether a reasonable amount of endometrial cells arrives in the pelvic cavity during menses.

In another study [55] that included 16 patients who underwent laparoscopy during menstruation, the peritoneal fluid was red colored in 100%. The peritoneal fluid cell fraction was contained in at least 90% cells that stained positively for vimentin (known to stain positively for endometrial stromal cells and mesothelial cells) and cytokeratin (known to stain positively for endometrial epithelial cells and mesothelial cells) and in 56% of cells that stained positively for BW 495/36 (proposed to be a selective marker for endometrial epithelial cells) [55]. In the authors' laboratory, they have not been able to confirm that monoclonal antibody BW 495/36 is specific for endometrial epithelial cells. In the study [56], peritoneal fluid contained only occasionally intact gland-like endometrial structures. These data are in accordance with the previous study [54] and leave open the possibility that the peritoneal fluid cells that stain positively for vimentin, cytokeratin, and BW 495/36 are shed mesothelial cells. To the best of the authors' knowledge, no other articles have been published to assess the correlation among red stained peritoneal fluid, presence of peritoneal fluid endometrial cells, and menstruation in women with or without endometriosis.

During nonmenstrual phases of the cycle, the prevalence of peritoneal fluid endometrial cells varies between 0% and 19% and increases to 23% to 67% after hysteroscopy or uterotubal flushing [53]. Uterine flushing introduces a new unquantified, nonphysiologic variable (increased intrauterine pressure during flushing), however, and the clinical significance of endometrial cells in peritoneal fluid after this procedure is questionable [53]. It seems that there are many problems with study design, including inadequate documentation of the cycle phase, variable preparation of peritoneal fluid cells (cytospin, cytoblock, in vitro culture), absence of a clear morphologic definition of endometrial epithelial and stromal cells, and lack of adequate immunohistochemical markers that identify specifically endometrial epithelial, endometrial stromal, and mesothelial cells. Further studies are needed in women with a normal pelvis and in patients with endometriosis to quantify the amount and implantation potential of these cells at various stages of the menstrual cycle [53].

Differences between eutopic endometrium and myometrium in women with and without endometriosis

There is increasing evidence that the following genes and gene products could be expressed aberrantly in endometrium or endometriotic tissue from women with endometriosis [57]: aromatase, steroidogenic factor-1, 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2, inactivation of estradiol), endometrium bleeding factor, hepatocyte growth factor, Homeobox (Hox) genes HoxA-10 and HoxA-11, leukemia inhibiting factor, Matrix Metalloproteinase (MMP)-1 and MMP-11, and progesterone receptors.

Aromatase mRNA has been detected in endometriotic implants and in much lower quantities in eutopic endometrial samples of women with moderate to severe endometriosis, but not in eutopic endometrium from women without endometriosis [58]. Steroidogenic factor-1 is present in endometriotic tissue but not in endometrium. It stimulates aromatase activity and acts as a stimulatory transcription factor [59]. Aromatase converts androstenedione of adrenal origin to estron, which is reduced to the more active estradiol in endometriotic tissue. Endometriotic tissues lack the enzyme that inactivates estradiol, 17β-HSD-2, which leads to increased local concentrations of estradiol [60]. In turn, estradiol induces prostaglandin synthase, which results in increased concentrations of Prostaglandin E (PGE)2, a potent stimulator of aromatase, and creates a positive feedback loop in favor of continuous estrogen formation in endometriotic tissue [61,62].

Homeobox (Hox) genes, HoxA-10 and HoxA-11, and α_v β-3 integrin, normally upregulated during the window of implantation, are both downregulated during this period in women with endometriosis [63,64]. Preliminary studies in mice [65] and in vitro [66] also suggest that leukemia inhibiting factor and Hepatocyte Growth Factor (HGF) may be respectively downregulated and upregulated in women with endometriosis. More work must be done in human endometrium from the luteal or menstrual phase, however, to detect differences quantitatively between women with and without endometriosis in the expression of the previously mentioned genes and other important factors in the pathogenesis of endometriosis, including MMPs, Tissue Inhibitor of Matrix Metalloproteinases (TIMP)s, estrogen receptors, progesteron receptors, tumor necrosis factor-α (TNF-α), interleukin-8, and integrins. The use of gene and protein arrays could be helpful in this search, but much attention must be paid to interindividual differences, intraindividual differences during the menstrual cycle and between different menstrual cycles, score, and stage of endometriosis. A sufficient number of patients with well-defined disease and cycle characteristics are necessary before it is possible to reach any meaningful conclusions.

New research efforts also are needed to assess the role of myometrium in the pathogenesis of endometriosis, quantitating the role of endometrial junctional zone and uterine contractility and intrauterine pressure during different phases of the cycle in patients with and without endometriosis. High-resolution ultrasound together with advanced image and data analysis is needed to achieve success in this endeavor. In vitro studies are needed to test the hypothesis that myometrium

can control endometrial growth, which is also important to study the pathogenesis of adenomyosis.

Integration of epidemiology and genetics

In a recent paper [67], Cramer and Missmer proposed the integration of epidemiology and genetics by the identification of an ''endometriosis phenotype.'' This phenotype could consist of early menarche, short cycles, painful periods, subfertility, and possibly tall stature, which could be explained by genetic factors that predispose to poor endowment of germ cells and canalization defects of the cervix. As candidates for genetic markers are identified, particular genotypes can be correlated with these clinical factors, even if a formal diagnosis of endometriosis has not been made [67].

To advance knowledge in the area of basic genetic research, Barbieri and Missmer [68] suggested using high-resolution karyotyping of ovarian endometriosis cyst epithelium (obtained by laser dissection of small groups of cells) and identifying nonrandom genetic arrangements to determine if peritoneal endometriosis lesions are monoclonal or polyclonal, identify the genes involved in the somatic mutations in ovarian endometriosis epithelium, perform transcript profiling between ovarian endometriosis, peritoneal endometriosis, and eutopic endometrium at different stages of the menstrual cycle, and develop cell lines from ovarian endometriosis epithelium for gene expression and protein synthesis experiments [68].

Immunologic factors, inflammation, and tumor necrosis factor-α blocking agents in the prevention and treatment of endometriosis

Although retrograde menstruation seems to be a common event in women, not all women who have retrograde menstruation develop endometriosis. The immune system may be altered in women with endometriosis, and it has been hypothesized that the disease may develop as a result of reduced immunologic clearance of viable endometrial cells from the pelvic cavity [69,70]. It has been reported that endometriosis can be caused by decreased clearance of peritoneal fluid endometrial cells because of reduced natural killer activity and decreased macrophage activity [71]. Decreased cell-mediated cytotoxicity toward autologous endometrial cells has been reported to be associated with endometriosis [3,72–74]. These studies used techniques that have considerable variability in target cells and methods, however [75,76]. Whether natural killer cell activity is lower in women with endometriosis than in women without endometriosis is controversial. Some reports demonstrated reduced natural killer cell activity [16,74,77–79], whereas others found no increase in natural killer cell activity, even in women with moderate to severe disease [3,74,75]. There is also great variability in natural killer cell activity among normal

individuals that may be related to variables such as smoking, drug use, and exercise [75].

