Endometriosis-related Infertility

A Comprehensive Manual

Simone Ferrero
Editor

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Preface

In addition to the distressing pain symptoms, individuals afflicted with endometriosis often face the challenge of infertility, a condition that necessitates precise diagnosis and adept management to optimize their chances of conception. The intricate nature of endometriosis-related infertility, stemming from the distortion of pelvic anatomy, inflammatory processes within the pelvic cavity, and adverse effects on ovarian follicles and ovulation, remains only partially understood. This book delves into the nuanced impact of various manifestations of endometriosis, including ovarian endometriomas and deep infiltrating endometriosis, on reproductive potential.

A significant emphasis is placed on the utilization of ultrasonography for the diagnosis of endometriosis in individuals struggling with infertility, alongside advanced imaging techniques to evaluate tubal patency. The therapeutic landscape, encompassing intrauterine insemination and in vitro fertilization, will be explored in depth to illuminate their roles in overcoming endometriosis-induced reproductive hurdles. Furthermore, the consequences of surgical intervention for endometriosis on natural fertility and the efficacy of assisted reproductive technologies will be thoroughly examined.

Additionally, this volume sheds light on the influence of endometriosis on endometrial receptivity and introduces the critical concept of fertility preservation for women diagnosed with this condition. Designed as an essential resource, this book aims to equip reproductive surgeons, sonographers, and IVF specialists with comprehensive insights into the multifaceted relationship between endometriosis and infertility.

I extend my heartfelt gratitude to the esteemed international experts whose contributions have enriched this work, making it an indispensable tool for healthcare professionals dedicated to the care of those affected by endometriosis.

Genova, Italy — Simone Ferrero

Contents

The Epidemiology of Infertility in Women with Endometriosis

Nicola Berlanda, Francesca Chiaffarino, Elena Roncella, Giovanna Esposito, and Fabio Parazzini

Endometriosis is a common disease, affecting about 10% of women of reproductive age. The main symptoms are pelvic pain, menorrhagia, dysmenorrhea; further, endometriosis may reduce fertility. Frequently cited statistics report that about 30% of women with endometriosis have a diagnosis of infertility [1]. Other studies have reported a frequency of endometriosis among infertile women ranging from 20 to 50% [2, 3]. The strength of the association between endometriosis and infertility is variable; it has been suggested that the extent of disease impacts the degree of reduced spontaneous fertility [4].

The causes of infertility in women with endometriosis are not completely understood, but distorted pelvic anatomy, endocrine and ovulatory abnormalities, altered peritoneal function, and hormonal and cell-mediated functions in the endometrium are factors that can explain the association.

In this chapter, we have briefly reviewed the main epidemiological data on the relationship between endometriosis and infertility, focusing on data on the frequency of infertility among women with endometriosis, the frequency of endometriosis among women with infertility, and finally the determinants of infertility among women with endometriosis. Further, a brief paragraph will address the impact of infertility on the quality of life of women with endometriosis.

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1 Frequency of Infertility among Women with Endometriosis

In the "normal" couples, the monthly probability of conception, i.e. fecundability, is about 0.15–0.20. This value decreases to 0.02–0.1 per month in women with endometriosis [5–7] and this disease is also associated with a lower live birth rate [8].

The more simple way to analyze the relationship between infertility and endometriosis is to investigate the frequency of infertility among women with endometriosis. However, despite the impressive number of papers published every year on endometriosis, only a few epidemiological studies offer information on this issue. The most quoted data are from the Nurses' Health Study II prospective cohort study. This study reported 1721 cases of laparoscopically confirmed endometriosis among women with no past infertility during a 10-year follow up. Among these, 1340 women were never infertile whereas 361 (21%) women reported an infertility evaluation as well as a laparoscopic confirmation of endometriosis. The overall incidence rate of endometriosis was 237/100,000 person-years and did not begin to decrease significantly until women were in their late 30 s to early 40 s. The corresponding values of age-adjusted incidence rate of diagnosis of laparoscopically confirmed endometriosis among women with a history of infertility were 1380/100,000 person-years [9].

In a prospective study conducted by Prescott et al., among the 58,427 eligible women included in the analysis, 3537 (6%) reported a diagnosis of laparoscopically confirmed endometriosis. Among them, 83% were parous by the age of 40 and, of these, 15% reported ever use of clomiphene or gonadotrophin to stimulate ovulation, and 2% reported ever use of IVF. In that cohort study, women with a history of endometriosis have a higher risk for incident infertility compared with women without a history of endometriosis [hazard ratio (HR) 2.12, 95% confidence interval (CI): 1.76–2.56] [10].

In line with these findings, recently in Canada, Singh et al. conducted a cross-sectional online survey of women aged 18–49. Out of about 2000 women with endometriosis, 22% reported infertility [11].

2 Frequency of Endometriosis among Infertile Women

Another way for quantifying the association between endometriosis and infertility is to analyze the frequency of endometriosis among infertile women. We have recently conducted a systematic review of the frequency of endometriosis in infertile populations [12]. We included studies that reported incidence or prevalence rates or ratios for infertile women. On the whole, 14 papers were included for a total of more than 6000 women [13–26] (Table 1).

The pooled estimated prevalence of endometriosis was 23.8% (95%CI: 16.1–31.5) in infertile women. However, this estimation has some limitations. First, since surgical visualization has been traditionally considered the gold standard for a diagnosis of endometriosis, it is possible that only a proportion of infertile women had laparoscopy. Second, large differences emerged in the prevalence among

Table 1 Main results of studies on the prevalence of endometriosis in women with infertility

References, country	Frequency of endometriosis (%)
[15], USA	43 (43/100)[a]
[20], Spain	34.5 (259/750)
[25], Nepal	2.5 (5/200)
[16], Pakistan	16.8 (134/796)
[21], Belgium	47 (104/221)
[13], Poland	9.6 (145/1517)
[14]. Malta	23 (74/437)
[22], India	48.4 (180/372)
[24], Australia	6.6 (5/76)
[26], Nigeria	24 (33/141)
[18], Pakistan	11 (9/80)
[19], USA	9.5 (68/717)[b]
[23], USA	55 (276/502)
[17], Pakistan	11 (11/100)

[a]Cases with endometriosis/total cases
[b]Endometriomas are not included

studies ranging from 2.5% to 55%. These differences can be at least in part explained by different study designs. Some studies have recruited selected women and other studies had a small sample size. The direction of these biases is unclear, but in general, we can consider that about one out of three to four women with clinically evident endometriosis experiences infertility problems.

3 Risk Factors

Frequency of the disease apart, the goal of epidemiological studies is also to analyze the factors associated with a condition. A few studies have considered the factors associated with infertility among women with endometriosis. Similarly, very few data are available on the risk factors for endometriosis among infertile women.

4 Factors Associated with Infertility among Women with Endometriosis

4.1 Stage and Site of Endometriosis

The Revised American Fertility Society (AFS) scoring system is widely used to staging the endometriotic disease. Clinical data, however, have consistently shown that there is no clear relationship between the AFS staging and the infertility [27, 28].

It is a common thought that pelvic anatomy distortion can explain infertility in patients with severe forms of endometriosis. Further, pelvic/peritubal adhesions

could affect tube patency, oocyte release and capture by the fimbriae, ovum pickup, and ovum transport. Moreover, it has been suggested that women diagnosed with advanced endometriosis have a smaller follicle count, maybe due to surgical treatment damaging the ovarian tissue [29]. In this perspective, Adamson and Pasta proposed the use of the "Endometriosis fertility index" that takes into account, with the ASRM score, the functional status of the fallopian tubes, ovaries, and fimbriae and some clinical characteristics such as woman's age, duration of infertility, and previous pregnancies [30]. This index has been shown to be a useful tool for predicting reproductive prognosis after ASRM staging, underlining the role of tubal status on the risk of infertility [30, 31].

However, infertility in women with early endometriosis, where pelvic anatomical distortions are not present, involves other mechanisms, such as the alteration of the peritoneal, follicular, and endometrial microenvironments which can cause damage to folliculogenesis, ovulation, oocyte quality, endometrial receptivity, and, even, sperm function [32, 33]. Due to the plurality of the possible mechanisms leading to infertility and to the frequent coexistence of different phenotypes of endometriosis, it is difficult to assess the risk of infertility specifically for deep, ovarian, and peritoneal disease. Recently, a specific mechanism for ovarian endometriosis to cause infertility has been demonstrated in a mice model, consisting in an iron-mediated oxidative stress of ovarian follicles [34]. Further studies are advisable to assess whether ovarian endometriosis is more frequently associated with infertility as compared to the other locations of the disease.

A risk factor for infertility in women with endometriosis may be represented by adenomyosis. In a recent study by Decter et al. [35], among women undergoing surgery for endometriosis, those presenting five or more ultrasonographic features of adenomyosis had a two-fold risk of infertility as compared to those who did not [odds ratio (OR) 2.31, 95%CI:1.20–4.45, $p = 0.012$].

4.2 General Characteristics of the Woman

In the previously quoted cohort study by Prescott et al., the increased risk of endometriosis-associated infertility was apparent only among women <35 years of age and those of normal weight (BMI < 25 kg/m^2) [10].

5 Risk Factors for the Endometriosis Associated with Infertility

The main recognized risk factors for endometriosis are nulliparity, never oral contraceptive use, and regular menstrual cycles [36]. A few studies have analyzed the role of these factors on the risk of endometriosis associated with infertility in comparison with asymptomatic endometriosis or endometriosis associated with pain.

In two case–control studies conducted in Italy during the last decade of the previous century, regular menstrual cycles and oral contraceptive use increased the risk of endometriosis associated with infertility and the estimated ORs were largely similar to those associated with the risk of painful endometriosis [37–39]. Calhaz-Jorge et al., among 1079 subfertile women, reported that risk factors for the presence of endometriosis were race, obesity, irregular menstrual cycles, intensity of menstrual flow, dysmenorrhea, chronic pelvic pain, obstetric history, oral contraceptive pill use, and smoking habits, i.e. the general risk factors for endometriosis [40]. These findings suggest that the epidemiological profile of endometriosis associated with infertility is similar to that of endometriosis associated with pain.

5.1 Impact of Infertility on the Quality of Life of Women with Endometriosis

Another important aspect of the relationship between endometriosis and fertility is the impact of infertility on the quality of life of women with endometriosis. Recently, Missmer et al. have published a narrative review. The authors have identified seven studies that address the impact of endometriosis on the fertility component of the life course [41]. The authors reported that "the experience of infertility adds to the burden of endometriosis, negatively affecting psychological health, marital relationships, social interactions (e.g. avoiding friends and relatives with children), and financial status (due to fertility treatment) as well as causing feelings of stigmatization and hopelessness." Moreover, some young women with endometriosis worry about finding a significant other who will be accepting a possible infertility [42]. In particular, the potential risk of infertility associated with endometriosis impacts the family planning [43]. Some couples may be pushed to search for a pregnancy earlier than they had planned, inducing anxiety.

6 Conclusion

Infertility is a condition commonly associated with endometriosis. Epidemiological data suggest that the risk of infertility is about two times or more higher among women with endometriosis in comparison with the general population. However, not all women with endometriosis had infertility: about 25% of women with clinically evident endometriosis will experience infertility during their life, and conversely about 25% of infertile women will be diagnosed with endometriosis.

If we are able to quantify the relationship between endometriosis and infertility, the mechanisms of this relationship are poorly understood. The functional status of the fallopian tubes, ovaries, and fimbriae is probably the most important determinant of the reproductive prognosis in women with endometriosis, but this evaluation

requires invasive procedures. Older age and no previous births (primary infertility) are clinical determinants of poor prognosis, but their clinical impact is limited due to the large proportion of “old,” nulliparous women among women with an incident diagnosis of endometriosis or infertile women.

Fertility preservation (e.g. egg freezing) among reproductive-age women with endometriosis has been suggested [44]. A more clearer understanding of the relation between endometriosis and infertility and, in particular, the identification of risk factors of poor reproductive prognosis among women with a diagnosis of endometriosis may be useful to offering personalized counseling and therapeutic options to women with endometriosis.

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Endometriosis and Infertility: The Comorbidities

Tommaso Capezzuoli, Flavia Sorbi, Silvia Vannuccini, Roberto Clarizia, Marcello Ceccaroni, and Felice Petraglia

1 Introduction

Infertility is one of the most important symptoms in women with endometriosis. Endometriosis-related infertility is associated with ovarian damage, altered endometrium, alteration of the pelvic cavity due to inflammation, and adhesions with distortion of pelvic architecture and inflammatory changes in peritoneal fluid [1]. The prevalence of infertility in women with endometriosis is very high and the disease is one of the main causes of female infertility. The monthly fecundity rate in endometriosis is reduced from 15–20% to 2–10%; an advanced stage of disease correlates with a greater decline of this rate. In patients undergoing laparoscopy for infertility, the prevalence of endometriosis is at least 30%, confirming the relevant impact on women's reproductive life [2].

The present chapter will review the coexistence of gynecological [adenomyosis, uterine fibroids, and polycystic ovarian syndrome (PCOS)] or systemic (immune, inflammatory, and psychiatric and neurological disorders) comorbidities (Fig. 1)

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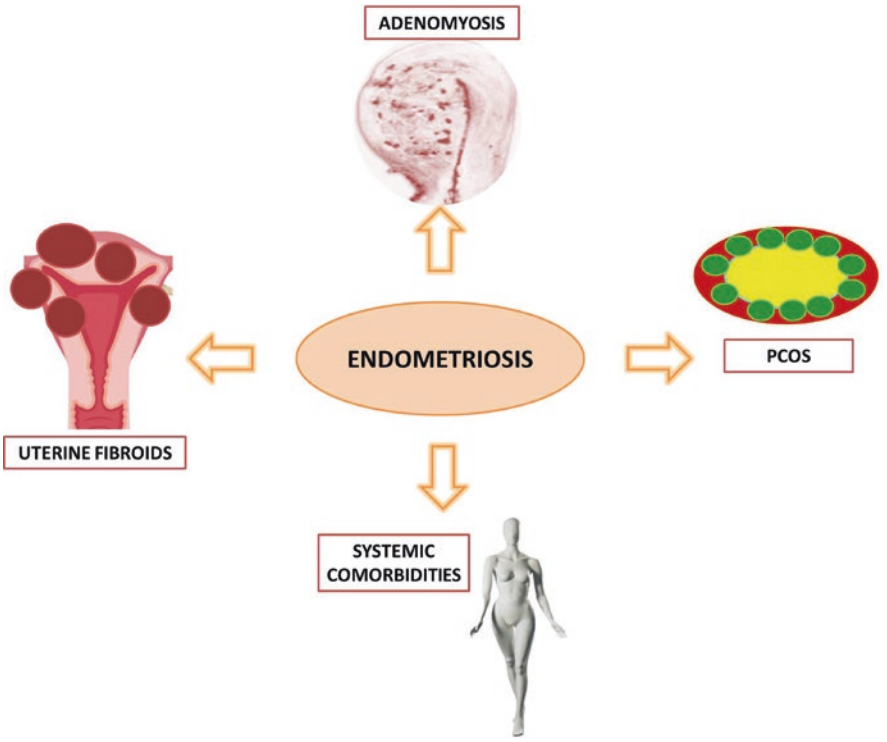

Fig. 1 Gynecological and systemic comorbidities of endometriosis

which may influence fertility and reduce the chance of onception in women with endometriosis.

The coexistence of gynecological and systemic comorbidities can in fact contribute in different ways to associated infertility. Therefore, the evaluation of these comorbidities is crucial in the management of endometriosis-associated infertility [3, 4].

1.1 Gynecological Comorbidities

1.1.1 Adenomyosis

Adenomyosis is characterized by the presence of endometrial glands and stroma in the myometrium and dysmenorrhea and heavy menstrual bleeding (HMB) are the main symptoms [5]. In the past few years, several studies have shown the presence of adenomyosis in patients with endometriosis.

Trans-vaginal ultrasound (TVUS) evaluation of women before undergoing laparoscopic surgery for pelvic pain highlights a strong association between uterine

adenomyosis and stage IV endometriosis [6]. Naftalin et al. observed a 20.9% prevalence of adenomyosis by using TVUS in a general population of patients attending a gynecological clinic; adenomyosis was associated with an older age, higher gravidity and parity, and presence of pelvic endometriosis [7].

Di Donato et al. showed a prevalence of 21.8% in patients undergoing surgery for endometriosis, detecting a statistically significant association with parity, age, dysmenorrhea intensity, and the presence of deep infiltrating endometriosis (DIE) [8]. A higher prevalence of adenomyosis was found by Eisemberg et al., who observed an 89.4% prevalence of TVUS signs of adenomyosis in women with a history of surgery for endometriosis [9]. Lazzeri et al. found a 47.8% prevalence of adenomyosis in women with DIE, influencing significantly the pre- and post-surgical dysmenorrhea severity [10]. A similar prevalence of adenomyosis (59.9%) was detected by using magnetic resonance imaging (MRI) in symptomatic women younger than 42 years, undergoing surgery for endometriosis [11]. Capezzuoli et al. evaluated the coexistence of uterine disorders by TVUS in patients with endometriosis and history of infertility, with a prevalence of adenomyosis in 21.2% of patients [3].

Adenomyosis-related infertility is caused by aberrant uterine contractility, abnormal myometrial activity, and deranged endometrial milieu with altered expression of implantation factors [5, 12]. Adenomyosis affects fertility in a very strong way by reducing the fertility rate and increasing the abortion rate, as described by a pioneer study in baboons [13] and recently confirmed [14].

1.1.2 Uterine Fibroids

Uterine fibroids are present in 5–10% of infertile women, but they represent the unique cause of infertility only in 2–3% and, in particular, when determining distortion of the uterine cavity, alteration to the endometrial and myometrial blood supply, deviation or obstruction of the tubal ostia, and impaired implantation [15, 16].

The association between uterine fibroids and endometriosis is less clear and most of the studies showed histological prevalence of uterine fibroids in women with endometriosis undergoing surgery. Uimari et al. [17] detected uterine fibroids in 25.8% of patients undergoing surgery for endometriosis and, conversely, in 19.6% of patients operated for uterine fibroids. According to another surgical report, premenopausal women requiring a hysterectomy for benign uterine disorders had endometriosis and adenomyosis in 40.4%, endometriosis and uterine fibroids in 22.7%, and both conditions in 34.1% [18]. In a similar report on women undergoing surgery for benign gynecological disease, the coexistence of endometriosis with uterine fibroids, adenomyosis, and benign ovarian cysts was 28%, 43.5%, and 50%, respectively [19].

Coexisting uterine fibroids and endometriosis were identified in 21.2% of patients undergoing laparoscopy myomectomy [20].

When evaluated by TVUS in infertile endometriotic patients [3], the prevalence of uterine fibroids in women with endometriosis was 3.1%, while the prevalence of uterine fibroids associated with adenomyosis was 14.6%.

1.1.3 PCOS

PCOS results from a vicious circle of androgen excess favoring abdominal and visceral adipose tissue deposition that induces insulin resistance and compensatory hyperinsulinemia, further facilitating androgen secretion by the ovaries and adrenal glands. This cyclical pathogenetic interaction between insulin resistance, hyperinsulinemia, and hyperandrogenism, in combination with hypothalamic-pituitary dysfunction, leads to further ovarian dysfunction that can result in anovulation and infertility. Similar mechanisms are involved in infertility related to metabolic syndrome [21].

The association between endometriosis and PCOS is less studied. In a recent retrospective cohort study and meta-analysis [22], the prevalence of asymptomatic endometriosis in women undergoing laparoscopic ovarian drilling for Clomiphene-resistant polycystic ovary syndrome was 7.7%. PCOS is associated with lower endometriosis stages (I and II) at the American Society for Reproductive Medicine (ASRM) classification [23].

1.2 Systemic Comorbidities

Endometriosis is a benign endocrine disorder but inflammation and immune factors should be considered in the pathogenetic mechanisms. Epidemiological studies show that women with endometriosis are often affected by systemic comorbidities, including immune, inflammatory, psychiatric, and neurological disorders [24–27].

1.2.1 Autoimmune Diseases

Systemic autoimmune diseases can interfere in several ways with female fertility, with general and specific mechanisms. Patients with systemic autoimmune diseases have less children than expected in the general population. Some of these women do not have children, some others report a prolonged time to pregnancy resulting in smaller family size than they expected. The disease itself and the musculoskeletal limitations linked to it can impair sexual function and psychologically impact woman desire. In addition, in several systemic autoimmune diseases, also the poor body image, the related to poor self-esteem, and depression can influence the personal and sexual relationships of these women [28].

Women affected by endometriosis present an increased prevalence of several autoimmune diseases. The presence and the growth of endometrial cells in the peritoneal cavity promote oxidative stress and inflammation. Endometriosis is in fact

characterized by an inflammation milieu with an increased production of metalloproteinases, prostaglandins, and cytokines, such as interlukein-6 and tumor necrosis factor (TNF) that promote the adhesion of endometrial tissue on ectopic surfaces. Moreover, women with endometriosis exhibit altered immune surveillance with depressed cell-mediated immunity and higher humoral immune response. Moreover, a genetic predisposition HLA DQ7-related is suggested for endometriosis and several gene polymorphisms are found both in endometriosis and autoimmune diseases as well as some genetic alleles involved in the release of autoantibodies [29].

Altered cell-mediated immunity is also involved in the development of the Celiac disease because the disease pathogenesis is characterized by a critical role of interleukin-18 (IL-18) and interferon-c (IFN-c) in inducing and maintaining Th1 responses after gluten exposure. Similarly, a Th1 imbalance with involvement of IL-18 and IFN-c has been reported in endometriosis and it has been shown that IL-18 is a key cytokine in developing the pathogenesis of endometriosis [29].

The relationship between systemic lupus erythematosus (SLE) and endometriosis could be related to ANA autoantibodies production, detected also in endometriosis [24]. Moreover, the similarities between the underlying humoral immune dysfunction observed in SLE and endometriosis and the similar direction of associations between hormonal risk factors in these two diseases may explain the strong concomitancy [30]. The premature ovarian failure observed in SLE patients is linked to immunosuppressive drug treatment but also the associated autoantibodies can directly affect the male and female gonads. In women, anti-ovarian antibodies described as linked to ovarian aging and autoimmune oophoritis leading to impaired ovarian function were reported in SLE patients and linked to premature menopause. Moreover, menstrual irregularity and ovulatory cycles are reported in SLE patients with high disease activity [28].

Humoral immunity can explain the correlation with autoimmune thyroid diseases. A higher reactivity of some autoantibodies (e.g., anti-thyroid peroxidase antibodies) in patients with endometriosis has been found. Another possible link between endometriosis and autoimmune thyroiditis (in this case Grave's disease) could be identified in an alteration in the expression of the estrogen receptor beta gene (ESR2), which is an important modulator of the immune system as an regulator of cytokine expression, antigen presentation, and B-cell lymphopoiesis [29, 31, 32]. Hypothyroidism associated with autoimmune thyroiditis can impair fertility by decreasing levels of sex-hormone-binding globulin and increasing the secretion of prolactin (ovulatory dysfunction from inadequate corpus luteal progesterone secretion associated to the altered secretion of gonadotrophin-releasing hormone) [28].

1.2.2 Inflammatory Diseases

Women with endometriosis have an increased risk of inflammatory bowel diseases, even after 20 years from diagnosis. In a large Danish cohort study, women with endometriosis had an increased risk of Chron's disease and ulcerative colitis with a standardized incidence ratio of 1.5 (95% CI 1.3–1.7) and 1.6 (95% CI 1.3–2.0), respectively [33]. In epidemiological studies with a control group, the proportion of

inflammatory bowel diseases in patients with endometriosis varied from 2% to 3.4%, compared to 0%–1% of the control group. Endometriosis and inflammatory bowel diseases are characterized by similar features and symptoms. In the case of concomitancy, this results in an increased risk of delayed or indeterminate diagnosis. Inflammatory cytokines and dysregulation of the immune system are key features of endometriosis and inflammatory bowel diseases. Both conditions overlap not only in symptoms but also in the potential mechanism of disease pathogenesis. In patients where endometriosis and inflammatory bowel diseases coexist, the symptoms can be atypical and cyclic, and fibrosis, caused by chronic inflammation, can contribute to obstruction of the intestinal lumen [34]. Women with Crohn's disease have normal or only slightly reduced fertility, whereas those with ulcerative colitis have normal fertility [35]. The low fertility rate is rather because of voluntary childlessness than severe disease, perianal involvement, and ileal pouch-anal anastomosis surgery [36].

Women with endometriosis are at a 1.4–1.6 higher risk of myocardial infarction/coronary disease. The data may be correlated with high levels of oxidative stress markers, elevated inflammatory factors, and oxidative stress markers in affected women. Part of the associations was found to be statistically accounted for by endometriosis treatments that are risk factors for cardiovascular diseases, such as hysterectomy/oophorectomy and earlier age at surgery following endometriosis diagnosis [24, 37, 38]. Moreover, women with endometriosis present a higher risk of hypercholesterolemia with RR 1.25 (95% CI, 1.21–1.30) and hypertension with RR 1.14 (95% CI, 1.09–1.18) [38, 39]. These data may be associated with the altered hormonal and chronic systemic inflammatory milieu typical of endometriosis. Conversely, elevated low-density lipoprotein in hypercholesterolemia and chronic systemic inflammation resulting from hypertension may increase the risk of endometriosis [39].

Considering intra-pelvic inflammation conditions, superficial endometriosis prevalence is increased in women undergoing emergency surgery for appendectomy [40]. Moreover, endometriosis patients seem to present a higher prevalence of pelvic inflammatory disease (PID), above all, in the case of high-stage disease [41]. Infertility can result from PID because the infection can cause severe damage to the fallopian tubes, including loss of the ciliary epithelial cells of the fallopian tube and occlusion of the tube [41]. Finally, also bladder pain syndrome and recurrent interstitial cystitis (BPS/IC) seems to be associated with endometriosis. BPS/IC and endometriosis share common pathogenetic mechanisms including inflammatory changes through several potential mediators such as chemokines or cytokines [42].

1.2.3 Mental Health Disorders and Migraine

A great vulnerability to psychiatric disorders is described in endometriosis patients. There is, in particular, a tendency to contract affective or anxiety disorders as well as panic–agoraphobic, somatoform, and substance use disorders. Endometriosis with pelvic pain, infertility, and psychic vulnerability usually leads to disability and

a markedly lower quality of life for women of reproductive age. Thus, the burden of endometriosis is not limited to the symptoms and dysfunctions of the disease; it extends to the social, working, and emotional spheres, leading to severe impairment of global functioning and significant disruption of daily life [26, 43]. Finally, endometriosis seems to be associated with a higher risk of migraine. In a recent study, adolescents with endometriosis were more likely to experience migraine (69.3%) than those without endometriosis (30.7%) [44].

2 Conclusions

Endometriosis is a complex disease and it is often associated with various gynecological and systemic comorbidities. The concomitant presence of these disorders has a synergistic effect in determining the worst quality of life in affected women and interferes with fertility. A common pathogenesis between endometriosis and some of these diseases (adenomyosis or systemic autoimmune diseases) supports the concept of infertility as a syndrome with various clinical aspects.

The diagnosis of concomitant gynecological and systemic conditions affecting fertility is critical to define a more comprehensive counseling and a better plan for fertility desire. The identification of coexistent gynecological diseases allows to plan a medical or surgical pretreatment. The association of endometriosis with systemic autoimmune conditions is a well-known cause of infertility and/or subfertility that needs to be taken into consideration when difficulties in conception are reported.

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Impact of the Endometriomas on the Ovarian Follicles

Paul J. Yong and Mohamed A. Bedaiwy

1 Introduction

This chapter will focus on the biological impact of ovarian endometriomas on ovarian structure and function, which may lead to infertility. We will begin with a brief overview of the etiology of ovarian endometriomas, and then review potential biological mechanisms including (a) anatomical distortion and other non-ovarian mechanisms; (b) endometrioma fluid and cyst wall; (c) iron metabolism, oxidative stress, and local inflammation, and their relation to abnormalities in granulosa cells and follicular fluid; and (d) pathways leading to a reduction in oocyte quantity. There will be a focus on the published literature specific to ovarian endometriomas, rather than endometriosis in general. These mechanisms are illustrated in Fig. 1.

2 Etiology of Ovarian Endometriomas

There are several hypotheses for the genes of the endometrioma cyst wall [1]. One hypothesis is metaplasia of invaginated mesothelial inclusions, where mesothelium covering the ovary invaginates into the cortex and subsequently undergoes coelomic metaplasia. A second hypothesis is that superficial implants invaginate into the ovarian cortex, for example, where the ovary becomes attached to adjacent non-ovarian endometriosis, followed by invagination into the ovarian cortex. A third hypothesis is that adjacent non-ovarian endometriosis invades a corpus luteum. Regardless, the resulting endometrioma has a mean cyst wall thickness of 1.4 mm,

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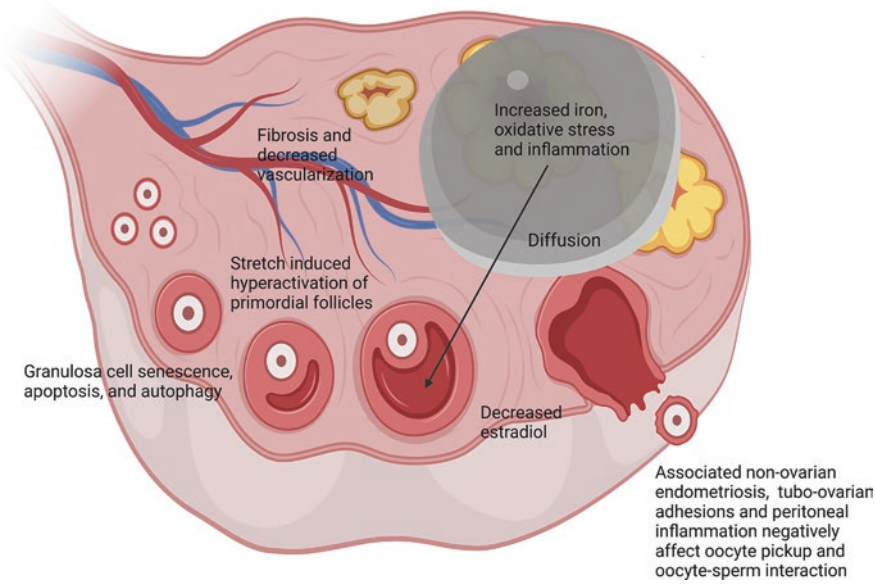

Fig. 1 Biological effects of ovarian endometriomas on ovarian follicles. Ovarian endometriomas are associated with increased iron, oxidative stress, and inflammation, which can diffuse to surrounding follicles and reduce oocyte quality. Granulosa cells demonstrate higher rates of senescence, apoptosis, and autophagy, which can lead to decreased estradiol production. Endometriomas also induce surrounding ovarian fibrosis and decreased vascularization, as well as stretch-induced hyperactivation of primordial follicles, which reduces oocyte quantity. Around endometrioma-affected ovaries, the presence of non-ovarian endometriosis, tubo-ovarian adhesions, and peritoneal inflammation also reduce fertility. It should be noted that this is a simplified diagram only; for example, the ovarian endometrioma would consist of chocolate fluid, endometrial epithelium/stroma cyst wall, as well as fibrosis. Created with BioRender

with the endometriosis epithelium/stroma penetrating the cyst wall only 0.6 mm on average [2].

While a full account of the biological studies of ovarian endometriomas is beyond the scope of this chapter, a brief review of recent novel methodological approaches will be provided. In a review of epigenetic studies of endometriomas [3], epigenetic alterations were noted in histones H3 and H4, and notably hypomethylation of steroidogenic factor-1 (SF-1) that binds promoters of steroidogenic acute regulatory protein (STAR) and aromatase. The latter was replicated in a genome-wide methylation study of endometrial stromal cells from endometriomas [4]. Other genes have been found to be hypomethylated or hypermethylated in ovarian endometriomas in another genome-wide analysis by Borghese et al. [5], although only a specific subset of epigenetic events were correlated to nearby gene expression.

Furthermore, somatic cancer driver mutations and other somatic genomic events in the epithelium of endometriosis (without cancer), including endometriomas, were recently reviewed [6]. In ovarian endometriomas, a variety of abnormalities

have been noted such as chromosome abnormalities (e.g. chromosome 17 aneuploidy, as well as a variety of chromosome arm gains or losses using comparative genomic hybridization), areas of loss of heterozygosity (e.g. 10q23.3), loss of BAF250 (*ARID1A*) immunohistochemistry expression in a proportion (8–19%) of endometriomas, and recurrent somatic cancer driver mutations in endometriosis epithelium in work done by Suda et al. (e.g. in *KRAS* and *PIK3CA*) [7, 8]. The biological implications of these somatic genomic events remain unclear, but as they are characteristic of malignancies, they may promote invasion or invagination of endometriosis cells into the ovary.

Sanchez et al. [3] reviewed the literature for microarray gene expression studies on ovarian endometriomas specifically in comparison to the eutopic uterine endometrium. They found that endometriomas had comparatively higher expression of hydroxysteroid 11beta-dehydrogenase that converts cortisone to cortisol; phospholipase A2 group II and group V that produce arachidonic acid precursor for prostaglandins; apolipoprotein E expressed by macrophages; peroxisome proliferator-activated receptor gamma that regulates cytokine transcription; as well as complement proteins (C1R, C3, and C7), cytoskeletal components actin alpha2 and myosin 11, and various major histocompatibility complex molecules.

Finally, Hayashi et al. [9] generated a mouse model of ovarian endometriomas, where uterine tissue was implanted in the ovaries of syngeneic mice. They found that the endometrioma-affected ovaries had elevated iron levels and more oxidative stress in follicles, accompanied by a reduction in FSH expression. The role of iron and oxidative stress in endometriomas and surrounding follicles will be explained in more detail below.

3 Anatomic Distortion and Other Non-ovarian Mechanisms

Endometriomas may be associated with tubo-ovarian adhesions and non-ovarian endometriosis (particularly, deep endometriosis), resulting in anatomic distortion that negatively affects the ability of the tubal fimbriae to capture the ovulated oocyte. Endometriomas and endometriosis, in general, are also associated with peritoneal inflammation (e.g. elevated IL-1beta, IL-6, and tumor necrosis factor) that may affect tubo-ovarian function and also hinder sperm motility and oocyte–sperm interaction [10]. The increase in peritoneal inflammation may also potentially impair oocyte quality [11]. Moreover, the peritoneal fluid has evidence of oxidative stress due to iron from shed blood from endometriosis lesions and from retrograde menstruation, which contributes to the inflammation in the peritoneal fluid that surrounds the ovary [10]. If macrophages take up the iron, then the iron not be accessible to ferritin, which further increases oxidative stress [12]. In addition, it is also plausible that endometriosis (and endometriomas) may affect endometrial receptivity and implantation, if there is an increase in eutopic endometrial inflammation in endometriosis (e.g. related to increased aromatase producing higher estradiol) [13],

with perhaps another mechanism being anterograde flow of endometriosis-associated inflammatory peritoneal fluid into the endometrial cavity.

4 Endometrioma Fluid and Cyst Wall

Cellular and molecular features of endometriomas were extensively reviewed by Sanchez et al. [1], who divided their review into the endometriosis fluid, the cyst wall and other cellular elements lining the inside of the endometrioma, and the local environment around the endometrioma. One hypothesis is that the endometrioma fluid itself, which arises from repeated bleeding into the cyst from the endometrioma cyst wall, is toxic to surrounding ovarian tissue. Similar to peritoneal fluid, the endometrioma fluid may have an increase in iron that can mediate an increase in oxidative stress and subsequent inflammation (e.g. IL-8). There may also be an imbalance among activins, inhibins, and follistatin, as well as changes in soluble adhesion molecules, in endometrioma cyst fluid. Unlike other cysts, endometriomas are not surrounded by a true capsule such that there is less of the barrier of diffusion from the endometrioma to surrounding ovarian tissue and follicles [14]. This local diffusion of molecules from the endometrioma is supported by the observation of an increase in total iron and ferritin in the follicular fluid of follicles proximal to the endometrioma compared to follicles distal to the endometrioma and from the contralateral ovary [15].

For the cyst wall, there are regions of endometriosis epithelium/stroma, but there can also be the presence of metaplasia and regions of the cyst wall being replaced with fibrotic tissue, as well as surrounding hemosiderin macrophages (particularly M2 macrophages) that may support endometriosis lesion growth [1]. It has been postulated that iron-mediated oxidative stress, such as in the endometrioma fluid, is one mechanism that can predispose to the somatic cancer driver mutations seen in ovarian endometrioma epithelium [1].

5 Iron, Oxidative Stress, and Inflammation

Before moving on to a discussion of changes in granulosa cells and follicular fluid, the relationship among iron metabolism, oxidative stress, and inflammation will be reviewed. Gupta et al. reviewed proteomic studies of the role of oxidative stress in infertility, including in endometriosis [12]. Reactive oxygen species arise from mitochondrial respiration (electron transport chain), and when antioxidants cannot clear these reactive oxygen species, the result is oxidative stress. Reactive oxygen species lack electrons which makes them reactive with surrounding molecules, with examples being hydrogen peroxide, hydroxyl radicals, and superoxide anion. Iron can be a cause of reactive oxygen species, due to its ability to shift between Fe2+

and Fe3+ forms [16], and is important in endometriosis due to shed blood in endometrioma fluid, in peritoneal fluid and via retrograde menstruation.

Anti-oxidants can be enzymatic (e.g. superoxide dismutase and glutathione oxidase) and non-enzymatic (e.g. Vitamins A and E, zinc, and selenium) [12]. There is a balance between reactive oxygen species and anti-oxidants: a homeostatic level of reactive oxygen species being important for physiological processes during ovulation such as resumption of meiosis I and formation of the dominant follicle, while anti-oxidants promote resumption of meiosis II. Thus, either excessive or inadequate reactive oxygen species may negatively affect reproduction. Specifically, oxidative stress results when reactive oxygen species exceed anti-oxidant activities, with the oxidative stress in endometriomas then resulting in an increase in pro-inflammatory cytokines [1].

6 Granulosa Cell Abnormalities

Huo studied granulosa cells with associated endometriomas for evidence of mitochondrial abnormalities [17]. They found evidence that endometrioma-associated granulosa cells had fewer mitochondria, more abnormal morphology, and lower ATPase and proteins involved in oxidative phosphorylation. There was also a higher level of cell-free mitochondrial DNA in follicular fluid in endometriosis cases compared to controls that were in turn inversely associated with cell-free mitochondrial DNA in granulosa cells. The authors interpreted these findings as suggesting a negative impact on oocyte quality, particularly as mitochondrial DNA has been correlated with embryo quality. Urs et al. [18] found that endometrioma-affected ovarian granulosa cells had less mitochondrial mass and membrane potential and less expression of STAR and 3beta-hydroxysteroid dehydrogenase (which together were correlated with decreased follicular estradiol), in comparison to different control groups. There was also an increase in apoptosis of cumulus cells in the endometrioma group.

Another study examined granulosa cells from patients with endometrioma and studied the role of endoplasmic reticulum stress [19]. There was evidence of endoplasmic reticulum stress (e.g. increased expression of unfolded protein response and phosphorylated endoplasmic reticulum stress sensor proteins). In functional culture studies, hydrogen peroxide (a feature of oxidative stress) promoted the expression of unfolded protein response in cultured granulosa cells, as well as apoptosis-associated caspase 8 and caspase 3. Therefore, oxidative stress in the ovary due to endometrioma may lead to endoplasmic reticulum stress and apoptosis in granulosa cells. Similarly, lipidomic profiling showed an increase in sphingolipids and phosphatidylcholines in endometrioma-affected follicular fluid, which could also be involved in apoptosis [20].

Recently the role of autophagy (catabolic process to recycle cell components) in granulosa cells with endometrioma was investigated [21]. They found that these granulosa cells had increased autophagy and expression of Beclin-1 (a mediator of

autophagy) and that these patients had an increase in serum progesterone in the late follicular phase that may be a marker of poorer oocyte quality. In functional studies, they showed that Beclin-1 promoted progesterone expression through the degradation of low-density lipoprotein.

Li et al. [22] examined the nuclear factor-κB (NF-κB) pathway and found that granulosa cells in patients with endometriomas had higher NF-κB binding activity. They also examined telomerase activity, which was inversely related to NF-κB binding levels. In cultured granulosa cells, tumor necrosis factor-alpha (TNF-alpha) reduced human telomerase reverse transcriptase (hTERT) and telomerase. The authors hypothesized that in the presence of ovarian endometriomas, there may be higher TNF-alpha that increases NF-κB pathway activation and reduces telomerase activity in granulosa cells, resulting in increased granulosa cell senescence. Given the importance of granulosa cells in promoting aromatase, this granulosa cell senescence, apoptosis, and autophagy may together account in part for the observation of decreased estradiol concentrations in endometriosis [11].

Recent studies have utilized innovative technologies to study granulosa cells in the presence of endometriomas. Notarstefano et al. [23] used infrared and Raman microspectroscopy on luteinized granulosa cells and found indirect evidence for oxidative stress and lipid/carbohydrate metabolism abnormalities, both in the endometrioma-affected ovary and in the normal contralateral ovary, in comparison to control ovaries. Da Luz et al. examined the transcriptome of cumulus cells from endometriosis patients with or without endometrioma, compared to controls, using RNA sequencing [24]. There were 461 differentially expressed genes between endometrioma cases and control, and 66 between endometriosis (non-endometrioma) cases and controls. These differentially expressed genes were involved in oocyte competence including oxidative phosphorylation, mitochondrial functioning, and steroid metabolism. Interestingly, there were no differentially expressed genes comparing endometriosis cases with or without endometrioma. Another study [25] involved microRNA profiling in cumulus cells and found that miR-532-3p was significantly lower in stage III/IV endometriosis compared to stage I/II and to the infertile control group (only five cases per group). The authors noted that this microRNA-regulated pathway is involved in oocyte competence and oocyte meiosis.

7 Follicular Fluid Abnormalities

In general, there is evidence that the follicular fluid in ovaries affected by endometriomas may be associated with increased oxidative stress (e.g. mediated by iron) and inflammation (e.g. IL-8 and IL-12) that lead to decreased oocyte quality [11]. It should be noted that one study did not find a difference in oxidative stress in endometriomas [26], while another did find evidence for an increase in ferritin and reactive oxygen species pathways using a proteomic tandem mass spectrometry approach in endometriomas [27]. Li et al. [28] also sampled follicular fluid in patients with stage III and IV endometriosis (anatomic subtypes not specified) and

found the endometriosis group to have decreased transferrin and iron overload and, using a mouse model, demonstrated that this may contribute to abnormal oocyte maturation. Another study found increased ferritin in the affected ovary compared to the contralateral normal ovary, but no difference in iron [29].

This iron overload and subsequent oxidative stress leads to local inflammation. Mao et al. [30] found that the follicular fluid cytokine profile in patients with a history of endometriosis compared to controls showed some that were elevated (e.g. IL-14, IL-13, IL-3, and IL-1alpha) and some were decreased (e.g. IFN-gamma). Yland et al. [31] recently profiled cytokines in follicular fluid in patients with endometriomas compared to controls. They found that a set of cytokines that were hypothesized to be abnormal in endometriosis (e.g. IL-6, IL-8, and IL-1beta) were generally elevated in endometrioma-affected ovaries (and, in some cases, the contralateral normal ovary in the same patient) compared to control ovaries. Toll-like receptors (TLRs) and associated inflammation have also been investigated in ovarian endometriosis [32]. In follicular fluid of endometrioma-affected ovaries, there was an increase in cytokines such as IL-6 and IL-8, and, in cell pellets from the follicular fluid, there was an increase in TLR1, 5, 6, 7, 8, 10, as well as NF-κB, IL-10 and transforming growth factor-beta (TGF-β).

It should be noted that mitochondrial superoxide dismutase (SOD2) is an antioxidant that converts superoxide to hydrogen peroxide that is subsequently detoxified [33]. Imbalances between enzymes may result in imbalances in reactive oxygen species, and, in fact, the accumulation of hydrogen peroxide may promote cell proliferation. Thus, while SOD2 has an anti-oxidant effect, there is some evidence that it can promote tumor cell proliferation and progression perhaps via hydrogen peroxide. In this study [33], endometriomas had increased expression of SOD2 (in response to increased oxidative stress), and, in endometrial primary cell cultures, there was evidence of SOD2-promoting cell proliferation and migration.

Finally, a microRNA profiling study was done on follicular fluid from 30 patients with ovarian endometriomas compared to controls [34]. The authors found that miR-451 was decreased in endometriosis, and, in functional studies, inhibiting miR-451 in human and mouse oocytes negatively affected oocyte and embryonic development with possible involvement of the Wnt pathway.

8 Reduction in Oocyte Quantity

The above mechanisms can reduce oocyte quality, as evidenced by changes in morphology, the spindle apparatus, and the mitochondrial content of the cytoplasm [11]. For example, Ferrero et al. [35] examined metaphase II oocytes from patients with ovarian endometriomas compared to healthy egg donors. Single-cell RNA sequencing was performed. They found numerous differentially expressed genes, typically overexpression, for oocytes from both the affected ovary and the normal contralateral ovary, in comparison to the egg donors. These genes were involved in a variety

of processes such as cell growth, oxidative stress, and steroid metabolism, with particular enrichment for the mitochondria.

However, endometriomas may also reduce oocyte quantity [36]; for example, a prospective longitudinal study found that a larger reduction in markers of ovarian reserve in women with endometrioma-affected ovaries compared to controls [37]. As well, follicle density is lower in ovaries with endometriomas compared to the unaffected contralateral ovary [38], and, more so, in comparison to other non-endometriosis benign cysts [39].