In contrast, endometriosis also can be considered a condition of immunologic tolerance versus ectopic endometrium, which essentially is self-tissue [69]. One may ask why viable endometrial cells in the peritoneal fluid would be a target for natural killer cells or macrophages. Autotransplantation of blood vessels, muscles, skin grafts, and other tissues is known to be successful in humans. There is no in vitro evidence that peritoneal fluid macrophages actually attack and perform phagocytosis of viable peritoneal fluid endometrial cells. Finally, high-dose immunosuppression can increase slightly the progression of spontaneous endometriosis in baboons [35]. There is no clinical evidence that the prevalence of endometriosis is increased in immunosuppressed patients, however. The fact that women with kidney transplants under chronic immunosuppression are not known to have increased infertility problems can be considered as indirect evidence that these patients do not develop extensive endometriosis.

Substantial evidence suggests that endometriosis is associated with a state of subclinical peritoneal inflammation, marked by increased peritoneal fluid volume, increased peritoneal fluid white blood cell concentration—especially macrophages with increased activation status—and increased inflammatory cytokines, growth factors, and angiogenesis-promoting substances [69]. Recently, it has been reported in baboons that subclinical peritoneal inflammation occurs during menstruation and after intrapelvic injection of endometrium [28]. A higher basal activation status of peritoneal macrophages in women with endometriosis may impair fertility by reducing sperm motility, increasing sperm phagocytosis, or interfering with fertilization [80,81], possibly by increased secretion of cytokines such as TNF-α [82–84]. The cytokine TNF also may facilitate the pelvic implantation of ectopic endometrium [9,73]. The adherence of human endometrial stromal cells to mesothelial cells in vitro has been shown to be increased by the pretreatment of mesothelial cells with physiologic doses of TNF-α [85]. Macrophages or other cells may promote the growth of endometrial cells [86,87] by secretion of growth and angiogenetic factors such as epidermal growth factor [87], macrophage-derived growth factor [88], fibronectin, and adhesion molecules such as integrins [89]. After attachment of endometrial cells to the peritoneum, subsequent invasion and growth seems to be regulated by metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) [90,91].

There is increasing evidence that local inflammation and secretion of prostaglandins is related to differences in endometrial aromatase activity between women with and without endometriosis. Expression of aromatase cytochrome P450 protein and mRNA was observed in human endometriotic cells but not in normal endometrium, which suggests that ectopic endometrium produces estrogens, which may be involved in the tissue growth interacting with the estrogen receptor [92]. Inactivation of 17β-estradiol has been reported to be impaired in endometriotic tissues because of deficient expression of 17β-HSD-2, which is normally expressed in eutopic endometrium in response to progesterone [60]. Finally, the inappropriate aromatase expression in endometriosis

lesions can be stimulated by prostaglandin-2 but also leads to local production of E2, which also stimulates PGE2 production and results in a positive feedback system between local inflammation and estrogen-driven local growth of ectopic endometrium [61].

Environmental factors and dioxin

There is an increasing awareness of potential links between reproductive health and infertility and environmental factors. There is support for the idea that lifestyle exposures that might raise or lower estrogen levels could affect risk, including a decreased risk associated with smoking and exercise and an increased risk associated with caffeine or alcohol use [67]. Recently, one publication [93] evaluated occupational exposures and risk of endometriosis in 281 infertile and 216 fertile women and reported a significant association between endometriosis and exposure to video display terminals (OR 3.7, 95% CI 1.5–9.1) but not to solvents (OR 2.1, 95% CI 0.96–4.72) or dusts (OR 3.6, 95% CI 0.99–13.28).

Much attention also has been paid to the potential role of dioxins in the pathogenesis of endometriosis, but the issue remains controversial in women. In humans, the Seveso Women's Health Study correlates prospective individual 2, 3, 7, 7-tetrachlorodibenzo-p-dioxin (TCDD) data with reproductive endpoints, such as the incidence of endometriosis, infertility, and decreased sperm quality after the 1976 explosion of a factory in Seveso (Italy) that resulted in the highest levels of TCDD exposure recorded in humans [94], but so far no data have been published. A recent case-control study failed to show an association in the general population between endometriosis and exposure to Polychlorinated Biphenyls (PCBs) and chlorinated pesticides during adulthood. No differences in mean plasma concentrations of 14 PCBs and 11 chlorinated pesticides were found between women with and without endometriosis [95]. In a recent prospective controlled study [96], the authors found a statistically nonsignificant association between exposure to dioxin-like compounds and the occurrence of endometriosis in infertile women (crude OR = 4.3; $P = 0.187$). After adjusting for body mass index and alcohol consumption, the risk increased slightly to OR = 4.6 ($P = 0.188$). There was no confounding by age, ovulatory dysfunction, caffeine intake, smoking, or exposure to non-coplanar PCBs. In this study, the lack of a statistically significant association between exposure to dioxin-like compounds and endometriosis could be explained either by the lack of a real association or by the lack of power to prove such an association. To detect a fourfold increase (OR = 4) with a power of 90% and a significance level of 0.05, 85 cases and 85 controls would be required. By increasing those figures by 10% (for multiple regressions), a sample size of 100 patients with endometriosis and 100 controls is required. It was concluded that additional studies, including a larger group of women with and without endometriosis, are needed [96].

Recent studies have evaluated genetic mechanisms that may play a role in dioxin exposure and the development of endometriosis. Transcripts of the

CYP1A1 gene, a dioxin-induced gene, were reported to be significantly higher (ninefold) in endometriotic tissues than in eutopic endometrium [97]. Other investigators [98], however, reported a similar expression of AhR (arylhydrocarbon receptor) and dioxin-related genes (semi-quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)) in the endometrium from women with or without endometriosis. In Japanese subjects [99], no association was found between endometriosis prevalence or severity and polymorphisms for arylhydrocarbon receptor repressor, arylhydrocarbon receptor, arylhydrocarbon nuclear translocator, or CYP1A1 genes. Based on these data in humans, insufficient evidence supports the association between endometriosis and dioxin exposure. More studies that integrate toxicologic and epidemiologic expertise are needed in large and well-controlled prospective studies in different geographic areas.

Primates

An initial retrospective case control study reported that the prevalence of endometriosis was not statistically different (Fisher exact test, $P = 0.08$) between monkeys chronically exposed to dioxin during 4 years (11/14, 79%) and nonexposed animals (2/6, 33%) after a period of 10 years, but a positive correlation was found among dioxin dose, serum levels of TCDD and dioxin-like chemicals, and severity of endometriosis [15,51].

In the meantime, two prospective studies have been published to evaluate the association between dioxin exposure and development of endometriosis in rhesus monkeys. In the most recent study [100], monkeys exposed during 12 months to low-dose TCDD (0.71 ng/kg/d) had endometriosis implants with lower maximal and minimal diameters and similar survival rate when compared to endometriotic lesions in unexposed controls, which suggested no effect of dioxin on endometriosis. After 12 months exposure to high-dose TCDD (17.86 ng/kg/d), however, higher diameters and higher survival rate of endometriosis implants were observed in exposed rhesus monkeys compared to nonexposed controls. The second randomized, controlled study performed in 80 rhesus monkeys that compared no treatment and 0, 5, 20, 40, and 80 μg of Aroclor 1254/kg body weight/d during 6 years reported endometriosis in 37% of controls and 25% of PCB-treated monkeys based on laparoscopy and necropsy data [101]. No association was observed between endometriosis severity and PCB exposure. These data in primates question the importance of dioxin exposure in the development of endometriosis, except at high doses. It would be interesting to study the effect of short-term ex vivo exposure of menstrual endometrium to different doses of dioxins on the extent of peritoneal adhesion after intrapelvic injection in baboons [28].

Rodents

Continuous exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin inhibited the growth of surgically induced endometriosis in ovariectomized mice treated with high-dose estradiol, and no correlation was observed between dose of TCDD and

survival of endo implants, adhesions, and serum E2 levels [102]. In ovariectomized mice induced with endometriosis, similar stimulating effects of estrone and 4-chlorodiphenyl ether (4-CDE) were observed on survival rates of endometriotic, which suggested an estrogen-like effect of 4-CDE [103]. It is important to note that potential mechanisms that mediate TCDD action to potentially promote endometriosis in rodents are complex and probably different in rats and mice. The mouse seems to be a better model to elucidate these mechanisms [103], but both models have important limitations.

Is endometriosis limited to a pelvic gynecologic disease?

Endometriosis should be suspected in women with subfertility, dysmenorrhea, dyspareunia, or chronic pelvic pain. Endometriosis may be asymptomatic, however. There is growing evidence that endometriosis may be associated with significant extrapelvic health problems, but more and better designed prospectively controlled studies are needed to assess these links. For example, endometriosis can be associated with significant gastrointestinal symptoms (eg, pain, nausea, vomiting, early satiety, bloating and distention, altered bowel habits) and a characteristic motility change (ampulla of Vater duodenal spasm, a seizure equivalent of the enteric nervous system) together with bacterial overgrowth [104]. In one study that evaluated the risk of cancer after hospitalization for endometriosis, the overall cancer risk was 1.2 (95% CI 1.1–1.3), with significant excesses reported for breast cancer, ovarian cancer, and hematopoietic malignancies, especially non-Hodgkin's lymphoma [105]. Preliminary evidence from an Endometriosis Association Survey analyzed at the National Institutes of Health [106] suggests an association of endometriosis with autoimmune diseases, hypothyroidism, fibromyalgia, chronic fatigue syndrome, or asthma. Additional research is needed to assess the strength of the link between endometriosis and nonpelvic or systemic disease.

Increasing patient awareness of endometriosis and quality-of-life issues

The average delay between onset of pain symptoms and surgically confirmed endometriosis is long: 8 ± 8 years in the United Kingdom and 12 ± 9 years in the United States [107]. A recent report [108] in a single specialized center published a diagnostic delay in the diagnosis of endometriosis of 6 and 3 years in women with pain and women with infertility, respectively. During the last 15 years, a steady decrease of diagnostic delay and a decline in the prevalence of advanced endometriosis at first diagnosis were reported in that center [108]. At the same time, patient awareness about endometriosis has increased, probably as a result of the efforts of the Endometriosis Association, an international nonprofit organization aimed at globally educating and supporting patients with endometriosis.

For many patients, endometriosis becomes a chronic disease that affects quality of life because of incapacitating pain, emotional impact of subfertility, anger about

disease recurrence, and uncertainty about the future regarding repeated surgeries or long-term medical therapies and their side effects. There is a need to examine endometriosis, at least in a subset of highly symptomatic women, as a chronic disease. Quality-of-life issues should be addressed and studied using reliable and valid questionnaires regarding pain, mood, and depression [109].

Does endometriosis cause subfertility?

In a recent review [10], the authors critically assessed the many arguments that support the hypothesis regarding a causal relationship between the presence of endometriosis and subfertility. Briefly, these arguments are as follows:

- There is an increased prevalence of endometriosis in subfertile women when compared to women of proven fertility.
- There is a reduced monthly fecundity rate in baboons with mild to severe (spontaneous or induced) endometriosis when compared to baboons with minimal endometriosis or a normal pelvis.
- There is a trend toward a reduced monthly fecundity rate in infertile women with minimal to mild endometriosis when compared to women with unexplained infertility.
- There is a dose-effect relationship: a negative correlation between the revised American Fertility Society (r-AFS) stage of endometriosis and the monthly fecundity rate and crude pregnancy rate. There is also a negative correlation between the r-AFS stage of endometriosis and the cumulative pregnancy rate after surgery.
- There is a reduced monthly fecundity rate and cumulative pregnancy rate after donor sperm insemination in women with minimal to mild endometriosis when compared to women with a normal pelvis.
- There is a reduced monthly fecundity rate after husband sperm insemination in women with minimal to mild endometriosis when compared to women with a normal pelvis.
- There is a reduced implantation rate per embryo after in vitro fertilization in women with moderate to severe endometriosis when compared to women with a normal pelvis.
- There is an increased monthly fecundity rate and cumulative pregnancy rate after surgical removal of minimal to mild endometriosis [110].

Additional prospective studies are needed to compare the Monthly Fecundity Rate (MFR) and Cumulative Pregnancy Rate (CPR) after insemination with good quality sperm from donor or male partner during the natural (unstimulated) cycle in women with medically or surgically untreated minimal to moderate endometriosis but open fallopian tubes and in women with unexplained infertility. Additional well-powered multicenter, randomized trials should be performed to confirm that surgical treatment of minimal to mild endometriosis increases MFR and CPR.

During these trials, patients should not be informed after surgery whether they had a diagnostic laparoscopy or laparoscopic ablation of endometriosis.

Is endometriosis a progressive disease?

Endometriosis seems to be a progressive disease in a significant proportion (30%–60%) of patients [111]. During serial observations, deterioration (47%), improvement (30%), or elimination (23%) was documented over a 6-month period [53]. In another study, endometriosis progressed in 64%, improved in 27%, and remained unchanged in 9% over 12 months [112]. A third study [113] in 24 women reported disease progression in 29%, disease regression in 29%, and no change in 42% over 12 months. Follow-up studies in baboons [35] and women [114] with spontaneous endometriosis over 24 months have demonstrated disease progression in all baboons and in six of seven women. More studies are needed in women and baboons, however, with a longer follow-up and without any intervening medical or surgical interventions that might affect menstrual cyclicity or intrapelvic inflammation.

Surgical treatment: what is deep endometriosis and what are the real cumulative recurrence rates for moderate to severe endometriosis?