Both oxidative stress and fibrosis induced by the associated local inflammation in endometriomas may lead to follicular depletion and decreased oocyte quantity [10]. A reduction in ovarian cortical stromal vascularization may also contribute [10]. In the presence of endometriomas, there may also be an increase in early follicular development and subsequent atresia [10]. Di Nisio et al. found that the ovarian cortex adjacent to an ovarian endometrioma had higher expression of apoptosis-associated caspase 8, and also of p53 that is involved in the regulation of oxidative stress response and apoptosis [40]. Altogether these mechanisms may lead to a "burnout" of follicles and decreased ovarian reserve [10].

Notably, Takeuchi et al. utilized a mouse model of endometriosis and oocytes from ovaries with endometriomas [41]. In the mouse model, there was a decrease in primordial follicles and an increase in primary, secondary, and antral follicles, suggesting elevated primordial follicle activation. In human oocytes from ovaries with endometriomas, there was an activation of the phosphoinositide 3-kinase (PI3K)–protein kinase B (Akt) pathway that when inhibited in a mouse model, increased the primordial follicles. Therefore, endometriomas may be associated with overactivation of primordial follicles mediated via the PI3K-Akt pathway, leading to "burnout" and a decrease in ovarian reserve.

The decrease in primordial follicles in endometrioma-affected ovaries may involve the Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) pathway known to be involved in primordial follicle activation [42]. In particular, YAP/TAZ are regulated by tissue stiffness and stretching. Thus, the stretching caused by an ovarian endometrioma may mechanotransduce YAP/TAZ that leads to the hyperactivation of primordial follicles, although the authors note that there are likely multiple pathways involved than just simple stretching of ovarian tissue. For example, they hypothesize that endometriomas may release reactive oxygen species and inflammatory factors that can promote the PI3K/Akt pathway, which can lead to hyperactivation of primordial follicles that further promote a reduction in ovarian reserve.

Regarding the environment around the endometrioma, reactive oxygen species may promote local tissue fibrosis, a change in follicular pattern, and vascular alterations [1]. Fibrosis results in a reduction in follicles and cortex-specific stroma and may also negatively affect follicular development. The loss of stroma is also important due to its role in providing blood supply to primordial follicles. This fibrosis and reduction in vascularization further compound the decrease in oocyte quantity.

9 Conclusion

In conclusion, endometrioma-affected ovaries are characterized by anatomic distortion and several pathophysiological changes including increased iron-mediated oxidative stress and inflammation. Together, these pathways may impair oocyte quality and quantity (Fig. 1). These biological observations have potential implications for clinical management, in terms of the potential long-term ongoing effects of an unoperated endometrioma on ovarian structure and function (due to oxidative stress and inflammation), and whether these effects can be attenuated by hormonal therapy or are in any way altered by surgical removal.

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Fertility Prediction in Patients with Endometriosis (Endometriosis Fertility Index)

Tingfeng Fang and Wenjun Wang

1 Introduction

Endometriosis remains an enigmatic disease. Pain and infertility are the primary presenting symptoms in the patient with endometriosis. The incidence rate of endometriosis is 6–10% in reproductive-aged women and 21–47% of them are subfertility [1]. The average incidence rate of endometriosis in infertile women is about 30% (if surgically investigated), and it rises to roughly 50% if these women have moderate-to-severe dysmenorrhea [2].

In terms of fertility, four factors are required for conception: the male sperm, the female oocyte, the functional uterine cavity and the patent tube. The prediction of a women's future fertility usually needs to be taken into account, including appropriate ovarian reserve, a patent tube, and a functional uterine cavity. Multi-factors potentially lead to the infertility of women with endometriosis: anovulation, anatomical changes in the pelvic floor, the adhesions in fallopian tubes that impair its transport function, and it has been demonstrated that the endometrioma intrinsic presence is correlated with decreased ovarian reserve (a decreasing quality and quantity of oocytes), especially in bilateral endometriomas [3, 4].

To date, four endometriosis classifications have been built up to provide a measure of the severity of the endometriosis, a prediction for future fertility, and a degree of pain. The earliest one is the revised American Fertility Society (r-AFS) or the revised American Society for Reproductive Medicine (r-ASRM) classification in 1996 [5], which is longevity and universal familiarity. The following is the Enzian classification for deep infiltrating endometriosis (DIE) in 2005 [6]. The third is the American Association of Gynecological Laparoscopists (AAGL) classification in 2007, which is more focused on pain and surgical difficulty [7]. The latest one is the

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endometriosis fertility index (EFI), which is used to predict pregnancy rates (PRs) after endometriosis surgical staging [8]. However, it has been demonstrated that, except for the EFI, the current classification systems have little prognostic value [9]. They rely on sole surgical findings while the EFI includes more important clinical variables which may reflect the probability of infertility.

2 The Current Commonly Used Endometriosis Classification Introduction

The Consensus of World Endometriosis Society (WES) in 2014 advised that "until better classification systems are validated, all women with endometriosis undergoing surgery should have an r-ASRM (or possibly, when published, AAGL) score and stage completed, women with deep endometriosis should have an Enzian classification completed, and women for whose fertility is a future concern should have an EFI score completed, and documented in the medical/surgical records" [9].

However, this consensus also indicated that "the classification systems in current use continue to attract criticism from women with endometriosis and those providing care for them because of the poor correlation with disease symptoms as well as a lack of predictive prognosis and, to date, unclear pathways of treating pelvic pain and infertility based on its classification" [9].

3 The American Society for Reproductive Medicine (r-ASRM) Classification Background

The first version of the AFS classification was established in 1979, as several authors had demonstrated that no correlation existed between PRs and the severity following treatment in this classification, further recommendations were then created to revise the AFS classification [7]. It was revised in 1985 [10]; the revised version presented more detail in observing and documenting the number of lesions, extent, size, and severity of adhesion. This version was republished in 1996 adding instructions and color illustrations to ensure consistency in describing the appearance of the disease. It was mainly set to predict the pregnancy chance after treatment. The DIE was not considered in this scoring system [5].

4 Limitations of the r-ARSM Classification

Despite several revisions in the current r-ARSM system, some limitations still have been found in this classification. Four different stages are pronounced (stage I:minimal, 1–5 points; stage II, mild, 6–15 points; stage III, moderate, 16–40 points;

stage IV, severe, >40 points) in this classification, but information on the lesion location is not provided. Moreover, the r-ARSM classification mainly depends on morphological descriptions with the arbitrary stage demarcation by point score and the wide score range [7]. Potential observer errors may exist resulting from the observer's subjective scoring [11]. It cannot effectively predict PRs in infertile patients [12] and pelvic pain [13]. To date, the r-ASRM staging system is still the most commonly used classification for endometriosis, which is still the best tool for physicians and surgeons to communicate the severity of the disease. Because of its widespread clinical use and prevalence in describing the surgical appearance of endometriosis, it is retained in the endometriosis classification [9].

5 The EFI Background

As a complement to r-AFS classification that can better diagnose the fertility status associated with endometriosis, the EFI, first proposed by Adamson and Pasta in 2010, can be used to accurately predict the probability of natural pregnancy for women following the surgical staging of endometriosis. This simple scoring system was established by prospectively collecting detailed clinical and surgical data of 579 infertile patients with endometriosis and then testing its predictive value on a cohort of 222 patients. The result revealed that the EFI is a simple, robust, and validated clinical tool for PRs prediction in women with a surgical documented endometriosis [8]. The EFI score combines historical factors and surgical factors, and the score ranges from 0 to 10, with a score of 0 indicating the poorest prognosis and a score of 10 indicating the best prognosis. The historical factors account for five scores based on patient's characteristics including age, years infertile, and history of a prior pregnancy. The surgical factors account for another five scores based on calculating the least function (LF) score of adnexa (fallopian tubes, fimbria, and ovaries) by the surgeon, the endometriosis lesion score, and total score in r-ASRM classification (Fig. 1).

It was found that the LF score was the most important contributor among all the EFI score variables [14]. The LF score of the bilateral tube, fimbria, and ovary was performed by the surgeon, where a score of 0 representing absent or nonfunctional; a score of 1 representing severe; a score of 2 representing moderate; a score of 3 representing mild dysfunction; and a score of 4 representing normal. If an ovary is absent on the one side, the lowest score on the other side with the ovary is doubled to obtain an LF score [8], a detailed description is shown in Table 1.

The EFI has been externally validated for its predictive value of endometriosis-associated fertility by over 24 studies [15]. The type, duration, and cost of treatment can be decided based on EFI for a patient before considering assisted reproductive technology (ART) procedures after endometriosis surgery. EFI also provides a guarantee for the patient with a good prognosis and avoids waste of time and treatment for the patient with a poor prognosis [7]. As only a part of patients enable attempts at ART therapy after endometriosis, the EFI can bring great benefit to most patients

LEAST FUNCTION (LF) SCORE AT CONCLUSION OF SURGERY

Score		Description		Left	Right
4	=	Normal	Fallopian Tube		
3	=	Mild Dysfunction			
2	=	Moderate Dysfunction	Fimbria		
1	=	Severe Dysfunction			
0	=	Absent or Nonfunctional	Ovary		

To calculate the LF score, add together the lowest score for the left side and the lowest score for the right side. If an ovary is absent on one side, the LF score is obtained by doubling the lowest score on the side with the ovary.

	Left		Right		LF Score
Lowest Score		+		=	

ENDOMETRIOSIS FERTILITY INDEX (EFI)

Historical Factors			Surgical Factors		
Factor	Description	Points	Factor	Description	Points
Age			LF Score		
	If age is ≤ 35 years	2		If LF Score = 7 to 8 (high score)	3
	If age is 36 to 39 years	1		If LF Score = 4 to 6 (moderate score)	2
	If age is ≥ 40 years	0		If LF Score = 1 to 3 (low score)	0
Years Infertile			AFS Endometriosis Score		
	If years infertile is ≤ 3	2		If AFS Endometriosis Lesion Score is < 16	1
	If years infertile is > 3	0		If AFS Endometriosis Lesion Score is ≥ 16	0
Prior Pregnancy			AFS Total Score		
	If there is a history of a prior pregnancy	1		If AFS total score is < 71	1
	If there is no history of prior pregnancy	0		If AFS total score is ≥ 71	0
Total Historical Factors			Total Surgical Factors		

EFI = TOTAL HISTORICAL FACTORS + TOTAL SURGICAL FACTORS:	Historical	+	Surgical	=	EFI Score

Fig. 1 Endometriosis fertility index surgery form. Reprinted from Adamson, G.D., & Pasta, D.J. (2010) Endometriosis fertility index: the new, validated endometriosis staging system. *Fertility and Sterility*, *94*(5):1609–1615, with permission from Elsevier

with fertility desire. To date, none of the other endometriosis classifications except the EFI shows any correlation with PRs after surgery [7, 9]. Clinicians should manage postoperative fertility in women with endometriosis according to EFI score (i.e. women with lower EFI score should be timely offered ART treatment as an option after surgery) [16]. A recent meta-analysis has also confirmed that the EFI score has a good performance in predicting the pregnancy rate beyond in vitro fertilization (IVF) [11]. The EFI comprehensively analyzes the multi-factors of endometriosis-related infertility, guides clinicians in making individualized treatment, and subsequently prompts to improve outcomes of endometriosis. Although the LF score may be differences in interpretations by different observers, a recent study has confirmed that EFI can be reliably reproduced by independent observers, further supporting its use in routine clinical practice for postoperative fertility counseling/management in a patient with endometriosis [17]. Ferrier et al. evaluated a cost-effectiveness perspective for surgically documented endometriosis-associated infertility with the stratification of the EFI score. The results indicated that immediate IVF/ICSI in women with EFI scores 0–3 was much costly and more effective. After one-year natural conception attempts failed, continuing natural conception attempts in

Table 1 Descriptions of least function terms

Structure	Dysfunction	Description
Tube	Mild	Slight injury to serosa of the fallopian tube
	Moderate	Moderate injury to serosa or muscularis of the fallopian tube; moderate limitation in mobility
	Severe	Fallopian tube fibrosis or mild/moderate salpingitis isthmica nodosa; severe limitation in mobility
	Nonfunctional	Complete tubal obstruction, extensive fibrosis, or salpingitis isthmica nodosa
Fimbria	Mild	Slight injury to fimbria with minimal scarring
	Moderate	Moderate injury to fimbria, with moderate scarring, moderate loss of fimbrial architecture, and minimal intrafimbrial fibrosis
	Severe	Severe injury to fimbria, with severe scarring, severe loss of fimbrial architecture, and moderate intrafimbrial fibrosis
	Nonfunctional	Severe injury to fimbria, with extensive scarring, complete loss of fimbrial architecture, complete tubal occlusion or hydrosalpinx
Ovary	Mild	Normal or almost normal ovarian size; minimal or mild injury to ovarian serosa
	Moderate	Ovarian size reduced by one-third or more; moderate injury to ovarian surface
	Severe	Ovarian size reduced by two-thirds or more; severe injury to ovarian surface
	Nonfunctional	Ovary absent or completely encased in adhesions

Note: 0 = absent or nonfunctional; 1 = severe; 2 = moderate; 3 = mild dysfunction; 4 = normal. If the ovary is absent on the one side, all the ovulation will occur from the ovary on the other side. In this situation, the LF score is obtained by determining the function score on the side with the ovary and then doubling it. Reprinted from Adamson, G.D., & Pasta, D.J. (2010) Endometriosis fertility index: the new, validated endometriosis staging system. *Fertility and Sterility*, *94*(5):1609–1615, with permission from Elsevier

women with EFI scores 9–10 was strongly dominant; delayed IVF/ICSI was more costly and more effective in women with EFI scores 0–7. They concluded that the EFI is a useful score to help a couple decide on different care pathways—natural conception, immediate or delayed IVF/ICSI after considering the healthcare cost [18]. In China, young women (age ≤ 30 years) with r-ASRM stages I and II and EFI score ≥5 were recommended to expectant management for 6 months under the guidance of the Chinese Medical Association; women with EFI score ≤4 and high-risk infertile factor (age > 35 years, infertile years >3 years, especially primary infertility, serve endometriosis, pelvic adhesion, incomplete lesion excision, and oviduct obstruction) were recommended to treat aggressively with IVF-ET [19].

6 Limitations of the EFI

First, the importance of adnexal function has been emphasized in endometriosis by the ESHRE and NICE guidelines [20, 21]. A possible limitation of EFI is the lack of ovarian reserve parameters. Studies have demonstrated that unilateral or bilaterality endometrioma sizes are significantly correlated with ovarian reserve [22, 23]. As the EFI serves as a reference for guiding the post-surgery patients about their fertility prognosis counseling, the time to ART treatment should take into the ovarian reserve. Serum anti-Müllerian hormone (AMH) as an effective marker for ovarian reserve has been proven by numerous studies [24–26]. It deserves further discussion whether adding serum AMH as a variable into the EFI score predicts reproductive capability more accurately. Second, the uterine abnormality is a factor of pregnancy prediction which is not included in the EFI. As clinically significant severe uterine abnormality is uncommon in endometriosis patients, Adamson proposed that "deficiencies in the reproductive function of the gametes or uterus will obviously affect the prognosis and must be considered separately as fertility factors, just as they would with any patient with any other type of disease" [7].

7 The Enzian Classification Background

The Enzian classification was established in 2005 to supplement the r-AFS score concerning the description of DIE, especially the retroperitoneal structures [6]. Advantages of the Enzian stage system include that it provides precise morphological description (e.g. anatomical location) of involved retroperitoneal structures; and suspected involvement of DIE can be well described preoperatively by using the Enzian classification [27]. Recent studies have shown a strong correlation between the MRI-based Enzian score for Deep Infiltrating Endometriosis (DIE) and intraoperative findings [28, 29]. This correlation is valuable for effective communication between radiologists and gynecologists when assessing surgical complexity and estimating the operating time.

8 Limitations of the Enzian Classification

Since the Enzian staging system is seen as more complicated to use compared with the r-ASRM score, it is mainly used in German-speaking countries with a poor level of international acceptance [15]. Only a few studies on the classification have been published in international journals. No current data exist to study whether the Enzian classification is associated with clinical symptoms [27].

9 Predicting Non-IVF Pregnancy Rate in Women with Endometriosis

As mentioned above, over 24 studies have demonstrated that EFI is an effective tool in predicting non-IVF pregnancy after endometriosis surgery. Some studies revealed that the cut-off of the EFI score for predicting a non-IVF pregnancy ranged from 5 to 7 [30, 31]. The cumulative non-IVF pregnancy rate of women with EFI ≥ 5 in the first 2 and 3 years after surgery was 50–66% versus 26–33% in women with EFI < 5 [31, 32]. The cumulative pregnancy rates (PRs) at 12 months after surgery ranged from 17% to 46% for EFI scores 0–3 and were 63% for EFI scores 9–10 in cases of Endometriosis Fertility Index (EFI) [14]. The EFI can also accurately predict the live birth of endometriosis in r-ASRM stages III and IV. The estimated cumulative non-IVF live birth rate at 5 years was 0% at an EFI score of 0–2, rising steadily to 91% at an EFI score of 9–10; while among women receiving ART treatment, the live birth rate increased steadily from 38% to 71% in the same EFI score strata [33]. Cook and Adamson's study presented additional information to assist the physicians and patients in understanding prognosis after endometriosis diagnosis at laparoscopy. As shown in Tables 2 and 3, they defined EFI score as four treatment levels (I–IV) based on monthly fecundity data, with treatment levels and recommendations ranging from "attempt non-IVF conception for at least 1 year" to "refer to ART center for IVF" [34]. Although EFI aims to predict PRs in infertile patients with laparoscopic surgery, a recent new study attempted to estimate EFI before surgery, the necessary information was obtained through clinical examination, gynecological ultrasound, and hysterosalpingo-foam sonography for tubal patency testing, and the results revealed that the EFI can be estimated accurately according to mere clinical and ultrasound information, this means that the EFI could be used as a tool to guide doctors and patients to make individualized treatment among surgery, ART, or other fertility management options [35].

Table 2 Treatment levels and recommendations

Treatment level	Monthly fecundity	Treatment recommendation
I	>3%	Attempt non-ART conception for at least 1 year
II	2–3%	Probable attempt non-ART conception, consider role of IVF
III	1–2%	Probable IVF, refer to a reproductive endocrinologist for fertility management
IV	<1%	Refer to ART center for IVF

Note: reprinted from Cook, A.S., & Adamson, G.D. (2013) The Role of the Endometriosis Fertility Index (EFI) and Endometriosis Scoring Systems in Predicting Infertility Outcomes. *Current Obstetrics and Gynecology Reports*, *2*(3):186–194, with permission from Springer Nature

Table 3 EFI score and treatment level

EFI	Treatment level		
	Year 1	Year 2	Year 3
0–3	IV	IV	IV
4	III	IV	IV
5	III	III	IV
6	II	II	III
7	I	II	II
8	I	II	III
9 and 10	I	I	IV

Note: Cook, A.S., & Adamson, G.D. (2013) The Role of the Endometriosis Fertility Index (EFI) and Endometriosis Scoring Systems in Predicting Infertility Outcomes. *Current Obstetrics and Gynecology Reports*, *2*(3):186–194, with permission from Springer Nature

10 Predicting ART Pregnancy Rate in Women with Endometriosis

Although the EFI does not consider to predict the PRs in women who underwent ART treatment after endometriosis surgery, two studies attempted to analyze the predictive value of EFI in IVF pregnancy, the results still revealed a good correlation between EFI and IVF pregnancy [36, 37]. One study in China analyzed 199 consecutive women with surgically documented endometriosis receiving IVF treatment. The results showed the cut-off EFI score for predicting IVF pregnancy was 6. The clinical pregnancy rate was 28.6% in women with an EFI score of ≤5, which was significantly increased to 53% in women with an EFI score ≥ 6. A higher number of antral follicle count, oocytes retrieved, and implantation rate were found in women with an EFI score ≥6 than women with an EFI score ≤5 [37]. Garavaglia et al. evaluated the predictive value of the EFI score for cumulative ART cycles pregnancy outcome in 44 women with previous attempts to obtain a natural pregnancy after surgery, the result showed the best cut-off point for ART pregnancy was 5.5, and the clinical pregnancy rate in women with an EFI score ≤5 was 5.6% [36].

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Spontaneous Ovulation in Patients with Endometriosis

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1 Introduction

The establishment and the progression of endometriosis are associated with ovulation and ensuing menses. Exposure to menses and associated retrograde bleeding is one of the critical factors related to an increased risk of endometriosis. Therefore, oligo-anovulation (as encountered in women suffering polycystic ovary syndrome, PCOS) might theoretically lessen the likelihood of developing endometriosis. Based on this background, some studies investigated the prevalence of oligo-anovulation in patients with endometriosis.

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Endometriomas are among the most common manifestations of endometriosis, affecting between 17% and 44% of women suffering from this disease [1]. These cysts contain "chocolate" fluid that is generally thought to arise from the accumulation of menstrual debris deriving from the shedding of the active implants inside the cyst. The cysts contain a high concentration of cellular damage-mediating factors, proteolytic enzymes, inflammatory molecules, reactive oxygen species, and iron [2]. These molecules could be a potential source of toxicity for the surrounding healthy tissue and, therefore, have detrimental effects on ovarian physiology. It has been hypothesized that endometriomas may interfere with ovulation. Therefore, several studies investigated whether endometriomas influence the rate of ovulation in the affected ovary.

This chapter will summarize the available data on ovulation characteristics in women with endometriosis.

2 Ovulation in the Animal Models of Endometriosis

Studies on the effects of experimental endometriosis on infertility have used animals with endometrial autografts placed throughout the pelvic peritoneum [3, 4]. In these studies, infertility was associated with failure of ovulation. Schenken and Asch investigated the effect of surgically induced endometriosis on the reproductive performance of New Zealand White rabbits [3]. Endometrium obtained from one uterine horn was surgically implanted into the peritoneum. Adipose tissue was implanted in another group of animals which served as a control. The induction of endometriosis significantly impaired fertility rates (25%) compared with the control group (75%). The primary cause of infertility in the endometriotic group was the failure to ovulate since only four of the eight animals showed stigmata of ovulation. However, because only two of the four ovulatory animals became pregnant, an effect on ovum transport, luteolysis, or induced abortion after implantation cannot be excluded.

Another study investigated the effect of endometriosis on follicular rupture [5]. Endometrial tissue was **autografted to New Zealand White rabbits. Endometrium was surgically implanted into the peritoneal cavity or the rectus muscle. Human chorionic gonadotropin was administered to induce ovulation. The viability of the implants was demonstrated histologically. The number of corpora lutea and stigmata was counted during three subsequent laparotomies. Ovaries were removed during the last laparotomy, and ovarian serial sections were examined. In rabbits with peritoneal-induced endometriosis, the percentage of stigmata/corpora lutea was significantly decreased. The macroscopic study was confirmed by histological examination. Indeed, a high incidence of entrapped oocytes was found in rabbits with peritoneal endometriosis. Extraperitoneal endometriosis did not affect ovulation. These data suggested that endometriosis induces a failure of follicular rupture. After the excision of endometriosis, no failure to ovulate was observed, suggesting

that the effect of endometriosis on the ovulation disappeared with the removal of endometriotic implants.

Another study performed in the animal model investigated whether ovarian endometriosis impairs ovulation [6]. The authors induced ovarian endometriosis in Virgin New Zealand White rabbits. Endometrial tissue was placed in one ovary, and adipose tissue was placed in the contralateral ovary as a control in a randomized fashion. Ovulation was induced with human chorionic gonadotropin, and ovulation points were counted before and after induction of endometriosis. Periovarian adhesions were graded according to their density and the extent of ovarian surface affected. A significant decrease in ovulation points was observed in ovaries with endometrial tissue but not in ovaries that contained adipose tissue. Periovarian adhesions decreased the number of ovulation points in ovaries with adipose or endometrial tissues. In the absence of adhesions, a near-significant decrease in the number of ovulation points was observed in ovaries with endometrial tissue. Still, no change was evident in ovaries with adipose tissue. Multivariate analysis demonstrated that an increase in adhesion severity was correlated with a decrease in the number of ovulation points, but endometrial tissue was not. In the rabbit model, the authors concluded that minimal ovarian endometriosis impairs ovulation primarily through a mechanism related to periovarian adhesions.

3 Oligo-Anovulation in Women with Endometriosis

In a study published more than 40 years ago, Soules et al. investigated the incidence of anovulation in women with endometriosis [7]. In a series of 350 women with endometriosis (77% of whom were confirmed by histology), these authors found that 17% exhibited anovulation or oligo-ovulation patterns. Among women with endometriosis and oligo-anovulation, the distribution according to disease severity was as follows: 39% had mild endometriosis, 59% moderate, and 2% severe endometriosis. These authors concluded that endometriosis and anovulation could coexist.

A study including 21 infertile women with laparoscopically documented minimal-mild endometriosis investigated follicular development and ovulation [8]. Of the 27 cycles studies, 24 (89%) appeared to be endocrinologically normal and ovulatory. Luteinized unruptured follicle (LUF) occurred in one cycle (4%). One further patient exhibited abnormal endocrinology with evidence of premature ovulation over two (8%) consecutive cycles. This study indicated that most women with minimal-mild endometriosis have endocrinologically regular menstrual cycles and that luteinized unruptured follicles occur infrequently.

Some studies investigated the prevalence of endometriosis in women with PCOS. An American retrospective study reported that among 102 infertile patients with PCOS diagnosed according to the Rotterdam criteria, 73 (71.5%) had endometriosis at laparoscopy [9]. About 40% had ASRM stage I endometriosis, 41% stage

II, 12% stage III, and 7% stage IV. A more recent retrospective cohort study investigated the prevalence of endometriosis in PCOS patients who did not suffer pain symptoms and underwent laparoscopic ovarian drilling for clomiphene citrate resistance [10]. Endometriosis was present in 16.9% of the patients. Around 86.6% of the patients had ASRM stage I endometriosis, and the remaining patients (13.2%) had stage II endometriosis. In a meta-analysis, the pooled prevalence of endometriosis in clomiphene citrate-resistant PCOS patients was 7.7% [10]. These data suggest that the prevalence of endometriosis in anovulatory women with PCOS is similar to that of the general population.

More recently, a French cross-sectional study investigated the prevalence of oligo-anovulation in women with and without endometriosis [11]. The study included 354 women with histologically proven endometriosis and 474 women in whom endometriosis was surgically ruled out. There was no difference in the rate of oligo-anovulation between women with endometriosis (15.0%) and controls (11.2%). Oligo-anovulation was observed in 18.2% of patients with superficial peritoneal endometriosis, 10.6% with ovarian endometrioma, and 16.6% with deep infiltrating endometriosis.

4 Impact of Endometriomas on Spontaneous Ovulation

Ovarian endometrioma may affect ovulation by several mechanisms. The inflammatory reaction caused by the endometrioma may have a negative effect on ovulation. In addition, the presence of an ovarian cyst may cause mechanical damage to the growing follicle by thinning and stretching the cortical tissue and disturbing the vascularization of the ovary.

Maneschi et al. [12] investigated the functional morphologic features of the ovarian cortex surrounding benign cysts. The study included 48 women who underwent surgical excision of benign ovarian cysts. The ovarian cortex was not morphologically altered in the presence of mature teratomas ($n = 13$) and benign cystadenomas ($n = 9$). In contrast, endometriomas ($n = 32$) were associated with microscopic stromal implants and reduced follicular number and activity. Follicular maturation up to the antral stage was observed less frequently in the cortical tissue surrounding the endometriomas than in that surrounding mature teratoma and benign cystadenomas. Moreover, there was no evidence of follicles in 16% of the specimens obtained from women with endometriomas.

Over the last 15 years, several studies investigated the impact of ovarian endometriomas on spontaneous ovulation and reported contradictory results. A retrospective study including 28 infertile women with unilateral endometriomas showed that the rate of ovulation (mean ± standard error of the mean, SEM) in the affected ovary was 34.4% (±6.6%) [13]. When the endometriomas had the largest diameter < 4 cm, the rate of ovulation in the affected ovary was 41.0% (± 8.0%). In contrast, when the endometriomas had the largest diameter ≥ 4 cm, the rate of ovulation in the affected ovary was 26.8% (±10.9%). All the patients included in the study underwent

laparoscopic cystectomy. After surgery, there was a significant decrease in the ovulation rate in the affected ovary (16.9 ± 4.5%). This decrease was observed when the endometriomas had the largest diameter < 4 cm but not when it was ≥4 cm.

An Italian prospective single-center study including women with unilateral endometriomas investigated the rate of ovulation in the affected ovaries [14]. The criteria for inclusion in the study were the presence of one or more endometriomas (with largest diameter ≥ 10 mm), no previous adnexal surgery, and regular menstrual cycles (24–35 days). Study patients underwent serial transvaginal ultrasonographic examination starting on days 6–10 of the menstrual cycle. Ovulation was defined as the presence of a growing leading follicle and subsequent development of a corpus luteum. The study included 70 women, and the mean age (± SD) of the patients was 35.0 (±4.5) years. Ovulation occurred in the affected ovary in only 31% of the cases. When the side of the endometrioma was considered, the study showed that the left ovary was less vulnerable than the right one; in fact, the ovulation rate was reduced only when the endometrioma was located on the right ovary. The significant limitations of the study were that patients were recruited only for one menstrual cycle and that the sample size was relatively small.

An Italian single-center prospective study investigated if ovarian endometriotic cysts influence the rate of spontaneous ovulation in the affected ovary [15]. The study included women of reproductive age desiring to conceive, with an ultrasonographic diagnosis of a unilateral ovarian endometriotic cyst with a diameter of ≥2 cm. The patients included in the study had no history of infertility. Study patients had regular menstrual cycles, and male partners had a normal semen analysis. Study patients underwent serial transvaginal ultrasounds to assess the side of ovulation starting on days 6–8 of the menstrual cycle for up to six ovulatory cycles. The ovulation was defined by the presence of a growing leading follicle and the subsequent development of the corpus luteum. Two hundred forty-four women were included in the study. The mean (± SD) age of the study population was 34.3 (±4.9) years. One hundred and ninety-eight (81.1%) patients had single endometrioma, 37 (15.2%) had two endometriomas, and 9 (3.7%) had three endometriomas. At baseline, 166 patients (55.5%) had endometriomas with a largest diameter of ≥40 mm, and 45 (15.1%) had endometriomas with a largest diameter of ≥60 mm. A total of 1311 cycles were evaluated. It was impossible to identify the ovulation in 112 cycles (8.5%). There was no significant difference in ovulation rate between the healthy (50.3%) and the affected ovary (49.7%). The ovulation rate between the affected and the healthy ovary was not affected by endometriomas' laterality, number, and size. The rate of ovulation in the affected and the healthy ovary was not affected by deep endometriosis. Following the six spontaneous ovulations monitored during the study, 105 patients conceived (43.2%). There was no significant difference in the side of ovulation (healthy or affected ovary) when the patients conceived. The high pregnancy rate observed in the current study may be explained by the fact that the patients had unilateral endometriomas, no history of infertility, no risk factors for tubal disease (such as a history of pelvic inflammatory disease), and their male partners had a regular semen analysis.

5 Endometriosis-Associated Comorbidities and Ovulation Disorders

Several studies have underlined the influence of chronic pelvic pain and infertility on the quality of life and psychological well-being of women with endometriosis. Nonmenstrual chronic pelvic pain (CPP), dysmenorrhea, dyspareunia, pain at ovulation, dyschezia, dysuria, and infertility often affect the psychological and social functioning of patients with endometriosis [16]. For this reason, endometriosis is considered a disabling condition that may significantly compromise social relationships, sexuality, and mental health. Women with endometriosis report anxiety, depression, and other psychiatric disorders.

High levels of anxiety and depression can activate the hypothalamic-pituitary-adrenal (HPA) axis or suppress the hypothalamic-pituitary-thyroidal (HPT) and hypothalamic-pituitary-gonadal (HPG) axes in women. The HPA exerts an inhibitory effect on the female reproductive system when activated by stress corticotropin-releasing hormone (CRH). CRH receptors have been identified in most female reproductive tissues, including the ovary. There is evidence that CRH inhibits hypothalamic gonadotropin-releasing hormone (GnRH) secretion, and glucocorticoids inhibit pituitary luteinizing hormone and ovarian estrogen and progesterone secretion. Reproductive CRH regulates ovulatory functions with an inflammatory component [17]. These effects are responsible for the "hypothalamic" amenorrhea of stress, anxiety, and depression observed in endometriosis. Taken together, stress, anxiety, and depression may impact the ovulation of women with endometriosis. There may also be an association between stress-induced anovulation and increased risk of cardiovascular diseases in endometriosis. However, there are just a few data in the literature about the influence of psychological factors and psychiatric comorbidities on the effectiveness of ovulation in women with endometriosis. Although fertility can be restored with exogenous gonadotropins, fertility management alone will not permit recovery of the HPA and HPT axes.

6 Molecular and Metabolic Changes in Follicular Fluid in Patients with Endometriosis

The composition of the follicular fluid is correlated with oocyte development, which may also impact ovulation. Inflammatory cytokine concentrations are higher in follicular fluid in endometriosis. However, significantly higher vascular endothelial growth factor (VEGF) concentrations in follicular fluid do not affect the IVF outcome in these patients [18]. One of the most recent studies revealed that there is a follicular fluid-specific metabolic profile in deep infiltrating endometriosis depending on the presence of an associated ovarian endometrioma. Mitochondrial dysregulation with a modified balance between anaerobic glycolysis and beta-oxidation could affect ovulation in endometrioma phenotypes [19]. It needs to be clarified if

inflammatory and metabolic changes in the micro-environment of oocytes affect the ovulation in patients with endometriosis, and if different phenotypes of endometriosis variously influence spontaneous ovulation.

7 Conclusion

Endometriosis may impair fertility through multiple pathways, including peritoneal inflammation and endocrine derangements, which reduce oocyte competence [20]. Studies performed in the animal model of endometriosis and humans investigated the impact of endometriosis on ovulation. Approximately 20 years ago, studies conducted in rabbits suggested that endometrial autografts placed throughout the pelvic peritoneum caused infertility by interfering with ovulation [3–6]. Based on this background, some studies investigated if ovarian endometriomas influence the frequency of ovulation in the affected ovary. Although initial studies suggested that ovulation occurs less frequently in ovaries with endometriomas [13, 14], a prospective Italian study with a large sample size (244 women with unilateral endometriomas) demonstrated that ovulation is not affected by the presence of endometrioma [15]. In addition, the number and the size of endometriomas do not influence ovulation [15]. Research on follicular fluid-specific cytokines, metabolic profiles, and clinical findings of ovulation may give us a better understanding of spontaneous ovulation in patients with endometriosis. It should also be investigated if there is a correlation between ovulation and associated comorbidities, infertility, and the severity of pain.

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Endometrial Receptivity in Women with Endometriosis

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Abbreviations

4OHE1	4-Hydroxyestrone
4OHE2	4-Hydroxyestradiol
ART	Assisted reproductive techniques
EFT	Endometrial function test
lncRNA	Long non-coding RNA
miRNA	microRNA
ncRNA	Non-coding RNA
PCOS	Polycystic ovary syndrome
PUFA	Polyunsaturated fatty acids
RIF	Recurrent/repeated implantation failure
TLRs	Toll-like receptors

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Treg	T regulatory cells
uNK	Uterine natural killer cells
WOI	Window of implantation

1 Endometrial Receptivity

Successful embryo implantation is an absolute requirement for the reproduction of mammalian species. In humans, the implantation process involves complex cross-talk between the embryo and the maternal endometrium, all of which must occur within an optimal time-frame. The implantation process relies on three major milestones: the embryo's development into an implantation-competent blastocyst and the optimum embryo quality, the synchronized transformation of the uterus into a receptive stage, and the two-way dialogue between the blastocyst and the endometrium [1–3]. Disturbances in these bidirectional interactions are believed to present a major reason why over 60% of all pregnancies are terminated at the end of the peri-implantation period [1, 4, 5]. Indeed, in assisted reproductive techniques (ARTs), where the best-quality embryos are transferred, implantation remains the rate-limiting step in obtaining successful treatment results [6, 7]. Many studies are focussing on understanding the dynamic development of the endometrium into the receptive stage to unravel the endometrial factor in embryo implantation failures.

The human endometrium is a dynamic tissue that undergoes growth, differentiation, and regression throughout the menstrual cycle. All these processes are guided by the ovarian steroidal hormones oestrogens and progesterone, and different autocrine and paracrine factors [1, 8]. The main role of the endometrium is to provide an adequate space for embryo implantation and for further foetal growth. Although endometrium is non-adhesive to embryos in majority of the menstrual cycle, it becomes receptive during a spatially and temporally restricted time period in the mid-secretory phase, named as the window of implantation (WOI) [9, 10] (Fig. 1). The WOI was first introduced by Psychoyos in 1973, being defined as the delimited and accurately coordinated period of time in which the endometrium becomes receptive for the embryo to implant [11]. During the WOI, ovarian oestrogens and progesterone induce the endometrial cells to proliferate, differentiate, and secrete molecules that prepare the endometrium and, at the same time, can influence the development of embryo (Fig. 2). With these morphological and functional changes, the sources of hostility that normally compromise the embryo attachment are removed [12–15]. Furthermore, the signalling factors required to nourish the developing embryo during the first weeks of pregnancy are provided in this time frame [16]. Classically, the WOI is considered to occur 8–10 days after the ovulation and is expected to last around 48–72 h [17, 18].

Paradoxically, human embryos are capable to implant in different human tissues except for the human endometrium that is not receptive [18]. Indeed, the displacement of this narrowly delimited period of time has been reported in some patients

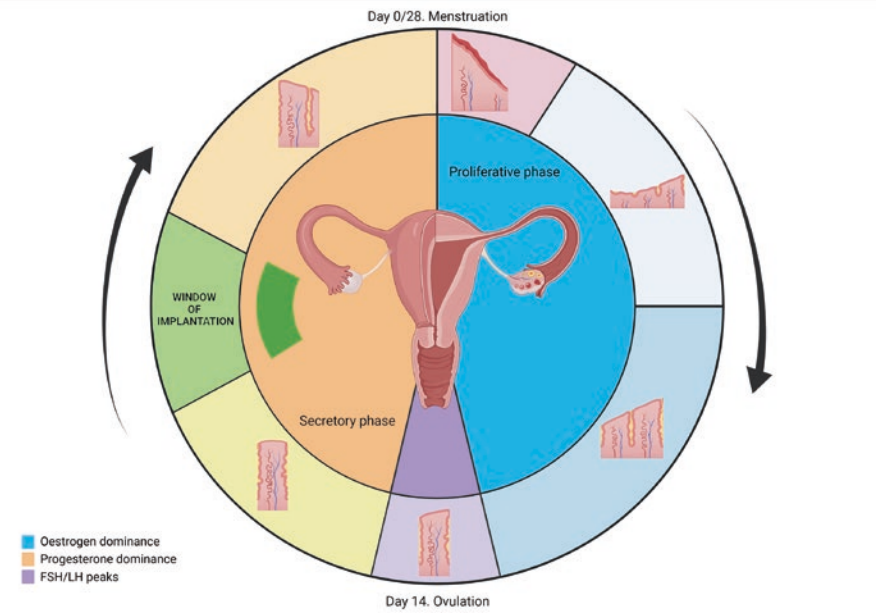

Fig. 1 The main phases in the uterine cycle in a typical 28-days cycle. The external part of the figure highlights the endometrial transformation across the cycle. Window of implantation (green) is restricted to a period of 48–72 h during the mid-secretory phase. Subphases in the endometrial cycle are also highlighted (pale red: menstruation; pale blue: early proliferative; blue sky: advanced proliferative; purple: ovulation; yellow: early secretory; green: mid-secretory; orange: late-secretory). Inner circle layers denote the hormone predominance in each phase: oestrogens (blue) in the proliferative phase; progesterone (orange) in the secretory phase; Follicle-stimulating hormone (FSH) and luteinising hormone (LH) peaks at the ovulation. Figure is created using BioRender.com

with certain inflammatory or anatomic conditions, including endometriosis, and is postulated as one origin for the embryo implantation failure, as WOI shift precludes embryo implantation and can thereby lead to infertility or pregnancy loss [19–22]. There is an active research ongoing to understand better the molecular changes leading to endometrial receptivity and successful reproductive outcomes. In this chapter, we summarise the main findings of the research on endometrial receptivity in women who are suffering from endometriosis.

2 Methods for Assessing Endometrial Receptivity

The complex changes in the endometrium throughout the menstrual cycle have boosted research for identifying informative biomarkers for predicting endometrial receptivity status [23]. The first reports concerning the assessment of the receptive phenotype of the endometrium date from 1950, when Noyes established the criteria

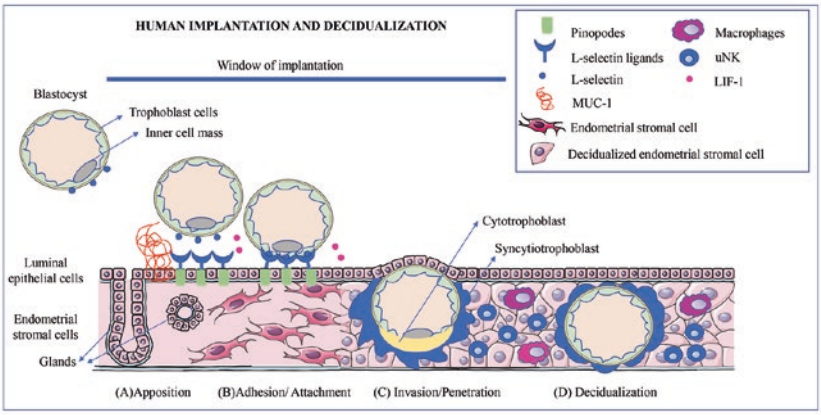

Fig. 2 Molecular changes in the human endometrium during embryo implantation. Human implantation can be divided into apposition, adhesion/attachment and invasion/penetration. (a) Selectins are expressed during apposition, and pinopodes expressing L-selecting ligands can be observed at this point in the endometrial epithelium. (b) During adhesion/attachment, L-selectin ligands mediate the interaction between the embryo and the maternal interface. (c) Invasion phase consists of the penetration of the blastocyst into the endometrial stroma; the cytotrophoblasts and syncytiotrophoblast cells appear during this phase. (d) In decidualisation, the blastocyst is completely embedded into the endometrial stroma, where the activity of macrophages and uterine natural killer cells is elevated. Reproduced with permission from Ochoa-Bernal and Fazleabas (2020) [213]

based on the histological evaluation of the endometrial biopsies [17]. These criteria are still used 70 years after its publication. However, this technique has evoked criticism due to the high intra- and inter-observer variabilities associated with several studies questioning its utility [24–27]. Furthermore, the diagnosis may vary depending on the histological variations at the moment of the biopsy collection [23], which constitutes another major limitation of this routinely used technique [28]. In consequence, some alternative parameters based on the endometrial morphology and subendothelial blood flow have been assessed, however, with no success in prognosing receptivity status [29, 30]. Identifying endometrial receptivity biomarkers has also been investigated in terms of single molecular and biochemical levels, where potential biomarkers such as growth factors, cytokines, chemokines, lipids, or adhesion molecules, among others have been proposed [18]. However, no success in their implementation in the clinical practice has been reached so far [15, 23, 31].

The genomic information obtained from the Human Genome Project boosted a revolution in the current molecular biology techniques and the development of the high-throughput omics technologies (e.g., genomics, epigenomics, transcriptomics, proteomics, and metabolomics), which encompasses a myriad of resources aimed at studying massively molecular profiles and changes between groups or individuals [23]. The use of the high-throughput omics technologies seems to be a powerful tool for the biomarker discovery, nevertheless research in human endometrium is

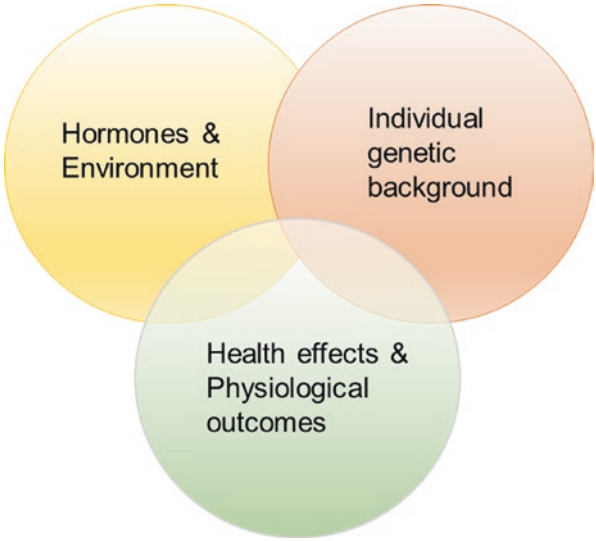

Fig. 3 Unravelling endometrial functions in endometriosis are complex and challenging as endometrial hormonal regulation and environment are interacting with the individual genetic background, health effects, and physiological outcomes

complex and challenging, as the endometrium is cyclically regulated by hormones and different factors, and together with the individual's genetic background can result in different biological responses (Fig. 3). Nevertheless, the omics technologies have significantly increased the understanding of the complex molecular processes of the endometrial physiology and pathophysiology, as for instance the molecular characterisation of the gene expression profiles across the menstrual cycle or the molecular changes underlying endometrial receptivity and implantation failure [32]. The application of omics studies, specifically transcriptomics (i.e. the gene expression profile) analyses, has enabled the identification of many endometrial receptivity biomarkers [33] with a successful effort to develop endometrial receptivity tests applicable for clinical use (Table 1). Analysing the transcriptomic profile of the receptive phase endometria would predict whether the woman has an optimal endometrial receptivity or whether it is displaced and adapted endometrial maturation protocol could be used together with the estimation of the best embryo transfer day in ARTs. With its wide use, the debate of its utility in a clinical setting in the assessment of endometrial receptivity among infertile patients is actively ongoing [38–40].