It has become clear that surgical treatment is at least partially effective in the treatment of endometriosis-associated subfertility [110] and pain [115,116]. In a prospective, controlled, randomized, double-blind study, surgical therapy has been shown to be superior to expectant management 6 months after treatment of mild and moderate endometriosis [116]. In women with mild and moderate disease treated with laser, 74% achieved pain relief. Treatment was least effective in women with minimal disease. There were no reported operative or laser complications [116]. One year later, symptom relief was still present in 90% of women who initially responded [113]. Patients with severe disease were not included because it was previously shown that surgery resulted in pain relief in 80% of patients who did not respond to medical therapy [115]. These results suggested that laser laparoscopy may be effective for the treatment of pain associated with mild to severe endometriosis. In women with minimal endometriosis, laser treatment may limit progression of disease. Few data are available on the long-term effectiveness of surgical treatment and long-term recurrence rates, however.

Endometriosis tends to recur unless definitive surgery is performed. The recurrence rate is approximately 5% to 20% per year and reaches a cumulative rate of 40% after 5 years. The rate of recurrence increases with the stage of disease [117–120], the duration of follow-up, and the occurrence of previous surgery [118]. The likelihood of recurrence seems to be lower when endometriosis is located only on the right side of the pelvis than when the left side is involved [121].

In a recent placebo-controlled randomized controlled trial [122], postoperative low-dose cyclic oral contraceptive use resulted in a significantly lower cumulative recurrence rate after 1 year, but not after 2 to 3 years. In women treated with a second operation for recurrent endometriosis [123], the cumulative recurrence rate was comparable after laparoscopy or laparotomy. It has been estimated that pain will recur within 5 years in approximately one in five patients with pelvic pain treated by complete laparoscopic excision of visible endometriotic lesions [124].

It is important that several biases may be present in the literature. First, it is well known that surgeons tend to overestimate the benefit of their interventions and minimize the importance of complications or recurrences. Second, only a few studies [124] report a complete and long-term follow-up of their patients using life table analysis. If a patient does not come back after surgical treatment for endometriosis-associated pain, she may feel better, but she also may feel that her condition has not improved at all. Third, most published studies come from a few individuals and centers with long-standing expertise in endometriosis surgery [123–125], and the recurrence figures presented in these studies may underestimate the average recurrence rates in gynecologic practice. Finally, it is not well established whether ovarian stimulation for in vitro fertilization that results in supraphysiologic estradiol levels results in a higher recurrence rate. In a recent study the authors reported that the cumulative recurrence rate of endometriosis (using life table analysis) after pelvic surgery for moderate to severe endometriosis was approximately 50% after 3 years but was not significantly affected by temporary high estradiol levels in women, which suggests that ovarian hyperstimulation for Intrauterine Insemination (IUI) or in vitro fertilization is not a risk factor for endometriosis recurrence [126].

Finally, it is important to clarify the term "deep endometriosis." Precise identification of the area of surgery is important in research and education [127]. Martin [127] suggested that the definition of retrocervical endometriosis could be used to describe the pouch of Douglas and retroperitoneal and vaginal fornix endometriosis behind or beneath the cervix with no rectal involvement. Rectovaginal endometriosis, in contrast, could be defined as the presence of deep endometriotic lesions on the rectal and the vaginal areas of the pouch and could include involvement of the rectovaginal septum [127]. Correct distinctions between these clinical presentations are important for surgical reasons (retrocervical endometriosis is easier to treat than rectovaginal endometriosis) and to understand better the pathogenesis and spontaneous evolution of endometriosis.

Medical treatment: from disease suppression to medical prevention and eradication?

The current medical treatment of endometriosis is based on hormonal suppression that induces atrophy of ectopic endometrial implants and interrupts the cycle of stimulation and bleeding. Reactivation of endometriotic lesions and

recurrence of symptoms can be expected in most patients after cessation of medical therapy, however. Conception is either impossible or contraindicated during medical treatment of endometriosis. Medical treatment with progestins, danazol, gestrinone, or gonadotropin-releasing hormone agonists is effective in treating pain associated with endometriosis, as shown in several prospective, randomized, placebo-controlled, double-blind studies [17,128–130]. Disadvantages of medical therapy over surgical therapy include the high cost of hormone preparations, the high prevalence of side effects, and the higher recurrence rate of endometriosis. There is a need to invent new medical strategies to prevent the development of endometriosis. There is hope that selective progesterone antagonists [131,132] and aromatase inhibitors [88,133,134] may offer new hormonal therapies, but further research must be conducted before the benefit of these drugs can be demonstrated and compared to the existing products. Selective progesterone receptor modulators have the potential to selectively suppress estrogen-dependent endometrial growth and induce a reversible amenorrhea without adverse systemic effects of estrogenic deprivation [135]. It seems that spiral arteries, which are unique to the primate endometrium, are the primary targets damaged or functionally inhibited by antiprogestins and selective progesterone receptor modulators.

The real challenge is to invent totally nonhormonal medications that may selectively prevent the development of endometriosis or suppress existing endometriotic lesions without suppressing ovulation and are safe during the first weeks or months of conception, which opens the possibility that these drugs could be used by women with endometriosis who wish to become pregnant. Based on new insights in the pathogenesis of endometriosis, it has become clear that pelvic inflammation, increased macrophage activation, pelvic angiogenesis, and invasion of the extracellular matrix (MMP, TIMPs) associated with endometriosis are potential targets for endometriosis therapy. The selective manipulation of macrophage function blockade of cytokines associated with inflammation, vascular growth factors, and peritoneal MMPs could offer totally new ways to prevent and treat endometriosis.

One of the most promising areas seems to be the selective blockade of TNF-α activity. In rats with experimental endometriosis, recombinant human TNF-α binding protein can reduce 64% of the size of endometriotic-like peritoneal lesions [136]. Similarly, a recent prospective randomized placebo- and drug-controlled study in baboons showed that recombinant human TNF-α binding protein effectively inhibits the development of endometriosis and endometriosis-related adhesions [39].

However promising these drugs seem to be, one must bear in mind that so far, most studies that evaluated the use of drugs such as interferon-α [137], interleukin-12 [138], loxoribine and levamisole [139], anti-Vascular Endothelial Growth Factor (VEGF) [140] and MMP inhibitors [141] have conducted tests only in rodents. It is essential that these drugs be tested for efficacy, safety, and teratogenicity during preclinical trials in nonhuman primate models, such as the baboon, before clinical studies are initiated in women.

Assisted reproduction and endometriosis

Current evidence suggests that patients with endometriosis have a poorer ovarian response and need a higher dose of gonadotropins in in vitro fertilization or Intracytoplasmatic Sperm Injection (ICSI) programs but not reduced endometrial implantation [142,143]. Currently, it remains unclear whether the presence or degree of endometriosis is associated with impaired oocyte quality, fertilization rate, and implantation rate. Future studies that evaluate the association between endometriosis and reproductive outcome after assisted reproduction should be prospective and include the following information [144–146]:

- Accurate and recent laparoscopic description of the stage of endometriosis
- Date, number of, and time interval between surgeries
- Ultrasound evidence of endometriosis, confirmed by cytology or histology when endometriotic cysts are aspirated during oocyte aspiration
- Effectiveness of interim suppressive therapy between diagnosis and treatment with assisted reproduction
- Reliability and date of negative diagnosis
- Clear definition of implantation rate, pregnancy rate, abortion rate, and baby take home rate per started cycle, per oocyte aspiration, and per embryo transfer
- Analysis that takes into account additional fertility factors, such as age, duration of infertility, and other causes of infertility.

Summary

Future research in endometriosis must focus on pathogenesis studies in the baboon model, the early interactions between endometrial and peritoneal cells in the pelvic cavity at the time of menstruation, and potential differences between eutopic endometrium and myometrium in women with and without endometriosis. More integration is needed between the areas of epidemiology and genetics. Pelvic inflammation in women with endometriosis could be the target for new diagnostic and therapeutic approaches. Important questions remain regarding the relationship between endometriosis and environmental factors. Systemic and extrapelvic manifestations of endometriosis must be analyzed carefully, and better tools are needed to measure quality of life in women with chronic pain caused by endometriosis. Most current evidence supports a causal relationship between endometriosis and subfertility, and the spontaneous progressive nature of endometriosis has been demonstrated in 30% to 60% of patients. Recurrence of endometriosis after classic medical and surgical therapy is a major and underestimated problem, especially in women with advanced disease. Integrated clinical and research teams are needed that combine expert medical, surgical, and holistic care with state-of-the-art research expertise in immunology, endocrinology, and genetics to discover new diagnostic methods and medical treatments for endometriosis.

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Obstet Gynecol Clin N Am
29 (2002) 599–611

OBSTETRICS AND GYNECOLOGY CLINICS of North America

Reducing death from cervical cancer Examining the prevention paradigms

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The impact of cervical cancer on the lives of women worldwide is indisputable. Cervical cancer is the third most common cancer in the world and the second most common cancer and leading cause of death from cancer in women in developing countries (where 80% of new cases occur). Almost 200,000 women died from this disease in 2000 [1]. The regions of highest prevalence of invasive cervical cancer include developing countries in Latin America, Asia, Southeast Asia, Africa, and the Caribbean; nearly 400,000 cases are diagnosed worldwide [2]. The cost of screening women in 5-year intervals and the health services rendered after a risk factor is established is approximately $100 per disability-adjusted life-year (DALY) compared with $2600 per DALY expended to treat or palliate cancer should we forego screening [1]. Most developed countries like the United States saw dramatic reductions in the incidence and death rate from cervical cancer following the implementation of an organized screening program [3–5]. Fig. 1 shows that in the United States the incidence and mortality rates associated with uterine and cervical cancer might have been decreasing prior to the implementation of organized Papanicolaou (Pap) smear-based screening programs. Other as yet unidentified factors might also have contributed to the decrease. A woman's lifetime risk of developing and dying from invasive cervical cancer is nearly 1.0% and 0.3%, respectively [6].

To reduce the incidence of cervical cancer we must refine our ability to help at-risk women obtain health services by finding financial support for the services and raising awareness regarding health seeking and preventative health behaviors, especially for high-risk women. We might be driven to offer screening in an opportunistic, unscheduled manner in some cases. Outreach efforts in high-risk women can involve a single lifetime intervention in those reticent to undergo screening for cultural reasons, excessive fears, or because of lack of economic resources to acquire medical services [7,8]. These patients will require extra financial and clinical expenditure. Those who pay for, organize, and deliver

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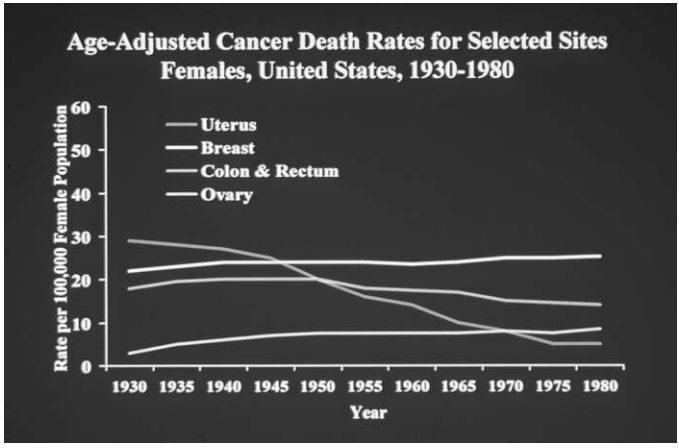

Fig. 1. Age-adjusted death rates for selected sites, females, United States, 1930–1988. (*Courtesy of* P.J. DiSaia, MD.)

screening services must consider providing the most sensitive measures to detect and eradicate disease in this group. If not, the massive investment in the infrastructure to reach, educate, and deliver services to these women is wasted. New cancer cases arise disproportionately in the underserved population, but the entire population must be considered when a comprehensive cancer prevention program is organized.

The majority of women in the US (see next section) gain access to the health care system and some have cancer precursors discovered and managed, yet our significant investment has not led to a measurable and sustainable reduction in the annual cervical cancer rate. We have witnessed a steady disease state over the last 15 to 20 years; the age-adjusted incidence rate has hovered around 8/100,000 women, and approximately 13,000 to 15,000 new cases of invasive cervical cancer and 3500 to 5000 deaths have occurred [9]. Obtaining knowledge, resources, and skills influence a preventative lifestyle and reduce a woman's lifetime risk through primary prevention and identifying those at risk. Appropriate care for the patient through secondary prevention—or prevention of the eventual outcome, death from cervical cancer through tertiary prevention—is the focus of this book.

Explaining the worldwide distribution and death rate from cervical cancer

Cervical cancer is the second leading cause of death from cancer in women worldwide, following lung cancer. The countries with the highest rates continue to be outside of the US in areas in the world that have poor resources regarding daily living (because of poverty) and health care delivery. The Pap smear has

been widely implemented in industrialized countries and desired in underdeveloped countries despite the fact that there were no randomized studies of its impact on incidence and mortality rates related to cervical cancer. The evidence of benefit therefore remains circumstantial. Nevertheless, most countries that have industrialized in the recent past have also seen an associated drop in the age-adjusted mortality rate following deployment of an organized screening program. The number of women screened per 100,000 is rarely or never reported along with the disease or mortality rate, but it should be inversely related to the disease rate. Table 1 shows the worldwide total incidence rate and crude death rate from invasive cervical cancer as reported in the Globocan 2000 World Health Organization database from the International Agency for Research on Cancer [10]. In the US, although the underserved population is at highest risk, approximately 40% to 50% of new cancers arise in women who have had at least one gynecologic screening examination and Pap smear within the prior 5 years.