In addition to the molecular dating tests that are highlighted in Table 1, the endometrial function test (EFT) and the inflammatory marker test (ReceptivaDX) have been developed and are being used in a clinical setting [41]. EFT assesses endometrial samples histologically and the developmental stage by quantitative immunohistochemistry (cyclin E and p27 detection) at cycle days 15 (early-secretory phase) and 24 (late-secretory phase) [41]. EFT limitations include the need for two biopsies and human interpretation of the results that may affect read-to-read reliability; however, the test has shown promising results [42]. ReceptivaDX test, on the other

Table 1 Commercially available endometrial receptivity tests based on molecular techniques

Test	Analysed transcripts	Technology used	Accuracy in detecting ER	Company	References
ERA	238	NGS	0.99758 sensitivity 0.8857 specificity	Igenomix	Díaz-Gimeno et al. (2011) [34]
BioER	72	NGS	–	Bioarray	–
ERMap/ER Grade	40	RT-qPCR	–	iGLS	Enciso et al. (2018) [35]
ERPeak	Variable	RT-qPCR + AI	96% accuracy	CooperSurgical	–
ERT	100	NGS + machine learning	93.3% accuracy	Yikon	–
Win-Test	11	RT-qPCR + algorithm	–	INSERM	Haouzi et al. (2012) [36]
Adhesio RT	10	RT-qPCR	–	OVO Clinic	Messaoudi et al. (2019) [31]
beREADY	67	TAC-seq patented technology	–	Competence Centre on Health Technologies	Altmäe et al. (2017) [33]; Teder et al. (2018) [37]
rsERT[a]	175	NGS	98.4% accuracy	–	He et al. (2021) [22]

NGS next-generation sequencing, *ER* endometrial receptivity, *AI* artificial intelligence
[a]Test not available commercially yet

hand, evaluates immunohistochemically an endometrial sample for an inflammatory marker BCL6 associated with endometriosis [41]. There are studies implying that treatment with laparoscopy for endometriosis following a positive ReceptivaDX test improves pregnancy outcomes [43, 44].

In conclusion, endometrium is a dynamic tissue with critical function and its receptivity assessment is complex. Different endometrial receptivity assessment tests are available, and the choice of the test is a matter of preference of the physician, patient, and clinic. Assessment of the receptivity requires invasive procedures is costly and time consuming; therefore, a shared decision between the patient and the clinician should drive the decision making considering that the testing is cost-effective.

3 Endometrial Receptivity in Endometriosis

Women with endometriosis often suffer from infertility, and the reduced pregnancy rates are frequently associated with the disease [45]. Couples with female partners suffering from endometriosis reach fecundity rates around 2–10%, while the normal

probability of success in achieving pregnancy fluctuates around 15–20% in endometriosis-free couples [46]. The underlying causes for the poorer reproductive outcomes could be a combination of distorted pelvic anatomy due to the inflammatory environment created by the disease, as well as further negative effects on oocyte quality and embryo development, and an unfavourable environment for embryo implantation [46, 47]. As endometriosis seems to affect the normal functioning of the endometrium, it is plausible to think that endometrial receptivity might be compromised in women suffering from the disease. While several studies are highlighting the changes in the receptivity in the eutopic endometria in endometriosis [47–49], other studies suggest no differences [50, 51].

The altered endometrial receptivity in the eutopic endometria of women with endometriosis is supported by the concept that endometriosis impacts cycle fecundity via the systemic and local inflammatory changes that take place as a consequence of the disease [48]. Inflammation has been proposed as a primary cause of unexplained endometrial receptivity defects [20, 48] but also as one of the main hallmarks of endometriosis [52]. Inflammatory changes are frequent in women with endometriosis, and the dys-regulation at many levels of inflammatory pathways is not rare among patients. Indeed, a recent systematic search and meta-analysis identified the enrichment of immune and defence pathways in the receptive phase endometria in women with endometriosis [49]. Thus, the alterations that take place at immune response levels in the eutopic endometrium may lead to the activation of the tissue, along with its escape from apoptosis, resulting in the displacement of implantation in endometriosis [53]. In fact, a previous matched cohort study demonstrated that pregnancy and live birth rates were considerably lower in women with endometriosis compared to control women when embryo quality together with woman's age and parity were controlled for [54].

Different morphological and molecular events could lead to the aberrant endometrial receptivity in endometriosis including structural and histological changes, somatic changes, extracellular vesicles, immune cells, stem cells, steroid hormone responses, different genes and molecular pathways, non-coding RNAs, and microorganisms that will be discussed further (Fig. 4). Additionally, there is some evidence that cell membrane ion channels have a role in the endometrial functions in endometriosis [55] and that longer telomeres and more active telomerase could be linked to this endometrial pathology [56–59]. In conclusion, the eutopic endometrium of women with endometriosis has been widely studied with regard to the dysfunctionality of steroid hormone responses, many candidate molecules, and omics analyses; however, the effect of endometriosis on the endometrial receptivity is still under debate.

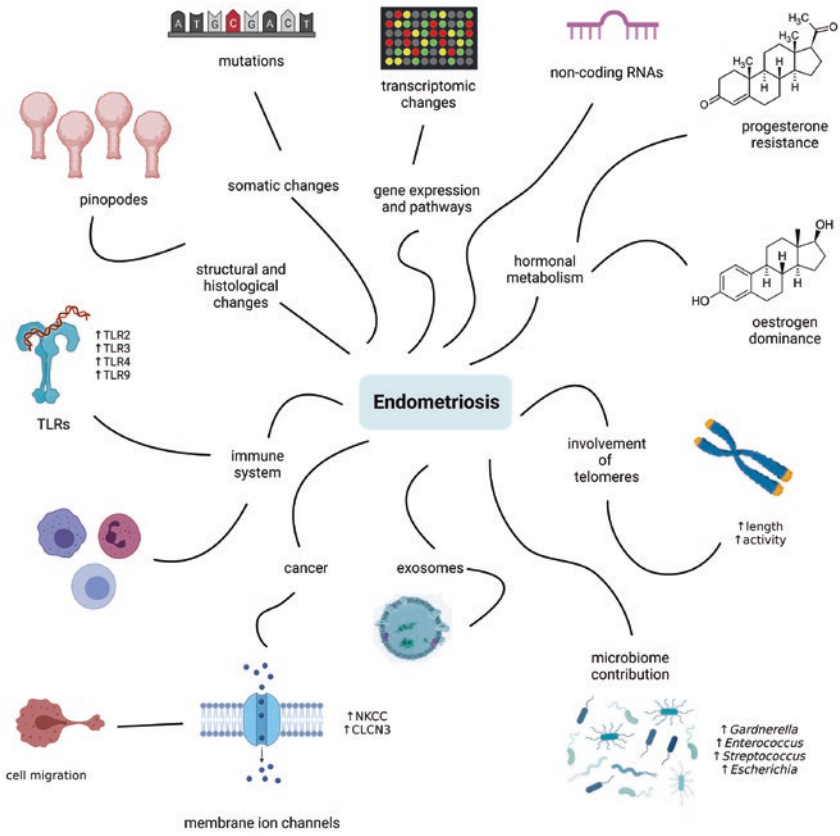

Fig. 4 Different mechanisms contributing to impaired endometrial receptivity in women with endometriosis: structural and histological changes in the number and activity of pinopodes; somatic changes; changes in immune system cell populations and innate immune system (activity of toll-like receptors (TLRs)); dys-regulation of the activity of membrane ion channels and cell migration; impairments in the hormonal metabolism (oestrogen dominance and progesterone resistance); longer telomeres and higher telomerase activity; microbial composition; gene expression profile and molecular pathways; exosomes, and non-coding RNA molecules. Figure is created using BioRender.com

3.1 Structural and Histological Changes in Endometriosis

In the early 2000s, the study of pinopodes was of interest and its detection was proposed as a possible marker of endometrial receptivity [60]. These microscopic structures emerge in the transition to the receptive phase endometrium, at the mid-secretory phase. In fertile women, the number of pinopodes resulted to be higher than in infertile women with repeated implantation failure [61], pointing to these specialised formations as necessary for the adhesion of the blastocyst to the human endometrium. The presence of pinopodes has also been assessed in women with

endometriosis, while no big differences have been detected between the presence and the developmental stage of pinopodes at the WOI in infertile women with endometriosis compared to healthy women [62, 63].

Another structural change in the endometrium that could be aberrantly regulated in endometriosis is decidualisation. Decidualisation is a process that results in morphological and functional changes to the endometrial stromal cells, the presence of decidual white blood cells, and vascular changes to maternal arteries, all of which are essential for endometrial preparation for embryo implantation and pregnancy establishment [64]. It has been proposed that one of the reasons why eutopic endometrium might underlie endometriosis-related infertility is its implication in defects in decidualisation [48]. Most of the changes that happen during the decidualisation are orchestrated by progesterone, whose metabolism is known to be impaired in women with endometriosis [65]. These changes may lead to aberrant decidualisation and, thus, negatively impact embryo implantation in women with endometriosis. Compared with endometrial stromal cells from control women, eutopic endometrial stromal cells from women with endometriosis showed impaired decidualisation [66]. The exact mechanisms through which the eutopic endometrium may contribute to an aberrant decidualisation are not known; however, some candidates such as the dys-regulation of progesterone metabolism by hormonal treatments or the activity of microRNAs such as miR-194-3p have been proposed [65, 67].

3.2 Different Cell Types in the Eutopic Endometrium

Endometriosis is associated with chronic inflammation and thereby with changes in the phenotype, activity, and function of immune cells [68]. Several studies are demonstrating that pathways involved in immune response evasion are dys-regulated in the eutopic endometria of women with endometriosis [49, 69] and that the increased systemic and localised inflammation in women with endometriosis is correlated with the imbalance within the immune cell populations [70]. The severity of endometriosis has been shown to modify the expression profile of immune cells, with differences found between the cell profiles of women with stages I and II versus III and IV endometriosis [71]. As the uterine immune niche involves different cell types with varying degrees of activation and communication among cells, characterisation of the immune niche is of great importance in unravelling endometrial functions and dysfunction in endometriosis [72]. There is little information, however, of the function and phenotypes of eutopic endometrial immune cells. Hereby we summarise an overview of the current knowledge in the topic.

Macrophages are key effector cells involved in tissue regeneration and required for endometrial functions. In the endometria of women without the disease, the fluctuation in the number and activity of macrophages seems to be regulated by oestrogens and progesterone throughout the menstrual cycle, with a modest increase in the secretory phase [73]. In endometriosis, some abnormalities in the presence of macrophages have been highlighted, where greater abundance of macrophages in

the eutopic endometria of women with endometriosis has been detected [72–74]. Nevertheless, there is no consistency in the results reported so far, and the possible implications of this dys-balance on endometrial receptivity await further studies [47].

Uterine natural killer (uNK) cells have a role in embryo implantation and successful pregnancy [72]. In endometriosis, it has been postulated that the reduction in the cytotoxic activity of these immune cells in peripheral blood may boost the development of endometriosis, as it would allow the accumulation of menstrual endometrial tissue in the peritoneal cavity [75]. The normal activity of uNK cells in healthy endometrium diminishes during the mid-secretory phase and is related to embryo implantation [76]. However, in the eutopic endometria of infertile women with endometriosis, uNK cells exhibited higher cytotoxicity at the WOI, which could lead to an inhospitable environment for embryo implantation [72, 75].

Regulatory T cells (Treg) also behave differently in the endometrium of women with endometriosis [77]. The normal activity of Treg in healthy endometrium is characterised by an increase in the proliferative phase and a decrease in the secretory phase to allow the embryo to implant [47]. In infertile women with endometriosis, however, an unusual Treg activity increase in the endometrium at the WOI has been detected, which may contribute to the implantation failure [78].

Dendritic cells play a pivotal role in the immune response in mucosal surfaces such as endometrium [47]. In normal pregnancy, they have been observed to be significantly increased during the first-trimester decidua in comparison with no pregnancy, suggesting an essential role in the interplay between the maternal immune cells and the trophoblast cells [79]. In animal models, the injection of dendritic cells into the peritoneal cavity induced endometriotic lesion formation, suggesting their contribution to lesion growth through angiogenic processes [80, 81]. Further, the increased activity of dendritic cells has been shown in the blood and endometrial tissue of women with endometriosis during the secretory phase when compared to control women, reflecting the consistently inflammatory tissue environment observed in endometriosis [82].

Mast cells are well-known contributors to homeostasis in the immune system [83]. The density and the activity of mast cells have been associated with endometriosis; however, the role of these immune cells in the etiopathogenesis of the disease is not clear [84]. It seems that oestrogen metabolism is influenced by the action of mast cells in endometriomas; however, the precise mechanisms have not been fully described so far [85].

Neutrophils are involved in all types of inflammatory conditions, ranging from acute, chronic, autoimmune, infectious, and non-infectious diseases [86], and they have been shown to play a role in the pathogenesis of endometriosis [87, 88], while the studies assessing neutrophils in eutopic endometria are lacking.

Eosinophils are major effector cells in the immune system, involved in defence and inflammatory processes. Populations of eosinophils have been detected in higher concentrations in the endometria of women with stage I and II endometriosis compared to healthy controls [71], while studies assessing these cells in the eutopic endometria are awaiting.

B-cells activity underlies the development of the humoral immune response through the production of antibodies against foreign antigens [47]. It has been claimed that B-cells might be involved in the development and function of nerve fibres in lesions and in eutopic endometrium of women with endometriosis [77]. However, no consensus has been reached on the levels of these immune cell proteins between women with and without endometriosis [89–92].

Also endometrial progenitor, stem cells, could have a role in the endometrial functions in endometriosis. Endometrial stem cells are shown to be associated with the development of the lesion at ectopic sites [93], while primary constitutive changes in these cells, when isolated from the eutopic endometrium, are still controversial [94]. In short, the information about the dynamics of endometrial stem cells in the eutopic endometria in women with endometriosis is scarce and more studies are required to bring more knowledge into this topic.

A pioneering study has performed the first single-cell RNA sequencing study of endometrial cells in endometriosis with the focus on receptive phase endometrium, where they identified nine clusters of stromal cells, nine clusters of epithelial cells, four of endothelial cells, and eight of immune cells including T-cells, NK-cells, macrophages, Th cells, and mast cells [95]. The biggest difference in the cellular proportion was noted in stromal (49% vs. 34%) and immune cells (8% vs. 15%) between the control group and women with endometriosis, where there were significantly more immune cells in endometria of women with endometriosis. Further, an increase in CD45+ leukocyte cells was detected in the secretory phase eutopic endometria in endometriosis, and the decrease of immune cells in the secretory phase that was noted in the control group was absent in endometriosis women, which may indicate an inflammatory environment during the receptive phase endometrium [95]. Interestingly, one cluster of epithelial cells that expressed PAEP and CXCL24, the endometrial receptivity markers [33], was absent in the eutopic endometrial samples in endometriosis in the receptive phase [95]. Altogether, these results provide novel insights into the endometrial immune microenvironment and aberrant endometrial receptivity among infertile women with endometriosis.

3.3 Exosomes in the Development of Endometriosis

Exosomes are a type of extracellular vesicles containing inner constituents of the cells that secrete them [96]. They mediate intercellular communication by trafficking signalling factors such as proteins, nucleic acids, and lipids [97]. The cell-to-cell communication mediated by exosomes seems to be involved in the pathogenesis of some inflammatory diseases, as they can affect cell function and behaviour when they are taken up by distant cells [96, 98] hinting at their possible involvement in endometriosis.

It has been postulated that exosome-mediated signalling may have an important effect on endometriosis progression, as extracellular vesicles could promote the formation of a pre-endometriotic niche and can regulate disease development

[99–102]. Further, exosomes derived from endometrial epithelial cells are important in the implantation in the context of endometriosis, as they carry molecules with targets required for embryo-endometrial interaction [33, 98]. Exosomes have also been isolated from the peritoneal fluid of women with and without endometriosis, and their concentration varied within the cycle phase and the disease stage [103]. Further, it has been described that the protein content between exosomes derived from eutopic versus ectopic endometria of women with endometriosis differed, with annexin A2 (ANXA2) being present in ectopic but not in eutopic endometrium-derived exosomes [104].

Exosomes also seem to modulate immune function in endometriosis through the modulation of the activity of specific immune cell types such as macrophages [105, 106]. Further, it has been shown that exosomes released in endometriosis lesions obtained from the eutopic endometrial tissue of women with endometriosis act to promote neuroangiogenesis [107]. The role of exosome-mediated inflammation and apoptosis through the use of miRNAs has been studied in the peritoneal fluid of women with endometriosis [108], with miR-138 as a vehicle acting on the regulation of nuclear factor (NF)-κβ and vascular endothelial growth factor (VEGF) proteins [109].

All these findings suggest that the development of endometriosis might be controlled, at least to some extent, by exosomes through the regulation of immune evasion, cell proliferation, angiogenesis, and invasion of the lesions, as it seems that the intercellular crosstalk mediated by exosomes may orchestrate cell fate by regulating the signalling pathways involved in the disease progression [98, 110, 111]. In conclusion, exosomes are considered an emerging novel biological concept in signal transduction and gene regulation, and their utility as potential biomarkers of endometriosis has been proposed [97, 110, 112].

3.4 Somatic Genomic Events

Non-inherited, the somatic genomic events present in endometriosis cells but absent from non-endometriosis cells have been a recent interest of research in endometriosis. The somatic events include genomic changes in DNA sequence at any level, as well as epigenetic modifications. Both ectopic and eutopic endometria of women with endometriosis have been detected to harbour genomic alterations [113]. In fact, it has been hypothesised that a potential explanation for the initial alteration of the eutopic endometrium in endometriosis could be the presence of somatic mutations in the epithelial and/or stromal endometrial cells [94]. Indeed, somatic cancer driver mutations have been described in a range of endometriosis lesions [114]. Despite the relevance of somatic genomic events in ovarian lesions, they do not appear to be crucial or significant in cells of the eutopic endometrium [115]. A recent study highlighted a somatic *ARID1A* mutation in epithelial cells in association with the upregulation of pro-angiogenic and pro-lymphangiogenic factors and remodelling of the endothelial cell compartment in endometriosis in the single-cell transcriptomics

analysis [116]. Some somatic mutations as PTEN loss have also been observed in the eutopic endometrium of women with endometriosis, although at lower mutant allele frequencies than those in the ectopic lesions [114]. While the somatic driver-like events as PTEN loss were also found in normal endometrial samples, which evidences the necessity of caution in the use of mutation-based early detection tools for screening endometrial samples in search of the disease state [117]. The presence of somatic mutations hint at dys-regulation of DNA damage response and DNA repair pathways as suggested in eutopic endometrial lesions [118]. Interestingly, it was previously shown that endometrial expression of genes involved in DNA damage response was modulated in women with endometriosis, which refers to the DNA damage-induced stimuli, either of higher strength or for longer duration in endometriosis [118]. Nevertheless, the causes underlying this dys-regulation are not fully determined yet [118].

3.5 Oestrogen and Progesterone Signalling and Dysfunctional Steroid Hormone Response

It is evident that the eutopic endometrium of women with endometriosis functions apparently normally and has comparable responsiveness to steroid hormones as in women without the disease. However, both animal and human studies using candidate markers analysis have demonstrated differential molecular regulation in endometriosis, suggesting progesterone resistance in eutopic endometrium and impaired endometrial receptivity [119–127].

The altered progesterone action and excessive oestrogen activity could affect endometrial receptivity while also promoting the pathogenesis of endometriosis as a disease [128]. It has been observed that changes in the eutopic endometria of women with endometriosis promote progesterone resistance and oestrogen dominance through aberrant cell signalling pathways and reduced expression of crucial homeostatic proteins (Fig. 5) [129, 130]. Progesterone is responsible for decidualisation and establishment of embryo implantation and together with oestrogens leads the uterine hormonal metabolism [131]. Previously, the down-regulation of the epithelial progesterone receptor was suggested as a requirement for the establishment of normal endometrial receptivity [132], and the impairment in the endometrial expression of progesterone receptors in women with infertility and endometriosis has been detected [133, 134]. Only small amounts of oestradiol and progesterone seem to be required at the secretory phase in women with normal endometrial functions [135], while too high or too low mid-secretory serum progesterone concentrations (<50 and > 99 nmol/L) associated with diminished implantation rates in cryopreserved embryo transfers [136].

In addition, the eutopic endometrium of women with endometriosis may be enriched in potentially genotoxic intermediates derived from the metabolism of oestrogens, such as 4-hydroxyestrone (4OHE1) and 4-hydroxyestradiol (4OHE2)

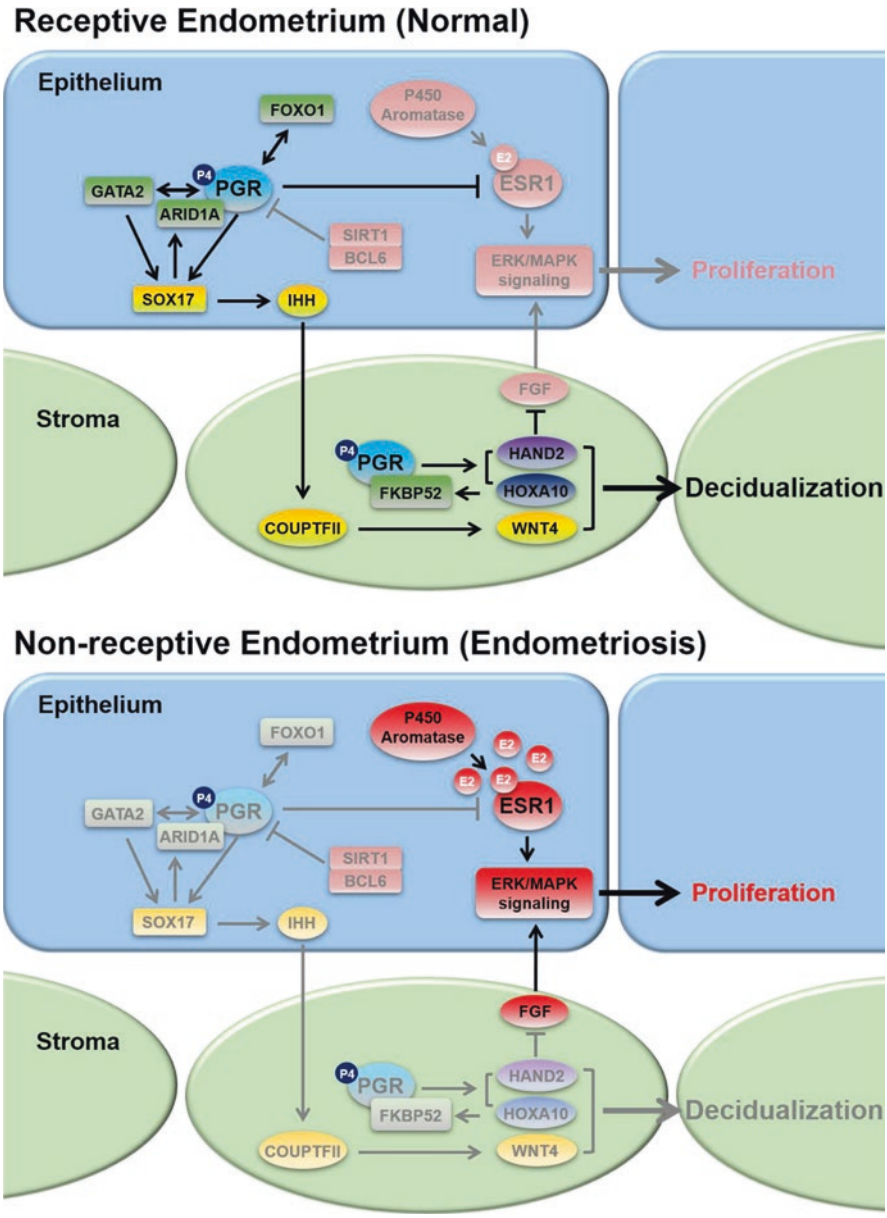

Fig. 5 Dys-regulation of the signalling pathways and transcriptional regulators involved in oestrogen (E2) and progesterone (P4) metabolism in the epithelial-stromal crosstalk in endometriosis. Characteristic oestrogen dominance and progesterone resistance observed in endometriosis result in epithelial transformation and impaired decidualisation, leading to compromised endometrial function. *ARID1A* AT-rich interaction domain 1A, *BCL6* B-cell CLL/lymphoma 6, *COUPTFII* chicken ovalbumin upstream promoter-transcription factor II, *E2* oestrogen, *ERK* extracellular signal-regulated kinase, *ESR1* oestrogen receptor 1, *FGF* fibroblast growth factor, *FKBP52* FK506 binding protein prolyl isomerase 4, *FOXO1* Forkhead box O1, *GATA2* GATA binding protein 2, *HAND2* heart and neural crest derivatives expressed 2, *HOXA10* homeobox protein-A10, *IHH* Indian hedgehog, *MAPK* mitogen-activated protein kinase, *PGR* progesterone receptor, *SIRT1* sirtuin 1, *SOX17* sex determining region Y box 17, *WNT* Wnt family member 4. Reproduced with permission from Marquardt et al. (2019)

[137]. Further, the concurrence with progesterone resistance with the dys-regulation of the activity of some proteins such as p65 and ARID1A may explain the biological alterations during the WOI in patients with endometriosis [138, 139].

Taken together, given the crucial role of these steroid hormones in the endometrial functions and etiopathogenesis of endometriosis, therapeutic approaches focussing on improving the expression of progesterone receptors may significantly affect final reproductive outcomes, paving the way to the development of strategies aimed to amend endometrial receptivity in women with the disease [134, 140].

3.6 Endometrial Transcripts and Molecular Pathways in Endometriosis

For understanding the endometrial physiology and to identify the biomarkers of endometrial receptivity in health and disease, the molecular changes that occur within the endometrium must be first well understood. The whole genome expression analysis, i.e., transcriptome analysis, is a direct reflection of gene expression in tissues. Thus, the transcriptome analysis of the eutopic endometrium of women with endometriosis has an invaluable potential in contributing to the understanding of local molecular events associated with the pathology. There is an active debate whether endometrial receptivity at a molecular level is dys-regulated in endometriosis or whether there are no transcriptional differences when compared to women without the disease. As the studies performed are on relatively small sample size, and there are no validation studies, it remains for future studies to bring knowledge into this debate. In fact, a recent meta-analysis that gathered transcriptome data of 125 women from eight different studies did not detect any significant differentially regulated genes, while pathway analysis identified chemotaxis and locomotion pathways enrichment, highlighting altogether that there are endometrial transcriptomic differences in women with endometriosis when compared to controls in the receptive phase, although the differences are small [49].

As women with endometriosis seem to demonstrate diminished endometrial receptivity [46], it is expected that detectable changes should also be seen on a molecular level. Several endometrial biomarkers such as aromatase, steroid hormones and their receptors, or cytokines are reported to be differentially expressed in the endometria of women with endometriosis compared with normal women [126, 141].

The homeobox genes A10 (*HOXA-10*) and A11 (*HOXA-11*) are the top endometrial receptivity molecules investigated in endometriosis [142, 143]. The evidence from human and animal studies indicates that reduced *HOXA-10* expression during the WOI in the eutopic endometrium of women with endometriosis may contribute to the infertility in these patients [144, 145]. Indeed, in women with endometriosis-associated infertility, endometrial biopsies obtained during the WOI exhibited a significantly lower expression of *HOXA-10* and *HOXA-11* when compared with

infertile women without endometriosis [143]. Moreover, these women presented alterations of the endometrial surface in terms of roughness, suggesting an adverse effect of the expression of these genes on endometrial remodelling [143]. One of the possible mechanisms that could lead to the dys-regulation of *HOXA-10* is hypermethylation [145].

Another set of molecules that have attracted research of endometrial receptivity in endometriosis is the expression of adhesion molecules such as integrins and annexins [143]. A decrease in the levels of annexins during the WOI in infertile patients with endometriosis has been associated with diminished endometrial receptivity [146]. A defective expression of integrin αvβ3 has been reported in women with endometriosis [147], and the dys-regulation of the adhesion molecules E-cadherin and beta-catenin in the mid-secretory endometrium of infertile women with endometriosis has been proposed as one of the potential molecular mechanisms of endometriosis-associated infertility [148]. Nevertheless, other studies have not associated integrin expression with endometrial functions in endometriosis [60, 62, 149].

Previous studies have detected gene expression changes during the WOI in eutopic endometria creating an inhospitable environment for embryo to implant due to dys-regulation of genes involved in embryonic attachment, stromal decidualisation, immune functions, and apoptotic responses that contribute to the pathophysiology of endometriosis-associated infertility [127, 150]. Several whole transcriptome studies covering the endometrial landscape of women with and without endometriosis during the mid-secretory phase have been performed [124, 133, 150–155]. However, the sample size covered by these samples is low, and there is little overlap and high variability between different studies, with a number of differentially expressed genes ranging from 26 [151] to 10,458 transcripts [152] (Table 2). Further, it is not clear whether the differential expression observed is a reflection of endometriosis-associated infertility or it shows the influence of the menstrual cycle instead [156]. In line with the controversy around these findings, other authors claim that the eutopic endometria of infertile women with endometriosis are transcriptionally similar to the healthy controls during the window of implantation, which may reinforce the belief that endometriosis does not affect endometrial receptivity [150, 155, 157]. On the other hand, a freshly published study performing single-cell RNA sequencing on eutopic endometrial epithelial and stromal cells detected markedly different transcriptome signatures between the two cell types in endometriosis suggesting that extensive transcriptional reprogramming is a core component of the disease process [158]. Further, a recent meta-analysis focussed on receptivity-specific genes at the receptive phase endometrium and identified dys-regulation of *C4BPA*, *MAOA*, and *PAEP* genes and enrichment of immune and defence pathways in women with endometriosis [49].

In addition, the use of transcriptomic techniques in combination with other high-throughput omics technologies, such as proteomics, has yielded advances in the research of endometriosis aetiopathiology and biomarker discovery. The endometrium of infertile women with endometriosis has been shown to exhibit a proteomic map enriched in proteins associated with immune responses that differ from healthy

Table 2 Endometrial transcriptome studies focussing in endometrial receptivity analysis in endometriosis. For each study, the technology used (microarrays or RNA-sequencing), the number of samples of women with endometriosis and control women, and the differentially expressed genes (DEGs) are shown

Study	Technology used	Endometriosis samples	Control samples	DEGs
Kao et al. (2003) [133]	Microarrays	$n = 8$	$n = 7$	149
Matsuzaki et al. (2005) [151]	Microarrays	$n = 3$	$n = 3$	26
Burney et al. (2007) [124]	Microarrays	$n = 9$	$n = 8$	721
Tamaresis et al. (2014) [152]	Microarrays	$n = 28$	$n = 8$	10,458
Garcia-Velasco et al. (2015) [157]	Microarrays[a]	$n = 17$	$n = 5$	0
Ahn et al. (2016) [153]	Microarrays	$n = 8$	$n = 8$	91
Zhao et al. (2017) [154]	RNA-sequencing	$n = 8$	$n = 5$	72
Da Broi et al. (2019) [150]	RNA-sequencing	$n = 6$	$n = 5$	0
Wang et al. (2019) [159]	RNA-sequencing	$n = 6$	$n = 6$	294[b]
Poli-Neto et al. (2020) [71, 94]	Microarrays	$n = 102$	$n = 41$	231[c]
Celik et al. (2020) [160]	Microarrays	$n = 6$	$n = 6$	18
Joshi et al. (2021) [155]	Microarrays	$n = 8$	$n = 3$	0
Saare et al. (2022) [161]	TAC-seq technology[d]	$n = 8$	$n = 8$	33
Huang et al. (2023) [95]	Single-cell RNA-sequencing	$n = 6$	$n = 7$	NA

[a]Endometrial receptivity array (ERA) test was used for measuring gene expression
[b]Circular RNA were assessed for differential expression analyses
[c]Differentially expressed transcripts in comparison between control women and women with endometriosis stages I and II during the mid-secretory phase of the menstrual cycle
[d]Gene expression profiling of 57 genes included in beREADY® endometrial receptivity test based on TAC-seq technology

women [162]. Further, an integrated analysis of existing expression profile data on endometriosis-related tissues led to the identification of many differentially expressed genes and proteins among the ectopic, eutopic, and normal endometria of women with and without endometriosis, respectively [163]. In conclusion, the implementation of multi-omics approaches to the study of the eutopic endometria would contribute towards the identification of the mechanisms that have an impact on the fertility potential of women with endometriosis [164].

3.7 microRNAs and Other Non-coding RNAs in Endometriosis

Non-coding RNAs (ncRNAs) are important regulators of cellular functions and gene expression, and their role in many chronic conditions has been investigated [165–167]. Among these, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have mostly attracted the attention of researchers in endometriosis.

Multiple studies have detected altered expression levels of miRNAs in the eutopic endometrial tissue [99, 168], while other authors have not observed any differences in miRNA expression levels in eutopic endometria of women with endometriosis compared to women without the disease [150]. Despite the controversies, the role of some miRNAs in the context of endometriosis etiopathogenesis and associated infertility has been described. The role of miR-543 in endometrial receptivity and embryo implantation has been shown [169]. miR-543 levels were down-regulated at the WOI compared to the proliferative phase in the endometria of infertile women with endometriosis [170], suggesting that the dys-regulation of miR-543 may affect embryo implantation and may thus explain the pathogenesis of endometriosis-related infertility in women with endometriosis [169]. Also other miRNA molecules, including miR-142-5p and miR-146a-5p, have been detected with aberrantly enhanced expression in the endometria of women with endometriosis, highlighting possible candidate molecules impacting endometrial receptivity in infertile women with endometriosis [170]. Additionally, higher levels of miR-494-3p, miR-10b-3p, miR-125b-2-3p, and miR-1343-3p were detected in exosomes derived from endometrial stromal cells from patients with endometriosis when compared to women without endometriosis [171]. Additionally, these miRNAs were predicted to target the expression of the endometrial receptivity genes *HOXA10* and *LIF* [98, 171].

On the other hand, lower levels of miR-34a in ectopic and eutopic endometrial samples compared to normal endometrial tissue have been detected [172]. The miR-34a down-regulation may contribute to the pathogenesis of endometriosis through the modulation of the expression of genes involved in apoptosis such as *SIRT-1* and *FOXO-1* [172]. Also involved in the regulation of apoptotic processes, the down-regulation of miR-370-3p targeting *EDN1* expression has been shown in women with endometriosis [173].

LncRNAs regulate gene expression via controlling transcription and post-transcriptional processing. The differential expression of some lncRNAs such as H19 and MALAT1 has been detected in endometriosis highlighting lncRNAs role in contributing to the pathogenesis of the disease [174–179]. Animal studies are demonstrating the involvement of lncRNAs in endometrial receptivity in endometriosis. The molecular mechanisms, however, by which the non-coding RNA molecules are associated with endometriosis need further research [180].

3.8 Uterine Microenvironment—Metabolites

Metabolomics is a cutting-edge method that allows for the detection and analysis of small molecules with a molecular mass typically below 1200 Da. These molecules, known as metabolites, play crucial roles as intermediates and final products of cellular processes, which provide a snapchat of the functional phenotype [181]. Despite the growing number of metabolomic studies, the use of the whole metabolite profile analysis related to uterine health is still scarce.

Metabolomics has garnered significant interest as a potential tool for identifying key metabolites involved in the mechanistic pathways that contribute to the pathophysiology of endometriosis. While traditional approaches have been limited in their ability to fully capture the complex cellular processes underlying this condition, a metabolomic-based approach holds great promise, as several metabolites have been shown to be closely linked to endometrial cell proliferation, cell survival, and high levels of oxidative stress, all of which have been extensively studied in the context of endometriosis [182].

A recent whole metabolomic study analysing endometrial biopsies collected in the mid-secretory/receptive phase identified 925 metabolites from different chemical classes, being lipids the most abundant [183]. Additionally, infertile women with endometriosis and recurrent implantation failure (RIF) showed lower levels of polyunsaturated fatty acids (PUFAs) compared to women with no clear endometrial alterations (i.e., male factor and unexplained infertility). Particularly, dihomolinolenate (omega-3 or omega-6), linolenate (omega-3 or omega-6), and linoleate (omega-6) metabolites related to PUFAs metabolism were significantly reduced in women with endometriosis and RIF [183]. Similarly, another study detected eight lipids significantly altered in endometrial fluid from non-implantation cycles, showing six of them had lower levels in the endometrial fluid of women in whom implantation did not occur compared to successful implantation IVF cycles [184]. These results highlight a possible link between the metabolome signature and infertility diagnoses where altered endometrial functions are suspected.

Several metabolomics-based studies have analysed women with endometriosis and demonstrated differences between endometriosis patients and controls, suggesting several potential biomarkers of the disease [185–189]. Metabolites such as amino acids, lipids, and nucleotides among others were found to be significantly different between the groups [182, 190]. By leveraging the power of metabolomics, researchers may be able to gain new insights into the underlying mechanisms driving this condition and to understand its role in aberrant endometrial functions.

3.9 New Player in Endometrial Functions – Microbiota

Recent studies indicate that microbes inhabiting endometrium might have a role in the endometrial functions and uterine pathologies such as endometriosis [191–194]. Humans are colonised with more microbes than human cells in the body [195, 196], and as more knowledge regarding the human microbiota (the collection of the microorganisms residing in/on the body) is acquired, the clearer it becomes that it has a significant effect on human physiology [197]. The majority of bacterial communities co-exist in a synergetic relationship with the human host; however, an imbalance in this relation may result in a disease [198]. Female reproductive tract, specifically vaginal milieu, is known to have an active microbiota, containing >90%

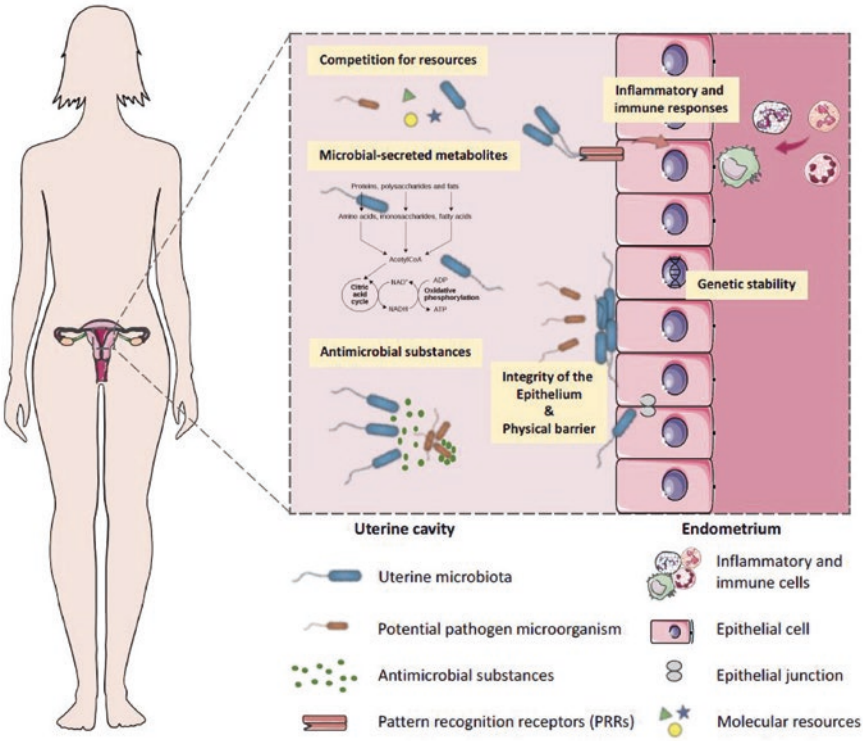

Fig. 6 Hypothetical endometrial microbiota–host interplay in the uterine cavity. Microorganisms could impact uterine stability: through genomic and epigenetic alterations; microbial-secreted metabolites may regulate the growth of specific bacterial species; and the competition for resources established among different species may compromise uterine stability. Endometrial microbial homeostasis is probably regulated through three main mechanisms: (1) the presence of epithelial cells conforming a barrier through junctions limiting the exposure of bacterial communities to immune system; (2) antimicrobial peptides controlling infection; and (3) detection and killing of bacteria by the endometrial lymphocytic defence. Reproduced with permission from Molina et al. (2020)

of *Lactobacillus*. Despite this, until recently the uterus was assumed to be sterile, with microbial colonisation being only as part of an infection or pathological process.

The influence of the microorganisms on immunomodulation and the development of several inflammatory diseases is well stablished [199]. Much is known about how the gut microbial composition maintains the integrity of the gastrointestinal epithelial lining as well as immune homeostasis, preventing bacterial translocation, which can cause low-grade systemic inflammation [197]. Conversely, little is known about the presence and composition of the microbes in the uterus and its role in the endometrial receptivity in health and disease (Fig. 6) [200]. Considering the altered inflammatory status in endometriosis, postulating that microbes are involved in the disease is logical. Indeed, women with endometriosis have a higher incidence of chronic endometritis, more severe pelvic inflammatory disease, a higher risk of surgical site infection after hysterectomy, and a higher incidence of lower genital tract infection [201]. In fact, a hypothesis of 'bacterial contamination' in endometriosis has been proposed [202], where the lipopolysaccharide inflammatory mediator could be the initial trigger and the bacterial 'contamination' its source in the intrauterine microenvironment that could lead to the growth regulation of endometriosis [203]. It is also probable that the microorganismal pathogens activate the immune response by binding to the host receptors. Indeed, aberrant regulation and expression of the innate immune system members Toll-like receptors (TLRs) have been linked to the pathogenesis of endometrial diseases [53]. Particularly, the eutopic endometrium of women with endometriosis has been shown to express higher levels of TLR3 cascade genes when compared to the control women [53]. Additionally, TLR2, TLR4, and TLR9 seem to be increased in the endometrial tissue and peritoneal fluid of women with endometriosis [204–206].

The microbiome (the genetic material of the microbiota) based studies are demonstrating that microbiome profiles in the endometrium differ significantly in women with endometriosis when compared to healthy women, where bacterial taxa such as *Gardnerella*, *Enterococcus*, *Streptococcus*, *Pseudomonas, Acinetobacter, Vagococcus, Sphingococcus*, and *Escherichia* are more prevalent in endometriosis [192, 207–211], while a decrease in *Lactobacillus* among women with endometriosis has been reported [207, 212]. In contrast, another study found a variety of bacteria including *Lactobacillus*, *Gardnerella*, *Streptococcus*, and *Prevotella* being the most abundant in the endometria from women with endometriosis [209]. Altogether, the performed studies are barely comparable and the endometrial 'core' microbial composition in health and disease still needs to be established. What seems to be in accordance is that the microbial composition differs from that of healthy controls and could have a role in the endometrial functions and disease development; however, the mechanisms are unknown.

4 Conclusions

Despite recent innovations, endometrial receptivity remains the 'black box' in assisted reproduction and we do not have a conclusive answer whether and to what extent endometrial receptivity is affected in women with endometriosis. What is evident is that women with endometriosis are twice as likely to be infertile and experience pregnancy loss than women without the disease, where different factors could play a role, including the receptiveness of the endometrium. Results from different studies and observations support the concept that endometrial defects exist in women with endometriosis, which include the inflammatory nature of this disease, accompanied by excessive oestrogen action and progesterone resistance, activated signalling pathways involved in proliferation and cell survival, presence of microbes, inadequate differentiation of the stroma, and remodelling of the endometrium among many other processes which altogether can lead to the changes in the eutopic endometria that interfere normal endometrial functions and thereby embryo implantation. In conclusion, further research is needed to understand better and in detail the effects of endometriosis on endometrial functions. Regardless, it appears increasingly that endometriosis has a negative effect on infertility treatment outcomes, and endometrial receptivity defects should, therefore, remain a relevant and vital part of the workup of couples with infertility.

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Assessment of Ovarian Reserve in Women with Endometriosis

Baris Ata, Engin Turkgeldi, and Uzeyir Kalkan

1 Ovarian Reserve and Endometriosis

Ovarian reserve is defined as an ovary's functional potential and is determined by both the quality and the quantity of oocytes it contains [1]. It is closely related to reproductive aging. Decline in ovarian reserve is a process that spans over decades, from fetal life until menopause. Consumption of the pool of non-growing follicles is thought to be the main mechanism for this process and it varies greatly among women [2]. The number of oocytes peaks at around 6–7 million oocytes in the 20th gestational week, which declines to 1–2 million at birth, 300,000–400,000 at puberty, 25,000 at age 40, and less than 1000 at menopause [3].

Although it is well established that oocyte quality deteriorates with age, this varies significantly among individuals as well [2]. Currently, there are no direct or indirect measure of oocyte quality available. Therefore, the term "ovarian reserve" currently refers to oocyte quantity in practice.

Information about ovarian reserve may prove helpful when counseling a woman in terms of her reproductive health, potential, and goals. The most common purpose is to predict the response to ovarian stimulation for women undergoing assisted reproductive technology (ART) [3]. It can also be useful when counseling women with endometriosis and planning the management of the disease. Ovarian reserve

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assessment helps in estimating the extent of ovarian damage by the disease per se or ovarian surgery.

There is building evidence that the presence of an endometrioma per se adversely affects ovarian reserve. When compared with tissue samples from ovaries with other types of benign cysts, a significant decline in the primordial follicle pool was observed in the ovaries of women with endometriomas [4]. In a longitudinal observational study of 80 people, anti-Müllerian hormone (AMH) levels, a reliable biochemical marker for ovarian reserve, were found to decline faster in women with endometrioma than those without (median decline −26.4% vs −7.4%, respectively) [5]. This finding was supported by a meta-analysis of 17 studies on the impact of endometrioma on ovarian reserve [6]. Data from 968 patients with endometrioma and 1874 without endometrioma were pooled and AMH was found to be significantly lower in women with endometriomas (mean difference −0.84 ng/ml, 95% confidence interval −1.16 to −0.52). The result was similar when women with endometriomas and other benign cysts were compared (mean difference −0.85, 95% confidence interval −1.37 to −0.32).