Table 1
Worldwide incidence and mortality from invasive cervical cancer

Cervix uteri: 2000 worldwide cancer cases and incidence rates				
	Incidence: cases	Incidence: crude rate	Mortality: cases	Mortality: crude rate
Eastern Africa	30206	24.4	15837	12.8
Middle Africa	6947	14.4	3799	7.9
Northern Africa	10479	12.2	5524	6.4
Southern Africa	5541	23.2	2906	12.2
Western Africa	13903	12.5	7154	6.4
Caribbean	6670	34.8	3143	16.4
Central America	21596	31.7	8690	12.8
South America	49025	28.1	18737	10.7
Northern America	14845	9.5	7070	4.5
Eastern Asia	51266	7.1	25639	3.5
Southeastern Asia	39648	15.3	20462	7.9
South Central Asia	151297	20.9	83678	11.5
Western Asia	3458	3.8	1765	1.9
Eastern Europe	35482	21.9	15180	9.4
Northern Europe	6049	12.6	3162	6.6
Southern Europe	10116	13.7	4011	5.4
Western Europe	13282	14.2	6207	6.6
Australia/New Zealand	1077	9.4	432	3.8
Melanesia	983	31.3	510	16.2
Micronesia	25	9.8	12	4.6
Polynesia	70	22.8	35	11.6
More developed countries	91451	15	39350	6.4
Less developed countries	379153	15.8	194025	8.1
World	470606	15.7	233372	7.8

From Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide, Version 1.0. IARC CancerBase No. 5. Lyon: IARC Press; 2001; with permission.

Approximately 75% to 85% of the US population have undergone screening within the prior 3 years. Approximately 60% of new invasive cervical cancer cases arise in women who are underscreened or underserved (never screened or not screened within 5 years; about 15% to 25% of the US population) [11]. The woman who is underscreened or underserved is also at increased risk for behaviors that lead to the development and harboring of neoplasic lesions on the cervix, which will not be discovered. By definition, underscreened women receive none or fewer screening examinations because of a wide variety of beliefs or circumstances, while underserved women desire but cannot obtain medical care because of financial or social barriers. Many experts state publicly that we should focus our financial and clinical resources to reach out to such women and provide medical care when screening can be accomplished [12]. If we are fortunate enough to reap success from this intervention, it is sadly rare for these patients to change their propensity to undergo additional future medical examinations and screening because of the same cultural, financial, or social barriers. If one paints an "all or none" picture regarding either investing in outreach or more accurate screening tests for cervical neoplasia, many experts have advocated staying the course with the existing Pap smear-centered strategy in lieu of investing in more expensive but more effective screening and triage technologies. Both the population at large and the cohort in which we have invested so heavily to screen and educate face the identical pitfall—the low single-test sensitivity of the Pap smear [13]. This is not a moot point. Failure to diagnose cancer in women is the leading cause of malpractice litigation against physicians, their health care setting, and their clinical laboratory [14]. The need to pair better screening rates with more effective screening—especially in patients in which we have invested time and funds to provide outreach and education services—offers the only effective opportunity to find the disease and alter its biologic behavior and natural history.

Should the screening methodology for the well-screened, unscreened, or underscreened groups be identical? It is irrefutable that a single Pap smear screening examination lacks sensitivity (51% to find all neoplastic lesions, 80% to 85% to find high-grade precursors) [13]. One could make an argument toward providing "extra" services for the underserved, but if the technology used to screen for cervical cancer is flawed and the flaw is reproduced with each visit, then one cannot overcome the failure rate of screening. In developing countries in Africa in which an organized Pap smear screening infrastructure exists, the screening rate might continue to be low [15]. The ability to effectively screen, educate, reach the older population and provide follow-up care continues to be a challenge in Latin America, Asia, and other developing countries. This might be because of a cultural barrier toward prevention with related health care interventions, lack of education regarding the link between precursor identification and subsequent progression to invasive cancer, misperceptions related to cost of care, or inadequate services to meet the volume of care required [16,17]. For this high-risk group that is less likely to receive care, providing more costly screening services using adjuncts to the Pap smear or primary colposcopy might be cost

effective if early detection of precursors that define the patient as being at-risk is paired with timely treatment or follow-up care. It is unfortunate that most demonstration projects related to adjunctive technologies instituted in developing countries document the high-risk status of women in these settings, but they cannot attain adequate funding for treatment and follow-up services for the long term.

Human Papilloma Virus as promoter or inducer of cervical carcinoma

Any strategy to improve the lives of women who have or who are at risk of developing cervical cancer would be suspect if it did not consider the massive challenge related to reducing the spread and effect of Human Papilloma Virus (HPV) infection. Exposure to the highly infective HPV seems to be a prerequisite for the development of invasive cervical carcinoma. Walboomers and associates have shown that 99.7% of cervical cancers worldwide show molecular evidence of HPV [18]. The virus is ubiquitous and is prevalent worldwide. Exposure, which results in lower genital tract infection, is most likely sexually transmitted. It might occur as a result of vertical transmission or by way of fomite transfer. Studies have shown that oncogenes E6 and E7 in the HPV viral genome alter the function of p52 and pRb, which affects the capability of the host to suppress damage in the host DNA or regulation of the cell cycle, increasing the risk for malignant transformation. Degradation of the human tumor suppressor gene products that inhibit cell division are the key contributors toward malignant transformation of the cervix [19]. These genes are p53 (which halts progression of the cell cycle in the G1 phase) and pRb (which halts progression of the cell cycle from the G1 phase to the S phase). While HPV-induced genomic transformation might be a prerequisite risk factor, the role of other carcinogens, hormone effects, or other cell alterations or humoral host defenses, presence of free radicals by way of coincident infection, and inflammation might be equally necessary in the ultimate development of invasive cervical cancer [20]. The link between exposure, outcomes, and the proposed mechanism of neoplastic transformation is strengthened by evidence from prospective studies that measured these variables. The elimination of risk factors and the subsequent regression or disappearance of precursor lesions over time also strengthens our theories of cause and effect related to these risk factors. Infection in the female genital tract with oncogenic HPV subtypes is common, yet death from invasive cervical cancer in relation to those who are exposed is fortunately rare. Moscicki and colleagues documented a high prevalence of HPV virus (496/601patients; 83% of the eligible study candidates at initial testing) yet transitory nature of the virus in the lower genital epithelium of predominantly young women. They also observed a 90% clearance rate of the virus within a follow-up period of 50 months in those who showed DNA evidence of the virus after entry into the study [21]. Many experts conclude that identification of women with HPV DNA integration into the human genome and

persistent infection who continue to harbor intraepithelial neoplasia is paramount to efforts to prevent cancer and save lives [22].