The possible mechanism for the decline in the ovarian reserve due to the presence of endometrioma was explained by Sanchez et al. [7]. Endometriomas have high concentrations of proteolytic enzymes, free iron, reactive oxygen species (ROS), 8-hydroxydeoxyguanosine (8-OHdG), and other inflammatory molecules. They proposed that these molecules infiltrate the surrounding healthy ovarian tissue, and along with transforming growth factor beta (TGF-Beta), they promote fibrosis and loss of cortex-specific stroma, which provides essential support for follicles and mediates nutrients and molecular signals. In addition, smooth muscle metaplasia and reduced angiogenesis are observed in the ovary. Eventually, follicular damage and loss are observed in the affected ovary.

Moreover, it is well established that endometrioma surgery, even when performed by experienced surgeons under ideal conditions, causes a significant and permanent decline in the ovarian reserve [8–11]. Thus, assessing ovarian reserve before and possibly after surgery can aid the clinician to set realistic goals for women undergoing endometriosis surgery.

Besides their possible detrimental effect on the ovarian reserve itself, endometriomas may also impair the utilization of ovarian reserve markers, resulting in misleading estimates of the ovarian reserve [12, 13].

Information on ovarian reserve can be valuable, even decisive, when counseling women with endometriosis about their reproductive goals and developing their management plan. Thus, ovarian reserve assessment should be a part of the routine evaluation of women with endometriosis, the vast majority of whom are in their reproductive years [8]. However, this can be a complicated task since endometriosis can affect both the ovarian reserve itself and methods for assessment [14, 15]. In this chapter, we aim to provide information about different methods of ovarian reserve assessment in women with endometriosis, because awareness of the strengths and limitations of each marker is required for a sound judgment of actual ovarian reserve.

2 Ovarian Reserve Markers

2.1 An Overview of Ovarian Reserve Markers

It is not possible to assess ovarian reserve directly, as this would require histological evaluation. Therefore, surrogate markers have been proposed for assessing ovarian reserve. These markers fall into two main categories, biochemical and ultrasonographic markers.

Biochemical markers include basal serum follicle-stimulating hormone (FSH) and estradiol, inhibin B, and AMH. While the first three are measured in the early-follicular phase (cycle days 2–4), AMH can be measured regardless of the cycle day.

Basal serum FSH concentration used to be the most commonly employed marker; however, its considerable intracycle and intercycle variabilities restrict its dependability, especially when only one measurement is available [16]. High basal serum FSH levels (10–20 IU/l) have high specificity (80–100%) but low sensitivity (10–30%) for predicting poor response to ovarian stimulation [17]. As the cut-off value for FSH draws near to 10 IU/l, sensitivity decreases further. Basal serum estradiol level alone is not used as a marker by itself but is measured for interpreting FSH levels. The variability and the low sensitivity of FSH and estradiol usually ask for repeat measurements in the consecutive months, which causes inconvenience and loss of time. The wide inter- and intracycle variabilities and low sensitivity of basal serum FSH and estradiol, and the possible need for multiple measurements have caused clinicians to prefer more practical and accurate methods to assess ovarian reserve.

Inhibin B, a glycoprotein hormone primarily secreted by granulosa cells in preantral follicles, has also been proposed as an ovarian reserve marker, however, because inhibin B levels are increased during ovarian stimulation and show significant intra- and intercycle variabilities, it is not considered to be dependable marker of ovarian reserve [17].

Provocative tests such as the clomiphene citrate challenge test (CCCT) have been used to improve the accuracy of other biochemical tests; however, the American Society of Reproductive Medicine (ASRM) recommends that CCCT should be abandoned since it does not provide additional benefits in predicting spontaneous conception, poor ovarian response to ovarian stimulation, or IVF success [16].

Due to the limitations of the markers above, we will focus on the two most reliable and widely used measures of ovarian reserve, AMH and antral follicle count (AFC).

It is important to remind that all the currently available ovarian reserve markers are only capable of quantitative assessment and do not represent oocyte quality. It is also crucial to remember that they cannot predict the likelihood of either spontaneous conception or pregnancy following ART treatment [3]. They are mostly used for predicting response to ovarian stimulation, diagnosing decreased ovarian reserve, and monitoring the ovarian reserve in women with endometriosis and receiving cytotoxic treatments [18].

2.2 Anti-Müllerian Hormone (AMH)

AMH is a glycoprotein hormone mainly secreted by granulosa cells of primary, preantral, and early antral follicles. Its secretion stops once the early antral follicle reaches 2–6 mm in diameter [19]. AMH prevents the recruitment of primordial follicles into antral follicles and reduces the sensitivity of the growing follicles to FSH. Since it is not dependent on gonadotropins, it shows minimal intra- and intercycle variabilities and is mainly consistent [20]. Repeated studies showed that AMH levels were mostly consistent within the same cycle and consecutive cycles of the same woman [20–22].

An important caveat is that current oral contraceptive and gonadotropin-releasing hormone (GnRH) agonist use has been shown to decrease AMH levels [23], which can mislead the clinician. Yet, AMH levels return to their natural cycle values after discontinuation for at least 2 months. Therefore, it is important to question and consider hormonal contraception and GnRH agonist use when interpreting AMH results, especially when AMH values are lower than expected. Last of all, handling and storage of AMH samples can influence the test results [18]. Therefore, it may be wise to consider or investigate these factors in the face of unexpected or inconsistent results.

Studies performed on women undergoing ART showed that low AMH cut-off values (0.2–0.7 ng/mL) predicted poor response to ovarian stimulation with a sensitivity and a specificity of 40–97% and 78–92%, respectively [3]. However, it was not successful in predicting pregnancy.

ASRM suggests preferring serum AMH instead of basal FSH and estradiol measurements as a biochemical ovarian reserve marker, since AMH is more sensitive, reliable, and its decline precedes FSH rise [16]. It declares AFC and AMH to be equivalent markers of ovarian reserve.

In conclusion, AMH is considered as an accurate, consistent, and reliable ovarian reserve marker.

2.3 Antral Follicle Count (AFC)

AFC is the total number of all identifiable follicles measuring between 2 and 10 mm in diameter in both ovaries, counted using transvaginal ultrasonography [24]. It has been histologically shown that at each cycle, a proportion of the available primordial follicle pool advances to the antral follicle [25]. Therefore, it is assumed that AFC is correlated with the remaining follicle pool and is a reliable surrogate marker for ovarian reserve.

Recently, a consensus opinion was published as an effort to standardize and optimize AFC [26]. It was advised that AFC should be performed using a transvaginal ultrasound probe with a frequency of ≥7 MHz, by an experienced sonographer who has at least performed 20–40 exams her/himself. During the examination, the ovary

should occupy at least 50% of the screen along its largest axis and all follicles measuring 2–10 mm in diameter should be counted as the ovary is scanned from one end to the other. Currently, real-time 2-dimensional manual, 3-dimensional manual, and semi-automatic volume analysis methods produce similar and acceptable results; therefore, the choice is up to the sonographer [26].

Traditionally, to minimize the intracycle variations and to prevent the corpus luteum from hindering visualization, clinicians are advised to perform AFC in the early follicular phase [25]. Intracycle variation could reach up to 30% for AFC [27], yet it is unlikely to be significant enough to alter patient management. In a study of 79 women, AFC was performed during both early and late follicular phases, and the median AFC was measured significantly less in the late follicular phase [16 AFC (IQR 9–24) versus 13 AFC (IQR 7–21); respectively. $P = 0.001$]. However, agreement on gonadotropin starting dose and protocol choice based on both AFC measurements was good ($k = 0.75$), and both measurements' predictive value for poor ovarian response and ovarian hyperstimulation syndrome (OHSS) risk were similar. Thus, it was concluded that although AFC may be significantly different in early and late follicular phases, patient management or predictive value for poor ovarian response and OHSS are comparable; therefore, from a clinical point of view, measurements during both are acceptable.

The aforementioned consensus opinion declared that although it is easier to perform AFC in the early follicular phase, it can be performed any time during the cycle since intracycle variation is not important enough to change patient management, and it is much more convenient for both the women and the clinic [26].

AFC has a high specificity (73–100%) for predicting poor ovarian response when a cut-off value of 3–4 is set; however, its sensitivity is reported to be lower and variable (9–73%) [3].

Like AMH, AFC can be reduced with current oral contraceptive or GnRH agonist use [23], therefore, results should be interpreted in this light. Expected than lower counts may be repeated after discontinuing the medications for at least 2–3 months for confirmation [26].

In short, AFC is a simple and convenient marker for ovarian reserve in the general population.

Currently, AMH and AFC are considered as practical, reliable, and, having shown comparable accuracy in multiple studies, equivalent markers of ovarian reserve in the general population [16, 28]. Now, we will discuss the advantages and disadvantages of both markers in women with endometriosis.

3 Strengths and Limitations of AMH as an Ovarian Reserve Marker in Women with Endometriosis

An important advantage of AMH for evaluating ovarian reserve in women with endometriosis is being a biochemical marker. As such, it is not affected by anatomical distortion and imaging difficulties experienced for AFC in women with

endometriosis. Therefore, it may provide a more realistic estimate of the actual reserve compared to AFC [8, 14, 15].

Second, AMH shows little intra- and intercycle variabilities as explained above [20–22]. This makes it a convenient choice as it can be measured at the patient's visit, regardless of the cycle day. Moreover, repeat measures are not necessary. Both of these properties save time and resources for the woman and the clinic. Last of all, in a study involving 77 women followed for 3–4 consecutive cycles showed that, although both AFC and AMH showed acceptable intra- and intercycle variations, AMH had significantly smaller variations than AFC [27].

Third, it is possible that changes in AMH may manifest earlier than the other markers. In a retrospective national cohort study of 1749 childhood cancer survivors, it was reported that many women, especially those in younger age groups, showed lower AMH levels when AFC, FSH, and inhibin B were normal [29]. The authors interpreted that the decline in AMH starts to decline earlier than other markers.

On the other hand, the main drawback of AMH compared to AFC is that it cannot provide side-specific information. This can be important in the presence of unilateral endometriomas and when trying to estimate the possible changes in ovarian reserve after surgical or medical treatment. Yet, from a practical point of view, a woman's total ovarian reserve is more important when counseling about and planning her reproductive prospects. Side-specific information may be more relevant for research purposes.

Next, AMH is subject to some general limitations of biochemical markers. Currently, there are 21 immunoassay method–platform combinations [30]. While efforts for harmonization and unification of these are underway, it is not fully established yet [18]. Therefore, clinicians should be cautious when interpreting AMH test results from different laboratories and should check the kit used. Handling and storage conditions may also affect AMH test results. In a study, it was shown that samples cryopreserved in −20° to – 80 ° C had lower results than those freshly tested [31]. However, this may not be clinically significant since the average difference was only 0.2 ng/ml.

A recent systematic review and meta-analysis on the effect of endometrioma surgery on AFC and AMH, involving 14 studies and 650 women, reported similar AFC but significantly reduced AMH (reaching −54% in 9–18 months) after surgery [8]. The authors commented that, in accordance with the histological studies showing that some healthy ovarian tissue is unavoidably removed during endometrioma excision, the decline in AMH has more biological plausibility than a stable AFC following surgery. They hypothesized that AFC was possibly underestimated prior to surgery, resulting in misleading stability in AFC before and after surgery. Finally, they declared AMH should be the ovarian reserve marker of choice when counseling prior to endometrioma surgery, as it is more sensitive than AFC and able to recognize subtle changes in the ovarian reserve.

4 Strengths and Limitations of AFC as an Ovarian Reserve Marker in Women with Endometriosis

The most significant advantage of AFC over other ovarian reserve markers is that it can provide side-specific information. As discussed above, this may be more relevant for research purposes, but less important for clinical practice. Moreover, with the impaired assessment due to visualization problems stated below, the validity of the side-specific information it provides is questionable.

Another advantage of AFC is that it can be performed during the initial examination of women with endometriosis. Previously, we have discussed that despite the intra- and intercycle variabilities, AFC can be measured any day of the cycle. Thus, AFC can be assessed on the spot when a patient is already having an ultrasound examination. Integration of AFC to routine evaluation of women with endometriosis can benefit both patients and clinicians. Women can be counseled right after the pelvic ultrasound examination for endometriosis. This can avoid trips to the laboratory, preclude anxiety of waiting for the results, financial burden of a laboratory test, and time loss for both women and clinicians.

Difficulty in acquiring high-resolution images of an ovary affected by endometrioma is the most important limitation of AFC. Chronic inflammation promoted by the abundant free iron and ROS within the endometrioma was explained before [7]. Inflammation causes significant debris to accumulate within the cyst, which may reduce the quality of the images acquired considerably. Besides impairing visualization of the ovary and the antral follicles, fibrosis may also distort pelvic anatomy. This results in more difficult access to ovaries and increased distance between the probe and the ovaries, which impairs visualization further [32].

The difficulty in acquiring high resolution of the ovary with endometrioma introduces the risk of underestimating the actual ovarian reserve of the woman. Some initial retrospective studies showed similar AFC in women with endometriosis and those without [33, 34]. Later, several studies showed the contrary, and, in a recent meta-analysis of 9 studies, the mean AFC was found to be significantly lower in women with endometriosis than those without (−1.92, 95% CI –2.75 to −1.09; $I^2 = 81\%$; $P < 0.00001$) [35]. The same authors performed another meta-analysis of five studies and found the mean AFC to be lower in ovaries with endometriomas than unaffected ovaries in the same woman (−2.09; 95% CI –3.46 to −0.73; $I^2 = 85\%$; $P = 0.003$) [35]. However, it should be noted that both meta-analyses showed high heterogeneity, and the authors failed to comment if these outcomes were due to an actual difference in ovarian reserve or the underestimation of AFC due to visualization problems. Yet, given lower AMH levels observed in women with endometriosis, we think that it is the former. Lima et al. followed 37 women with unilateral endometriomas undergoing ovarian stimulation for ART. Initially, the median AFC was significantly lower in the ovaries with endometrioma than

those without [3 (interquartile range, 1–6) vs 5 (interquartile range, 2–6.5), respectively; $P = 0.001$]. However, the median number of oocytes was similar in the ovary with endometrioma [2 (interquartile range, 0.5–5)] and the unaffected ovary [2 (interquartile range, 0–4)] ($P = 0.6$). Thus, the authors concluded that AFC may be underestimated in ovaries in the presence of an endometrioma.

Another disadvantage of AFC is that it has higher intra- and intercycle variabilities compared to AMH. In a study comparing the intra- and inter-individual variabilities of AMH and AFC in 3–4 consecutive cycles of 77 subfertile women with regular menstrual cycles, although both AMH and AFC showed acceptable variation, AMH showed less intra- and intercycle variabilities than AFC [27]. Age-adjusted intraclass correlation coefficient (ICC) for intercycle variation between AMH [ICC, 0.89 (95% CI, 0.84–0.94)] and AFC [ICC, 0.71 (95% CI, 0.63–0.77)] was 0.18 (95% CI, 0.12–0.27). Age-adjusted ICC for intracycle variation between AMH [ICC, 0.87 (95% CI, 0.82–0.91)] and AFC [ICC, 0.69 (95% CI, 0.46–0.82)] was 0.18 (95% CI, 0.034–0.42).

As with all other ultrasound evaluations, AFC can be affected by the device and the operator, and it can show intra- and interobserver variabilities [24]. However, this is less likely to be an issue in the case of endometriosis since endometriosis evaluation is usually performed by an experienced sonographer using an adequate device.

Last of all, some women may not prefer the transvaginal route due to personal reasons. In these cases, there are two alternative routes. The abdominal route, while convenient for the patient, will provide substantially reduced image quality, especially in the presence of endometriosis and obesity AFC will be significantly misleading, if not totally inadequate. Second, the rectal route can be used. While this may give comparable image quality as the transvaginal route, it may be uncomfortable for the woman, and she might not prefer this route either.

5 Qualitative Aspect of Ovarian Reserve in Women with Endometriosis

The term “quality” of an oocyte should define the potential of that oocyte to generate a healthy live birth. The occurrence of a healthy live birth is dependent on too many factors and, to the best of our knowledge, there is no single marker of “oocyte quality” in this regard. In our opinion, several studies from assisted reproductive technology cycles suggest that endometriosis is unlikely to affect oocyte quality [36]. Blastulation and blastocyst euploidy rates, which can be considered the best surrogates of oocyte quality, are reported to be similar between women with and without endometriosis [37, 38]. Regarding endometriomas, Leone Roberti Maggiore and colleagues reported that oocytes from endometrioma-containing gonads are similarly likely to reach live birth as oocytes from unaffected gonads [39]. Given these findings, we think female age remains as the only reliable marker of “oocyte quality” in women with endometriosis.

6 Conclusion

Ovarian reserve is an ovary's functional potential and is determined by both the quality and the quantity of oocytes it contains. It is shown in multiple studies that ovarian reserve is adversely affected by the presence of endometrioma per se, and possibly even more with endometriosis surgery. Therefore, information on ovarian reserve can be a critical when planning the management of endometriosis. Currently, two markers are found reliable and convenient to assess ovarian reserve, AFC and AMH.

AFC is generally accepted as a reliable, convenient, and quick method for testing ovarian reserve in the general population. It is the only ovarian reserve marker that can provide side-specific information. However, problems due to anatomical distortions and problems in obtaining high-quality images in the presence of an endometrioma, it may underestimate the actual reserve. Thus, its value is limited in women with endometriosis.

On the other hand, AMH is not subject to problems with distorted anatomy and poor image quality with ultrasonography, therefore, it may be a better marker of ovarian reserve in women with endometriosis. Although it lacks side-specific information, most of the time, the total ovarian reserve is relevant when managing a patient. Therefore, AMH seems a more accurate and reliable marker than AFC in women with endometriosis than AFC and should be preferred when assessing the ovarian reserve in this population. When AFC is used, the possibility of underestimation in endometrioma-containing ovaries should be remembered.

Strengths and limitations of AMH and AFC for the assessment of ovarian reserve in the presence of endometriosis are summarized in Table 1.

Table 1 Strengths and limitations of anti-Müllerian hormone and antral follicle count in the presence of endometrioma

	Strengths	Limitations
Anti-Müllerian hormone (AMH)	• Marker with lowest intra- and intercycle variabilities • Provides more reliable comparison of pre- and post-surgery periods • May detect decline earlier than other markers	• Additional cost and visit to the laboratory, result not immediately available • May be affected by handling and storage conditions • Possible discrepancies in results due to different assays, not completely standardized
Antral follicle count (AFC)	• Provides side-specific information • Can be performed on the spot and simultaneously when evaluating endometriosis • No additional cost or waiting time	• Ovarian reserve may be underestimated due to anatomical distortion and difficulties in obtaining high-quality images • Acceptable intra- and intercycle variations but more than those of AMH • Requires appropriate equipment and experienced sonographer, but probably will not be a problem for sonographers competent enough to evaluate endometriosis • Transvaginal route may not be preferred by some women

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Assessment of Tubal Patency in Women with Endometriosis

Fabio Barra, Marco Crosa, Francesco Rosato, Giulio Evangelisti, and Simone Ferrero

1 Introduction

Endometriosis can affect the fallopian tubes in different ways (Fig. 1). Endometriotic implants may be located on the serosal surface of the fallopian tubes. This is a form of peritoneal endometriosis, and these implants, at laparoscopy, may appear as white, red, or black lesions. Alternatively, endometriotic tissue may implant along the mucosal layer of the fallopian tube. Bleeding into these implants may result in the development of hematosalpinx [1].

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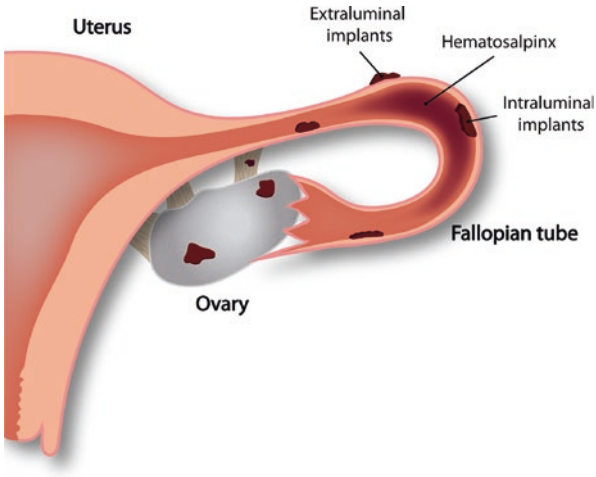

Fig. 1 Schematic representation of fallopian endometriosis

The assessment of tubal patency is similar in women with endometriosis and those with other causes of infertility. Therefore, in women with endometriosis, the patency of the fallopian tubes can be assessed by hysterosalpingography (HSG), hystero-salpingo contrast sonography (HyCoSy), and laparoscopy with transcervical dye instillation. This chapter will describe the techniques used to assess tubal patency in women with endometriosis.

2 Hysterosalpingography

2.1 Technique

HSG is the fluoroscopic evaluation of the uterine cavity and fallopian tubes; it is performed by injecting an iodinated contrast medium through the cervical canal. It should ideally be performed between days 6 and 11 of the menstrual cycle to avoid peak menses and radiating a pregnancy. Neither serum nor urine pregnancy tests reliably exclude pregnancy during the first three weeks following the last menstrual period and are, therefore, not routinely performed.

The contraindications to HSG are contrast allergy, pregnancy, and active pelvic infection. Antibiotic prophylaxis is not routinely performed following HSG. The risk of infection is relatively low and is reported to range between 0.3 and 3.4% [2]. It is advisable to administer antibiotics when there are dilated tubes without spill or spilled contrast within locules because these findings are associated with an increased risk of post-procedural infection. The most used antibiotic regimen is doxycycline 100 mg orally, twice daily for 5 days.

HSG usually causes mild pain related to the placement of the catheter into the uterine cavity and to the uterine distention. Furthermore, the irritation of the peritoneum by the contrast medium can also cause pain. Non-steroidal anti-inflammatory drugs can be administered to treat cramping occurring during the procedure.

Iodinated contrast is injected into the uterine cavity and the fallopian tubes. Nonionic, water-contrast is preferred because the risk of allergic reaction is less than with ionic contrast. The amount of contrast injected is variable; it usually ranges between 10 and 30 ml. When HSG is performed in patients with a history of mild contrast allergy, a premedication with a steroid (oral prednisone 50 mg at 13, 7, and 1 h before contrast administration) and antihistamine (oral, intramuscular or intravenous diphenhydramine 50 mg 1 h before contrast administration) is advisable.

2.2 Hysterosalpingography in Endometriosis

An American retrospective study reviewed the HSG and laparoscopy performed in 50 infertile women (35 with endometriosis at surgery) [3]. Criteria for tubal abnormality included incomplete or absent filling and ampullary dilatation or convolution. Laparoscopy showed that only 10 (15%) of 68 tubes were affected by endometriosis. The sensitivity of HSG was only 40% (4 of 10), with a poor positive predictive value (PPV) of 21% (4 of 19) due to a large number of false-positive diagnoses. The study showed that endometriosis, regardless of its severity, rarely causes radiographic abnormalities in HSG. A Portuguese retrospective study investigated the role of HSG in the diagnosis of endometriosis [4]. The study included 30 patients submitted to laparoscopy (18 with endometriosis). Compared to laparoscopy, HSG revealed a sensitivity of 55.5%, a specificity of 75%, a positive predictive value of 77%, and a negative predictive value of 53% in diagnosing endometriosis. A Polish study compared the assessment of fallopian tube patency during HSG with laparoscopy results in infertile women with endometriosis [5]. Three hundred thirty-one infertile women with endometriosis were included in the study. At HSG, the bilateral tubal patency was observed in 51.7% of the patients, unilateral tubal patency in 36.7%, and bilateral occlusions in 11.6%. During laparoscopy, the bilateral tubal patency was observed in 36.7% of the patients, unilateral patency in 33.3%, and bilateral occlusions in 30.0%. The diagnostic compatibility of these examinations was 49.6% for both fallopian tubes and 34.2% for only one fallopian tube. A prospective Egyptian study assessed the role of HSG in predicting endometriosis [6]. The study included 86 infertile women who underwent laparoscopy; HSG was performed within 3 months before surgery. During the evaluation of HSG, the authors assessed if one tube was higher than the other (by drawing a transverse line over the uterine fundus) and if there was a coiling of one or both tubes so that C or S shapes could be identified. Around 41.9% of the patients had endometriosis at laparoscopy. The authors observed that two signs were helpful in the prediction of endometriosis. A higher left tube had a sensitivity of 86.1%, a specificity of 82.0%, a positive predictive value (PPV) of 77.5%, and a negative predictive value (NPV) of 89.1% in the prediction of endometriosis. The presence of bilateral C or S shapes of the tube had a sensitivity of 75.0%, a specificity of 66.0%, a PPV of 61.4%, and an NPV of 78.6% in predicting endometriosis. The authors explained the higher level of the left tube by the higher prevalence of endometriosis on the left

side of the pelvis. Endometriosis can cause scarring and shortening of the mesosalpinx, especially in the distal part of the tube. This shortening could lead to limited mobility of the tubes; thus, when filmed during HSG while the patient is lying supine, it appears higher than the fundus. In addition, this shortening makes the mesosalpinx so tight that the tube becomes coiled to fit into its narrow peritoneal covering, which can give the C or S-shaped coiling.

3 Hystero-Salpingo Contrast Sonography

HyCoSy involves a transvaginal ultrasound investigation of the fallopian tubes both before and after the injection of an echo-enhancing agent into the tubes via the uterine cavity.

HyCoSy does not require premedication because the procedure generally causes little discomfort. The patient is required to empty the bladder before the examination. After the patient is placed on the ultrasound table in the lithotomy position, a speculum is placed in the vagina, and the uterine cervix is cleansed with an antiseptic solution. A catheter is placed in the endocervical canal; a tenaculum is rarely required. The speculum is then removed, and the vaginal transducer of the ultrasound machine is introduced either anterior (in the case of the anteverted uterus) or posterior (in the case of the retroverted uterus) to the uterine cannula. The contrast is slowly injected through the cannula to distend the endometrial cavity; the uterus is scanned longitudinally during installation. The transducer is then turned 90° to scan the uterus transversely. Saline solution is anechoic and, therefore, cannot optimally delineate the fallopian tubes. However, it can be helpful to confirm that at least one tube is patent by visualization of the saline in the pouch of Douglas after infusion. Better contrasts to depict the fallopian tubes generate hyperechoic (positive) images of medium flow through the tubes to the abdominal cavity [7]. Air (hyperechoic) may be added to the saline solution to improve the visualization of the fallopian tubes. The main limitation of the air/saline HyCosy is that moving air bubbles and saline does not generate a clear and steady visualization of the fallopian tubes. Therefore, the performance of this technique in evaluating tubal patency largely depends on the expertise of the sonographer. The addition of color Doppler sonography, using saline as the contrast agent, increases the accuracy of assessing fallopian tubes. Several commercial hyperechoic contrast agents have been proposed to improve the performance of HyCoSy in tubal patency testing. The Exem Foam (hydroxyethyl cellulose, glycerol, and purified water; Gynaecologie, Delft, the Netherlands) is one such commercial agent. The HyCoSy method using foam is called hystero-salpingo-foam sonography (HyFoSy).

3.1 Hystero-Salpingo Contrast Sonography in Endometriosis

A retrospective study investigated the accuracy of HyCoSy in assessing tubal patency in infertile women with endometriosis [8]. One hundred twenty-six patients underwent HyCoSy and a laparoscopy (with dye test) within 6 months of the HyCoSy. The tubal patency was assessed by HyCoSy, and the findings were compared with the results of laparoscopy, which was considered the gold standard for the assessment of tubal patency. Forty-two patients (33.3%) had a diagnosis of pelvic endometriosis, and 84 (66.7%) had no endometriosis. In women with endometriosis, HyCoSy had a sensitivity, specificity, PPV, NPV, LR+, and LR- of 85%, 93%, 81%, 94%, 12.6, and 0.15, respectively. In patients without endometriosis, HyCoSy had a sensitivity, specificity, PPV, NPV, LR+, and LR- of 85%, 93%, 71%, 97%, 13.2, and 0.15, respectively. The diagnostic accuracy of HyCoSy was 91% in patients with endometriosis and 92% in patients without endometriosis.

A potential limitation of HyCoSy in women with endometriosis is that loculated spills can be difficult to identify in the setting of adhesions and endometriosis [9]. This is relevant because approximately 30% of women with endometriosis have tubal involvement at laparoscopy [1]. An Italian study investigated the performance of HyCoSy in assessing tubal patency in women with endometriosis [10]. The study was based on a retrospective analysis of a prospectively collected database. Two hundred seventy-three women were included in the study. HyCoSy showed bilateral tubal patency in 197 patients, unilateral tubal patency in 24 patients, and bilateral tubal occlusion in 52 patients. In 18% of the patients, adhesiolysis was required during laparoscopy before assessing tubal patency. The mean time to perform HyCoSy was 15.6 min. SSG had a sensitivity of 93.2% and a specificity of 99.2% for diagnosing tubal patency compared with laparoscopy. There was no significant difference in the performance of HyCoSy and laparoscopy in assessing tubal patency. These findings were confirmed when the analysis was restricted to patients with ovarian endometriomas. One hundred fifty-six patients did not complain of any pain during HyCoSy; mild pain (VAS score < 3) was reported by 62 patients; 38 patients complained of moderate pain (VAS scores 4-7), and 17 patients had severe pain (VAS > 7).

An advantage of HyCoSy in women with endometriosis is that patients may benefit from a one-step comprehensive ultrasonographic infertility evaluation assessing at the same time the presence of endometriosis [11] (Figs. 2, 3 and 4), the uterus and the uterine cavity, and tubal patency.

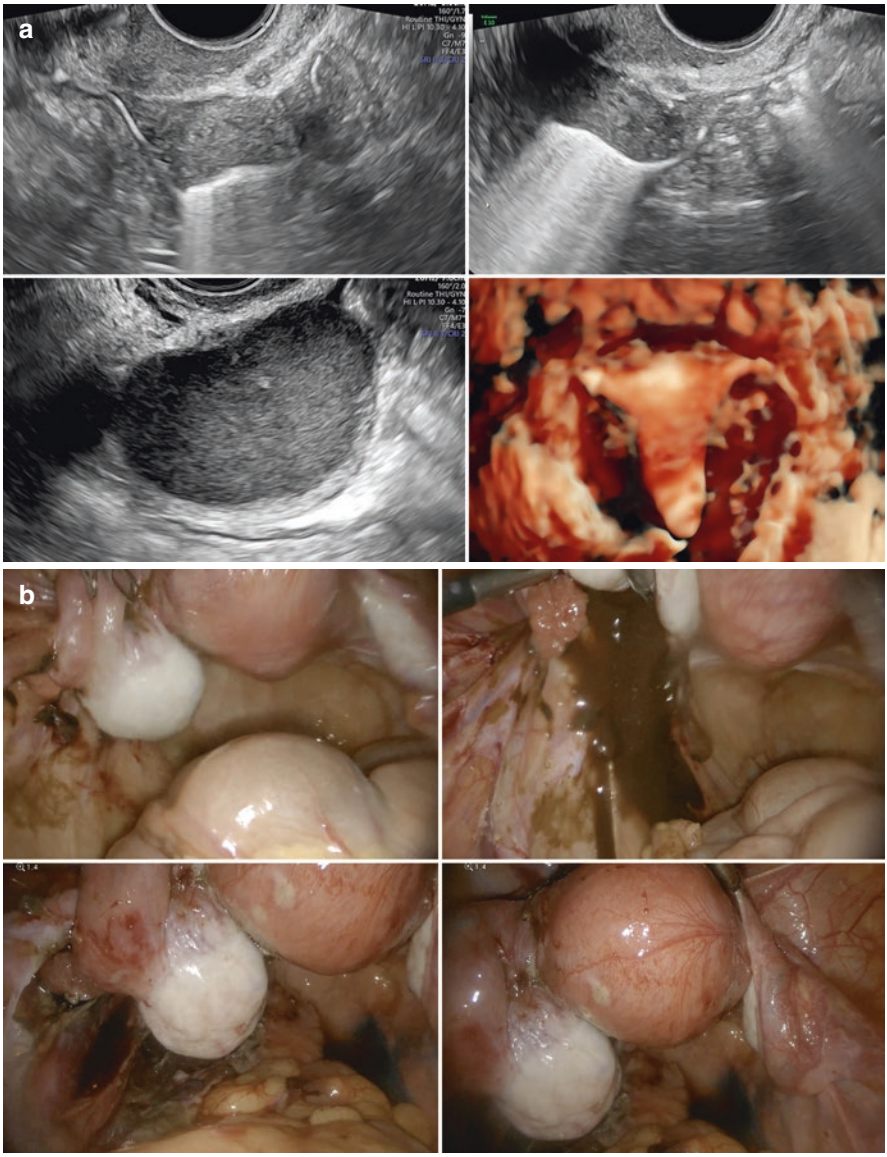

Fig. 2 Bilateral tubal patency demonstrated by HyCoSy and tubal patency test performed during laparoscopy. (**a**) HyCoSy in patients with left ovarian endometrioma (a largest diameter of 4.8 cm) demonstrating bilateral tubal patency. The exam is performed using the ExEm foam medium. Top left: right tube. Top left: left tube. Bottom left: endometrioma. Bottom right: 3D HD-live reconstruction. (**b**) In the same patient, laparoscopy confirms the presence of the left ovarian endometrioma and bilateral tubal patency

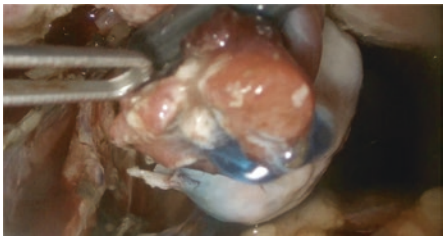

Fig. 2 (continued)

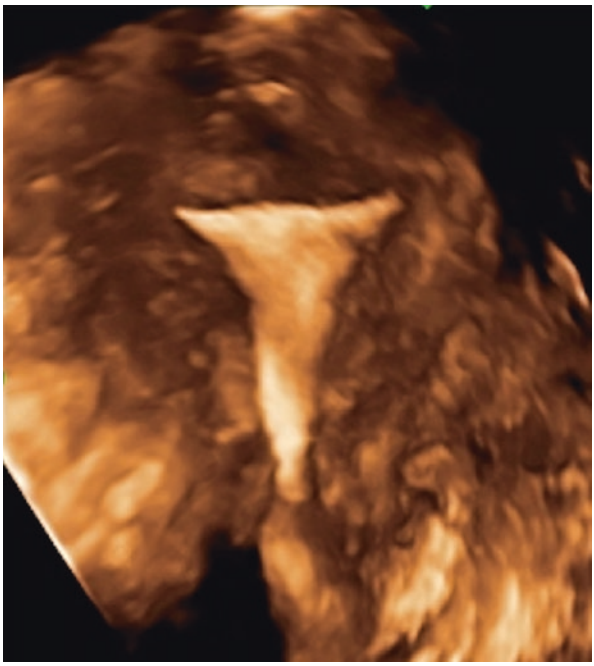

Fig. 3 HyCoSy demonstrates bilateral tubal occlusion

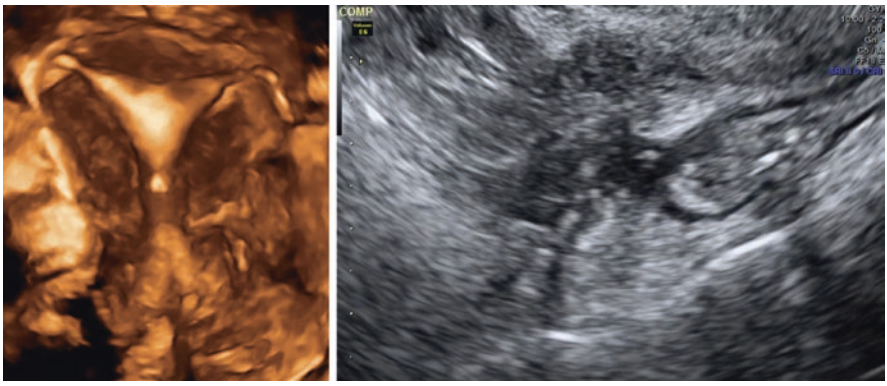

Fig. 4 HyCoSy demonstrates bilateral tubal patency in a patient with rectosigmoid endometriosis

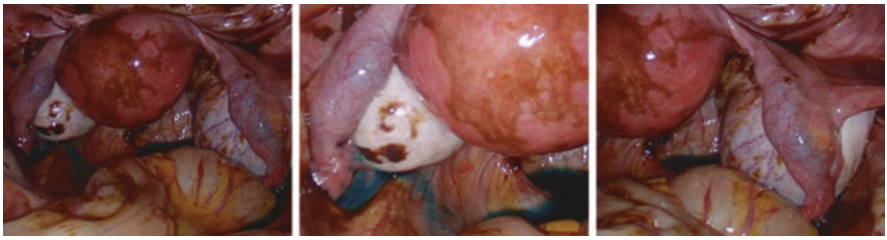

Fig. 5 Tubal patency test performed during laparoscopy for endometriosis demonstrates bilateral tubal patency

4 Laparoscopic Chromopertubation

Laparoscopic chromopertubation is performed by instilling a dilute solution of methylene blue dye into the uterine cavity and the fallopian tubes (typically through a uterine manipulator connected to a 50 mL syringe). Spillage of the dye from each tube is noted as a confirmation of tubal patency.

Ideally, chromopertubation should always be performed in women of reproductive age undergoing laparoscopy to treat endometriosis (Fig. 5). It is advisable to perform the chromopertubation specifically after the endometriosis treatment because the dye's spillage in the pelvic cavity may interfere with surgery; furthermore, the methylene blue dye may be used to check the integrity of the intestinal lumen after the excision of deep endometriosis.

A recent retrospective study showed that infertile women with endometriosis undergoing laparoscopic chromopertubation have a 34.3% risk of having a tubal occlusion; the bilateral tubal occlusion was observed in 9.5% of the patients [12]. These findings were confirmed by another retrospective study, including infertile women with endometriosis who underwent laparoscopic chromopertubation. Around 142/550 (25.8%) tubes were occluded in patients with endometriosis. Any kind of tubal occlusion (either unilateral or bilateral) was found more often in 35.3% of infertile women with endometriosis [13].

Laparoscopic chromopertubation has some advantages and disadvantages compared with other techniques. Laparoscopy allows the direct visualization of the fallopian tubes and enables the diagnosis of endometriosis, even in patients with superficial disease that may not be detected by transvaginal ultrasonography. Furthermore, laparoscopy allows therapeutic interventions during tubal patency evaluation (such as lysis of adhesions, removal, ablation, drainage of ovarian cysts, and excision of endometriotic lesions). The obvious disadvantages of laparoscopy are that it requires general anesthesia, has a higher risk of complications compared with less invasive procedures, causes more significant patient discomfort, and has higher costs [9].

5 Conclusion

When endometriosis involves the fallopian tubes, abnormal findings on HSG are often nonspecific and include tubal dilatation, blockage, convolution, and peritubal loculations [3, 14, 15]. For example, ampullary dilatation and hydrosalpinx usually indicate tubal or peritubal disease, but they may result from endometriosis or adhesions caused by endometriosis. The fallopian tubes may also be displaced secondary to adjected ovarian endometriosis, which can be accurately diagnosed by transvaginal ultrasonography. HSG allows to assess tubal patency in patients with endometriosis, but it does not diagnose endometriosis or assess its severity [4, 6]. Because of its invasive nature, laparoscopy should be reserved for those women needing surgery (such as strong suspicion of endometriosis, pelvic/adnexal adhesions, or tubal disease requiring treatment) and not only for the evaluation of the fallopian tubes. Given this perspective, HyCoSy should be the first-line investigation of tubal patency in women with endometriosis. It allows a one-step evolution of tubal patency, uterus (myometrium and endometrium), deep endometriosis, and ovarian endometrioma.

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Role of Fallopian Tubes in Endometriosis-Related Infertility

Simone Ferrero, Michele Paudice, Umberto Leone Roberti Maggiore, Francesco Rosato, and Ertan Saridogan

1 Introduction

The fallopian tubes play a crucial role in gamete transport, fertilization, and early embryo development. Gamete transportation is affected by contractions of the tubal musculature, ciliary activity, and the flow of tubal secretions [1]. Endometriosis may affect the fallopian tubes and cause infertility by several mechanisms, including distortion of pelvic anatomy and adhesions, infiltration of the fallopian tube wall, dilatation and/or blockage of the fallopian tube, and impairment of fallopian tube function without anatomical damage. This chapter will describe how the fallopian tubes may contribute to endometriosis-related infertility.

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2 Tubal Endometriosis

Endometriosis may directly affect the fallopian tubes (Fig. 1). Tubal endometriosis is a heterogeneous disease. Most commonly, endometriotic implants infiltrate only the tubal serosa or subserosa; this condition typically coexists with other pelvic lesions and should be considered peritoneal endometriosis [2] (Figs. 2 and 3). A pathologic study showed that lesions confined to the mucosa might differ from those infiltrating the muscular or serosal layers. Serosal lesions typically show an inflammatory reaction, hemosiderin deposition, and fresh bleeding. The lesions in the myosalpinx are usually multifocal; in these lesions, the endometriotic glands are typically dilated and atrophic, mainly surrounded by an inflammatory background. Finally, mucosal lesions show little or no inflammatory reaction. The expression of Cox-2, NF-kB, and VEGF of the ectopic endometrial stromal cells tends to increase in the progression from the inner to the outer part of the tubes. The expression of NF-kB and VEGF correlates with the microscopic findings of inflammation [3]. Ectopic endometrial tissue can grow into the tubal lumen; this form is called "intraluminal endometriosis" [4]. In some cases, the ectopic endometrial tissue may give rise to intraluminal polyps [5]. Repeated cycles of hemorrhage in superficial implants or intraluminal endometriosis may cause fibrosis, which can lead to retraction and enlargement of the tube, promoting the formation of hematosalpinx (Fig. 4). Rarely, fallopian tubes containing chocolate-like fluid may undergo torsion [6].

The incidence of tubal endometriosis has been reported to range between 0.29% and 14.48% [7–10]. The prevalence of tubal endometriosis may increase with the severity of the disease. In women with moderate to severe endometriosis, the prevalence of tubal endometriosis is as high as 60% [11]. Qi et al. analyzed the histologic

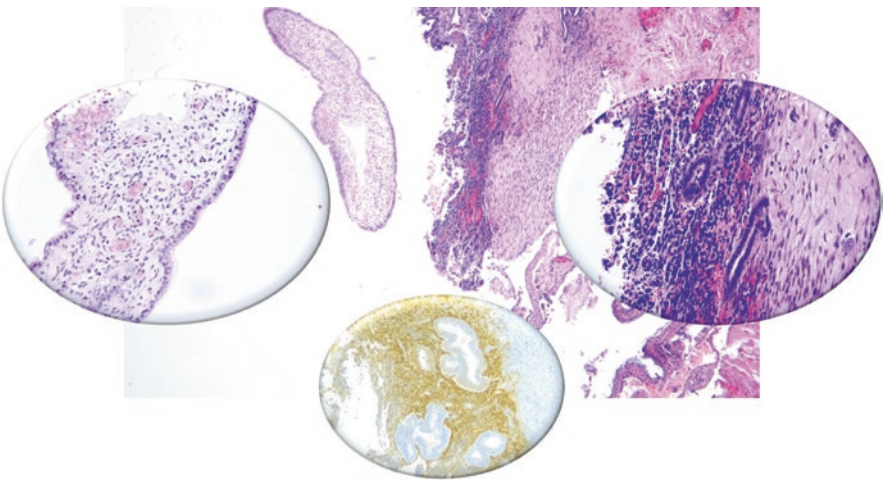

Fig. 1 Hematoxylin and eosin-staining demonstrates tubal endometriosis; immunohistochemical staining with CD10 confirms the diagnosis of endometriosis

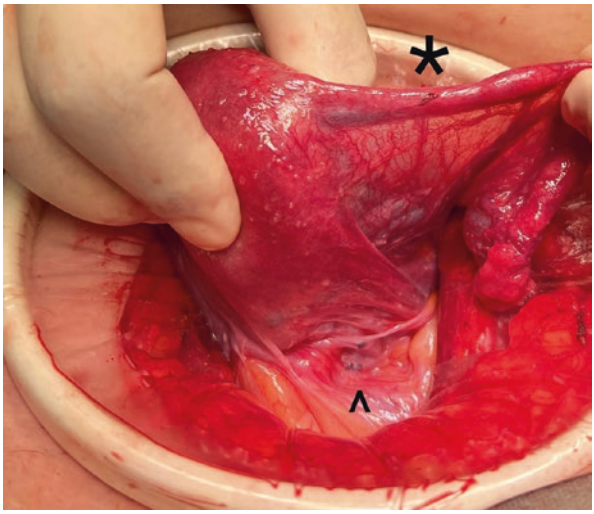

Fig. 2 Superficial tubal endometriosis diagnosed during laparotomic hysterectomy

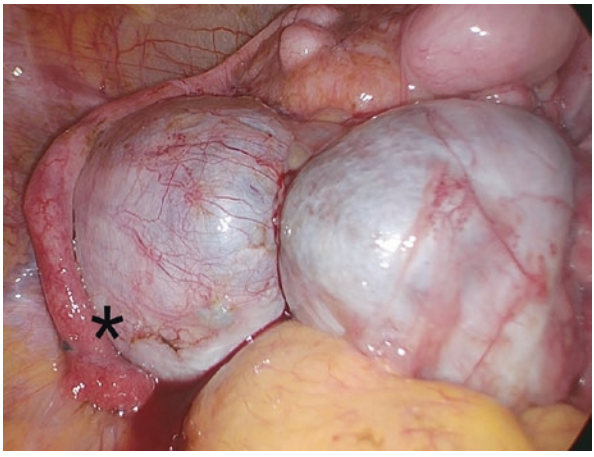

Fig. 3 Superficial tubal endometriosis diagnosed during laparoscopic excision of endometriosis

specimens obtained from 198 patients with histological diagnoses of endometriosis who underwent salpingectomy. The incidence of fallopian tube endometriosis was 37.4%, and 44% in patients with severe disease [9]. A retrospective cross-sectional study from the United States investigated the incidence of fallopian tube endometriosis in patients undergoing laparoscopy with a preoperative diagnosis of endometriosis, pelvic pain, infertility, and cystic adnexal mass. The incidence of macroscopic tubal endometriosis observed by direct laparoscopic visualization was 11% in patients who did not undergo salpingectomy and 12% in patients who underwent salpingectomy. However, the diagnosis of microscopic tubal endometriosis revealed by the histological examination was 42.5% after salpingectomy [12]. The clinical relevance of microscopic tubal endometriosis is still unclear [13]. It is unknown

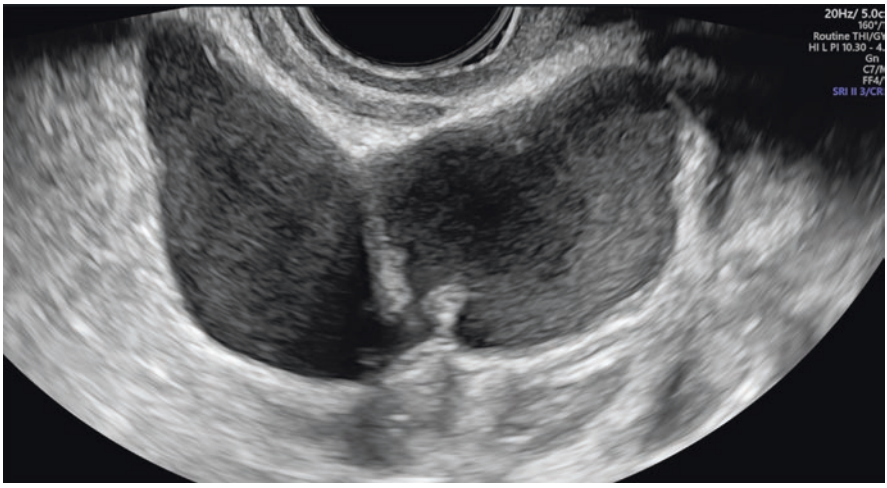

Fig. 4 Ultrasonographic appearance of hematosalpinx

whether microscopic endometriosis contributes to pain symptoms and abnormal tubal function or is only an epiphenomenon with no clinical relevance.