Cervical cancer prevention and public health

A comprehensive cervical cancer prevention program requires efficient recruitment, identification of the ''at-risk'' population, and risk reduction through primary, secondary, or tertiary preventative measures. The value of any health service intervention hinges upon doing the right intervention at the right time. In major public health preventative measures, the target of our intervention is the entire population and the intervention must be simple and practical to employ. The value of our effort is measured by the improvement in quality of care each service brings (as evidenced by outcome measures), factored by the quality of the services rendered (access, satisfaction, interpersonal educational experience) divided by the overall cost of those resources as a requirement to conclude that our activities are worth the investment [23]. Timing of the services rendered is also relevant. If a patient harbors a true cancer precursor lesion on her cervix but never suffers an untoward medical event from that lesion, should we ask if it was necessary to expend the services and money to find that patient? I find this question to be the linchpin that underlies the confusion in both clinicians and patients regarding cervical cancer prevention in this country and worldwide. When individual patient concerns are overshadowed by implementing public health measures for the good of the entire population, the distribution of services might not always be appropriate for every woman, just appropriate for the average citizen in the setting in which care is rendered. When governmental or private insurance programs dictate which services should be provided based on the ''common good'' of the population, some patients will be underserved unless the opportunity to pay for additional services or another opportunity to access such services is made available. Investment in the prevention of other more prevalent female malignancies in the US such as breast cancer has also reduced the funds available for research and services related to cervical cancer. We must ask appropriate questions around the debate regarding an investment in cervical cancer prevention technology and services. The key questions we hope to address in this text are ''How can we save the lives of women who suffer from cervical cancer?,'' ''Who is truly at risk of harboring or developing a true cervical cancer precursor?,'' and ''How do we find the patient and assure or actively alter the natural history of disease to prevent suffering and death from disease?'' We will also ask ''How do we safely evaluate, triage, or treat patients?,'' ''Can moving the discovery point earlier in the neoplasia continuum save lives?,'' '' Is primary prevention of precursor lesions feasible?,'' and ''What can we reasonably expect for our investment?''

The number of women at risk (ie, harbor cervical neoplasia) numbers in the millions in the US alone, and the probability that any one woman will die

from or suffer morbidity from malignant transformation is small, thus we are somewhat complacent about investing in preventative measures. Most community-based physicians delivering gynecologic or primary care services might see less than two cases of invasive cervical cancer during their entire career. Both insurers and providers of health services lament at the inefficiencies of the investment in finding precursors, measuring their malignant potential through adjunctive testing or focused services, treating high-risk candidates, following patients until regression of disease occurs, and the unnecessary treatment of some patients with precursors who are not destined to die from their disease.

Debate centers around defining the target population and the goal during the screening process. Should the strategy focus on identifying patients at an early stage who are at risk after an exposure to HPV (an infectious agent) and treating all patients with precursors? In contradistinction to treating all patients with precursors, should we use adjunctive measures to only find and treat patients who are exposed and possess high-grade precursors who are at the highest risk (at the time of discovery) of developing and dying from invasive cervical carcinoma? At this time we are unable to distinguish with certainty the ''true cancer precursor'' with a grave prognosis from the multitude of women who are diagnosed with cervical intraepithelial neoplasia. Myers and associates have lamented that improved sensitivity in screening for precursors cannot be effective until we are better able to predict the prognosis of the ''at-risk'' lesion [24]. Most precursors found following screening and diagnostic colposcopy and biopsy (in association with HPV infection) are not destined to undergo malignant transformation. Our need to reassure women of their true risk status is driven through quality assurance measures whose origin might have been the risk of malpractice litigation, regardless of the enormous volume or cost.

The need to reassure patients might be confused when HPV is brought into discussions with the patient regarding the natural history of cervical intraepithelial neoplasia (CIN) and cervical cancer. Discussions begin by explaining the relationship of antecedent infection with HPV and the subsequent development of cervical cancer as indisputable [25]. The concept that cervical cancer is a sexually contracted and transmitted viral infection that insinuates into and alters the cervical cell genome can be conveyed. One must advise that the establishment of low-grade cervical epithelial abnormalities might indicate a transitory and reversible effect in young women, but it might serve as a nest for a clone of cells thus infected to continue to proliferate toward malignancy [26]. Immune recognition can lead to regression of disease along the continuum from mild, moderate, or severe dysplasia; however, the probability of regression decreases as the grade of disease increases [27,28]. Regression is not guaranteed, and if the patient with early changes is discovered, regular follow-up evaluations to reassure the patient and clinician are warranted. Despite these discussions and reassurances, many patients choose to be treated at the time of initial diagnosis, including patients with earlier precursors and a more optimistic prognosis.

Identification of all precursors during screening: lessons from medical history

The debate regarding the utility of screening tests and treatment of patients with precursors is not novel to lower genital tract cancers. The history and controversy around the detection and treatment of tuberculosis (TB), another fatal disease related to an infectious agent (a bacillus, not a virus), paralleled what is being debated around the goals and needed measures related to cervical cancer in current times [29]. Three decades ago, medical experts debated the value of finding all patients at risk of harboring TB following exposure. Prior to the development of the Purified Protein Derivative (PPD) skin test and other tests of prior immune recognition, clinicians advised a chest radiograph examination annually as a means to find evidence of intrapulmonary TB. In drawing a parallel to the performance of annual Pap smears and the discovery of a high-grade lesion, a patient with an abnormal chest radiograph could be considered "high risk" because of the obvious risk associated with a pulmonary focus and reaction. The sensitivity of a single chest radiograph in detecting TB infection was analogous to the low sensitivity of a single Pap smear in detecting any grade of cervical intraepithelial neoplasia. A chest radiograph with evidence of pulmonary pathology would clearly deem the patient at "high risk" of dying from TB and would guide the clinician toward further sputum testing, treatment with chemotherapeutic drugs, and possible quarantine. Once instituted, a positive PPD skin test would trigger a more judicious use of the chest radiograph test. In most positive skin test cases, a negative radiograph was (and still is) more prevalent than discovering concomitant positive radiograph changes in the screening population. The positive PPD test and negative chest radiograph result was not as predictive of risk of dying from TB as the abnormal chest radiograph. The patient with a positive PPD alone would be deemed as being at low risk of developing active TB and dying from the disease. Patients who tested negative were assumed to be disease- and risk-free because the negative predictive value of the test is high in patients who are not anergic. One of the most successful examples in medical history of a secondary prevention intervention resulting in a dramatic decrease in mortality from a disease occurred when the strategy and goal for TB screening was changed from finding only the highest-risk precursor (active pulmonary TB) to finding all patients at risk (as defined by a positive skin test) and adding prophylactic antibiotic treatment for patients at low risk and therapeutic doses for those at high risk. Confidence that the treatments were, for the majority of patients, safe and effective allowed clinicians to use the most sensitive test in the interest of the entire population. Some patients still suffered untoward and potentially fatal effects from isoniazid and other antituberculosis chemotherapeutic agents, but almost all patients were still treated or offered chemotherapeutic prophylaxis under strict supervision [30]. This strategy continues today as TB skin testing has become near universal and tied to employment or governmental benefits. Unlike our model for the

management of cervical cancer precursors, clinicians are treating patients without histologic confirmation of TB disease—based solely on the positive PPD result—with potent drugs. In contrast, many gynecologists are reticent to use the existing treatment armamentarium for cervical cancer precursors, which are predominantly destructive measures (ablation or excisional therapies) that rarely cause major complications in those treated. The most common side effects of laser large loop electrosurgical excsion procedure (Lletz or LEEP), cold knife conization, or cryotherapy include hemorrhage, cervical stenosis, or infection [31]. Although no direct effect of these treatments on fertility have been proven, the relationship between the volume of tissue removed during excision and the risk of an ensuing cervical incompetence during future pregnancies has been documented [32,33].