Contradictory data have been reported on the lateral distribution of tubal endometriosis. Some studies reported a higher prevalence of left-sided tubal endometriosis than right-sided disease [14–16]. One study found a prevalence of tubal endometriosis of 58.5% in the right compared with 41.5% in the left [7].

3 Distortion of Pelvic Anatomy

Endometriosis may distort the pelvic anatomy and cause adhesions between the fallopian tubes, the uterus, ovaries, the pelvic sidewall, and the rectosigmoid on the left. These adhesions may entrap the tubes [17], thus impairing fertility due to the inhibition of ovum pickup or disturbed gamete/embryo transport in the fallopian tube. Furthermore, obstruction of the fimbrial end of the fallopian tube can lead to fluid stagnation in the lumen, forming hydrosalpinx. In addition, menstrual blood may become stagnant in the tubes because of retrograde menstruation, resulting in hematosalpinx. Haemato- or hydrosalpinges may also develop without distal tubal blockage due to fibrosis of the tubal wall and/or increased tubal secretions secondary to endometriosis-induced inflammation. In animal models of endometriosis, pelvic adhesions appear to contribute to the observed decreased fecundity noted in animals with advanced endometriosis [18]. A retrospective cohort study including 275 infertile women with endometriosis showed that the rate of fallopian tube occlusion increases significantly with the rASRM stage of endometriosis [19]. Furthermore, patients who previously underwent surgical treatment for

endometriosis may also have significant postoperative adhesions, which may be more severe if previous surgery was performed by laparotomy.

4 Influence of Endometriosis on Tubal Function

Endometriosis may also affect the fallopian tube ciliary beat frequency and muscle contractility.

A prospective observational study investigated uterotubal transport employing hysterosalpingoscintigraphy (HSSG) in women with and without endometriosis. The study included 56 infertile women with laparoscopically proven endometriosis and patent fallopian tubes and 52 control women with partners suffering from male factor infertility. HSSG was performed by administering 20 MBq technetium-99-marked microalbumin aggregates (a size of 5–20 Am, which imitates the size of sperm) diluted in 2 mL saline solution into the posterior vaginal fornix of the recumbent patient. Scans with a gamma camera were taken immediately after application and in various intervals of up to 30 minutes. For quantitative evaluation of HSSG, "regions of interest" were determined in both fallopian tubes to visualize the radioactivity concentration. The study demonstrated that endometriosis was significantly associated with reduced physiologic uterotubal transport capacity, either in the form of lack of transport or pathological transport towards the opposite side of the dominant follicle, compared with controls with male factor infertility. The data also showed diminished pregnancy rates even in women with normozoospermic partners [20]. The same authors studied utero-tubal transport in patients with adenomyosis. Patients with peritoneal endometriosis were divided into three study groups: women with evidence of adenomyosis at magnetic resonance imaging (MRI), women with at least one adenomyotic focus, and women with diffuse adenomyosis. The study showed that women with endometriosis and early adenomyosis start to develop hyperperistalsis. In contrast, dysperistalsis (reflected by a transport failure) prevails in patients with diffuse adenomyosis [21].

Fallopian tube motility is thought to be controlled by hormones and nerves. The isthmus of the fallopian tubes has a sphincter-like function, and it is more innervated than other segments of the fallopian tubes. Early embryos or unfertilized eggs seem to stay in the fallopian tube lumen for up to 80 hours due to increased isthmic tone and dense and tenacious mucus in the isthmus (mucus plug). This is thought to allow early embryonic development and the arrival of the embryo in the uterine cavity at the optimal time for implantation [22]. A study investigated nerve fiber density in the isthmus of the fallopian tubes of women with and without endometriosis. The tissues were immunochemically stained with protein gene product (PGP) 9.5, substance P (SP), neuropeptide Y (NPY), and vasoactive intestinal peptide (VIP). Women with endometriosis had a significantly decreased density of nerve fibers stained with PGP9.5, VIP, and NPY in the isthmus of the fallopian tubes compared with women without endometriosis. Furthermore, in women with endometriosis, reduced nerve fibers stained with PGP9.5 and SP in the serosal layer, NPY in the

muscular and mucosal layers, and VIP in the mucosal layer of the oviduct isthmus were all associated with the severity of the disease [23].

The interaction between spermatozoa and the epithelium of the isthmic region of the uterine tube is considered an essential part of the mechanisms of sperm transport to the site of fertilization and in preparing them for fertilization. A study investigated the sperm-binding characteristics of the epithelium from the uterine tubes of three groups of women: patients with pelvic endometriosis (not involving the uterine tubes), patients with endometriosis treated with gonadotropin-releasing hormone analogs (GnRHa), and controls. The authors observed that the sperm binding to the tubal epithelium might be increased in women with endometriosis, reducing the number of spermatozoa for fertilization; in fact, there were significantly more spermatozoa bound per unit area to the ampullary epithelium of the uterine tubes taken from women with a previous diagnosis of endometriosis. This effect was not seen in women on GnRHa, suggesting that suppression of endometriosis eliminated this detrimental effect [24].

It is well known that the peritoneal fluid of women with endometriosis contains higher levels of proinflammatory cytokines. Peritoneal fluid enters the fallopian tube and, because of the direct contact with the tubal epithelium, it can affect tubal transport. Ciliary activity is pivotal in gamete and embryo transfer in the fallopian tube. A study investigated the effect of peritoneal fluid from women with endometriosis on ciliary beat frequency in vitro using a well-established technique based on the changes in light intensity using contrast enhancement [25]. The peritoneal fluid was obtained at laparoscopy from six infertile women with endometriosis and six fertile controls with no evidence of endometriosis undergoing tubal sterilization. Normal fallopian tubes were obtained from 17 women undergoing hysterectomy because of uterine fibroids. At 24 hours of incubation with peritoneal fluid, the ciliary beat frequency was significantly lower in the incubations with peritoneal fluid from women with endometriosis than in controls. A further laboratory-based study demonstrated that interleukin-6 (IL-6) inhibits the ciliary action of the human fallopian tube in vitro, suggesting that IL-6 may be one of the mediators that reduce ciliary beat frequency in women with endometriosis [26].

A study from China investigated the ciliary beat frequency and muscular contractions in the fallopian tubes of women with endometriosis and adenomyosis [11]. Fallopian tube specimens were obtained from 20 women with uterine fibroids (control group), 20 women with adenomyosis without pelvic endometriosis, and 35 women with pelvic endometriosis (11 with ASRM stage III and 24 with ASRM stage IV) without adenomyosis (according to preoperative MRI). Furthermore, according to the histopathological findings, 24 of the 35 women with endometriosis had lesions involving the fallopian tubes (tubal endometriosis subgroup); the remaining 11 patients were included in the non-tubal endometriosis subgroup. The ciliary beat frequencies of the ampulla and the isthmus in patients with endometriosis were significantly lower than those of controls and patients with adenomyosis. Furthermore, ciliary beat frequencies of the ampulla and isthmus in tubal endometriosis cases were considerably lower than those of the non-tubal endometriosis and control subgroups. In both the ampulla and the isthmus segment, the percentages of

ciliated cells in patients with endometriosis were significantly lower than those of adenomyosis and control patients. In addition, the tubal endometriosis subgroup showed considerably lower rates of ciliated cells than the control and non-tubal endometriosis subgroups. Amplitude-to-weight ratios of longitudinal muscular contractility in patients with endometriosis were significantly lower than control values; patients with tubal endometriosis showed substantially lower values than controls and those with non-tubal endometriosis. Contraction frequencies in patients with endometriosis were considerably lower than those of control and patients with adenomyosis in both longitudinal and circular muscles; patients with tubal endometriosis showed significantly lower values than controls and patients with non-tubal endometriosis.

Another study from China investigated the miRNA expression profiles in tubal endometriosis by microarray analysis (four patients with tubal endometriosis and five controls). The authors identified 17 significantly differentially expressed miRNAs (4 upregulated and 13 downregulated). In addition, 4343 potential miRNA-target genes involved in tubal endometriosis were identified (fold change >1.5). Most identified genes were involved in the mTOR signaling pathway, SNARE interactions in vesicular transport, and endocytosis. Functional analysis showed that the mTOR pathway was connected closely to tubal endometriosis. These changes in the signal pathways may be involved in the pathogenesis of endometriosis-associated infertility [10].

In a subsequent study, the same authors tried identifying potential hub mRNAs/proteins of tubal endometriosis through integrated transcriptomic and proteomic analyses in four women with tubal endometriosis and four controls. The authors tried to identify significant pathways, cellular functions, and interaction networks involved in the initiation and progression of tubal endometriosis. The study used human fallopian tube epithelium and tubal fluid samples from patients with and without tubal endometriosis. Tubal epithelial cells were analyzed using microarray, and the tubal fluid was analyzed using quantitative label-free LC-MS/MS. The authors identified differentially expressed genes (DEGs) and differentially expressed proteins (DEPs) and determined common mRNAs/proteins. They observed 35 commonly deregulated mRNAs/ proteins, and ingenuity pathway analysis indicated that cellular movement, inflammatory response, and immune cell trafficking were significantly activated during the pathogenesis of tubal endometriosis. The authors also identified acute phase response signaling pathway activation as a unique pathogenesis signature of tubal endometriosis [27].

5 Conclusion

Tubal endometriosis lacking specific clinical symptoms and routine examinations (such as transvaginal ultrasonography and MRI) do not allow the diagnosis of tubal endometriosis in most patients unless dilatation of the tubal lumen occurs, resulting in hydro- or hematosalpinges [28, 29]. Up to 30% of women with endometriosis

exhibit some form of tubal involvement. In addition, in some patients with endometriosis, the visualization of the fallopian tube during laparoscopy may not be enough for accurate diagnosis of tubal endometriosis [13] because microscopic tubal endometriosis may be present in macroscopically normal fallopian tubes in up to 42–44% of patients [12]. Anatomical causes of tubal dysfunction in endometriosis may be tubal blockage, adhesions, and hydrosalpinx. These conditions can coexist and are often interlinked; therefore, it is difficult to distinguish the exact contribution of each cause of abnormal tubal function. In addition, fallopian tubes may have an abnormal function in women with endometriosis, such as altered ciliary activity and peristalsis and abnormal sperm binding to tubal epithelium.

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Role of Ultrasonography in the Diagnosis of Endometriosis in Infertile Women: Ovarian Endometrioma, Deep Endometriosis, and Superficial Endometriosis

Rodrigo Manieri Rocha, Mathew Leonardi, and George Condous

Abbreviations

TVS	Transvaginal ultrasound
MRI	Magnetic resonance imaging
IDEA	International Deep Endometriosis Analysis
DE	Deep endometriosis
MUSA	Morphological uterus sonographic assessment
IOTA	International Ovarian Tumour Analysis
O-RADS	Ovarian-Adnexal Reporting & Data System
ADNEX	Assessment of Different NEoplasias in the adnexa
OE	Ovarian endometriomas
OR	Odds ratio

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CI	Confidence interval
HyCoSy	Hysterosalpingo-contrast-sonography
LDT	Laparoscopic chromopertubation dye test
HSG	Hysterosalpingography
IVF	In vitro fertilization
SST	Site-specific tenderness
SE	Superficial endometriosis
POD	Pouch of Douglas
PVF	Posterior vaginal fornix
RVS	Rectovaginal septum
SPG	Saline-infusion sonoPODography

1 Introduction

The association between endometriosis and infertility has been suspected since Hippocratic observations [1]. Today, it is a well-established concept with wide acceptance within the scientific community [2, 3]. Apart from obvious occurrences such as bilateral tubal obstruction due to anatomical distortions, a growing body of evidence brings us closer to understanding the relationship between these two conditions [4]; however, the precise mechanisms that link endometriosis to infertility remain largely unknown [5, 6].

Regarding the diagnosis, in general, the transvaginal ultrasound scan (TVS) is paramount in women's health, especially in those experiencing infertility. It can diagnose a wide range of pathologies that could impair conception, from endometriosis to congenital uterine abnormalities, myometrial and endometrial diseases, and adnexal conditions [7]. Even with the possibility of a clear and reliable identification of endometriosis via ultrasound, the gold standard in confirming the presence of the disease has historically been histopathology confirmation of ectopic endometrial-like structures after a surgical biopsy [8]. Of course, performing laparoscopic surgery to obtain a diagnose is unappealing in this scenario and involves the risks of all invasive procedures. As such, there is a movement toward the non-invasive diagnosis of endometriosis, opposing the necessity of laparoscopy with biopsy for the final confirmation of the presence of disease. TVS, added to the clinical suspicion of endometriosis, is becoming the first choice in the investigation workup [9]. Although magnetic resonance imaging (MRI) could be included in the diagnostic arsenal [10], some characteristics make its utilisation with a less favourable performance, especially related to the availability of highly trained specialists that could bring consistent diagnostic accuracy [11].

2 Description of the Ultrasound Technique: International Deep Endometriosis Analysis(IDEA)

In 2016, the IDEA group proposed a standardised protocol for ultrasound diagnosis of endometriosis to create reproducible results regarding clinical performance and report consistency [12]. The protocol involved the subdivision of the ultrasound examination into four steps that can be performed in any order, guiding the meticulous pelvis examination to search for endometriosis within a dedicated structured examination, referred to here as deep endometriosis (DE) TVS (Table 1).

2.1 Step One—Uterus

Usually, the uterus is the first structure visualised, and its orientation to the pelvis (anteverted, axial, or retroverted) should be noted. It is possible then to identify the presence of uterine conditions that may affect fertility, including adenomyosis, fibroids, and intracavitary pathologies. The coexistence of one of those pathologies with endometriosis is common and cannot be overlooked [13]. Particular attention should be given to the uterus that is anteverted and retroflexed (cervix anteverted and fundus retroflexed, also known as the 'question mark sign') [14] (Fig. 1) due to its association with significant pelvic adhesions and DE [15]. It is also essential to describe uterine characteristics potentially related to adenomyosis using the descriptors from the Morphological Uterus Sonographic Assessment (MUSA) group's consensus opinion. It includes, among other details, the serosal contour of the uterus, the symmetry of uterine walls, and the characteristics of the junctional zone as the most important features related to pathologies [16].

Table 1 Systematic approach for the deep endometriosis dynamic ultrasound of the pelvis according to the IDEA consensus[a]

Routine evaluation of uterus and adnexa (+ sonographic signs of adenomyosis/ presence or absence of endometrioma)	First step
Evaluation of transvaginal sonographic 'soft markers' (i.e. site-specific tenderness and ovarian mobility)	Second step
Assessment of status of POD using real-time ultrasound-based 'sliding sign'	Third step
Assessment for DIE nodules in anterior and posterior compartments	Fourth step

[a]Guerriero S, Condous G, van den Bosch T, Valentin L, Leone FPG, Van Schoubroeck D, et al. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. Ultrasound Obstet Gynecol. 2016;48(3):318–32

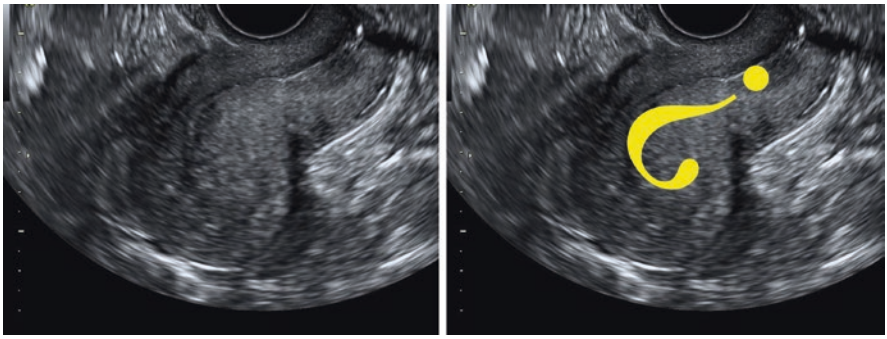

Fig. 1 Uterine orientation: uterus anteverted-retrofexed. The 'question-mark' sign

2.2 Step Two—Adnexa

Second, the adnexa is evaluated. Ovarian measures should be taken in three orthogonal planes (length is obtained in the midsagittal plane, thickness in the anteroposterior plane, and transverse diameter in the transverse plane). Ovarian masses, regardless of whether they are thought to be related to an infertility workup, can be associated with malignancies and should be described as per the consensus of the International Ovarian Tumour Analysis (IOTA) group consensus opinion, with consideration for the American College of Radiologists Ovarian-Adnexal Reporting & Data System (O-RADS) [17]. If an ovarian mass is identified and it cannot be adequately described using IOTA Easy Descriptors [18], one can apply the 'Assessment of Different NEoplasias in the adneXa' (ADNEX) risk assessment tool to adequately stratify the risks for potential malignancies and provide the necessary guidance for management. The ADNEX tool can be found online at http://www.iotagroup.org. The association between benign non-endometriosis ovarian masses and infertility is debatable, as is the surgical treatment, which may be harmful to future fertility [19]. Therefore, the detailed description and assessment of all ovarian masses on ultrasound are paramount.

One of the adnexal masses with a well-known association between its presence and infertility is ovarian endometriomas (OE). The exact mechanisms that yield infertility issues are still largely unknown but can be hypothesised have been related to a great variety of inflammatory substances and genetic impairments, markedly higher than in other benign masses and possibly not only related to mechanical stretching of the ovarian cortex [20, 21]. OE's' prototypical sonography findings are unilocular ovarian cysts with a 'ground-glass' appearance content fluid, with no detectable blood flow (Fig. 2) [22]. More than four locules and papillary structures or solid components are uncommon findings and are more often found in other benign or malignant ovarian masses [23]. It is important to remember that there is a potential higher epidemiological risk of the association between endometriosis and adnexal malignancies (with an adjusted odds ratio (OR) 2.80, 95% confidence

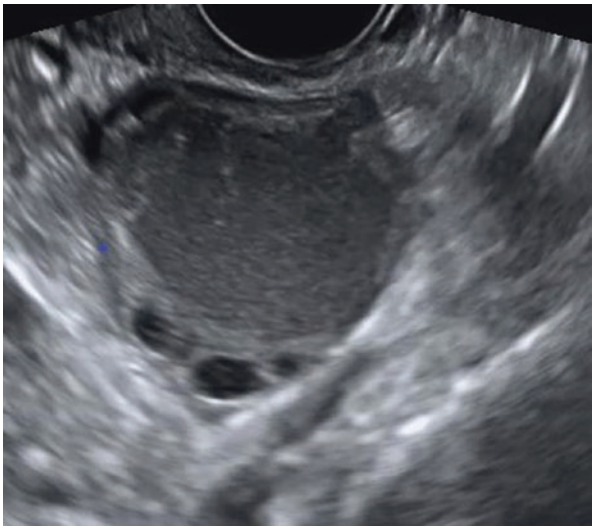

Fig. 2 Typical presentation of an OE: unilocular ovarian cyst, with 'ground glass' appearance content

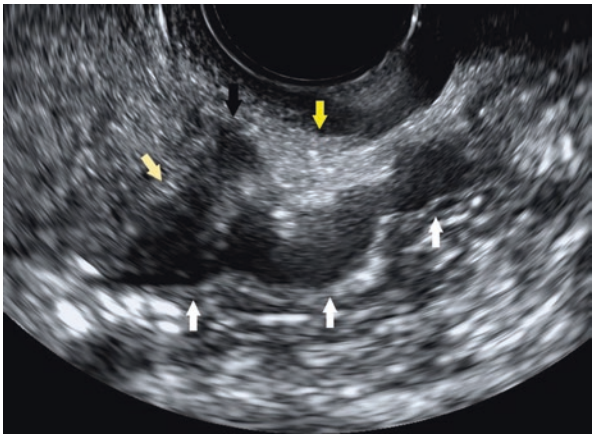

Fig. 3 OEs are markers for DE. The ultrasound image depicts, in a patient with bilateral OEs, the presence of DE in the posterior compartment. White arrows: bowel DE; yellow arrow: left uterosacral ligament (LUSL); black arrow: DE in the LUSL; light yellow arrow: DE in the torus uterinus

interval (CI) 1.84–4.27) [23]. Therefore, the assessment mentioned above for possible ovarian malignancies is mandatory and also apply to endometriomas.

Diagnosing ovarian endometriotic cysts with ultrasound is highly accurate, with 93% sensitivity (95% CI 97–99%) and figuring 96% specificity (95% CI 92–99%) [10], with 97% of interobserver agreement, when considered adnexal alterations [24]. On that account, ultrasound is considered the first-line diagnostic tool for OE [25]. Additionally, the presence of OE could be a marker for DE, given that the risk of the concomitant occurrence of other lesions in the posterior pelvic compartment could be as high as 98%, as well as bowel disease in 57% (Fig. 3) [26].

The detailed description of the number, size, and location (unilateral or bilateral) of OE (and other DE lesions) is an essential step of the meticulous evaluation of the patient with OE for the adequate planning of eventual surgical treatment. However,

the decision for surgery on OE should consider the potential harm to the ovarian reserve, the offer for possible fertility preservation techniques before the surgery, and the team's expertise involved in the procedure, among others [27]. In fact, if fertility is the focus, there is no established management of the OE regarding improvement on cardinal outcomes such as live birth rate [28]. Therefore, precise information, counselling, and informed consent for the potential treatments of people diagnosed with OE should be individualised and tailored to the patient's best interest [29, 30].

Progressing in the evaluation of the adnexa, the uterine tubes are assessed. The normal tubes are not usually visible during TVS; consequently, the presence of hydrosalpinx or haematosalpinx are considered pathological and should be described, not only considering DE as the primary aetiology but also due to the significant detrimental impact of tubal dilatation regarding fertility, possible infections, and neoplasms [31, 32]. Endometriosis implants can be seen on the tubes in 6% of women with endometriosis, and adhesions might be present in 26%. Small endometriosis implants might be challenging to visualise. If adhesions cause obstruction, hydrosalpinx may be seen. Intraluminal signs of bleeding represent haematosalpinx and are nonspecific; ectopic pregnancy, tumours, and torsion should be considered in the differential diagnosis [31]. Additionally, in an infertility setting, the assessment of the tubal patency might be indicated. The use of hysterosalpingo-contrast-sonography (HyCoSy) has become more popular as an alternative to the invasive laparoscopic chromopertubation dye test (LDT) and X-ray-based hysterosalpingography (HSG) for the assessment of tubal patency. Although tolerable and with significantly low complications rates [33], the presence of hydrosalpinx visible on ultrasound configures as a potential contraindication for such procedure if associated with acute adnexal inflammation processes [34]. The identification of tubal dilatations and other abnormalities is indispensable to fertility treatments planning, given the evidence that, in those cases, procedures such as salpingectomy and proximal tubal occlusion can optimise in vitro fertilisation (IVF) results [35].

2.3 Step Two—Soft Markers

The second step of the IDEA approach involves assessing 'soft markers', which are indirect findings that might represent endometriosis. According to IDEA, the two primary soft markers are site-specific tenderness (SST) and ovarian immobility [12]. Those features are evaluated in a dynamic fashion, where gentle pressure with the ultrasound probe is applied to the desired anatomical structure. During this step, it is essential to inform the patient about the probe's movements and request the patient's judgement on the subjective pain in various areas of the pelvis. At present, there is no validated score system related to SST, so we recommend using a dichotomised system of '0' for no pain and '1' for pain as per the IDEA protocol. From a

practical point of view, it is possible to assess SST towards the end of the TVS to avoid a DE scan interruption due to pain [36].

Ovarian immobility on TVS observed evaluating the expected mobilisation of the ovaries against the surrounding pelvic structures was significantly associated with the presence of endometriomas at surgery, with a prevalence of 52.7% for bilateral ovarian fixation compared with 7.3% for normal ovaries [37]. Reid et al. demonstrated that ovarian immobility was also significantly associated with ipsilateral pelvic pain, uterosacral ligaments (USL) and pelvic sidewall superficial endometriosis (SE), OE, posterior compartment DE, and pouch of Douglas (POD) obliteration [38]. Although unilateral ovarian immobility alone might not be associated with increased detection of DE [37], severe adhesions of the ovaries with the posterior compartment (the 'kissing ovaries' sign) [39] are a recognised marker for DE lesions in the pelvis and also the presence of rectosigmoid endometriosis (Fig. 4) [40].

Within an IVF scenario, the thorough evaluation of the relationship of fixed ovaries with the other pelvic organs is a piece of essential information for safety and planning of oocyte retrieval, to reduce the risks of injuries during the procedure and equally important to the arrangements on alternative assessment locations in the case of non-pelvic fixed ovaries [41].

Soft markers are also potentially associated with the detection of superficial endometriosis, and this concept will be discussed later in this chapter.

2.4 Step Three—Sliding Sign

The third step consists of evaluating the POD state for adhesions, another marker of severe endometriosis, using a dynamic test called 'sliding sign' [42]. The method involves the mobilisation of the uterus against the content of the posterior

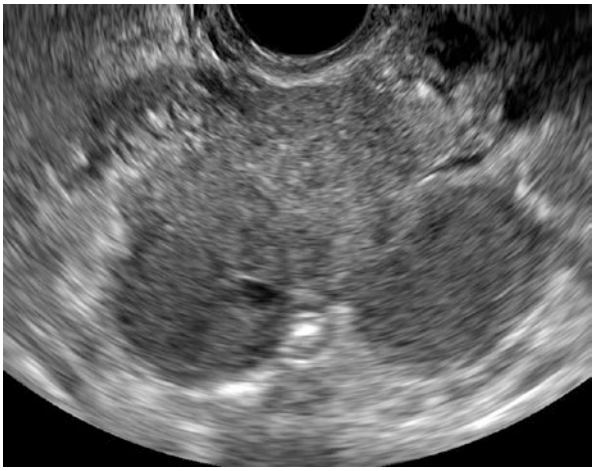

Fig. 4 'Kissing' ovaries. This ultrasound image depicts the right and the left ovaries (both of them containing an endometrioma) fixed medially at the level of the torus uterinus. This condition is a marker for other DE lesions

compartment, particularly the rectum at the level of the retrocervix and the rectosigmoid colon at the level of the uterine fundus. The objective is to observe and document how the uterus moves relative to the structures posterior to it. The technique differs slightly depending on uterine orientation. *On an anteverted uterus:* initially place gentle pressure against the retro-cervix using the transvaginal probe. Observe whether the anterior rectum glides freely across the posterior aspect of the cervix and posterior vaginal wall. Second, place one hand over the lower anterior abdominal wall and ballot the uterus between the palpating hand and the transvaginal probe. Assess whether the anterior bowel glides freely over the posterior aspect of the upper uterine fundus. *On a retroverted uterus:* place gentle pressure against the posterior upper uterine fundus with the transvaginal probe. Observe whether the anterior rectum glides freely across the posterior upper uterine fundus. By its turn, place one hand over the lower anterior abdominal wall and ballot the uterus between the palpating hand and the transvaginal probe. Assess whether the anterior sigmoid glides freely over the anterior lower uterine segment [36]. Fig. 5 shows a negative 'sliding sign'.

In normal anatomy, where the POD is non-obliterated, sliding between the posterior uterine wall and the bowel surface is identified. When the sliding sign is not identified, we classify this as a negative sliding sign, representing POD obliteration. POD obliteration may be a spectrum from focal or partial obliteration to complete obliteration, so we suggest evaluating for subtle areas of negative sliding sign in the context of a generally positive sign (i.e. midline, right or left of the midline, and combinations) [36]. Young et al. showed that the implementation of the sliding sign into the routine pelvic scans with videos acquired by sonographers and interpreted by physicians increased the identification of patients with DE from 2% to 6% ($p = 0.012$), with sensitivity and specificity improving from 36%/94% to 68%/98%, respectively [43]. The application of this technique represents one of the most critical elements of the sonographic evaluation of the pelvis, which is not possible to be implemented in other imaging modalities, such as MRI [10].

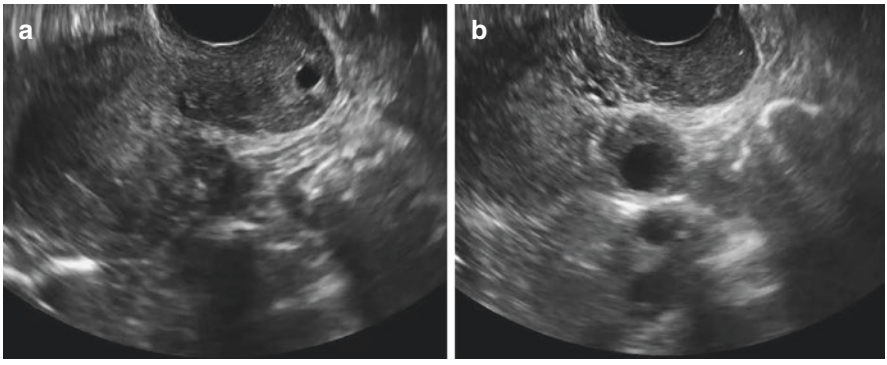

Fig. 5 Sliding sign (SS). The ultrasound pictures depict a negative SS: after gentle pressure with the ultrasound probe, it is possible to observe the adherent bowel and right ovary moving cranially as a single unit (**a**: without pressure; **b**: with pressure)

2.5 Step Four—The Direct Visualisation of Deep Endometriosis

The fourth and last step of the IDEA protocol involves the direct evaluation of DE nodules in the posterior and anterior pelvic compartments. Due to its much higher prevalence, the posterior compartment DE lesions involving the uterosacral ligaments, torus uterinus, and rectum/rectosigmoid colon should be prioritised. The rectovaginal septum (RVS) lesions are far less common, as are nodules in the anterior compartment along the urinary tract [44]. However, all areas should be evaluated. According to IDEA recommendations, all lesions should be documented and measured in three orthogonal planes [12].

In general, the most common sonographic feature of endometriotic nodules is hypoechogenic nodularity, contributing to the distortion of surrounding anatomy, including thickening. Therefore, the knowledge of the typical sonographic aspects of the pelvis is paramount. The posterior compartment's fundamental structures are the uterosacral ligaments (USL) and torus uterinus, the posterior vaginal fornix (PVF), the rectovaginal septum (RVS), and the bowel [12]. The IDEA group developed a schematic view of the pelvis, addressing the compartments' essential landmarks that should be thoroughly evaluated in a DE TVS (Fig. 6).

Normal USLs are hyperechoic band-like structures that can be identified from their medial origin on the torus uterinus and retrocervical area, and it extends lateroposteriorly until it reaches the presacral area (Fig. 7). Leonardi et al. have proposed a detailed approach to the identification of the USLs on DE TVS. Essentially, the ultrasound probe should be placed in the posterior vaginal fornix, maintaining the cervix anteriorly, whilst moving the probe laterally with a degree of rotation (clockwise for the right USL and anti-clockwise for the left) [45]. It is also essential to identify the fibrous and fatty tissue surrounding the uterosacral ligaments, corresponding to the parametrium, bordered laterally by the internal iliac vessels, medially by the uterus and inferiorly by the ureter (this area contains the uterine artery and the superficial uterine vein). Additionally, the paracervix, bordered laterally by the internal iliac vessels, medially by the upper two-thirds of the vagina and insertion of the USL (this area contains, among other structures, pelvic splanchnic nerves, part of the hypogastric nerve, and inferior hypogastric plexus) [46]. The lateral extension of endometriotic lesions in this area can compromise the parametrium and the paracervix, and consequently, bring alterations to the normal anatomy of the ureter, especially with nodules greater than 17 mm [47].

The identification of the anatomical position of the torus uterinus is given by drawing an imaginary line that connects the vesicouterine fold and the internal os. Following that line, the torus uterinus is located in the posterior aspect of the cervix. This step is particularly important, given the high association of DE in the torus uterinus with bowel disease and POD obliteration [12, 45].

Posterior vaginal fornix disease is suspected when hypoechoic nodularity (regular or irregular) is identified in the vagina, above a line passing along the lower border of the posterior lip of the cervix and below a line passing along the caudal

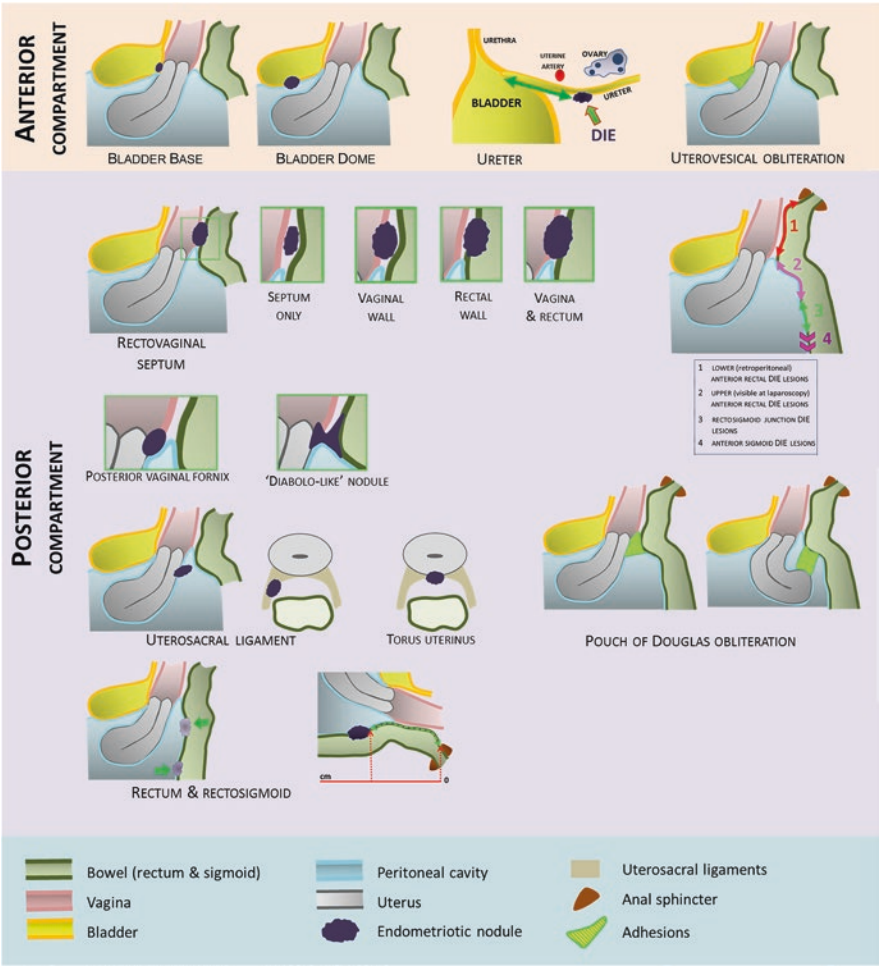

Fig. 6 Schematic overview of the anterior and posterior pelvic compartments deep endometriosis description according to the IDEA Consensus

end of the peritoneum of the lower margin of the POD. Posterior vaginal fornix nodules can occur in conglomeration with USL, torus uterinus, or bowel nodules. The RVS is defined as the narrow space between the vagina and the rectum in the retroperitoneum, below the line passing along the caudal end of the peritoneum of the lower margin of the POD. Nodules in this space are extremely rare but, when present, usually extend from the peritoneal cavity in the form of a bowel nodule [36] These lesions' retroperitoneal position makes them difficult to visualise during laparoscopy, and lower rectal resections are technically challenging, potentially associated with increased incidence of postoperative complications [48, 49].

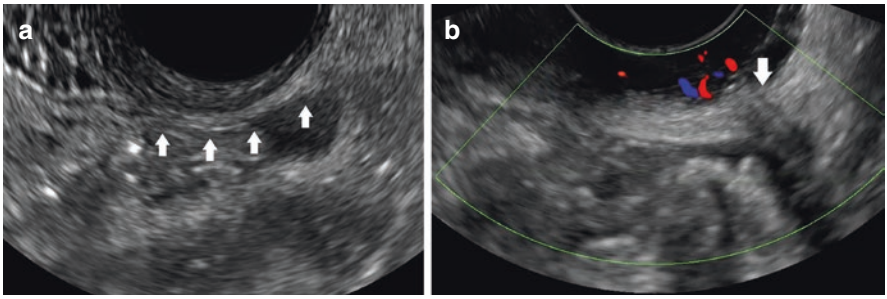

Fig. 7 (**a**) Expected appearance of the uterosacral ligament (USL) on ultrasound. The white arrows are positioned on the peritoneal aspect of the left USL. (**b**) Aspect of endometriosis within the left USL: hypoechoic image, with Doppler score 1, in contrast with the hyperechoic structure of the ligament

The remaining upper rectum lesions are commonly associated with other posterior compartment nodules, affecting the retrocervical area at the torus level. It is essential to remember the importance of identifying POD obliteration via the 'sliding sign' already performed in the third step. The key to identify bowel nodules is to follow the muscularis propria of the rectum from the anal verge cephalad. The two hypoechoic layers of the bowel's longitudinal and circular muscles are clearly visible, with a hyperechoic connective tissue layer dividing them. DE lesions usually are thickenings of the hypoechoic layers, which can present in different forms and shapes. All lesions should be measured in three orthogonal planes, and the distance from the anal verge should be noted to understand surgical implications [50]. Attention should be given to the presence of multicentric or multifocal disease, sometimes affecting also other segments of the rectosigmoid colon. The caecum-appendix area can be difficult to appreciate on TVS due to its distance from the probe so that alternative imaging modalities can be considered [12].

The anterior compartment primarily consists of the bladder, where DE will be considered when the nodule lesions affect at least the vesical muscularis layer. Superficial disease of the bladder or vesicouterine peritoneum might be present, including a possible impairment in the sliding sign also in this site. When assessing bladder DE, the most important feature to highlight is the distance of the lesion from the closest border to the meatus ureteralis, localising the nodule using the meatus as a reference point. Between the urethra and the ureteral orifices is the trigone region, up to the vesicouterine fold is the bladder base, and the remaining intra-abdominal segment of the bladder is the dome. Though they are often affected by posterior compartment disease, the ureters fall under the anterior compartment evaluation, and this is of extreme importance due to the risk of ureteral obstruction and silent kidney death. After identifying the urethra, the probe is angled laterally to the ureteral ostium, then followed in its portion within the detrusor muscle, up to its crossing at the level of the bifurcation of the common iliac vessels. Hydroureter should

be considered if the ureters measure more than 6 mm during their relaxation after physiological vermiculation [12]. Some advocate routine evaluation of the kidneys via transabdominal ultrasonography to assess for possible hydronephrosis [51].

3 Recent Advancements in Superficial Endometriosis

In addition to the concept of the three endometriosis phenotypes [52], being endometriomas and DE diagnosis characteristics on ultrasound well established, the next frontier in imaging for endometriosis is the diagnosis of peritoneal disease and superficial endometriosis. SE is defined as endometriosis less than 5 mm in depth lining the peritoneum or other areas in the abdomen (e.g. uterine serosa, ovaries), and it has been evasive to imaging for a long time [10].

Many investigators have sought to identify soft markers with the intent to establish associations with SE. Reid et al. showed that SST over the left adnexa was positively associated with superficial endometriosis of the left pelvic sidewall [38]. Robinson et al. evaluated the identification of the USLs (naming as the 'white line sign') and imaging alterations with the presence of SE. Hypoechoic nodules within the 'white line' showed a specificity of 82% (95% CI 66%–92%) in the detection of SE, and thickening of the USLs (cut-off 5.8 mm on the left and 6.1 mm on the right) showed a specificity ≥96%. However, those features should not be used as screening tests regarding SE [53]. Chowdary et al. also reported a significant association of USL thickening with SE [54].

Leonardi et al. have proposed that it is possible to visualise SE directly, but this often necessitates fluid within the pelvis. This state can be achieved artificially through a procedure called saline-infusion sonoPODography (SPG) or can be noted due to natural peritoneal fluid post-ovulation in the luteal phase (Fig. 8). Like the POD peritoneum and USLs, some pelvis areas will be more amenable to the assessment of SE due to the dependent nature of the fluid collection. For fluid to settle in the POD, there must be a non-obliterated POD state, depicted as a positive sliding sign [55].

The Practice Committee of the American Society for Reproductive Medicine is aware of the potential source of biases related to patients with early-stage endometriosis not included as a separated subgroup among couples with unexplained infertility [56]. Therefore, there is crescent importance on the research of the precise identification of possible SE, which could most likely change some of the definitions in this setting, possibly facilitating the most necessary comparisons between the different clinical presentations and improving the counselling regarding infertility treatments in the future.

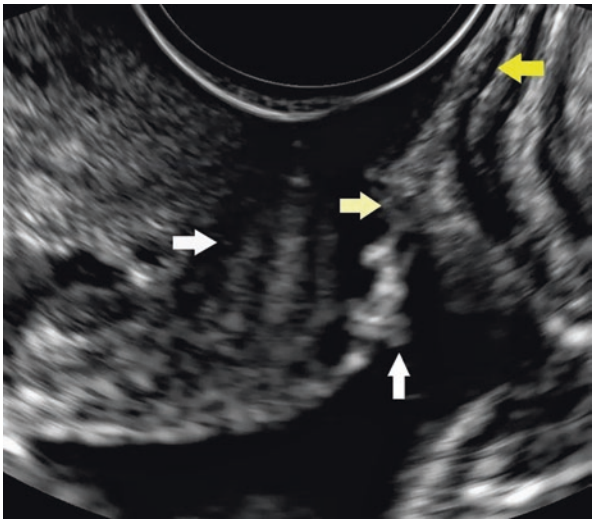

Fig. 8 Superficial endometriosis (white arrow) seen in the anterior aspect of the pouch of Douglas (POD). Light yellow arrow: apex of the POD; yellow arrow: rectovaginal septum; gray arrow: cervix

4 Learning Curve

Despite several additional steps from a conventional TVS, the IDEA protocol can be implemented within a feasible learning curve [57]. However, it is essential to highlight that the ultrasound learning curve is a dynamic process and depends on various factors, primarily individual characteristics of the operator, such as previous experience with TVS. The learning schedule should be tailored as well to attend to those particular needs and differences [58].

Indrielle-Kelly et al. demonstrated a positive learning curve for some areas of the pelvic endometriosis mapping as early as 35 scans within a gynaecology and obstetrics resident trainee setting, slightly better regarding the number of locations when compared with radiology trainees using MRI [57]. Apparently, it is possible for a sonographer trained in gynaecological ultrasound to obtain satisfactory performance over the primary sites for DE disease after examining approximately 50 patients with endometriosis that undergo surgery in a training setting [59].

It is essential to promote initiatives for the inclusion of dedicated protocols for the detection of endometriosis, including physicians and non-physicians service providers, with the potential to reduce the number of patients with a delayed diagnosis significantly due to eventual false-negative scans [60]. It is also possible to propose training programs that combine offline and hands-on settings to provide satisfactory learning curves within a relatively short period [61].

5 Final Considerations

Ultrasound imaging for endometriosis in the context of individuals with infertility contributes immensely to the diagnosis of endometriosis and can be considered as one of the most valuable tools in this setting. Consistency, agreement, and reproducibility of the imaging acquisition and report of the results are indispensable. It is essential to address that there were other protocols and techniques published before the IDEA consensus. However, the IDEA group brings together experts from around the globe and encourages a systematic, stepwise approach to diagnose and characterise endometriosis via ultrasound. External validation of the protocol is ongoing, with results from a large multicentre study to be available in due course [62]. The advantages of a precise diagnosis of endometriosis are crucial for managing such a complex condition as infertility, which requires high clinical and surgical expertise from the management team. We cannot emphasise enough the importance of the sonographers and sonologist's knowledge of pelvic anatomy, ideally correlated to significant surgical anatomy and procedural experience, associated with the female pelvis's normal and pathologic sonographic appearance. The correlation of the imaging with the surgical view can represent an unrivalled opportunity to increase the quality of diagnosis of endometriosis, including the presence of superficial disease.