Because of a higher prevalence of precursors in the women under 35 years of age and concern about altering the treated patient's reproductive potential, some questions exist about treating low-grade precursors with a wide range of destructive therapies. Many women with low-grade precursors can be offered conservative management without treatment. These patients are counseled regarding the possibility of regression through native immune recognition. The obligation of all clinicians who offer such a choice is to assure the patient that regression has occurred in a reasonable time period of observation (no greater than 1 year). Patients with persistent dysplasia or progression of their disease should be counseled to undergo treatment. In addition to direct ablation, cryotherapy might induce immune recognition and host defense against further neoplasia [34].

Although most patients with precursors can be found when we use the most sensitive screening tests, our ambivalence regarding treatment remains a barrier to effective secondary prevention until our concerns about treatment can be overcome. Finding and treating more women at an early precursor stage (when each patient's prognosis is uncertain, when the initial diagnosis of dysplasia is made) could effectively reduce the incidence and age-adjusted mortality rate from invasive cervical carcinoma. In the US, millions of predominantly young women would be identified as being at risk and the treatment would have to be safe, widely available, easy to administer, and affordable. The current treatment armamentarium of cryotherapy, excisional therapy, and laser therapy is viewed by many clinicians (and conveyed to patients directly or through the lay media) as destructive, potentially harmful, and fraught with side effects. The success and safety of treatment is also dependent of the expertise of a health care provider with specialized training and might not be available and affordable in all settings. CIN is a disease of young women for whom we are reticent to treat because of controversial effects on fertility or fecundity whereas it is the middle-aged and older women who predominantly die from invasive malignancy. Our aversion to treatment of CIN might persist until a universally effective primary preventative intervention (HPV vaccine) is developed that prevents the development of cervical intraepithelial lesions or development of a therapeutic vaccine that inhibits their progression towards malignancy. The vaccine strategy might not

be feasible, but the secondary TB prevention model was effective in the US as the risk of developing or dying from TB was eliminated in those who were tested and those who were deemed to be at risk were effectively treated. Hence the bacille Calmette-Guérin (BCG) vaccine, a vaccine made of a live, weakened strain of the TB bacteria, was never implemented in the US. In countries in which universal access to screening was not assured, the BCG vaccine has been the mainstay of prevention of TB [35].

Beyond diagnosis: efficient triage related to the prognosis of CIN lesions

In the patient who harbors CIN lesions, the presence of concomitant risk factors and the patient's health seeking behavior or access to care all influence the "natural history" or clinical course of cervical neoplasia. Any organized health service screening effort must take a holistic approach, drawing on medical, economic, cultural, and psychosocial information and rendering the best decision for every individual. These services are costly; the business of preventing cervical cancer is a significant medical expenditure in the US and worldwide. Approximately 10% of women screened in the US are deemed at risk, mostly because of minor or major Pap smear abnormalities (rarely caused by suspicious visual lower genital tract changes or a positive screening HPV test), and most are either referred for colposcopy or offered additional services. The services range from repeating the Pap smear within a few months of the initial abnormality or using an intermediate in vitro screening tool (Hybrid Capture II, Digene Corporation, Silver Springs, MD) or in vivo screening tool (Cervicography, National Testing Laboratories, St. Louis, MO) to guide the need for follow-up colposcopy. If speculoscopy is performed as part of the Papsure procedure (Watson Diagnostics, Corona, CA) coincident with the Pap smear at the initial screening visit, the result can further predict a woman's risk of harboring an "at-risk" lesion (ie, cervical cancer precursor or cervical cancer) and can result in more effective triage for diagnostic colposcopy. Referral of all patients with screening abnormalities for colposcopy is a possible option; however, the cost of colposcopy might be prohibitive. Colposcopy, additional clinic visits, cervical biopsy acquisition and evaluation, and the indirect costs associated with these interventions can exceed $2 billion annually in the US (and might be closer to $4 billion) [36]. Sadly, this screen, triage, and treat paradigm has been paralleled by only a slight decrease in the death rate (which might in fact be a result of improved treatments for cancer) and has not lowered the incident cases of cervical cancer in the US in the last 15 years. Much recent effort has been directed at finding lesions earlier and concomitantly evaluating their oncogenic propensity using biophysical or biochemical "markers" or measures. We seek to find the elusive at-risk lesion and render treatment based on the patient's prognosis with more certainty related to its potential toward malignant transformation.

Rationale behind this issue: perspectives on prevention

In an attempt to begin with the end in mind, the organization of this issue deals with the concrete and moves to future possibilities and long-range goals in the prevention of death from cervical cancer. Following this introduction, we describe factors associated with the financial burden to the medical infrastructure related to prevention. The focus is more general but is for the most part applicable to both invasive squamous cell cervical carcinoma and adenocarcinoma. We have divided this issue into three sections: tertiary prevention related to cancer, altering the natural history of patients with preinvasive disease during secondary prevention, and discussing the elimination of the risk of dying from cancer in patients who are unaffected (but might be at risk) through primary prevention. Because efforts in prevention revolve around the utilization of resources, the economics related to screening, triage, and treatment of cervical cancer is reviewed. When this context is framed, we will focus on the care of patients with established invasive carcinoma with the goal of improving survival rates. Established and emerging therapeutic interventions serve as the basis for tertiary prevention and are viewed in separate chapters. Secondary prevention relies on the identification of the at-risk patient and providing meaningful clinical and educational interventions that might alter the probability of developing cancer precursors or invasive carcinoma. Conventional cervical cytology has been the mainstay for clinicians to find patients with preinvasive and invasive disease. Its usefulness and pitfalls are discussed. New technologies to overcome a significant false-negative rate of screening associated with the conventional in vitro, cytologic-based Pap smear have emerged. One article deals with in vitro improvements including liquid-based cytology and testing for evidence of prior or current HPV infection. Another article focuses on more immediate clinician-rendered in vivo testing, during which the goal is bedside visual or biophysical identification of cervical neoplastic lesions that the Pap smear would otherwise miss. The majority of lesions missed that the in vitro test finds are because of sampling error, and the etiologies underlying this error are explored. Because a large number of women are found to be at risk following screening, evidence-based practice guidelines have been established to assist in their management [37]. Secondary prevention not only involves accurate screening but also triage of patients who are deemed to be at risk following an abnormal screening result or the identification of a preinvasive lesion through a diagnostic evaluation, usually colposcopy and biopsy. The decision to provide treatment or conservative follow-up care is based on medical evidence or consensus by experts. When a treatment is planned, the advantages and disadvantages and the effectiveness of available and proposed treatment modalities are conveyed to the patient and are covered in a separate article. We begin by identifying the ''at-risk'' population according to physical, biological, epidemiological, and genetic definitions of risk. The at-risk group who might be destined to develop CIN that progresses to carcinoma is distinguished from a low-risk group that is unlikely to be affected. In the last section, risk factors related to the development of cervical cancer are reviewed.

Meaningful lifestyle modifications or medical interventions such as vaccine therapy, which might contribute to lowering or eliminating the risk of developing cervical cancer are explored. The opportunities and barriers to effective primary, secondary, and tertiary prevention will be discussed. We hope this book will serve as a resource for those who plan or deliver cervical cancer prevention services for women worldwide.

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