From the patient's perspective, the health practitioners need to provide enough information to obtain reliable informed consent to complete this sort of diagnostic test. Clear information related to the benefits and risks should be provided; nevertheless, patients should understand that the imaging procedure can bring transient discomfort and pain due to the scan's dynamic features, especially related to the 'sliding sign' and the search for soft markers. The four steps of the IDEA protocol do not necessarily have to be performed in the same order. However, it is possible to recommend that sonographers and sonologists should consider being consistent with the same order through every scan to increase the likelihood of identifying all the fundamental details. It seems reasonable to assume that the steps related to discomfort should be performed close to the overall procedure's end, avoiding the scan's premature interruption.

As a practical approach, to increase the overall quality of the reports, the IDEA consensus suggests describing potential DE lesions in detail, including:

- echographic features;
- precise anatomic location;
- size in three orthogonal planes;
- correlation with surrounding organs and structures.

Despite the recent advancements in the technique, endometriosis might still be present, even in the absence of DE and SE on the ultrasound scan.

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Surgical Treatment of Endometriomas: Impact on Ovarian Reserve

Sabrina K. Rangi, Natalia C. Llarena, and Tommaso Falcone

1 Introduction

Endometriosis is a chronic inflammatory condition defined by the occurrence of estrogen-sensitive, ectopic endometrial tissue. The condition affects as many as 10% of reproductive-aged women, and 50% of those with infertility or pelvic pain. Ovarian endometriomas represent one of three categories of endometriosis, the other two being peritoneal disease and deep infiltrating lesions. These subtypes of endometriosis can occur independently or together, and all three can result in pelvic pain and infertility. The pathophysiology of each has overlapping and unique components that lead to the specific phenotype.

Endometriomas are characterized by a cyst wall lined by endometriotic tissue that results in the production of menstrual debris and the accumulation of "chocolate fluid" inside the cyst. Inflammation surrounding the endometriotic cyst leads to the formation of a fibrotic capsule and dense adhesions between the ovarian cortex and the cyst, which complicate surgical excision [1]. Although no single theory fully explains the pathogenesis of endometriomas, hypotheses include retrograde menstruation followed by invagination of the ovarian cortex at the site of a superficial endometriotic implant, ceolomic metaplasia of invaginated ovarian epithelium into endometrial tissue, and invasion of functional ovarian cysts by endometriotic implants [2]. These pathologic changes occur against a background of immune dysfunction and inflammation that propagates and sustains endometriosis, and in part explains why the majority of women experience retrograde menstruation but do not develop endometriosis [3].

Women with endometriomas are at risk for diminished ovarian reserve, both because of the pathophysiology of the disease and because of iatrogenic injury

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associated with surgical intervention. Baseline levels of anti-Mullerian hormone (AMH) are lower in the presence of an endometrioma, particularly when both ovaries are affected [4]. Additionally, histologic evaluation of ovaries affected endometrioma show increased atresia and primordial follicle activation, likely due to local inflammation [5]. In order to minimize further depletion of the ovarian reserve, surgical intervention for endometriomas should be undertaken judiciously, be performed by experienced endometriosis surgeons, and utilize meticulous technique that minimizes electrosurgery. The discussion that follows will review the literature regarding the impact of surgical treatment of endometriomas on ovarian reserve and surgical approaches that minimize ovarian injury.

2 Preoperative Considerations: Selecting Appropriate Surgical Candidates

Minimizing iatrogenic injury to the ovarian reserve begins with careful selection of surgical candidates. Indications for surgical management of an endometrioma include pain, desire for spontaneous conception, need for a pathologic diagnosis, concern for malignancy, or the presence of a lesion that is large or increasing in size [1]. Although medical therapy may have a role in the management of small lesions (<3–4 cm in size), recurrent endometriomas, or in women who wish to avoid surgery, medical management is suppressive or preventative and cannot effectively treat an endometrioma [1, 6, 7]. Additionally, the role of medical therapy in women who desire pregnancy is limited by the fact that hormonal therapies inhibit ovulation.

Although a number of approaches to the surgical management of endometriomas have been described, including aspiration, ablation, excision, and sclerotherapy, ovarian cystectomy is typically the treatment of choice. Cystectomy results in higher rates of symptom resolution and lower recurrence rates than aspiration or ablation [8]. For women with endometriomas who desire spontaneous conception, randomized trial data demonstrate a clear benefit of excision. A 2008 Cochrane review of two randomized clinical trials demonstrated that cystectomy of endometriomas larger than 3 cm significantly improved spontaneous pregnancy rates compared to ablation (OR 5.21, 95% CI 2.04 to13.29) [8].

In contrast, there is a limited evidence to support surgical management of an endometrioma in the setting of assisted reproductive technology (ART). The potential benefits of endometrioma removal prior to in vitro fertilization (IVF) include improved access during oocyte retrieval and prevention of endometrioma spillage that can lead to infection or contamination of oocytes; however, neither cystectomy nor aspiration results in improved pregnancy outcomes [9]. Several meta-analyses have evaluated fertility outcomes in women with endometriomas undergoing surgery followed by IVF compared to IVF alone. The most recent was a meta-analysis from 2015 that included 33 studies, including three randomized controlled trials. The results failed to demonstrate a difference in either live birth rate or clinical

pregnancy rate in women with surgically treated versus untreated endometriomas undergoing IVF/ICSI [10]. Two other meta-analyses also showed no significant improvement in pregnancy rates after surgical treatment of an endometrioma prior to IVF [11, 12]. Although surgical treatment of an endometrioma may be considered prior to IVF in order to manage symptoms, improve ovarian access, or reduce the risk of cyst rupture, asymptomatic endometriomas should not be routinely excised prior to ovarian stimulation.

The decision to pursue surgical management of an endometrioma must take into account the patient's treatment goals (management of pain, infertility, or both), age, surgical history, disease severity, and whether the patient is a candidate for a trial of spontaneous conception. Patients should be counseled preoperatively about the potential for injury to the ovarian reserve. Additionally, women at particularly high risk for damage to the ovarian reserve, including those of advanced reproductive age, women with baseline diminished ovarian reserve, or those with bilateral endometriomas, may benefit from preoperative counseling regarding fertility preservation [13]. Fertility preservation may involve oocyte or embryo cryopreservation prior to laparoscopy, or ovarian tissue cryopreservation at the time of surgery.

3 Untreated Endometrioma and Ovarian Reserve

At baseline, the presence of an endometrioma has been shown to have detrimental effects on ovarian physiology and ovarian reserve [14]. The presence of an endometrioma results in mechanical stretch and mediates damage on a cellular level through its inflammatory contents [15]. These molecular-level changes appear to have an effect on the ovarian reserve. Histologic evaluation demonstrates significantly lower follicular density in the cortex of an ovary with an endometrioma when compared to the unaffected contralateral ovary (mean ± SD = 6.3 ± 4.1/mm3 vs 25.1 ± 15.0/mm3) [16]. Additionally, when compared to other benign ovarian cysts including mature teratomas, cystomas, and dermoid cysts, endometriomas are associated with reduced follicular number and activity [17, 18].

A prospective cohort study evaluated AMH in 30 women with endometriomas compared to 30 healthy controls, and showed that women with endometriomas had significantly lower AMH levels (4.2 ± 2.3 versus 2.8 ± 2.2 ng/mL, respectively, $P = 0.02$) at baseline [19]. Additionally, AMH levels in women with bilateral endometriomas were lower than in those with unilateral endometriomas (0.55; IQR: 0.59 vs. 2.00; IQR: 2.80; $p < 0.001$) [4, 20]. Although AMH levels decline with age in women with endometriosis, women with bilateral endometriomas show a weaker correlation between age and AMH than healthy women; one study showed that median AMH levels in 18–22 year olds with bilateral endometriomas were as low as 0.82 compared to 4.24 in healthy controls (0.82; IQR: 1.12 vs. 4.24; IQR: 1.24; $p = 0.036$) [4]. There are several studies that show no difference in ovarian reserve in women with an endometrioma [21–23]. However, these studies did not report AMH levels and instead investigated ovarian responsiveness to hyper-stimulation,

oocyte quality, and oocyte retrieval during IVF treatment. Additionally, many of the studies had limited sample sizes and included patients with small endometriomas (2–3 cm).

In addition to affecting the ovarian reserve, some data suggest that endometriomas also impact oocyte and embryo quality. Embryos derived from patients with endometriosis develop more slowly and show higher rates of arrested and abnormal growth [24, 25]. Additionally, studies of donor oocytes support the hypothesis that oocyte quality is diminished in endometriosis. When healthy women undergo transfer with embryos obtained from women with moderate-to-severe endometriosis, implantation and pregnancy rates are reduced compared to those with endometriosis who receive embryos from healthy controls [26–28]. Overall, evidence points to an intrinsic effect of the endometrioma on ovarian reserve and oocyte quality.

4 Impact of Surgery on the Ovarian Reserve

The benefits of surgical intervention for endometriomas must be weighed against the risk of ovarian injury. Numerous studies have demonstrated that surgical treatment can cause additional damage to healthy ovarian tissue and a further decline in ovarian reserve [20, 29, 30]. The primary mechanisms of iatrogenic harm include excessive removal of ovarian cortex, thermal injury to the ovarian parenchyma, and injury to the gonadal vasculature [31]. A meta-analysis from 2012 that included 237 patients evaluated serum AMH in women with endometrioma undergoing excision. Overall, AMH decreased significantly by 38% postoperatively (weighted mean difference (WMD) −1.13 ng/mL; 95% confidence interval (CI) −0.37 to −1.88) [32]. A subgroup analysis demonstrated that the surgical impact on ovarian reserve is even more pronounced for bilateral endometrioma. While serum AMH fell 30% in patients with unilateral endometrioma, it declined by 44% in women with bilateral endometrioma [32]. Similarly, a systematic review from 2012 evaluated the change in serum AMH after surgical excision of endometrioma. Out of the 11 studies, 9 studies demonstrated a statistically significant reduction in serum AMH after surgical intervention [33]. Overall, the available evidence strongly demonstrates a decline in ovarian reserve following endometrioma excision.

Whether the postoperative decline in ovarian reserve is transient or permanent has also been studied; however, data are mixed. A meta-analysis performed by Raffi et al. that included two studies demonstrated a significant decrease in AMH at 6–9 months postoperatively (WMD −1.49 ng/mL; 95% CI −0.86 to −2.12; I2 = 58%) [32, 34, 35]. A prospective cohort of 25 women undergoing cystectomy similarly demonstrated a sustained decline in serum AMH of 24% at both 1 and 6 months postoperatively ($p < 0.01$) [36]. However, a prospective cohort study of 116 women showed a significant postoperative decline in AMH at 1 month, but partial recovery of AMH levels by 6 months [20]. Recent data from elective oocyte vitrification in women with endometriosis provides some data on IVF outcomes following endometrioma surgery [37]. Although there were no differences in

outcomes according to endometriosis stage, in women under the age of 35, a history of endometrioma excision was associated with decreased oocyte yield and live birth rates [37]. These data are consistent with the postoperative decline in AMH and suggest the possibility of a lasting impairment in ovarian reserve following surgery.

Repeat surgery for endometrioma in the fertility patient should be avoided when possible, as it has minimal fertility benefit and can cause increased damage to the ovarian reserve compared to the initial surgery. Several case–control studies have demonstrated that repeat surgery results in a greater decrease in AMH or antral follicle count when compared to the initial surgery. Additionally, the volume of the affected ovary decreases after a repeat procedure [38, 39]. As such, women whose primary treatment goal is pregnancy should undergo IVF rather than repeat surgery.

It is clear that measures of ovarian reserve decline after endometrioma excision, particularly in cases of repeat surgery or bilateral endometrioma excision; however, beyond the implications for women undergoing IVF, the clinical significance of this decline is unclear, as AMH levels poorly correlate with spontaneous pregnancy rates [40]. Additionally, some evidence suggests partial recovery of AMH levels 6 months postoperatively [20]. Nevertheless, the potential for injury to the ovarian reserve is an important consideration when selecting surgical candidates, sequencing surgery and IVF, and counseling patients about the risks and benefits of laparoscopy.

5 Minimizing the Iatrogenic Effects of Surgery on the Ovarian Reserve

5.1 Cystectomy

Attention to surgical technique at the time of ovarian cystectomy can minimize ovarian injury associated with surgery (Table 1). First, the plane between the endometrioma and the ovarian cortex must be carefully established to minimize injury to healthy cortex. The endometrioma is typically surrounded by a fibrotic capsule that can make this dissection challenging. Gentle traction–countertraction should be used to peel the endometrioma from the surrounding cortex (Fig. 1). Although it is

Table 1 Tips and tricks for endometrioma excision

Tips and tricks for endometrioma excision
• Hydrodissection with vasopressin can assist in separating the cyst wall from the ovarian stroma
• Forceful tissue separation causes “stripping” of ovarian follicles; instead use controlled traction and counter traction to minimize tissue trauma
• Consider using energy forms with low penetration such as plasma energy when needed to cut the attachment of the cyst
• Bipolar electrosurgery should be used sparingly. Consider the use of suture or hemostatic sealants as alternatives to electrosurgery for hemostasis

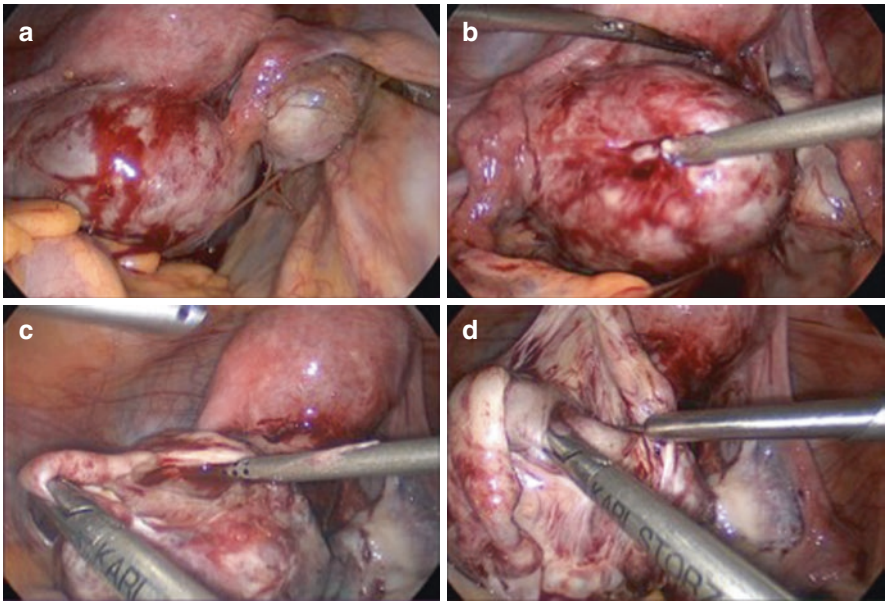

Fig. 1 Endometrioma excision. A left-sided ovarian endometrioma is shown with adhesions to the posterior uterus (**a**). The adhesions of the endometrioma to the posterior uterus are taken down bluntly (**b**). The cyst is grasped using laparoscopic Allis forceps and the plane between the cyst and the ovarian cortex is developed. The suction irrigator is used for blunt dissection (**c**). Using gentle traction–countertraction, the cyst is dissected away from the cortex (**d**).

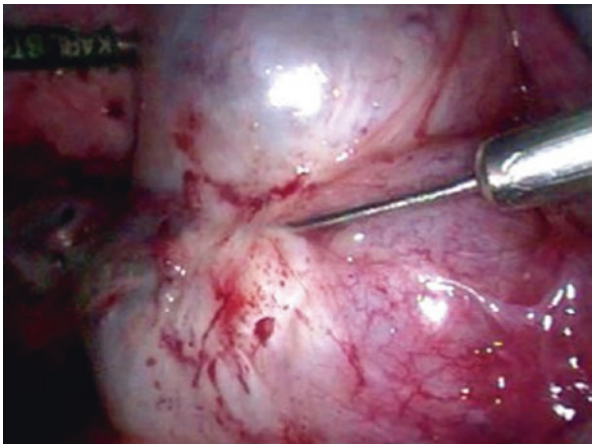

Fig. 2 Hydrodissection using dilute vasopressin

not FDA-approved for this indication, dilute vasopressin can also be used to develop the plane between the cyst and cortex through hydrodissection and reduce bleeding (Fig. 2) [13]. Particular care should be taken around the ovarian hilum, where bleeding and follicular injury often occur. Electrosurgery may be used sparingly for

hemostasis; however, evidence from randomized trials demonstrates a benefit of suture or hemostatic sealants over electrosurgery to minimize injury to the ovarian reserve [41–44].

5.2 Ablation

Although cystectomy results in improved rates of pain resolution and reduced recurrence rates compared to ablation, it also results in greater injury to the ovarian reserve than ablative approaches. Ablation, therefore, may have a role in the treatment of endometriomas in women at particularly high risk of diminished ovarian reserve (Fig. 3). Ablation can be achieved through multiple energy sources, including monopolar, bipolar, plasma, and CO_2 laser; however, ablation achieved via CO_2 laser or plasma energy results in less thermal injury to the ovary than ablation performed with monopolar or bipolar electrosurgery [45, 46].

It is unclear, however, whether the added benefit of preserving ovarian reserve with ablation via plasma energy translates to improved fertility outcomes. A prospective cohort study compared pregnancy outcomes in 104 women with endometriomas after ablation with plasma energy compared to cystectomy [47]. Although

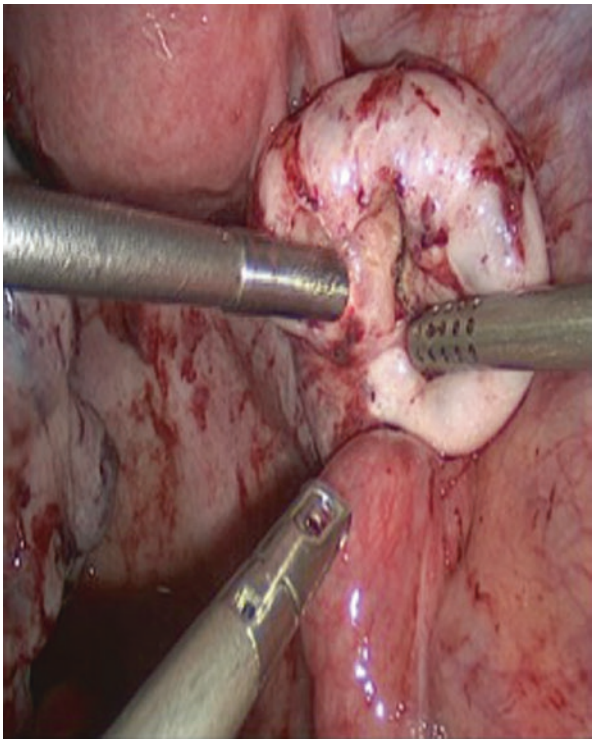

Fig. 3 Ablation using plasma energy

the study found no differences in pregnancy rates within 36 months postoperatively, the cohort that underwent plasma ablation was significantly older than those treated with excision, had a higher revised American Fertility Society (rAFS) score, higher rates of deep infiltrating endometriosis, increased rates of colorectal lesions and posterior cul de sac obliteration. The differences in age and endometriosis stage in the plasma group suggest that this cohort was at increased risk of infertility at baseline, and the benefits of plasma ablation may be underestimated by this study. Further randomized trials are needed to investigate plasma ablation, and its impact on ovarian reserve and fertility outcomes.

Because of the potential for reduced ovarian injury with ablation, several studies have investigated ablation combined with either medical therapy or excision. Donnez et al. describe a 3-step approach to the treatment of large endometriomas (> 3 cm): (1) laparoscopy with biopsy and drainage of the cyst to confirm the diagnosis of endometriosis on pathology, (2) 12 weeks of a gonadotropin receptor hormone (GnRH) agonist to shrink the endometrioma, and (3) laparoscopic ablation of the residual cyst via CO_2 laser [48]. Data from a small randomized trial comparing this three-step ablative method to cystectomy demonstrate a smaller postoperative decline in AMH and a recurrence rate of 8% after 2 years of follow-up [34]; however, the need for multiple laparoscopies limits the utility of this approach.

Combined ablation/excision techniques obviate the need for multiple laparoscopies and appear to reduce the risk of ovarian injury compared to complete excision. The combined technique, first described in 2010 by Donnez et al., comprised two steps: partial cystectomy of 80–90% of the endometrioma followed by laser vaporization of the remaining tissue [49]. The cyst is excised until the hilum is reached, where bleeding and follicular damage often occur, and the remainder of the cyst wall is ablated. This technique was initially evaluated through a prospective cohort study of 52 women. Six months after surgery, ovarian volume and antral follicle count (AFC) of the operated and non-operated ovaries were measured by transvaginal ultrasound. There was no significant difference in the ovarian volume or AFC between the operated and non-operated ovary 6 months after surgery (7.64 ± 2.95 cm^3 and 6.1 ± 3 versus 7.99 ± 5.33 cm^3 and 6.2 ± 4.8, respectively). The recurrence rate was low at 2% [49]. These results provide evidence that a combined approach allows for a better preservation of the ovarian reserve than cystectomy while minimizing recurrence risk; however, this study is limited by its small sample size and lack of randomization.

A randomized trial comparing combined cystectomy/ablation to complete excision failed to demonstrate a benefit of the combined approach [50]. In this study, one ovary was randomly assigned to treatment with cystectomy and the contralateral ovary was treated with a combination of excision and ablation with bipolar electrosurgery. Follow-up at 1, 3, and 6 months showed no difference in AFC between groups; however, the study was underpowered with only 51 patients [50]. Larger randomized trials are needed to further evaluate the combined cystectomy/ablative technique and its impact on ovarian reserve.

5.3 Method of Hemostasis

After excision of the endometrioma, subsequent bleeding from the cyst bed requires hemostasis. Traditionally, bipolar electrosurgery is used; however, despite its common use, bipolar energy can result in destruction of ovarian follicles adjacent to the cyst wall and thermal injury to the ovarian blood supply. Alternative methods to control bleeding have been studied, including suture and hemostatic sealants. Randomized trial data suggest a benefit of these agents over electrosurgery to reduce injury to the ovarian reserve.

A 2016 RCT compared bipolar electrosurgery to suture in 109 patients undergoing surgery for a unilateral endometrioma [42]. In the bipolar electrosurgery group, 40 W of current (Richard Wolf, Germany) were used to achieve hemostasis after cystectomy. In the suture group, 2–0 polyglican absorbable suture (Vicryl; Ethicon Inc., New Jersey, USA) was used to control bleeding. The suture was used to reapproximate the edges starting around the ovarian hilus to the peripheral tissue in a running fashion. At 3 months postsurgery, serum AMH levels were significantly lower and FSH levels were significantly higher in both study arms. When comparing the two techniques, the suture group had a significantly higher serum AMH and a significantly lower FSH level than the bipolar group ($p < 0.001$). Additionally, there was a significantly greater rate of decline in serum AMH levels in the bipolar electrosurgery group (53.42 ± 15.28) versus the suture group (15.94 ± 18.55) [42]. A similar RCT of 100 women undergoing surgery for bilateral endometriomas showed that postoperative FSH levels were significantly higher when hemostasis was obtained through bipolar electrosurgery (20–30 W) compared to suturing at 3 and 6 months postoperatively, but not at 12 months [51]. Additionally, there was a larger decline in AMH in the bipolar electrosurgery group (20–30 W) compared to the suture group; however, this difference did not reach statistical significance [51]. Both studies demonstrate a significant decline in ovarian reserve following endometrioma surgery, regardless of which method of hemostasis was used; however, overall these data favor suture over electrosurgery to maintain the ovarian reserve (Fig. 4).

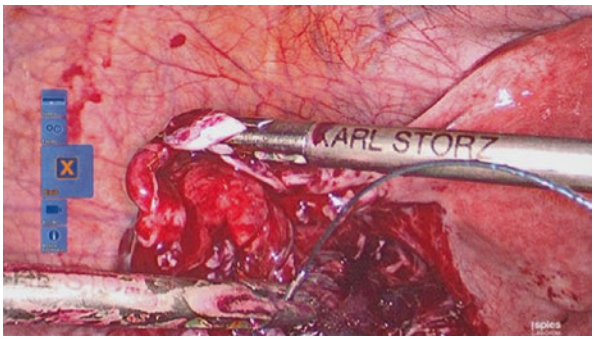

Fig. 4 Use of suturing for hemostasis

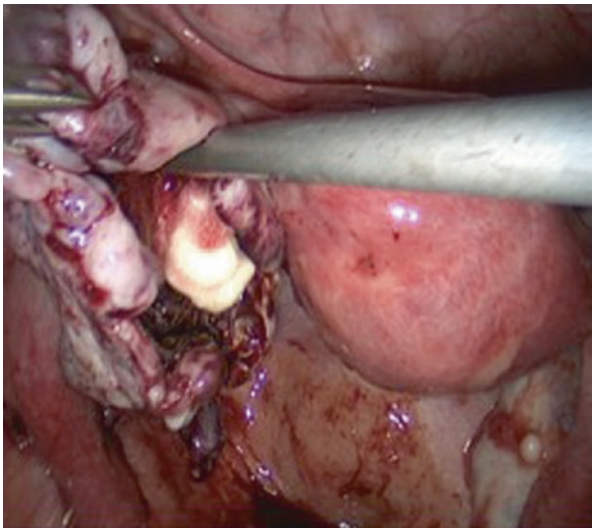

Fig. 5 Use of hemostatic sealant

An alternative to thermal energy and suturing includes the use of topical hemostatic agents to control bleeding (Fig. 5, Table 2). Most sealants are composed of a mixture of bovine-derived gelatin matrix and a human- or bovine-derived thrombin solution. These two components work synergistically to achieve hemostasis. When the solution comes into contact with blood, the gelatin swells around the wound and serves as a tamponade. The high concentration thrombin then acts to reinforce the matrix by initiating the formation of a fibrin clot [41, 43].

The FDA approved the use of hemostatic agents in 1999, but the first pilot studies of its use in endometrioma surgery were not published until 2009 [41, 52, 53]. These initial studies demonstrated that time to hemostasis with FloSeal, a hemostatic agent composed of collagen granules and thrombin, is comparable to conventional bipolar electrosurgery. Since these early studies, several randomized controlled trials have demonstrated a benefit of hemostatic sealants over bipolar electrosurgery for preservation of the ovarian reserve. In 2009, Raga et al. demonstrated a significantly smaller postoperative decline in AMH at 3 month postcystectomy when hemostatic sealants are used compared to bipolar electrosurgery [54]. Similarly, a small randomized trial of 30 patients showed that when hemostasis was achieved through hemostatic sealant rather than bipolar electrosurgery, AMH levels were significantly higher 1 month postoperatively; however, the difference was no longer significant at 3 months [44]. Greater recovery in AMH levels was seen in the bipolar electrosurgery group compared to the hemostatic sealant group (127 vs 29%, respectively, p: 0.0002), suggesting the possibility of a transient decline that recovers over time. It is important to note, however, that this second RCT had several limitations, including lack of statistical power and no intention-to-treat analysis [44]. The largest and most recent RCT investigating bipolar coagulation versus hemostatic sealants and their impact on ovarian reserve was conducted by Song et al. in 2014 [43]. The study was a prospective, multicenter RCT of 100 women

Table 2 Studies evaluating alternative hemostatic agents to electrosurgery

Study type	Method of hemostasis	Preoperative AMH (ng/mL)	AMH at 1 month postop (ng/mL)	AMH at 3 months postop (ng/mL)	Other results	Citation
Randomized trial ($n = 100$)	Bipolar	3.26	–	2.04	Rate of decline in AMH: 41.2%	Song et al.
	Hemostatic sealant (Floseal)	3.20	–	2.67	Rate of decline in AMH: 16.1%	
Randomized trial ($n = 30$)	Bipolar	3.66	1.64	2.84	–	Sonmezer et al.
	Hemostatic sealant	3.73	2.72	3.07	–	
Randomized trial ($n = 109$)	Bipolar	4.41	–	–	Percent decline in AMH: 53.42%	Asgari et al.
	Suture (2–0 polyglican)	3.1	–	–	Percent decline in AMH: 15.94%	
Randomized trial ($n = 100$)	Bipolar	2.3	–	–	No significant difference in % change in AMH levels between groups at 3, 6, and 12 months. The % increase in basal FSH was higher in the bipolar group than the suture group at 3 and 6 months.	Ferrero et al.
	Suture	2.7	–	–		

with benign ovarian cysts undergoing cystectomy, of which 56 had endometrioma. At 3 months postsurgery, there was a significantly greater decline in AMH in the bipolar electrosurgery group (41.2%; IQR, 17.2–54.5%) than in the FloSeal group (16.1%; IQR, 8.3–44.7%) ($p = 0.0004$) [43]. A 2015 meta-analysis that pooled several RCTs comparing suture or hemostatic sealants to bipolar electrosurgery and included 213 women upheld a benefit of suture or hemostatic sealants over electrosurgery [41]. Together, these data suggest that hemostatic sealants are a viable alternative to bipolar coagulation with similar surgical outcomes but less detrimental effects on ovarian reserve.

It is important to note that although there have been reports of serious complications associated with the use of hemostatic sealants, including small bowel obstruction (SBO) and thromboembolism, these outcomes are rare. Hemostatic agents can cause the formation of eosinophilic granulomatous tissue, which leads to adhesions and subsequently SBO [55–57]. Additionally, there are a handful of case reports of hemostatic sealant extravasation into the systemic circulation with resultant thromboembolism and disseminated intravascular coagulation [58, 59]. However, these cases were reported in patients undergoing spinal surgery. There have not been any

reports of thromboembolism related to pelvic surgery. Finally, there is a theoretical risk of viral transmission associated with human or bovine-derived plasma; however, there have been no reported cases of viral transmission to date [41]. Although the costs of hemostatic sealants are higher than for suture, this additional cost must be weighed against the potential for increased operative times associated with suturing [41].

5.4 Surgeon Experience

When considering additional strategies to improve ovarian reserve, the expertise of the surgeon may also play a role. Few studies have evaluated how a surgeon's level of training impacts ovarian architecture after laparoscopic excision of endometrioma. A multicenter, prospective trial that included 50 patients evaluated endometrioma cyst wall specimens after laparoscopic excision [60]. Specifically, the authors measured mean thickness of the cyst wall and the mean thickness of the ovarian tissue inadvertently removed were measured. The study evaluated experienced surgeons from four different centers of excellence compared to residents and found that the specimens excised by residents contained thicker ovarian tissue (0.49 ± 0.30 mm vs. 0.97 ± 0.29 mm, $P = 0.002$), suggesting that more healthy cortex was removed by the less experienced surgeons [60]. Additionally, retrospective data from an academic institution in Taiwan suggest improved live birth rates after cystectomy performed by an experienced surgeon compared to a trainee [61]. These results demonstrate that although the loss of ovarian tissue is inevitable, a surgeon proficient in endometriom surgery can minimize the loss of healthy ovarian tissue. Whether these differences have an impact on ovarian reserve is unclear given the lack of serum AMH measurement. However, it can be inferred that an experienced surgeon may be a better equipped to perform the meticulous technique required to remove an endometrioma and preserve normal ovarian architecture.

5.5 Anti-Adhesion Barriers

An additional area of consideration in pelvic surgery and the impact on fertility includes postoperative adhesion formation. Adhesions result in the distortion of pelvic anatomy and can lead to pelvic pain, adverse fertility outcomes, and small-bowel obstruction. In an attempt to prevent their formation, several anti-adhesion barriers have been created. The efficacy of these barriers and their impact on several measures including fertility and pain has been evaluated, and these studies offer an insight into a reasonable approach to preserving fertility and reducing other adverse outcomes associated with laparoscopic excision of endometrioma.

A Cochrane review published in 2015 evaluated four different anti-adhesion barriers used during pelvic surgery and their impact on pain, live birth rate, and postoperative adhesions [62]. The four barrier agents included expanded

polytetrafluoroethylene (Gore-Tex), oxidized regenerated cellulose (Interceed), sodium hyaluronate with carboxymethylcellulose (Seprafilm), and fibrin sheets. The review included 18 RCTs for a total of 1267 patients. Laparotomy was performed in eight of the studies and laparoscopy was performed in the remaining 10 studies. None of the 18 studies measured pelvic pain or live birth rate, which were the primary outcomes of the review. However, the results did demonstrate that oxidized regenerated cellulose (Interceed), expanded polytetrafluoroethylene (Gore-Tex), and sodium hyaluronate with carboxymethylcellulose (Seprafilm) were associated with a reduction in adhesion formation. One study showed that there was no reduction in the incidence of adhesion formation following the use of fibrin sheets [62]. It is important to note, however, that the majority of the evidence was deemed low to very low quality. Regardless, there were no observed adverse outcomes associated with the use of anti-adhesion barriers in pelvic surgery. It is important to note that the safety and effectiveness of anti-adhesion barriers have not been established for laparoscopic surgery. Although adhesion barriers are not FDA-approved in in the setting of minimally invasive surgery, their use in laparoscopic surgery has been described in the literature. Additionally, in order for adhesion barriers to be effective, meticulous hemostasis is imperative.

5.6 Postoperative Suppression

The role of medical management of endometriomas is limited since hormonal therapies inhibit ovulation and thus cannot be used in women who desire pregnancy. However, evidence shows that postoperative suppression therapy for at least 18–24 months has a role in preventing endometrioma recurrence [63]. A randomized control trial of 239 women demonstrated a significantly lower recurrence rate in women who received postoperative suppression with continuous oral contraceptive pills (OCPs) (8.2%) and cyclic OCP therapy (14.7%) compared to women who did not receive treatment (29%) [64]. In cases of recurrence, endometriomas were smaller at the time of diagnosis and had slower rates of growth in women receiving OCPs when compared to nonusers [64]. Preventing recurrence is an important factor to consider in surgical candidates since endometrioma surgery is known to decrease baseline AMH levels. Medical management can be considered in selected patients who have a delayed desire for fertility or who do not desire fertility. The European Society for Human Reproduction and Embryology (ESHRE) endometriosis guidelines recommend postoperative hormonal suppression to prevent endometriosis recurrence [65].

5.7 Alternative to Cystectomy: Sclerotherapy

An alternative technique to cystectomy includes chemical ablation with ethanol sclerotherapy. Ethanol sclerotherapy has a long history of use in other organ systems, but its use in endometrioma treatment was first described in 1988 [66]. The

initial technique involved transvaginal or transabdominal aspiration followed by sclerotherapy. However, this approach was associated with several risks including infection, bleeding, inflammation, and pelvic adhesion formation [67]. A laparoscopic approach to ethanol sclerotherapy has been described by De Cicco Nardone et al. [68]. First, the cyst is drained and washed repeatedly by creating a 5-mm puncture on the cyst with a monopolar coagulator followed by wash out with a central irrigation and suction system (Olympus HiQ+ suction and irrigation system; Olympus Winter & Ibe GmbH, Hamburg, Germany). After the outflow fluid is clear, a seal is created using an 8-Fr Nelaton Foley catheter to prevent spillage. The catheter is inserted into the puncture site; the balloon tip is filled with saline and then lifted to the puncture site. The cyst is then filled with a solution with methylene blue to evaluate for leaking. If there is no evidence of leakage, the solution is removed and replaced with 95% ethanol that is mixed with methylene blue for 15 min. Simultaneously, the pelvic cavity is filled with ringer's lactate solution to safeguard nearby organs in case of ethanol spillage [68].

The laparoscopic approach to ethanol sclerotherapy was evaluated by a single-center retrospective study of 53 women with endometriomas measuring 4–10 cm [68]. All patients received postoperative suppression therapy with continuous hormone therapy. Therapy was discontinued after 3 months in patients who desired fertility or stopped in patients with adverse effects. Recurrence occurred in 5 of the 53 patients (9%). The recurring cyst was on average smaller than the initial cyst (mean length of follow up was 31 months). Additionally, pregnancy occurred in 16 of the 28 women (57%) who desired pregnancy. Out of the 16 who achieved pregnancy, 14 conceived spontaneously and two conceived after in vitro fertilization. No major operative complications were observed [68]. The effect of ethanol sclerotherapy on ovarian reserve was not evaluated by this study; however, several studies evaluating sclerotherapy prior to in vitro fertilization stimulation have demonstrated no effect on antral follicle count, retrieved oocytes, embryo quality, or hormone levels when compared to infertile females without ovarian cysts [69–72]. While retrospective data are promising, there is a need for prospective studies and RCTs investigating the impact of ethanol sclerotherapy on ovarian reserve.

6 Conclusion

Women with endometriomas are at risk for diminished ovarian reserve, due to both the pathophysiology of endometriosis and iatrogenic injury. Although the surgical treatment of endometrioma negatively impacts measures of ovarian reserve, several evidence-based strategies can be used to minimize ovarian injury and, in experienced hands, surgical excision can be safely performed. First, decisions to pursue surgery must be made with the patient's treatment and fertility goals in mind. The routine excision of asymptomatic endometriomas prior to IVF does not improve IVF outcomes, and should be considered on an individual basis to improve ovarian access during retrieval or minimize the risk of cyst rupture. Additionally, repeat

surgery with the goal of optimizing fertility outcomes should be avoided. In women at risk for bilateral ovarian injury, such as those with bilateral endometriomas, consideration should be given to fertility preservation through oocyte, embryo, or ovarian tissue cryopreservation.

When surgery is indicated, the use of suture or hemostatic sealants can help to minimize the use of electrosurgery and thereby reduce the risk thermal injury to healthy cortex. Although excision of endometriomas remains the standard of care for optimizing spontaneous fertility, improving symptoms, and reducing recurrence rates, there may be a role for ablation or combined ablative/excisional approaches in women at particularly high risk of injury to the ovarian reserve. Ablative approaches may also be considered in those unlikely to conceive spontaneously. Finally, postoperative hormonal suppression in those not desiring immediate conception can help reduce the risk for recurrent endometrioma and the need for repeat surgery.

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Surgical Treatment of Deep Endometriosis: Impact on Spontaneous Conception

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1 Introduction

Deep endometriosis (DE) is defined arbitrarily as endometriosis infiltrating the peritoneum >5 mm [1] (Fig. 1). It may affect several locations, including the rectovaginal septum, uterosacral ligaments, bowel, bladder, ureters, vagina, and other extrapelvic sites. Most women with DE have severe pain, it is estimated that only 5% of DE patients are pain-free [2].

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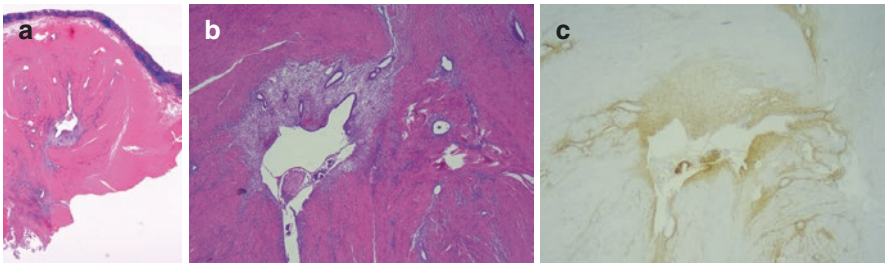

Fig. 1 Sigmoid deep endometriotic nodule. (**a**, **b**) Hematoxylin and eosin stain shows a deep endometriotic nodule infiltrating the muscularis propria of the sigmoid. (**c**) CD10 immunohistochemistry demonstrates the endometrial stroma in the same sigmoid endometriotic nodule

1.1 Fertility After Surgical Treatment of Endometriosis of the Posterior Compartment

Since the 1990s, several authors reported descriptive data on the pregnancy rates following surgical treatment of DE of the posterior compartment. These studies often included patients who underwent surgical treatment of rectosigmoid endometriosis. Intestinal involvement by DE has been estimated to occur in 5–25% of women with endometriosis [3].

Donnez et al. described a series of 231 patients who underwent laparoscopic treatment of rectovaginal DE [4]. One hundred and fifty-one patients were followed for >1 year, and 48 were infertile. Fifty-two percent of the infertile patients ($n = 25$) became pregnant the first year after surgery. Subsequently, Fedele et al. reported the long-term follow-up of 83 patients who underwent conservative surgical treatment of rectovaginal DE [5]. Seventeen infertile women became pregnant during follow-up, equivalent to 34% of those who wanted to conceive before surgery. Notably, the occurrence of pregnancy after surgery decreased the probability of pain recurrence and a new treatment. A study by Chapron et al. is the only one currently available reporting the fertility outcomes after laparoscopic treatment of DE infiltrating the uterosacral ligaments [6]. This study included 30 infertile women. Depending on the spread of endometriosis, ureterolysis and/or dissection of the lateral rectal fossa was performed. The mean duration of infertility was 35 ± 18.6 months. In this study, patients had no associated infertility factors (tubal patency at preoperative hysterosalpingography) and no associated male infertility. The overall intrauterine pregnancy rate (including birth and miscarriages) was 50.0% (three patients conceived after ovulation induction and one after in vitro fertilization (IVF)). The cumulative intrauterine pregnancy rate for the 14 pregnancies which occurred spontaneously was 48.5% at 12 months. The rate of spontaneous pregnancies was not significantly correlated with the revised American Fertility Society (rAFS) classification of the disease; it was 47.0% for patients with stage I or II endometriosis and 46.1% for patients with stage III or IV endometriosis.

In a retrospective study, Darai et al. examined fertility after laparoscopic colorectal resection for endometriosis [7]. The study included 34 women with colorectal endometriosis, of whom 22 wished to conceive. The mean follow-up after surgery was 24 months (range, 6–42 months). Ten patients (45.5%) conceived; two women conceived twice. Nine pregnancies were spontaneous, and three were obtained by IVF indicated for fallopian tube disorders. The median time to conceive after colorectal resection was 8 months (range, 3–13 months).

In a patient preference study, Vercellini et al. investigated whether the chance of conception is increased and the time-to-conception is reduced in infertile women with rectovaginal endometriosis undergoing conservative surgery compared with those on expectant management [8]. In this study, patients were aged <40 years and had not previous pelvic surgery. Forty-four patients underwent surgery, and 61 chose expectant management. The mean follow-up was 27 months for the subjects in the conservative surgery group and 24 for those in the expectant management group. Among the 44 women who had resection of rectovaginal endometriosis, 15 became pregnant, compared with 22 of the 61 women who chose expectant management. There was no significant difference in the 24-month cumulative probability of conception between patients who underwent surgery (44.9%) and those who chose expectant management (46.8%). Similarly, the two study groups had no significant difference in time to conception. Therefore, the authors concluded that surgical excision of rectovaginal DE does not improve the incidence of pregnancy and reduces the time to conception in women with endometriosis-associated infertility.

In a prospective study, Ghezzi et al. reported their experience with laparoscopic segmental bowel resection combined with exteriorization of the affected segment via a colpotomy incision to complete the resection [9]. This study included 33 patients; of the 13 who tried to conceive, 4 (30.8%) were successful, and none required assisted reproductive techniques (ART).

A prospective cohort study by Ferrero et al. assessed the pregnancy rate after bowel resection for DE [10]. The pregnancy rate was higher in women who underwent bowel resection by laparoscopy (57.6%) than in those who underwent laparotomy (23.1%). No significant difference was observed in pregnancy rate and mode of conception between women with different fertility statuses before bowel resection. Women who conceived were significantly younger than those who did not conceive; only 26.7% of women aged ≥35 years conceived after bowel resection. Uterine adenomyosis was more frequent in women who did not conceive than in those who conceived. Infertile women who conceived had a shorter length of infertility before surgery than those who did not conceive.

Meuleman et al. retrospectively reported the clinical outcome of patients who underwent CO_2 laser laparoscopic excision of colorectal DE [11]. Out of 56 patients included in the study, 33 wanted to conceive after surgery. Seven women conceived spontaneously, one after ovarian stimulation and intrauterine insemination, and eight conceived after IVF. Nearly all spontaneous (6/7) and four out of eight IVF pregnancies occurred the first year after surgery.

In 2010, Donnez and Squifflet analyzed the pregnancy rate after shaving of rectovaginal endometriotic nodules infiltrating the rectum [12]. The main steps of the

surgical procedure were the separation of the anterior rectum from the vagina, excision or ablation of DE after complete dissection of the nodule from the posterior part of the uterine cervix (systematically removing the posterior vaginal fornix), and vaginal closure. Five hundred women were included in the study, and 324 (64.8%) had a history of infertility. The duration of postoperative follow-up was 2–6 years (median 3.1 years). Among 388 women wishing to conceive after surgery, 221 (57%) naturally conceived during the follow-up.

Kavallaris et al. treated 55 patients with rectovaginal endometriosis (42 with a history of infertility) using a combined laparoscopic vaginal technique [13]. The procedure was started by vaginal excision of the involved vagina and part of the rectovaginal septum, which was left on the rectum. Parasigmoid and retrosigmoid-rectal spaces were developed laparoscopically before rectal transection using a laparoscopic stapling device. Among the 30 patients who were followed-up after surgery, 17 tried to conceive, and 11 became pregnant (7 spontaneously and 4 by IVF).

A French retrospective study including patients with DE compared the incidence of spontaneous pregnancies in women who underwent excision of DE and those who only underwent intraperitoneal surgery [14]. Women with anovulation, age $\geq$ 40 years, bilateral tubal occlusion, and severe oligozoospermia of the male partner were excluded from the study. Thirty-four patients preferred intraperitoneal surgery only, and 41 chose extensive surgical treatment. The pregnancy rate was similar in patients treated with intraperitoneal surgery (n = 6) and in those treated with extensive surgery (n = 8); the 12-month cumulative probabilities were 24.8%

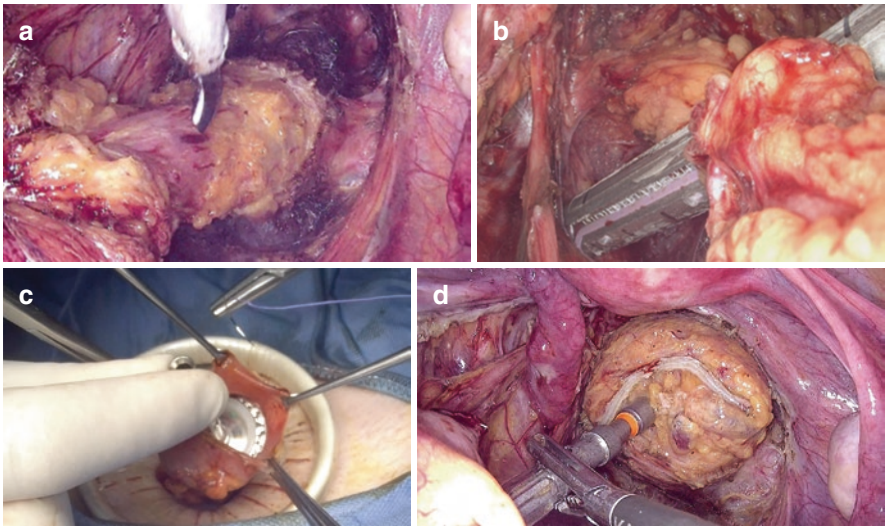

Fig. 2 Segmental bowel resection for endometriosis. (**a**) Preparation of the rectosigmoid for segmental resection. (**b**) The rectosigmoid is transected using a linear stapler. (**c**) The anvil of the circular stapler is inserted in the bowel lumen and secured with a purse-string suture performed extracorporeally. (**d**) End-to-end anastomosis is performed using a transanal circular stapler

and 11.4%, respectively; whereas the 24-month cumulative probabilities were, respectively, 24.8% and 23.2%. The perioperative surgical complication rate was higher in patients who underwent extensive surgery.

In a retrospective study by Jelenc et al. including 52 patients with rectal endometriosis, 8 out of 14 infertile women conceived spontaneously after surgery; two of these patients conceived twice [15].

In a retrospective study including 248 patients, Malzoni et al. evaluated the pregnancy rate after laparoscopic segmental bowel resection for DE [16] (Fig. 2) (Fig. 2). Among 72 patients who tried to conceive spontaneously, 44 achieved pregnancies with a mean (± SD) interval of 8.4 ± 4.1 months.

A prospective study by Minelli et al. included 357 consecutive patients who underwent colorectal resection for endometriosis [17]. Among 113 patients with preoperative infertility, 47 conceived during the postoperative follow-up, with 64 pregnancies (mean number of pregnancies per patient, 1.4). However, only 20% of the pregnancies were achieved spontaneously, while 80% were obtained after assisted reproductive techniques (ART).

Stepniewska et al. compared the reproductive outcomes of three group of patients: (1) patient who underwent surgery for endometriosis including colorectal segmental resection (n = 60); (2) patients with evidence of bowel endometriosis who underwent endometriosis removal without bowel resection (n = 40); and (3) patients who underwent surgery for moderate or severe endometriosis with at least one endometrioma and DE but without bowel involvement (n = 55) [18]. The pregnancy rate was lower among patients who tried to conceive spontaneously if bowel endometriosis was present and untreated during surgery. The differences in reproductive outcomes between groups were evident not only in the cumulative pregnancy rate but also in terms of the time necessary to conceive. The time to conceive was significantly shorter in patients undergoing colorectal segmental resection (696 days) than in those undergoing endometriosis removal without bowel resection (1417 days).

A prospective study by Darai et al. investigated the determinant factors of fertility after laparoscopic colorectal resection for endometriosis [19]. The study included 83 women (39 with infertility). The mean duration of infertility before surgery was 4 years (range, 2–10 years). The mean follow-up after surgery was 34 months (range, 6–68 months). Twenty-nine pregnancies were obtained, including 20 spontaneous pregnancies (69%) and nine pregnancies by IVF (31%). A relation was found between pregnancy rate and patient age. Reduction in pregnancy rate was correlated to the presence of adenomyosis, high ASRM total score, and exclusive laparoscopy compared to conversion to laparotomy for colorectal resection.

Subsequently, the same authors investigated in a randomized controlled trial (RCT) whether the surgical route of colorectal resection is a determinant factor for fertility [20]. Fifty-two patients were included in the study (23 with infertility). The mean follow-up was 29 months (range, 6–52 months). Among the 28 patients wishing to conceive, 11 (39.3%) became pregnant. The cumulative pregnancy rate for these patients was 45.1% at 52 months. For patients with or without infertility, the cumulative pregnancy rate was 37.6% and 55.6%, respectively, and the cumulative

spontaneous pregnancy rate was 13.3% and 36.5%, respectively. All the spontaneous pregnancies were observed in the laparoscopy group and in women aged <35 years. The median time to conceive spontaneously was 7.5 months (range, 1–18 months), and by ART 21 months (range, 14–24 months).

Vercellini et al. performed a systematic review to define the pregnancy rate in infertile patients before surgery for rectovaginal DE and who sought spontaneous conception [21]. Eleven studies were included in this review. Five hundred and seventy-one women desiring pregnancy after surgery for DE were identified, and 510 were infertile before surgery and sought spontaneous conception. There were 223 conceptions in the entire group of 571 women with pregnancy desire after surgery, with pregnancy rates varying from 19% to 65% with a weighted mean of 39% (95% C.I., 35–43%). However, only 123 pregnancies were reported among the 510 infertile patients who sought spontaneous pregnancy (weighted mean 24%, 95% C.I., 20–28%), with percentages varying from 10% to 41%. Several factors impacted the postoperative probability of conception. Age over 35 years, surgery via laparotomy, diagnosis of uterine adenomyosis at preoperative MRI, and the presence of endometriotic bowel lesions exerted a negative effect. In contrast, surgery via laparoscopy and bowel resection were favorable prognostic factors.

Meuleman et al. assessed the clinical outcome of women requiring laparoscopic excision of moderate-severe endometriosis with and without bowel resection [22]. The study included 203 patients, 148 (73%) wanted to conceive after surgery. At the end of the study, 51% (n = 75) of these patients had conceived (61 gave birth). Spontaneous conception occurred in 38% of the patients who underwent bowel resection and in 48% of those without bowel endometriosis.

Roman et al. assessed the postoperative outcomes of patients with rectal endometriosis managed by full-thickness disc excision [23]. Fifty patients were included in this study. Follow-up varied from 5 to 65 months. Among 20 patients with pregnancy intention, 16 achieved pregnancy (80%), 10 of them spontaneously (63%), and 6 by assisted reproductive technology (37%).

A prospective study by Roman et al. investigated the pregnancy rates in patients with ovarian endometriomas managed by ablation using plasma energy [24]. This study included women with colorectal endometriosis (n = 52) and those free of colorectal lesions (n = 72). The mean follow-up was 32 ± 18 months. Sixty-six percent of the patients in the group with colorectal endometriosis and 57.8% of those without colorectal involvement conceived during the follow-up. Among these conceptions, 15 (60%) in the group with colorectal endometriosis and 18 (69.2%) in the group without colorectal involvement were spontaneous.

More recently, Roman et al. reported the pregnancy rate of patients included in a RCT (ENDORE) [25]. This study included patients with DE infiltrating the rectum up to 15 cm from the anus, measuring more than 20 mm in length, involving at least the muscular layer in depth, and up to 50% of the rectal circumference. Twenty-five patients had conservative surgery (shaving or disk excision), and 30 had radical rectal surgery by bowel resection. Thirty-six patients tried to conceive during follow-up, ranging from 50 to 79 months. Among them, 23 patients had unsuccessfully attempted to conceive before surgery for more than 12 months (63%). At the end of

the follow-up, 29 patients achieved pregnancy (81%); among them, 17 conceived naturally (47% of women, 59% of conceptions), and 12 conceived using ART techniques (41% of conceptions). In the group of 23 infertile patients, 17 women achieved pregnancy (74%) and 9 conceived naturally (39%). Several women had more than one pregnancy (range: 0–3). The probabilities of achieving pregnancy at 12, 24, 36, and 48 months postoperatively were 33.4% (95% CI: 20.6–51.3%), 60.6% (44.8–76.8%), 77% (61.5–89.6%), and 86.8% (72.8–95.8%), respectively. Women advised to attempt natural conception achieved pregnancy significantly earlier than patients referred for ART. In infertile patients, the postoperative pregnancy rate was 74%, and 53% of conceptions were natural.

A recent retrospective cohort study reported the reproductive outcomes of 55 infertile patients who underwent surgical treatment of DE [26]. The patients had no plausible infertility factor, including abnormalities in the partner's semen analysis. There were 34 pregnancies (61.8%): 24 patients (70.6%) conceived spontaneously, and 10 (29.4%) by IVF. The interval between the operation and pregnancy was 10.3 ± 5.6 (1–26) months. Univariate analysis showed that a lower endometriosis fertility index (EFI) score (EFI < 8) was a risk factor for infertility.

1.2 Fertility after Surgical Treatment of Urinary Tract Endometriosis

Understanding the impact of bladder endometriosis and its treatment on fertility is challenging because these lesions are typically associated with other forms of the disease (such as ovarian endometriomas, uterine adenomyosis, superficial endometriosis, and other localization of DE) [27].

Few case series assessed fertility outcomes after bladder endometriosis removal. A French retrospective study reported the follow-up of 24 patients who underwent surgical treatment of bladder endometriosis (14 partial resections and 9 submucosal resections) [28]. The mean length of follow-up was 34.7 months (range, 3–108 months). Eleven patients tried to conceive after surgery (7 with a history of infertility before surgery); of whom 7 became pregnant (4 were infertile before surgery).

In a retrospective study, Kovoor et al. reported that 5 out of 10 (50%) infertile women with bladder endometriosis conceived naturally after the surgery [29]. One patient conceived by IVF. The time to achieve pregnancy was 3–12 months after surgery. Notably, 16 out of 21 patients included in this study had associated DE in the pelvis.

A retrospective observational study based on a prospectively collected database assessed the reproductive outcomes of women who underwent laparoscopic resection of bladder endometriosis [30]. A partial cystectomy was performed for lesions that extended into the mucosa. In all the other cases, deep excision of the detrusor muscle was performed. During surgery, all endometriotic lesions were treated. The

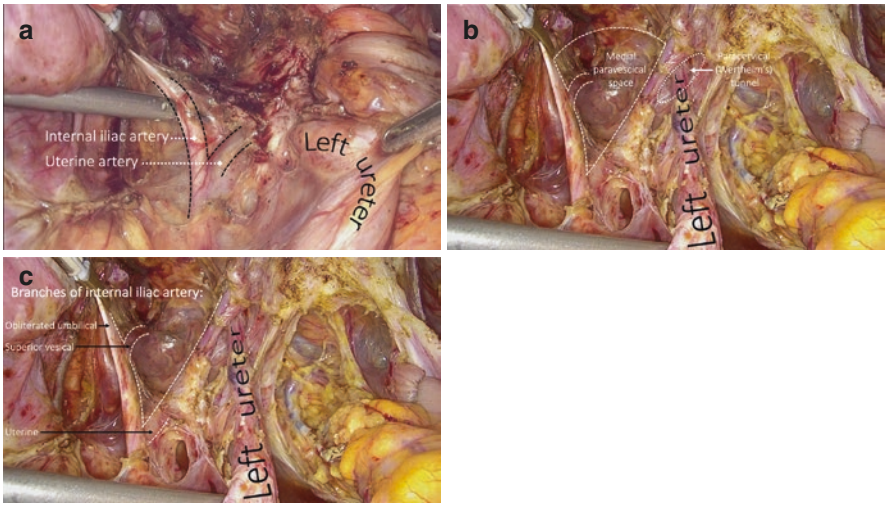

Fig. 3 (**a**) Periureteral nodule. (**b**, **c**) Ureter after excision of periureteral nodule

minimum follow-up after surgery was 36 months. Sixty-nine patients were included in this study. Of the 42 patients who wished to conceive, 35 patients (83.3%) conceived: 16 patients spontaneously (47%) and 18 patients after IVF (53%). No difference was observed in fertility outcome between patients treated by partial cystectomy and those treated by partial-thickness excision of the detrusor muscle.

Since ureteral endometriosis is often associated with other DE localizations, it is difficult to evaluate its independent effect on fertility [31] (Fig. 3). A retrospective analysis of prospectively collected data by Uccella et al. analyzed the fertility outcomes of 36 women who wished to conceive after laparoscopic ureterolysis for DE [32]. Twenty-six pregnancies were registered in 20 women (55.6% of patients who wanted to conceive after surgery); 6 of these women conceived by ART. Sixteen patients did not conceive despite their reproductive desire.

1.3 Surgery for Deep Endometriosis after Infertility Treatments

Few studies investigated the role of surgical treatment of DE in patients who previously failed infertility treatments.

An American retrospective case series reported the pregnancy rate in patients with previous IVF failure who underwent laparoscopic treatment of DE [33]. Twenty-nine patients were included in this study. Twenty-two patients conceived after surgery. Twelve patients conceived spontaneously and two with clomid/IUI; time to conception in these 14 patients ranged from 1 to 8 months after surgery. Seven patients conceived with additional IVF after surgery.

More recently, a French retrospective study investigated the pregnancy rates after surgical treatment of DE in infertile patients who failed at least 2 IVF/ICSI cycles [34]. Seventy-three patients were included in the analysis; the mean age of patients was 31.9 years, and the mean length of infertility was 48.4 months. The postoperative pregnancy rate was 43.8% (32/73), with a mean time from surgery to pregnancy of 11.1 months. Twenty-two percent of the pregnancies were spontaneous. Non-pregnant women had significantly more lesions involving the sigmoid colon and the rectum; endometrioma surgery was performed more frequently in the non-pregnant group. Multivariate analysis identified three variables associated with a lower probability of conception: ovarian surgery, age $\geq$ 35 years old and minor endometriosis stage. The management of colorectal DE and associated male infertility did not significantly impact the possibility of conception.

2 Conclusion

The decision to perform an excision of DE to improve fertility is highly debated. Ideally, firm evidence, preferably from randomized controlled studies, would be needed to offer surgery to women with DE. In these patients, the effectiveness of surgery as a fertility-enhancing procedure should be assessed on the rates of natural conception. Ideally, the best design to quantify the effect size of this type of surgery is a randomization to surgery (experimental arm) versus expectant management (control arm), considering the natural conception rate as the primary study objective. With this design, the incremental benefit of surgery over the background probability of conception could be quantified. However, RCTs are challenging to perform in this field. Patients with pain and a desire to become pregnant will be reluctant to leave the important decision about surgery to chance [35].

Most of the available data in this setting are based on retrospective or prospective observational studies. A recent Cochrane meta-analysis showed that the beneficial effects of surgery are too modest to justify the procedure, at least in women without pain symptoms [36]. Most uncontrolled published studies reported a spontaneous pregnancy rate after surgery for DE of approximately 40–50%. The long-term follow-up of patients included in the ENDORE RCT provided encouraging results on the impact of surgery for DE on fertility [25]. Forty-seven percent of the patients conceived spontaneously after surgery. Furthermore, in patients with preoperative infertility, the spontaneous pregnancy rate was 39.1%.

Most of the published studies have several limitations. The baseline fertility status of the patients is often not appropriately defined, and women who did not try to conceive before surgery are included in the analysis [21]. In some studies, it is difficult to discriminate between spontaneous conception and that resulting from ART; therefore, it is difficult to attribute conception exclusively to surgery [21]. Furthermore, it is impossible to discriminate the effect on fertility due to the treatment of DE and that due to the treatment of ovarian endometriotic cysts/superficial peritoneal implants. Only one study compared the incidence of spontaneous

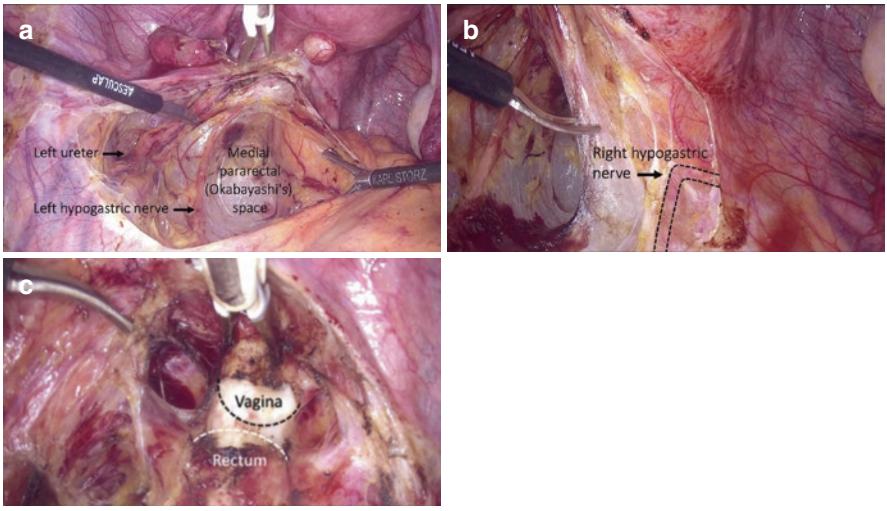

Fig. 4 Excision of deep endometriosis performed by experienced surgeons. (**a**) Identification of the anatomic structures in the pararectal space. (**b**) Identification of the right hypogastric nerve. (**c**) Dissection of the rectovaginal space

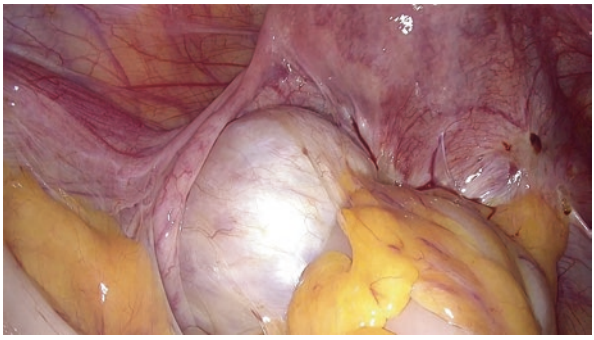

Fig. 5 Infertile woman with ovarian left endometrioma and deep endometriosis

pregnancies in women who underwent excision of DE versus patients receiving only intraperitoneal surgery, the results showed similar pregnancy rates in the two study groups [14]. Finally, publications are likely to be performed by referral centers with highly experienced surgeons particularly concerned with conserving patients' ability to conceive after surgery (Fig. 4). Thus, these results might not easily be reproducible. Lower pregnancy rates may occur if less experienced surgeons perform surgery without complete excision of DE [18].

Most infertile women with DE can be managed with either surgery or in vitro fertilization (IVF). If pain symptoms are tolerable, IVF may be offered instead of surgery. Primary IVF has the advantages of shortening the delay in conception, preventing the risk of postoperative ovarian reserve impairment, and the negative impact of potential postoperative complications. However, IVF may have a higher

cost, and unoperated patients may face some peculiar additional risks during the procedure and pregnancy (such as bowel occlusion or subocclusion, endometrioma rupture, and spontaneous hemoperitoneum during pregnancy) [37, 38]. Unbearable pain in women wanting a spontaneous pregnancy and refusing IVF still constitutes an indication for excisional treatment of DE in infertile subjects. Surgery must also be performed in patients with bowel and ureteral stenosis. In general, surgery has the advantage of relieving pain symptoms but has the disadvantage of potentially decreasing ovarian reserve in patients with concomitant endometriomas (Fig. 5). Furthermore, surgery for DE carries a substantial risk of significant complications, mainly when bowel surgery is performed. Some complications (such as a pelvic abscess) may theoretically impair fertility. However, a retrospective study showed that the pregnancy rate among women who wished to conceive after a severe complication of surgery for colorectal endometriosis was 41.2% (spontaneously for 80%, after ART procedure for 20%) [39].

Infertile patients with DE must be carefully informed on the actual probability of postoperative spontaneous conception avoiding general overestimations, and of the potential morbidity associated with the proposed intervention [21]. If surgery is performed, the procedure must be completed by laparoscopy to increase the chance of spontaneous conception [20]. In general, the type of treatment should be tailored to the patient considering the intensity of pain symptoms, history of previous surgery, women's age, ovarian reserve, presence of large endometriomas, tubal patency, and semen analysis.

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Intrauterine Insemination in Women with Endometriosis

Simone Ferrero, Umberto Leone Roberti Maggiore, and Luca Bernardini

1 Intrauterine Insemination in Endometriosis

Intrauterine insemination (IUI) is a noninvasive first-line assisted conception technique that involves depositing a processed semen sample in the upper uterine cavity. Controlled ovarian stimulation, particularly with low-dose gonadotropins, with IUI offers significant benefit in terms of pregnancy outcomes compared with natural cycles or timed intercourses while reducing complications associated with controlled ovarian stimulation (such as multiple pregnancy and ovarian hyperstimulation syndrome). With or without ovarian stimulation, IUI has been widely used to treat endometriosis-related infertility.

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1.1 *Efficacy of IUI in the Treatment of Endometriosis-Related Infertility*

In the past, several studies investigated the effectiveness of IUI in treating endometriosis-related infertility. However, most of these studies have limitations such as retrospective design and small sample size (Table 1). Some studies suggested that women with endometriosis have lower pregnancy rates than those with other infertility disorders [1–3].

An Italian randomized trial including 49 women with a laparoscopic diagnosis of revised American Fertility Society (rAFS) stage I/II endometriosis and infertility compared three cycles of ovarian stimulation combined with IUI (24 patients) with six months of expectant management (25 patients). The pregnancy rate per cycle was 14.8% in the patients treated with IUI and 4.5% in the untreated group [4]. Subsequently, a randomized controlled trial evaluated the efficacy of IUI combined with ovarian stimulation versus no treatment in infertile women with stages I–II endometriosis. Three hundred and eleven cycles were performed in 103 couples. Live birth followed 14 of 127 (11%) superovulation and lUI cycles and 4 of 184 (2%) no-treatment cycles. The live birth rate was 5.6 times higher in the treated couples than in the couples who tried to conceive spontaneously (95% C.I., 1.8–17.4) [5]. Another randomized study compared IUI combined with ovarian stimulation with urine LH-timed IUI alone in 57 couples with minimal or mild endometriosis. The biochemical pregnancy rate was 5.1 times higher in patients treated with IUI

Table 1 The pregnancy rate in patients with endometriosis treated with IUI

		Number of patients	Pregnancy rate per patient (%)	Pregnancy rate per cycle (%)
Yovich and Matson [1]	Stages I–II	33	15.2	7.7
	Stages III–IV	23	8.7	4.1
Fedele et al. [4]	Stages I–II	24		14.8
Omland et al. [2]	Stages I–II	49	16.3	
Nuojua-Huttunen et al. [19]	Stages I–II			6.5
Tavmergen Göker et al. [20]	Stages I–II	39	5.1	
	Stages III–IV	17	23.5	
Lodhi et al. [21]	Stages I–II	55	50.9	29.4
	Stages III–IV	14	35.7	31.3
Werbrouck et al. [8]	Stage I	41		21.0
	Stage II	17		18.9
Monsour et al. [3]		114	41.2	14.3
Van der Houwen et al. [9]	Stages III–IV	65	23.1	6.1
Cai et al. [11]	Women with endometrioma	56	14.3	9.2
Zhang et al. [15]	Stages I–II pretreated with GnRH-a	41		15.3
	Stages I–II	56		11.8

and ovarian stimulation than in those treated with IUI alone (95%CI 1.1–22.5) [6]. A prospective cohort study compared the outcomes of IUI combined with ovarian stimulation in 49 patients with stages I–II endometriosis and 119 with unexplained infertility. The pregnancy rate was significantly higher for patients with unexplained infertility (33.3%) than for those with endometriosis (16.3%). Furthermore, an increased number of multiple gestations was obtained in the unexplained infertility group resulting in a significantly higher implantation rate per cycle compared with the endometriosis group [2].

A Mexican study compared the efficacy of IUI in patients with stages I–II endometriosis, stages III–IV endometriosis, and without endometriosis. The pregnancy rate per cycle was significantly lower in patients with stages III–IV (5.6%) than in those with stages I–II (22.7%) and in those with unexplained infertility (25.7%) [7]. A retrospective study investigated the pregnancy rate after controlled ovarian hyperstimulation in patients submitted to surgical excision of stage I ($n = 41$) or stage II ($n = 17$) endometriosis. There was no significant difference in the clinical pregnancy rate per cycle between patients with stage I (21.0%) and stage II (18.9%) endometriosis. Furthermore, the clinical pregnancy rate per cycle was similar in patients with stages I–II endometriosis and those with unexplained infertility (20.5%). Also, the cumulative live-birth rate was similar in patients with stage I endometriosis (70.2%), stage II endometriosis (68.2%), and unexplained infertility (66.5%) [8]. A retrospective study investigated the efficacy of IUI in women with surgically confirmed American Society for Reproductive Medicine (ASRM) stages III–IV [9]. Sixty-five patients receiving 245 IUI treatment cycles were included in the study. In 69.2% of patients, IUI was performed without ovarian stimulation in the first three cycles, followed by IUI with ovarian stimulation; 30.8% of the patients were immediately treated with IUI with ovarian stimulation. Fifteen pregnancies were accomplished after IUI. Significantly higher pregnancy rates were found in patients assigned to IUI with ovarian stimulation than those assigned to three IUI with natural cycle followed by IUI with ovarian stimulation. A prospective non-randomized study compared IUI combined with controlled ovarian stimulation and expectant management in surgically treated endometriosis patients. Two hundred and eighteen patients underwent laparoscopy, and 20 patients underwent laparotomy. One hundred patients (42.0%) had stages I–II endometriosis, and 138 had stages III–IV endometriosis (58.0%). The cumulative pregnancy rate was significantly higher in patients treated with IUI (53.4%) than those without treatment (38.5%). Similarly, a significant difference was observed in the live birth rates (48.3% versus 34.2%). The cumulative pregnancy rate was significantly higher in patients with endometriosis stages I–II (64.6%) than in those with endometriosis stages III–IV (45.6%) [10]. A recent retrospective study using propensity matching analysis evaluated the efficacy of IUI in women with endometrioma-associated infertility [11]. IUI was performed in natural or stimulated cycles. Fifty-six women with endometrioma were matched to 173 women with unexplained infertility. For women in the endometrioma group, 39.3% had undergone prior surgery for endometriomas before IUI treatment, and the remaining 60.7% were diagnosed based on ultrasonography. The 56 women from the endometrioma group underwent 87 cycles of IUI (45 natural cycles and 42 cycles with stimulation). The 173 women with unexplained infertility underwent 280 cycles of IUI (152 natural cycles and 128 cycles with stimulation).

The per-cycle clinical pregnancy rate was lower in women with endometrioma than those with unexplained infertility, though this was of borderline statistical significance (9.2% vs. 17.9%, $p = 0.06$). The subgroup analyses of IUI with or without stimulation also yielded comparable results. Compared with natural cycles, IUI with stimulation cycles seemed to result in a slightly higher pregnancy rate per cycle in the group with endometriomas (11.9% vs. 6.7%, $p = 0.40$), though the differences were not significant. No significant difference between the two strategies was observed in the subset of unexplained subfertility (18.8% vs. 17.1%, $p = 0.72$). All pregnancies occurred within the first two cycles of the IUI program for women with endometrioma-associated subfertility. Among women with endometriomas, there were no differences in the size and number of unilateral or bilateral endometriomas between women who conceived and those who did not. Women who had surgical removal before IUI had similar cumulative pregnancy rates to those without surgical treatment (13.6% versus 14.7%).

A systematic review with meta-analysis evaluated the efficacy of IUI in patients with stages III–IV endometriosis. Nineteen studies were included in the analysis. The calculated weighted mean clinical pregnancy rate was 13.4% (95% CI, 7.4%–19.4%) per treatment cycle and 32.7% (95 C.I., 21.3%–44.0%) per patient. Nine studies reported on live births. The calculated weighted mean live birth rate per cycle and per patient was 5.6% (95% C.I., 3.0%–8.2%) and 20.3% (95% C.I., 11.2%–29.4%) [12].

2 Pretreatment with Gonadotropin-Releasing Hormone Analogs before IUI

Hormonal suppression had been proposed before infertility treatment to decrease the levels of proinflammatory cytokines and abnormal oxidation damage to ovarian follicles and to improve implantation rates. However, few studies investigated the role of hormonal suppression before IUI in patients with endometriosis.

Rickes et al. investigated the role of pretreatment with gonadotropin-releasing hormone analogs (GnRH-a) before IUI in 63 patients with ASRM stages II to IV endometriosis. Patients received monthly goserelin over 5 or 6 cycles. Pregnancy rates were significantly higher in patients treated with GnRH-a ($n = 27$; 89%) than in those who did not receive the treatment ($n = 22$; 61%) [13]. In a randomized controlled trial, Kim et al. compared standard down-regulated IUI cycles (2 weeks GnRH-a) versus an ultralong down-regulated IUI cycle (6 weeks GnRH-a). The clinical pregnancy rate per cycle was significantly higher in the ultralong protocol (48.7%) than in the long protocol (26.8%). The miscarriage rates were 21.1% in the ultralong protocol group and 18.2% in the long protocol group. There was no significant difference between the two groups concerning clinical pregnancy rate per cycle in patients with stage I or II endometriosis. In patients with stage III or IV endometriosis, the clinical pregnancy rate per cycle was significantly higher in the ultralong protocol group than in the long protocol group [14]. Van der Houwen et al. investigated the role of long-term pituitary down-regulation with GnRH agonist

before IUI in patients with surgically confirmed stages III–IV endometriosis treated by IUI; 31 patients underwent pretreatment and 34 directly started IUI treatment. Long-term pituitary down-regulation with GnRH agonist before the first IUI cycle resulted in nonsignificantly higher odds of achieving an ongoing pregnancy: 35.0% in treated patients compared with 21.3% in women who directly started with IUI treatment [9]. A retrospective study investigated the effectiveness of GnRH-a therapy before IUI in patients with endometriosis. All patients had a previous laparoscopy demonstrating ASRM stage I or II endometriosis and two patent fallopian tubes. Forty-one patients received 3.6 mg of goserelin every four weeks for 1–3 cycles. Fifty-six patients did not receive pretreatment with GnRH. The clinical pregnancy rate was significantly higher in patients treated with GnRH-a than in those that did not receive the treatment (15.3% vs. 11,8%). There was a trend for a higher live birth rate in patients who received the treatment with GnRH-a (12.9%) than in those who did not (10.0%), but the difference did not reach statistical significance. Patients pretreated with GnRH-a had a similar incidence of multiple pregnancies, miscarriage, and ectopic pregnancy [15].

3 Safety of IUI in Women with Endometriosis

Physicians and patients may be reluctant to perform IUI directly with ovarian stimulation due to the fear that ovarian hyperstimulation may increase the risk of endometriosis progression or recurrence after surgery. Limited data are available on the recurrence of endometriosis after IUI combined with controlled ovarian stimulation [12].

A retrospective cohort study tested the hypothesis that the cumulative endometriosis recurrence rate after fertility surgery of stage III or IV endometriosis is increased in women exposed to very high estradiol levels during ovarian stimulation for IVF compared with a control group of women exposed to less high estradiol levels during ovarian stimulation for IUI. The study included 67 patients with endometriosis stage III or IV who underwent laparoscopy and subsequently started fertility treatment with either IVF only ($n = 39$), both IVI and IUI in different cycles ($n = 11$), or IUI only ($n = 17$). At 21 months after the start of ovarian stimulation, the overall cumulative endometriosis recurrence rate was significantly lower in patients treated with IVF only (7%) or in women treated with both IVF and IUI in different cycles (43%) than in those treated with IUI only (84%). The median peak estradiol values were significantly lower in the patients treated with IUI than in those treated with IVF only or with both IVF and IUI in different cycles. There was no correlation between the cumulative peak estradiol per patient and the recurrence of endometriosis [16].

Van der Houwen et al. investigated the safety of ovarian stimulation in 65 patients with surgically confirmed stages III–IV endometriosis treated by IUI. Recurrence of endometriosis was defined as a recurrence or increase in patients' complaints within 12 months after the last IUI treatment attempt. The cumulative endometriosis

recurrence rate was 36.5% for patients treated with three IUI with natural cycle followed by IUI with ovarian stimulation, and it was 72.3% in patients treated with IUI with ovarian stimulation. Surprisingly a significantly higher 12-month cumulative endometriosis recurrence rate was found in patients treated with GnRH agonist before the first IUI cycle versus patients without GnRH agonist pretreatment [9].

4 Conclusions

Several studies reported pregnancy rates after IUI with or without superovulation in women with endometriosis. However, it is difficult to summarize the finding of these studies because some studies included patients who had only diagnostic laparoscopy, others included patients who underwent surgical excision of endometriosis, and in other studies, it is unclear whether patients underwent surgical treatment of endometriosis. Furthermore, some studies included women with unexplained infertility, some presumed to have minimal endometriosis [17].

Compared with expectant management, IUI effectively improves fertility in women with stages I–II endometriosis [5, 18]. Furthermore, IUI preceded by controlled ovarian hyperstimulation in these patients results in higher pregnancy rates than IUI alone [6]. IUI may be less efficacious in patients with stages III–IV than those with stages I–II [7], possibly because the anatomic distortion caused by endometriosis may impair fertility. A systematic review with meta-analysis found that, in patients with stage III-IV endometriosis, the clinical pregnancy rate per IUI cycle is 13.4%, while the clinical pregnancy rate per patient is 32.7%. The authors concluded that IUI could be a possible treatment in patients with stages III–IV endometriosis [12]. However, IUI's role in managing patients with stages III–IV endometriosis remains unclear because of the limited available data obtained from retrospective studies with small sample sizes [18]. The recent European Society of Human Reproduction and Embryology (ESHRE) guidelines state clinicians may perform IUI with ovarian stimulation instead of expectant management or IUI alone in patients with rASRM stage I/II endometriosis. IUI with controlled ovarian stimulation could be considered in patients with rASRM stage III/IV with tubal patency, although the value of this treatment is uncertain [18].

Limited data on the role of hormonal suppression of endometriosis before IUI are available. One small-sized randomized controlled trial suggested that pretreatment with GnRH-a improves pregnancy rates in patients with stages III–IV endometriosis [14]. However, the potential improvement in pregnancy rates must be balanced with the adverse effects of treatment and the delay in the treatment, which is particularly relevant in patients with poor ovarian reserve. Therefore, pretreatment with GnRH-a cannot be recommended before IUI, also considering this treatment's relatively low success rate [18].

In general, the choice of performing IUI in women with endometriosis should be based on several variables, including the age of the patients, the ovarian reserve, the severity of endometriosis, and the potential distortion of adnexal anatomy, the

quality of the semen, the preference of the patients. Future studies should investigate the effectiveness of IUI in patients with endometriosis according to the Endometriosis Fertility Index.

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Hormonal Therapies before In-Vitro Fertilization in Women with Endometriosis

Antoine Naem and Antonio Simone Laganà

1 Introduction

Endometriosis occurs mostly in women of the childbearing age [1], and affects almost all aspects of the patients' life [2]. Although it could be asymptomatic in a considerable proportion of patients [3], pain symptoms—like dysmenorrhea and dyspareunia—are the most commonly reported complains [4]. Endometriosis-related infertility is another debilitating sequel of endometriosis that affects approximately 25–50% of patients [5]. Additionally, an overall of 50% of infertile patients were found to have endometriosis [6]. It was estimated that couples with endometriosis are 3 times less likely to conceive spontaneously each month when compared to healthy controls [7]. The exact mechanism by which endometriosis affects the female fertility remains to be elucidated. However, current evidence indicates that endometriosis-associated infertility is multifactorial. Endometriosis was shown to impair folliculogenesis, oocytes quality, and embryogenesis [8, 9] through proinflammatory environment, oxidative stress, and the accompanying hormonal dysregulations [10–12]. It was demonstrated that patients with endometriosis have an increased number of activated macrophages in the peritoneal cavity [13]. Hyperactivated macrophages in turn mediates the fibroblasts recruitment through increased secretion of growth factors and cytokines [14]. Indeed, patients with endometriosis were found to have increased intraperitoneal concentrations of IL-6, IL-8, IL-10, IL-12, and tumor

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necrosis factor-α (TNF-α) [15–17]. Furthermore, these patients were shown to have increased intraabdominal iron deposits and heme concentrations due to a predisposing insufficiency in the peritoneal detoxifying system [18]. Increased intraperitoneal heme concentration in patients with endometriosis could aggravate the oxidative stress, and lead to further activation of macrophages [19, 20]. Adhesiogenesis and fibrosis are well-known consequences of the chronic intrapelvic inflammation that distort the normal pelvic anatomy and physiology [21]. Patients affected by endometriosis were found to have abnormal tubal transport [22], increased uterine peristalsis [23], insufficient physiologic changes in the spiral arteries, and abnormal placentation [24]. Ovarian reserve is also influenced by endometriosis [25]. Patients who have ovarian endometriomas were found to exhibit an accelerated rate of follicular loss [26, 27], and decreased follicular density in the area surrounding the cyst [28, 29]. Moreover, the follicular fluid of patients with endometriosis was found to contain elevated levels of reactive oxygen species (ROS) and cytokines [30]. In addition, impaired follicular steroidogenesis due to decreased aromatase expression in the granulosa cells of patients with endometriosis was also reported [31]. In contrast, the eutopic endometrium of endometriotic patients express higher aromatase enzymes, unlike the endometrium of their healthy comparators [32, 33]. It is noteworthy that the normal mid-cycle increase in the expression of the homebox A10 (HOXA10) gene—a gene closely related to integrin expression and implantation [34, 35]—is absent in patients with endometriosis [36]. These complex and interwoven mechanisms harden the management of endometriosis-related infertility. Although surgery could restore the normal pelvic anatomy and excise the endometriotic lesions, it remains unable to reverse the hostile peritoneal microenvironment and to improve the immune homeostasis within the peritoneal cavity [37, 38]. The spontaneous pregnancy rate following surgery for endometriosis was reported to be 24.8–58.6% [39, 40]. On the one hand, it was estimated that 25 therapeutic laparoscopies should be performed to achieve one live birth in patients with endometriosis [41]. On the other hand, surgery itself could have a detrimental effect on fertility by impairing the ovarian vasculature [42], and provoking a thermal injury to the healthy ovarian tissue surrounding the cyst [43]. Furthermore, the lack of a cleavage plane in ovarian endometriomas impose an increased risk of resecting normal ovarian tissue when the endometrioma stripping technique is applied, as shown by various studies [44, 45].

For these reasons, in-vitro fertilization-embryo transfer (IVF-ET) is now widely used to optimize the fertility outcomes of patients with endometriosis. IVF-ET helps in overcoming the hostile inflammatory microenvironment, and therefore, avoiding the detrimental impact of endometriosis on the oocytes, sperms, and the fertilized oocyte. However, reports on outcomes of IVF-ET cycles demonstrate decreased ovarian responsiveness and poorer outcomes in patients with endometriosis in comparison with tubal factor infertility patients [46]. It was estimated that patients with endometriosis are 35% less likely to conceive after an IVF-ET cycle. These patients also have significantly decreased fertilization rates, implantation rates, and lower number of retrieved oocytes. Patients with stages III and IV endometriosis according to the revised classification of the American Society for Reproductive Medicine (r-ASRM) [47] have poorer outcomes than patients with

stages I and II endometriosis [46]. These deteriorated results indicate that the real cause of the endometriosis-related infertility is beyond anatomy, and the detrimental effects of endometriosis on oocytes and embryos may presist, even when assisted reproductive technology is used. While surgery and ovarian suppressive medications are successfully used in treating the endometriosis pain symptoms [48], their use for optimizing the IVF-ET outcomes remains controversial. Surgical excision or ablation of superficial endometriotic lesions is expected to have minimal effect on the outcomes of IVF-ET cycles, if any [49]. Additionally, a recent Cochrane review indicated that surgical resection of ovarian endometriomas has no clear benefits on IVF-ET outcomes [50]. A more recent meta-analysis also demonstrated that surgical excision of ovarian endometriomas has no benefits on IVF-ET outcomes in terms of the number of retrieved oocytes, clinical pregnancy, and live birth rates [51]. Conversely, endometrioma cystectomy exposes patients to an increased risk of lowering their ovarian reserve, and responsiveness to controlled ovarian stimulation (COH) protocols [52]. Nevertheless, the sole surgical approach that has an established benefit on the IVF-ET outcomes is the resection of hydrosalpinges whenever they are found [53].

On this basis, there was a general tendency toward using hormonal suppressive therapies prior to IVF-ET cycles in order to improve fertility outcomes. Prolonged ovarian suppression was generally accepted as an efficient mean for improving IVF-ET outcomes in patients with endometriosis, especially when considering its excellent safety profile [54, 55]. The most commonly used pharmacological drug groups in endometriosis-related infertility management are the Gonadotropin-releasing hormone (GnRH) analogs, progestogens, and aromatase inhibitors. GnRH analogs suppress ovulation through pituitary desensitization, leading to a decrease in the secretion of endogenous gonadotropins, and eventually decreasing the circulating endogenous ovarian estradiol [56]. Since endometriosis is estrogen-dependent, lesion remission is achieved with this hypoestrogenic status. Progestins, such as medroxyprogesterone acetate (MPA) and dienogest, induce decidualization of the endometriotic lesions, leading to their atrophy and remission [57]. Moreover, increased exposure to dienogest induces the down-regulation of endometrial estrogen receptors-β (ER-β), and the up-regulation of estrogen receptors-α (ER-α) and restoring the normal balance between the progesterone receptor isoforms [58]. A similar effect of MPA on the progesterone receptors was also reported [59]. Therefore, synthetic progestins could restore the normal balance between the estrogen and progesterone receptors in the eutopic endometrium.

Aromatase inhibitors are newly introduced agents that inhibit the function the cytochrome P450 aromatase, which is a key enzyme in the biosynthesis pathway of estrogen [60]. On the one hand, these agents are mostly used as an add-back therapy to GnRH analogs to induce a deeper hypoestrogenic environment [33]. On the other hand, aromatase inhibitors could cause a rebound increase in Follicular-Stimulating Hormone (FSH) and lead to the formation of ovarian cyst [61]. Aromatase inhibitors interfere with the extra-ovarian synthesis of estrogen in the adrenal glands and skin. In addition, aromatase inhibitors could suppress the aberrant aromatase activity in the endometriotic implants [62]. Therefore, these could break a substantial positive

feedback loop for the endometriosis activity that consists of aromatase, estrogen, cyclo-oxygenase-2 (COX-2), and prostaglandin (PG) E2 [62].

In this chapter, we aimed to review the current knowledge and available clinical evidence regarding the use of each of these hormonal treatments before IVF-ET cycles in patients with endometriosis, while taking in consideration their efficacy, tolerability, and their associated adverse effects. We also aimed to discuss the possible mechanisms by which these treatments could affect the female fertility and IVF-ET outcomes.

2 GnRH Analogs and the Endometriosis-Related Infertility

2.1 Overview of the GnRH Analogs Uses and Mechanism of Action

GnRH agonists were studied for a considerable period of time, and their efficacy in managing the endometriosis-related pain was proven in several reports [63, 64]. Furthermore, the GnRH analogs are being used regularly in ovarian stimulation protocols for infertile patients undergoing IVF-ET cycles. These agents can guarantee a synchronized follicular growth and development [65]. There are two types of GnRH analog protocols used in ovarian stimulation. The first is the short protocol, where small doses of GnRH analogs are given to the patient in the second or third day of the follicular phase of the stimulation cycle. The second one is the long protocol, where small amounts of GnRH analogs are started in the mid-luteal phase of the menstrual cycle preceding the IVF-ET cycle directly. However, a special protocol for patients with endometriosis was suggested over 30 years ago, which is the ultralong or prolonged GnRH agonist protocol, where GnRH agonists such as leuprolide, triptorelin, or goserelin, are administered for at least 2–3 months before starting the ovarian stimulation. In this chapter, we will refer to the last protocol with "the ultralong protocol," to avoid confusion.

The use of the ultralong GnRH agonist protocols goes back to 1990, when Dale et al. [66] administered buserelin for 4–6 months to treat the endometriosis-related infertility in two patients. The rationale behind administering GnRH agonists for a long period is achieving a prolonged and deeper suppression of the pituitary gland, which will lead subsequently to hypogonadotropic hypogonadism. Therefore, the ovarian steroidogenesis and ovulation will be suppressed, and thus, creating a hypoestrogenic status. The decreased estrogen concentration will result in the remission of endometriosis and associated intra-pelvic inflammation [4]. The ultralong administration of GnRH agonists is thought to neutralize the peritoneal inflammation through decreasing IL-1, and other inflammatory cytokines in the peritoneal fluid [67]. This in turn will be reflected in a better follicular microenvironment where less inflammation, and oxidative stress are present. In fact, patients that were treated with buserelin for 3 months had significantly lower TNF-α and

8-hydroxy-2′ deoxyguanosine (8-OHdG) in their follicular fluid. Moreover, a significant increase in the follicular fluid melatonin was observed in these patients [68]. Melatonin is a free-radical scavenger and anti-oxidant that tends to protect the oocytes from the ROS within the follicles [69, 70]. It was hypothesized that the increased levels of TNF-α may decrease the melatonin concentration, resulting in oocytes and embryos of impaired quality [68]. GnRH agonists were also proven to decrease the endometriotic nodules size and vascularization [71]. Furthermore, the prolonged administration of GnRH agonists resulted in an increase in the proapoptotic molecules, and a decrease in the anti-apoptotic activity, which eventually induce the endometriotic cells apoptosis [72]. Indeed, prolonged administration of GnRH agonists was postulated to improve the endometrial receptivity in many ways. The prolonged medically-induced amenorrhea is thought to increase the endometrial receptivity. It was reported that prolonged administration of GnRH agonists can increase the endometrial pinopodes, and restore the $\alpha_v\beta_3$ vitronectin expression [73, 74]. Surrey et al. [75] demonstrated that patients with negative $\alpha_v\beta_3$ vitronectin could have IVF-ET outcomes similar to patients with positive $\alpha_v\beta_3$ vitronectin expression when treated with depot–leuprolide for 2 months. Moreover, the suppressed inflammation and subsequent decrease in the cytokines concentrations could improve the endometrial receptivity, since these were found to interfere with implantation [76, 77]. GnRH agonists were also found to inhibit the P450 aromatase in the eutopic endometrium due to the hypoestrogenic state [78]. This could also contribute to the increased receptivity since increased P450 aromatase mRNA expression in the endometrial cells was found to interfere with implantation [79]. In addition, it was suggested that GnRH agonists may have a direct effect on the luteinized ovarian granulosa cells. This conclusion was mainly led by the facts that some patients develop functional cysts even when GnRH agonists are being taken [80, 81], and the isolation of high affinity GnRH receptors in the granulosa cells [82]. However, the clinical relevance of these observations is yet to be determined.

Despite the established and the proposed benefits of the ultralong GnRH agonist protocols, their implications in the clinical practice remain controversial. On the one hand, the prolonged use of GnRH agonists is not tolerable by all patients due to the menopausal symptoms, like hot flashes, vaginal dryness, decreased libido, and bone loss [48]. On the other hand, excessive suppression by the ultralong protocol could impair the ovarian responsiveness to human menopausal gonadotropins (HMG) or recombinant Follicular-Stimulating Hormone (r-FSH), and reduce the oocytes quality [83]. Moreover, the live birth rate was found to decrease with the increasing dosage of r-FSH [84]. In the following paragraph, we will review the available pieces of evidence about the use of the ultralong protocol in patients with endometriosis.

2.2 The Ultralong GnRH Agonist Protocol and IVF-ET Outcomes of Patients with Mild, Moderate, and Severe Endometriosis

In 2006, Sallam et al. [85] published a systematic review investigating the feasibility of the prolonged ovarian suppression using GnRH agonists in patients with endometriosis. The authors concluded that 3–6 months of suppression using GnRH agonists could increase the odds of the live birth rates, and pregnancy rates by nine and four folds, respectively. However, this review was subject to many criticisms because it only included 3 trials [86–88], and the live birth rate was concluded depending on only one study [86]. More recently, another Cochrane review conducted by Georgiou et al. [89] was published. The latter review analyzed the data of 640 patients which was extracted from 9 clinical trials. Conversely, this review concluded that prolonged GnRH agonists administration may decrease the live birth rate by 52%. Moreover, the clinical pregnancy rate, mean number of retrieved oocytes, and mean number of the resulting embryos were found to be unaffected by the ultralong protocol [89]. The great discrepancy found between these two reviews are worth questioning. By taking a closer look to the methodologies followed by Sallam et al. [85] and Georgiou et al. [89], a part of the disagreement could be resolved. The different strategies in dealing with missing data could account for the different results. Georgiou et al. [89] followed the famous quote "What is not documented, is not done," and considered every unreported data as a negative finding when examining the outcomes. In contrast, Sallam et al. [85] considered all the viable pregnancies reported by Dicker et al. [86] ended in live births, and based their conclusions solely on the results of the later study. It is noteworthy that Georgiou et al. [89] excluded the previously mentioned study [86] from the birth rate analysis because it is does not fit with the Zegers-Hochschild definition of the live birth [90]. Both Cochrane reviews [85, 89] concluded the effect of the ultralong GnRH agonist protocol on live birth rate on the basis of one study, that differed in each analysis [86, 91]. Moreover, the study of Dicker et al. [86] consisted of patients with severe endometriosis only, unlike the study of Rodríguez-Tárrega et al. [91] that included patients with different disease severity. In line with the latter study, Kaponis et al. [92] demonstrated in their multicentric randomized trial that patients with mild-to-moderate endometriosis did not benefit from the ultralong treatment. In both studies, the fertilization rate was higher in patients treated with ultralong protocol [91, 92]. Another randomized trial conducted by Decleer et al. [93] investigated the potential benefits of 3-month regimen of goserelin in patients with peritoneal endometriosis. Similarly, there was no statistically significant difference in the mean number of M-II oocytes and the pregnancy rate were similar in both groups. On the contrary, prolonged suppression had caused decreased ovarian responsiveness to gonadotropins stimulation, since all three studies reported higher HMG/FSH doses, and longer stimulation period in patients treated with the ultralong protocol [91–93]. This is especially true for patients with impaired ovarian reserve after endometrioma cystectomy, as reported by Zhao and their colleagues [94]. In their study,

significantly longer ovarian stimulation was observed in patients who received the prolonged triptorelin suppression protocol, and comparable pregnancy rates to patients who received the regular long protocol [94]. Nonetheless, Surrey et al. [88] reported that the ongoing pregnancy rate was higher in patients who received a 3-month suppression with depot–leuprolide, whereas the dosage and duration of administration of HMG did not differ significantly between the two groups. It should be noted that the previous study included only 51 patients in the final analysis, 25 of them in the treatment group, and 21 out of 25 had severe endometriosis [88]. From a different perspective, Prasad et al. [95] suggested that each r-ASRM stage of endometriosis should be treated with different durations of GnRH agonist protocol administration. The authors suggested that patient with stage I endometriosis should be treated with a conventional short GnRH agonist protocol, while patients with stage II endometriosis should be treated with a single dose of the long-acting depot GnRH agonist prior to ovarian stimulation; conversely, patients with stage III and stage IV endometriosis should be treated with two and three doses of depot GnRH agonist, respectively. This strategy was reported to achieve similar IVF-ET outcomes to those of patients with tubal factor infertility [95]. In line with the previous observations, Rickes et al. [87] reported no significant benefit from using the ultralong protocol in patients with stage II endometriosis. Whereas patients with stage III/IV endometriosis treated with the ultralong protocol had a pregnancy rate almost 2 times greater than the pregnancy rate of the untreated comparators [87]. As noticed, it is becoming clearer that ultralong GnRH agonist protocols are of limited effectivity in mild cases of endometriosis. However, the situation is more controversial for patients with more severe disease. Van der Houwen et al. [54] investigated the potential benefits of administering depot–leuprolide acetate in patients with stage III/IV endometriosis compared to no treatment. Ongoing pregnancy rates after fresh embryo transfers were similar between groups. Conversely, ongoing pregnancy rates were higher in patients treated with the ultralong protocol. Similarly, Surrey et al. reported improved reproductive outcomes in endometriosis patients with different severities of the disease who received a 3-month GnRH agonist protocol right after the ovum pick-up and before cryopreserved-thawed embryo transfer [96]. However, these benefits could be attributed to the process of cryopreservation of embryos, since it was suggested to have an inherent positive effect on fertility outcomes [96–98]. Conversely, Tamura et al. [68] did not report any significant benefit gained from administering the ultralong protocol in 11 patients with severe endometriosis, when compared with untreated controls. In contrast, Dicker et al. [86] reported favorable outcomes of patients with severe endometriosis that were treated with the 6-month suppression with triptorelin. The authors reported that the ultralong protocol group resulted in higher number of oocytes retrieved and embryos transferred, with higher clinical pregnancy rate per cycle, and per transfer, when compared to patients receiving the conventional ovarian stimulation protocol [86]. Similarly, Marcus et al. [99] reported significant increase in the pregnancy rate in patients treated with goserelin for 2–7 months when compared to untreated controls. Furthermore, their study indicated that patients who were treated with four or more doses of goserelin had higher odds for clinical pregnancy than patients treated

with less than four [99]. Finally, a recent meta-analysis showed that the ultralong protocol yielded higher fertilization rate than the short protocol [100]. Most importantly, it was suggested that the ultralong suppression with GnRH agonists could be more beneficial for patients with stage III/IV endometriosis when compared with the conventional long protocol (RR = 2.04, 95% CI: 1.37–3.04). In contrast, analyzing non-randomized controlled trials studies did not show any additional benefit of using the ultralong protocol over the conventional short protocol [100].

3 Progestins in the Endometriosis-Related Infertility Treatment

3.1 General Characteristics and Indication of Progestins in Patients with Endometriosis

Progestins are the newer generation of synthetic progestogens. Dienogest and MPA are the most commonly used progestins in the management of endometriosis-related pain and infertility. Dienogest is a fourth generation progestin that exhibits strong progestational characteristics with minimal androgenic and antiestrogenic effects [101, 102]. On the other hand, MPA is a 17-hydroxyprogesterone derivative that exhibits strong antiandrogenic and antiglucocorticoid effects [103]. These agents are supposed to suppress endometriosis through inducing elevated local progesterone concentrations in the endometriotic lesions [57]. As previously mentioned, dienogest was found to provoke the decidualization of endometriosis, leading to shrinkage and atrophy of the endometriotic lesions [57]. When compared with GnRH agonists, dienogest was found to possess stronger cytoreductive effects on endometriosis as well [104, 105]. In addition, dienogest demonstrated remarkable anti-inflammatory, antiangiogenic, and cytokines inhibitory effects that could help in ameliorating the hostile peritoneal microenvironment [106–108]. Both dienogest and MPA were found to be effective in managing the endometriosis-related pain symptoms. Dienogest was found to be as effective as GnRH agonists in controlling chronic pelvic pain in patients with endometriosis [108, 109]. Depot–MPA on the other hand showed comparable outcomes to those of depot–leuprolide acetate in controlling endometriosis-related pain [110]. Both agents showed higher effectiveness when compared to placebo [103]. The most common adverse effects associated with the use of these progestins are vaginal breakthrough bleeding, breast discomfort, headache, and weight gain [102, 103]. In general, these consequences are more remarkable when progestins are used in pain management for a prolonged period of time (≥6 months).

More recently, progestins were also reported to improve fertility outcomes in patients with endometriosis, and other infertile patients. Progestin-primed ovarian stimulation (PPOS) showed favorable outcomes when used in IVF-ET cycles [111]. The use of progestins in ovarian stimulation protocols is thought to block the

premature luteinizing hormone (LH) surge by inhibiting the endogenous GnRH surge induced by estradiol [112]. Progestins can also effectively reduce the risk of moderate to severe ovarian hyperstimulation syndrome (OHSS) [55, 113]. Indeed, progesterone treatment was reported to improve the activity of matrix metalloproteinases (MMPs) in patients with endometriosis [114]. A previous study reported that patients with endometriosis had abnormally increased activity of MMP-1 and MMP-2, and an abnormally decreased activity of tissue inhibitor of matrix metalloproteinase-1 were observed in endometriosis patients. These abnormalities were neutralized after progesterone supplementation [114]. Furthermore, progestin-primed ovarian stimulation protocols resulted in higher number of oocytes retrieved, embryos obtained, and comparable clinical pregnancy and live birth rates when compared to the conventional GnRH analog protocols [111]. Therefore, these agents could optimize the cycle outcomes while maintaining a perfect safety profile reportedly. Nevertheless, progestins were reported to potentially have unfavorable effects on fertility outcomes. Progestins were reported to suppress the follicular growth, induce follicular atresia, and inhibit the recruitment of primordial follicles [115–117].

Although progestins were proven to be a good alternative to GnRH agonists in terms of pain management and ovarian stimulation, their effectiveness when used in prolonged ovarian suppression prior to IVF-ET cycles in patients with endometriosis are not established yet. More recent studies investigated the benefits of certain types of progestins in optimizing the fertility outcomes in patients with endometriosis. Some studies investigated whether the prolonged administration of dienogest and MPA prior to IVF-ET cycles could improve the fertility outcomes in patients with endometriosis. Others investigated whether progestin-primed ovarian stimulation protocols that contain MPA specifically could benefit patients with advanced stage endometriosis compared to the conventional stimulation protocols. In the following paragraphs, we provide a thorough description and discussion of the current findings regarding the use of progestins prior to IVF-ET cycles in patients with endometriosis.

3.2 Prolonged Progestins Administration Prior to IVF in Patients with Endometriosis

The use of progestins in managing the endometriosis-related infertility have gained more attention in recent years. The majority of studies investigated the effectiveness of dienogest administration for a period of 2–6 months prior to initiating ovarian stimulation.

In a prospective randomized controlled trial, Tamura et al. [118] investigated the effects of oral dienogest administration for 3 months on IVF-ET outcomes in patients with severe endometriosis. The control group consisted of untreated endometriosis patients with equivalent r-ASRM classification. Patients treated with

dienogest were found to have poorer ovarian responsiveness in terms of antral follicles count and serum estradiol levels. The total amount of gonadotropins used for stimulation was also increased in the dienogest group. The number of retrieved oocytes, the fertilization rate, the pregnancy rate, and the live birth rate were significantly lower in patients treated with dienogest. However, it should be noted that the authors administered estrogen and progesterone to induce the withdrawal bleeding [118]. This procedure is thought to be responsible for the biased IVF-ET outcomes in the dienogest group [119, 120]. Conversely, a more recent randomized controlled trial conducted by Khalifa et al. demonstrated the effectiveness of dienogest in improving fertility outcomes in patients with endometriosis [120]. The study compared a 3-month dienogest pretreatment before IVF-ET cycles to a 3-month prolonged protocol of depot–leuprolide acetate. Both groups showed comparable cycle outcomes in terms of the number of mature oocytes, number of transferred embryos, fertilization rate, and clinical pregnancy rate [120]. The authors also demonstrated that dienogest pretreatment was significantly cheaper and more tolerable than using the prolonged leuprolide protocol [120]. Although this trial used a greater sample size, and followed more robust methodology, the results should be carefully interpreted. Khalifa et al. [120] included patients of all endometriosis stages, while Tamura et al. [118] only included patients with severe endometriosis. According to a recent multicenter randomized controlled trial [92], the prolonged GnRH agonist protocol has no benefit in managing patients with mild–to-moderate endometriosis, unlike patients with advanced endometriosis stages [100]. Therefore, it is very likely that the results of Khalifa et al. [120] are confounded by including patients with different degrees of disease severity. In other words, it is not clear whether dienogest is effective in all endometriosis stages, or only in patients with severe endometriosis since they represented 70% and 73% of the dienogest and GnRH agonist groups, respectively [120]. A subgroup analysis would have helped giving a better explanation of the result. Moreover, both studies were not blinded, and did not adjust for the presence of adenomyosis, male factor infertility, or other comorbidities that could influence the IVF-ET outcomes [118, 120]. Results from another two prospective cohort studies also favored the prolonged use of dienogest in patients with endometriosis prior to ovarian stimulation [119, 121]. Muller et al. [121] compared the use of dienogest for 6 months to triptorelin, and to direct ovarian stimulation. The authors reported that patients who were referred directly to ovarian stimulation had lower number of antral follicles and cumulus–oocyte complexes compared to patients treated with dienogest. The clinical pregnancy and live birth rates were 2.5 and 3 times higher in patients pretreated with dienogest, respectively, when compared to those that were referred directly to IVF-ET. No significant difference between patients pretreated with dienogest, and patients pretreated with the prolonged GnRH agonist protocol in terms of the gonadotropin stimulation dose, duration of stimulation, number of oocytes retrieved, number of A-class embryos, clinical pregnancy rates, and live birth rates [121]. Similarly, Barra et al. [119] also compared the IVF-ET outcomes of patients with unoperated ovarian endometriomas who received a 3-month pretreatment, to those of patients who did not. The total dose of HMG used, the stimulation duration, number of retrieved

oocytes, number of metaphase II (MII) oocytes, and the A-class embryos were comparable between the two study groups [119]. However, the authors reported a significantly increased cumulative implantation rates, clinical pregnancy rate, and live birth rate in the dienogest group in comparison with the non-treated group. It is noteworthy that the largest endometrioma diameter, and its size were found to decrease significantly following dienogest treatment. Finally, the authors found that patients pretreated with dienogest with endometriomas ≥4 cm had a significantly increased number antral follicles, MII oocytes, blastocysts and transferred embryos when compared with patients who did not receive the dienogest pretreatment with the same endometrioma size [119]. Studies reporting on the efficacy of MPA pretreatment in patients with endometriosis are very few. With our search, we were able to identify one study addressing this issue [122]. However, the study consisted of patients who had unstimulated IVF-ET cycles and were treated with MPA for 2 months before the cycle. Although the study design is weak, the reported results favored the use of MPA for infertile patients with endometriosis [122].

The use of progestins generally, and dienogest specifically, to treat the endometriosis-related infertility seems promising, cost-effective, and tolerable. However, a definitive conclusion is hard to be made because of the little number and limited external validity of the available studies.

3.3 Progestin-Primed Ovarian Stimulation in Patients with Endometriosis

Progestin-primed ovarian stimulation is a relatively new strategy introduced by Yanping Kuang's group in 2015 [112]. Although it was investigated more extensively in the general population of infertile patients [111], only few studies reporting on its effectiveness in improving the fertility outcomes of patients with endometriosis are present. To the best of our knowledge, only 4 studies reporting on the use of progestin-primed ovarian stimulation protocols are published to date.

In 2017, Guo et al. [55] published their results of a retrospective study comparing the IVF-ET outcomes of endometriosis patients who were treated with MPA as a part of progestin-primed ovarian stimulation protocol in comparison with the conventional short protocol. This study included patients with moderate–to-severe endometriosis with ovarian endometriomas. Oral MPA was started on the third day of the menstrual cycle and continued until the trigger day. On trigger day, patients who were treated with MPA and HMG were found to have deeper LH suppression, and lower serum progesterone levels. While serum estradiol gradually increased during stimulation, reflecting a good ovarian responsiveness to stimulation. The mean HMG dosage was lower in patients receiving the conventional short stimulation protocol, whereas the mean stimulation duration was shorter in patients receiving MPA with HMG. No patients experienced premature ovulation and moderate–to-severe OHSS in the MPA group [55]. Although the number of retrieved

oocytes did not differ significantly between the study groups, the mature oocytes rate, and high-quality embryos per oocyte rate were higher in patients who were treated with MPA. Nevertheless, the fertilization rate, cleavage rate, implantation rate, and clinical pregnancy rate did not differ significantly between groups. The cycle cancellation rate was slightly but insignificantly higher in patients receiving MPA during ovarian stimulation [55]. However, these results are hard to be interpreted due to several reasons. First of all, patients treated with MPA had their ovarian endometriomas removed through either cystectomy or aspiration, unlike the controls whose ovarian endometriomas were kept during stimulation. Second, it should be noted that IVF-ET outcomes were suboptimal in the two study groups. Third, this study also included cryopreserved-thawed embryo transfer, which could possibly act as a confounder [96]. Finally, the control group in this study consisted of patients with severe endometriosis that were treated with a short stimulation protocol. As previously mentioned, the conventional short protocol was proven to be ineffective in managing patients with stage III/IV endometriosis [86, 100]. Therefore, we are uncertain whether the authors included an appropriate comparator to investigate the effectiveness of progestin-primed ovarian stimulation protocol in managing these patients. In another prospective study, the same investigators investigated the effectiveness of the same MPA and HMG protocol for managing stage III/IV endometriosis patients with surgically removed ovarian endometrioma [123]. Unlike their previous work, this study included tubal infertility patients who were treated with the same progestin-primed ovarian stimulation protocol as controls. The number of retrieved oocytes, mature oocytes rate, fertilization rate, implantation rate, and clinical pregnancy rates were comparable between the two groups. Interestingly, a subgroup analysis demonstrated that patients with surgically removed endometrioma had lower number of dominant follicles on trigger day, and retrieved oocytes [123]. Thus, confirming previous reports on the detrimental effect of surgery on ovarian responsiveness [52]. In an open-label randomized non-inferiority trial, Guo et al. [124] also demonstrated that progestin-primed ovarian stimulation using MPA resulted in deeper LH suppression, more retrieved oocytes, and more MII mature oocytes when compared with dydrogesterone and progesterone. Similar to their previous reports [55, 123], no patients had premature LH surge [124]. Finally, birth defects rate and general pregnancy outcomes of endometriosis patients treated with MPA were found to be comparable to those of endometriosis patients treated with the conventional GnRH analogs [125].

Although preliminary results seem encouraging, it is hard to draw a conclusion regarding the use of MPA in ovarian stimulation protocols for infertile patients with endometriosis.

4 Aromatase Inhibitors

4.1 General Implications of Aromatase Inhibitors in Endometriosis

The third generation aromatase inhibitors, such as letrozole and anastrozole, are effective agents that act directly on suppressing the activity of the P450 aromatase. These agents are selective, with excellent bioavailability of 99.9% when administered orally. Their effects are reversible due to their relatively short half-life [126]. Aromatase inhibitors attain a potency of decreasing the circulating estrogen levels by 97%, which makes them excellent candidates in managing estrogen-dependent diseases, like endometriosis and ER-positive breast cancer, where hypoestrogenemia is mandatory [60, 126]. Besides the profound hypoestrogenic status induced by suppressing skin and adrenal aromatase, these agents have direct effect on endometriosis because aromatase was found to be intensely expressed in the endometriotic lesions [62]. P450 aromatase maintains the local biosynthesis of estrogen in endometriosis, which in turn induces the production of PGE2 by COX-2. Elevated concentrations of PGE2 on the endometriotic lesions level stimulate the aromatase activity to produce more estrogen [62, 127]. Moreover, PGE2 was thought to upregulate the oxytocin receptor expression, and induce the local production of oxytocin in the endometriotic cells [128]. Oxytocin receptors activation will lead to increased production of PGF2-α [129]. Indeed, PGE2 and PGF2-α are substantial elements in the endometriosis-related pain pathophysiology [130]. Furthermore, the eutopic endometrium of patients with endometriosis is thought to express a similar positive-feedback loop to that of endometriosis, which is mediated mainly by P450 aromatase, estrogen, oxytocin, and prostaglandins [128]. This notion is supported by the observation of abnormally expressed P450 aromatase in the endometrium of patients with endometriosis, unlike the endometrium of healthy women [33, 61]. Therefore, inhibiting the aromatase activity would result in the deactivation of complex and interwoven feedback loops that promote the endometriosis proliferation and progression on the one hand, and could also decrease the expression of the oxytocin receptors on the level of the eutopic endometrium, on the other hand. Decreased oxytocin activity on the level of the endometrium would result in less uterine peristalsis, and thus, would optimize the implantation conditions in patients seeking fertility [131]. In line with this speculation, suppression of the aromatase activity of cultured endometriotic cells resulted in significant decrease in their proliferation potential [132]. Similarly, administering letrozole to patients with rectovaginal endometriotic nodules resulted in a significant reduction in the lesion's size, and resolved the associated pain symptoms [133]. Additionally, letrozole administration for postmenopausal endometriosis patients was effective in reducing the lesions size and pain symptoms, with an acceptable safety profile [134]. In a prospective randomized trial, administration of GnRH agonist and anastrozole for 6 months resulted in lower recurrence rate at 24-month of follow-up when compared with the treatment with GnRH agonist alone [135]. Moreover, there was no significant difference

in the postmenopausal quality of life between the two groups. Although a greater bone loss was observed in patients treated with GnRH agonist and anastrozole, the mean loss of baseline bone mass at 24 months of follow-up did not differ significantly between groups, and none of the treated patients had osteoporosis [135]. As noticed, letrozole and anastrozole are rarely prescribed solely when managing endometriosis. This is mainly due to their limited ability to suppress ovarian estrogen synthesis [27]. In contrast, letrozole when administered alone was found to stimulate ovulation, or even provoke ovarian cysts formation [135]. In fact, letrozole was found to be as effective as clomiphene citrate for superovulation induction [136]. For these reasons, aromatase inhibitors are typically administered as an add-back therapy to either GnRH agonists or progestins.

To date, letrozole was found to have a favorable impact on the fertility outcomes of patients with endometriosis. In a retrospective study, Miller et al. [32] reported that all patients with type 2 integrin deficiency had endometriosis, while 80% of patients with type 1 integrin deficiency had endometriosis. Among patients with integrin deficiency that were treated with letrozole, 66.7% had their integrin level corrected. Patients with type 2 integrin deficiency who were treated with letrozole had significantly higher pregnancy rates when compared to those who did not take letrozole [32]. In other words, letrozole significantly improved the fertility outcomes in patients with endometriosis who were proven to have integrin deficiency. In contrast, another study reported that adding letrozole to the ovarian stimulation protocol had no extra benefits on the IVF-ET cycle outcomes in patients with ovarian endometriomas [137]. It is noteworthy that aromatase inhibitors were shown to induce early antral cavity formation in-vitro [138]. Therefore, caution should be taken when determining the criteria of ovulation triggering. It was suggested that ovulation should be triggered when the dominant follicles reach 19 mm in diameter [139].

4.2 Aromatase Inhibitors to Improve IVF-ET Outcomes in Patients with Endometriosis

To the best of our knowledge, only four studies investigated the effectiveness of aromatase inhibitors as a pretreatment in patients with endometriosis undergoing IVF-ET, only one of them is a randomized trial [140], but unfortunately of suboptimal quality.

In a multicentric retrospective cohort study, Cantor et al. [27] investigated the benefits of letrozole when coadministered for 2 months with depot–leuprolide acetate, compared with administering depot–leuprolide acetate alone. The study included patients who had ovarian endometriomas diagnosed radiologically with previously failed IVF-ET cycle. A greater increase in the antral follicles count, number of retrieved oocytes, number of mature oocytes, and number of 2 pronuclei embryos were observed in patients receiving letrozole with depot–leuprolide

acetate. Moreover, the clinical pregnancy rate and the live birth rate were significantly higher in patients who received letrozole. In addition, a significant reduction in the endometrioma's diameters was observed in patients receiving depot-leuprolide acetate with letrozole. The authors claimed that patients treated with letrozole complained more often of bone mass loss [27]. In line with these results, another study reported favorable impact of a 2-month pretreatment with letrozole and depot–leuprolide acetate on the IVF-ET outcomes of patients with previous history of recurrent implantation failure [141]. Patients pretreated with letrozole and leuprolide had significantly more mature oocytes, blastocysts, day-3 embryos, and higher pregnancy rates when compared with patients who did not receive any previous treatment. However, the results of this study are controversial since it included patients without previous diagnosis of endometriosis. In 2009, Lossl et al. [137] reported the IVF-ET outcomes of 20 patients with ovarian endometriomas pretreated with goserelin and anastrozole for 69 days. The study did not contain a control group, and the results were generally unfavorable. However, 75% of patients experienced a decrease in the cyst's size, with a mean decrease of 29%. The authors reported that only one patient had flare phenomenon with ovarian cysts formation [137]. Finally, in a randomized controlled trial, Alborzi et al. [140] compared the IVF-ET outcomes between three groups of endometriosis patients who were treated laparoscopically. The authors did not report any significant difference in the cycle outcomes and pregnancy rates between patients who received letrozole, triptorelin, and the untreated controls. It should be noted that this study was an open-label trial, with a relatively small sample size, and a tendency toward selection and detection biases. Therefore, drawing a conclusion on the benefits of the use of aromatase inhibitors prior to IVF-ET cycles in patients with endometriosis is not possible yet. The two described retrospective cohort studies included patients with ovarian endometriomas diagnosed radiologically, with no further information on the endometriosis stage [27, 137]. The study by Lossl et al. [137] included cryopreserved embryo transfers, which could confound the results as previously mentioned [96]. In addition, the authors did not report any data concerning the previous fertility status in the included patients. The trial by Alborzi et al. [140] was underpowered to detect any significant difference in the IVF-ET outcome parameters. Moreover, the authors did not specify the r-ASRM classification of endometriosis in the included patients, and did not provide information about their previous fertility status. Therefore, the external validity of the current studies is extremely limited, and there is a need for prospective randomized controlled trials investigating the effectiveness of aromatase inhibitors pretreatment on fertility outcomes of patients with endometriosis scheduled to have IVF-ET.

5 Discussion

The issue of whether to administer a precycle hormonal treatment to patients with endometriosis is one of the most debated topics in reproductive medicine. Although it has been studied for more than 30 years, finding a definitive answer remains challenging. The more studies we have, the more controversies we get. Throughout this chapter, we provided a comprehensive and critical review of the available pieces of evidence concerning the use of each of the hormonal suppressive therapies prior to IVF-ET cycles in patients with endometriosis. Although we are still far from making any clinical recommendation, we know more than ever what should be studied in deeper details, and what should be abandoned. Despite that our current knowledge of the effectiveness of these hormonal treatments does not add much value to our daily practice, it is substantial to lead future research in the direction of what appears promising, cost-effective, and tolerable. This is especially true when knowing that a high quality, multicentric, randomized controlled trial took 15 years to be completed [92]. In fact, spending another 15 years researching things that seem ineffective, or do not rely on solid and robust evidence in a field already struggling from being unfunded sufficiently, is truly a waste of time and resources.

Our first step toward a more feasible research is identifying which patients with endometriosis are infertile, who require treatment, and how to treat them. Indeed, 25–50% of patients with endometriosis are infertile, means that 50–75% of them are fertile [5]. Therefore, identifying the differences between fertile and infertile patients with endometriosis, and the associated etiological processes with the endometriosis-related infertility is of utmost importance to find effective treatments. It should be kept in mind that endometriosis is a multifactorial, systemic disease, with genetic and epigenetic basis [142, 143]. Moreover, it was suggested that each type of endometriosis is an independent disease that has its proper regulatory pathological signaling [143]. It is quite possible that this postulation is true, and the discrepancy in reproductive outcomes is simply the consequence of different diseases. Moreover, when taking the important role of epigenetics in the pathogenesis and pathophysiology of endometriosis, it seems plausible to postulate that different genetic/epigenetic dysregulations alter the responsiveness of endometriosis to different hormonal therapies, and to the same therapy in different subpopulations. For example, despite the important role of P450 aromatase in the endometriosis progression, it was found to be absent in certain lesions [33, 144]. Such variation will lead to different responses or different levels of responsiveness to aromatase inhibitors, with the optimal results obtained in patients whose endometriotic lesions express aromatase the most. Moreover, a decreased aromatase expression in the granulosa cells of patients with endometriosis through hypermethylation of the PI.4, and hypoacetylation of PII promoters of the gene *CYP19A1* was demonstrated [31]. This could partially mean that administering aromatase inhibitors to such patients will further suppress the aromatase function, and elevate the follicular fluid androgen, and therefore, impair folliculogenesis and the oocytes quality [145, 146]. We admit that identifying and targeting all the genetic/epigenetic dysregulations may

not be doable on the short term, but we insist on looking to endometriosis in a more comprehensive view. The etiological processes that are associated with endometriosis are way beyond hormonal dysregulations [10, 128] but, in contrast, the main focus of the current medical therapies is inducing a hypoestrogenic state to achieve lesion's remission. This applies to the GnRH agonists, and progestins as well. Although GnRH agonists were suggested to improve the endometrial receptivity through restoring the integrin expression [75], other studies failed to demonstrate this effect [147]. Therefore, their main action is suppressing ovarian steroidogenesis, and the circulation estrogen subsequently. This in turn may explain why these agents are ineffective in many cases of endometriosis-related infertility. In addition, He et al. [131] and others [23] have reported that patients with endometriosis have increased uterine peristalsis. The oxytocin concentrations and the oxytocin receptors were found to be upregulated in the endometrium of patients with endometriosis [128]. On that basis, the authors investigated whether administering atosiban prior to embryo transfer could improve the fertility outcomes by enhancing the implantation [131]. It was reported that the atosiban group had higher clinical pregnancy rate per cycle and the implantation rate were significantly higher in patients treated with atosiban. The study by He et al. [131] is an adequate demonstration of how targeting non-hormonal pathways could result in improved IVF-ET outcomes in patients with endometriosis. Moreover, aromatase inhibitors and progestins were found to affect non-hormonal pathways besides their effects on the hormonal profile of the estrogenic lesions as previously discussed. This in turn could explain the general tendency toward better fertility outcomes when these agents are administered to patients with endometriosis.

6 Conclusions and Future Perspectives

Hormonal therapies before in-vitro fertilization in patients with endometriosis are one of the most debated topics in reproductive medicine. The prolonged administration of GnRH agonists to patients with mild-to-moderate endometriosis should not be considered anymore since it was proven to be ineffective in many clinical trials and systematic reviews. In contrast, the ultralong ovarian suppression protocol for patients with severe endometriosis is of undetermined effectiveness, and further research is needed on this specific population of patients. Progestin-primed ovarian stimulation is another protocol that seems to be promising in managing patients with severe endometriosis and accompanying ovarian endometriomas. However, the current evidence is scarce and mostly based on single-center experience. Dienogest was proven to be cheaper and more tolerable by patients when used for 2 months prior to ovarian stimulation. Nonetheless, it is too early to recommend using it in the clinical setting due to the lack of high-quality evidence. The role of aromatase inhibitors in treating the endometriosis-related infertility is not established yet. Future research should focus on identifying the real pathophysiology of the endometriosis-related infertility, identifying which patients will benefit from

hormonal treatment, deciding the most effective agents that should be used, and determining the optimal timing and duration of therapy.

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IVF Stimulation Protocols and Outcomes in Women with Endometriosis

Jwal Banker, Henrique D'Allagnol, and Juan A. Garcia-Velasco

1 Introduction

Endometriosis is an estrogen-dependent disease of high morbidity, infertility being one of symptoms. Briefly, three different entities have been described, namely peritoneal, ovarian (endometrioma) or deep infiltrating, and these frequently coexist. Due to the lack of a reliable noninvasive method for its diagnosis, it is difficult to estimate its true prevalence. Studies report its prevalence to be about 10% in the general population and a contributing factor in causing infertility in approximately 40% of women. It is also estimated that about 50% of women with endometriosis have difficulty in getting pregnant [1].

Although a direct causal relationship with infertility cannot be made, it is shown that the fecundity rate of untreated women can go as low at 2% [2]. The impact exerted by the disease on oocyte quality/quantity and ultimately on the embryos makes this pathology a subject of constant study and interest for infertility specialists. It is assumed that this generalized disease causes damage due to the production of cytotoxic chemicals and also by disturbing the pelvic anatomy. Focal lesions like endometrioma can be more harmful due to its additional space occupying effect. It is also found that the disease itself and its surgery can damage the ovarian reserve and hence this disease is of interest [3].

Dr. Carl Wood of the Monash in vitro fertilization (IVF) team in Melbourne reported the first IVF pregnancy in 1973, although it resulted in an early miscarriage, started a new era. Medical history was made on July 25, 1978, with the birth of the world's first "test tube baby" by performing a natural cycle IVF. Trounson et al. in 1981 introduced ovarian stimulation (OS) in IVF and this led to higher pregnancy rates [4]. These ovarian stimulations consist basically of the

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administration of urinary or recombinant gonadotropins, used alone or in conjunction with Letrozole or Clomifene. Premature luteinizing hormone (LH) peak is usually prevented with the use of gonadotropin-releasing hormone (GnRH) analogs (agonists and antagonists) or more recently by the use of oral progesterone [5, 6]. Thus, discussions have arisen over the years about which is the best OS protocol for these patients with endometriosis when undergoing fertility treatments. This population also frequently undergoes ovarian surgery to remove the endometriotic cysts, and, therefore, may also present impairment of the ovarian reserve [6]. Optimizing treatments and seeking the best protocols in order to obtain satisfactory amounts of oocytes and embryos of good quality is crucial to achieve reproductive success.

Preparations prior to OS have also been proposed, with the aim of obtaining a more synchronous follicular development; limit the growth of the endometriotic implants and reducing the chronic pelvic inflammatory process, which supposedly could negatively impact treatments. These have also been used post OS but prior to performing a frozen embryo transfer (FET) with the same purpose. These protocols include the use of long periods of oral contraceptives, depot GnRH agonists, and/or even intrauterine hormonal devices [7, 8].

This chapter will have a special emphasis on the peculiarities and results of using the aforementioned protocols, comparing them with each other and with patients without endometriosis.

2 Background/Impact of the Disease

Decades after the first reports on the association between endometriosis and infertility, it has yet to be fully understood. Distortion of pelvic organs with a structural and functional loss of ovarian function due to toxic metabolites has been suspected to play an important role [9].

It was previously thought that just like every other pathology, surgical removal of this disease will also lead to a decline in its side effects including infertility. It is true to some extent as in some cases precise laparoscopic excision of the endometriotic lesions while avoiding damage to the normal tissues does reduce pain and improve quality of life. This is evident as spontaneous pregnancy after such corrective surgery in cases with severe endometriosis had reached even up to 73% in young patients. But, this might not be the case for all infertile women and there are strong drawbacks of the surgery as witnessed by the declining AMH levels. For this population, waiting for a spontaneous pregnancy might not be advisable due to their advanced maternal age, or other reasons [10]. Hence, they are subjected to OS to get early and promising results with a faster and maybe even cheaper time and cost to pregnancy rates.

A meta-analysis in 2002 included 22 studies and compared results of over 2300 IVF cycle in women with endometriosis to more than 4300 controls. After adjusting for confounding factors, statistically significant reductions were found in implantation and pregnancy rates in patients with endometriosis, as well as a lower number

of oocytes obtained by ovarian stimulation. Another comparison carried out within the same work, assessed the impact of disease severity on reproductive outcomes, and concluded that patients who suffer from severe/advanced forms obtain significantly lower amounts of oocytes, in addition to even lower rates of implantation and pregnancy compared to minimal stages. This study therefore demonstrated that women with endometriosis have a reduction of up to 54% in pregnancy rates when compared to patients undergoing IVF for other reasons, such as tubal factor [11].

A more recent meta-analysis studied the reproductive outcomes in these women. The authors confirmed that endometriosis is associated with a considerable decrease in the likelihood of success for these patients in their reproductive treatments. The negative influence on the number of oocytes obtained, embryos generated, on the rates of fertilization and pregnancy in this population was evident. Severe forms of the disease had a negative effect on all treatment processes, and when present in the ovary, a significantly smaller number of mature oocytes are aspirated [9].

The impact exerted by the inflammatory cytokines present there on steroidogenesis and ovarian folliculogenesis seems to be evident, corroborating with the publications that showed lower oocyte mitochondrial content, anomalous oocyte morphology and higher rates of embryo granulation/fragmentation. The concentrations of reactive oxygen and interleukin species present in the follicular fluid are also associated with a higher percentage of immature oocytes [9, 11, 12].

After all efforts, the end result which is important for the patient is the cumulative live birth rate. To address that, a recent retrospective study by Boucret et al. compared 1124 COS cycles performed in patients with and without the disease. They too confirmed that patients with endometriosis had reduced AMH and AFC values, even without undergoing surgical procedures. Due to this low reserve, these women had significantly fewer oocytes retrieved and even fewer mature oocytes (7.0 vs 9.7 and 4.8 vs 6.9, respectively). As a result of this, they had fewer embryos formed. Though the maturation rate and cleavage rates were in the same in both groups, which signifies only a quantitative loss of ovarian function, the number of embryos which could be frozen for future use were less. Due to this, the affected group had a reduced cumulative live birth rate (32.1% vs 50.7%, $p = 0.001$) [13].

Hence, while these women frequently undergo OS and IVF, doubt still persists about the ideal stimulation protocol as there can be an ill effect on IVF outcomes as reflected by a lower oocyte yield and quality. Several studies report that women with endometriosis have high levels of oxidative stress markers and low levels of antioxidant markers even in the follicular fluid. This indirectly is supposed to create a lower number of good quality embryos which can hamper results. This assumption is supported by the finding that oocytes from women with endometriosis have a different profile related to oxidative stress and cell growth regulation. These also show a different transcriptome behavior when compared with controls [14]. Though this is true, its clinical relevance is questioned as American Society of Reproductive Medicine (ASRM) studies have shown that the not oocyte quality but only quantity is hampered and even the aneuploidy rates are similar [10]. Hence, early intervention with OS and IVF is still thought to be the best option to achieve a pregnancy for most infertile women (Fig. 1).

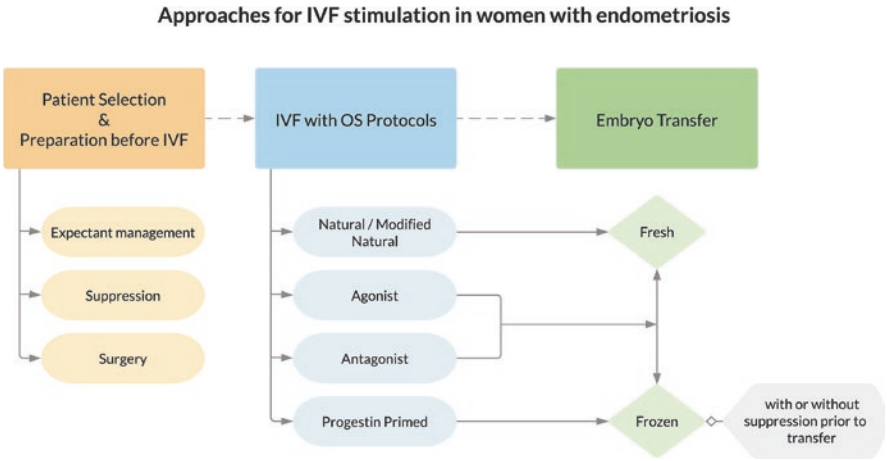

Fig. 1 Various approaches for women with endometriosis undergoing OS for IVF

3 Protocols and Results

Keeping the distinctive nature of this progressive disease in mind, fertility specialists have tried various OS protocols over the years in order to get good results.

3.1 Natural or Modified Natural Cycle IVF

IVF was originally performed in natural cycles with hCG trigger. In a Norwegian study, the authors tried this method in couples with minimal endometriosis associated infertility and compared it to patients with unexplained and tubal factor infertility. The prospectively recruited couples were given a maximum of 5 cycles with a natural IVF before proceeding to conventional IVF. In spite of having a lower pregnancy rate per initiated cycle, the pregnancy rate per embryo transfer was 23.5% in the endometriosis group and it was higher compared to the other groups [15]. The clinical relevance is questionable, but this method is a cheap and safe alternative when compared to conventional IVF.

3.2 GnRH Agonist Vs Antagonist Cycle IVF

The long protocols with GnRH agonist for OS were pioneers in contemporary reproductive medicine, being used in clinical practice even in the 1980s [16]. These treatments are based on the suppression of pituitary function by desensitizing their receptors, resulting in cycles with greater follicular synchrony, and decreased risk of

premature LH rise. In contrast, the recently implemented protocols for OS with GnRh antagonists cause suspension of the pituitary function immediately after its administration, culminating in shorter treatments and with lower necessary dosages of gonadotropins. Both these regimens are routinely used in fertility practice, but studies comparing these two in a specific endometriosis population are limited.

It was thought that the long GnRH agonist protocol would be helpful in cases with endometriosis as longer suppression could decrease the local inflammatory processes and could improve oocyte quality. As opposed to this, due to the short suppression with the antagonist protocol, these benefits were lost. Pabuccu et al. conducted a prospective randomized study to elucidate the differences in these protocols in 246 patients. These women were initially divided into 3 groups: those who had mild/moderate endometriosis confirmed by laparoscopy, those who underwent cystectomies prior to OS and women with ovarian endometriomas without surgical intervention. Results showed a nonsignificant improvement with the agonist cycle and they concluded that OS with both GnRH antagonist and GnRH-a protocols may be equally effective in patients with mild-to-moderate endometriosis and endometrioma who did and did not undergo ovarian surgery [17].

Another retrospective observational study published in 2013 also compared patients with endometriosis and infertility who underwent OS with these two protocols. In total, 1180 women who were diagnosed with endometriosis surgically or by ultrasound were analyzed, and when the confounding factors were adjusted, no strategy was shown to be superior with regard to pregnancy rates [5].

When analyzing whether the severity of endometriosis could predict the results of OS in different protocols, a retrospective study published 5 years later compared the use of GnRH agonists and antagonists in 386 patients with the disease, dividing them into two groups according to the severity of disease. In patients with grades I and II endometriosis, a higher percentage of clinical pregnancies and live births (42.8% vs. 26.7%) were reported using agonists. In patients with advanced disease, the overall results were worse, but they were equivalent among the protocols. All patients included in this study were diagnosed by videolaparoscopy and did not use any hormonal preparation in the 6 months prior to stimulation, thus reducing possible confounding factors. A shorter treatment time and gonadotropin dosages were reported by the group that used antagonists to suppress premature LH peaks, reflecting greater convenience during treatment [18].

In view of the relative frequency of surgical procedures to remove ovarian endometriotic cysts, a Beijing research group tried to prove the best strategy to perform OS in 342 patients undergoing cystectomy. These women were divided into three groups: those submitted to depot GnRH agonist protocol (3.75 mg agonist in the menstrual cycle prior to stimulation), flare cycles with GnRH agonist (0.1 mg agonist since the beginning of ovarian stimulation), or classical cycles with fixed-onset GnRH antagonists. Differences were not statistically significant. The number of oocytes and embryos obtained also did not differ between the groups studied [19].

Apart from these studies comparing the two protocols, Cao et al. recently performed a meta-analysis on the effectiveness of the GnRH-a ultra-long protocol, GnRH-a long protocol, and GnRH-a short protocol in infertile women with

endometriosis. As it was assumed that the longer the suppression, the better would be the results as the inflammation would be reduced. The analysis concluded that the GnRH-a ultra-long protocol can improve the clinical pregnancy rate of the patients with stages III–IV endometriosis. This conclusion was made only based on randomized controlled trials (RCTs) studies which were included in the analysis. However, subgroup analysis showed that the different down-regulation protocols provided no significant difference in improving clinical outcomes in the non-RCT studies. Hence, it is advised not to draw conclusions yet, as randomized studies would be beneficial [20].

Therefore, according to the small number of studies published with this purpose to date, it is not clear whether there is any significant difference in the results between OS cycles with protocols with GnRH agonists and antagonists in patients with endometriosis. Some studies indicate a higher amount of aspirated oocytes, implantation rates, pregnancy, and live birth with agonist protocols. However, no prospective study was able to show statistically significant differences between them, and thus both are considered equally effective in daily clinical practice. Antagonist protocols can result in lower rates of treatment dropout, given the lower amount of gonadotropins used and significantly shorter treatment duration.

The concept of freeze-all has also been challenged in women with endometriosis. It was hypothesized that the OS might generate further uterine inflammation, especially in the endometrium, and this might compromise successful embryo implantation. We have recently published a large retrospective analysis where we did not find any difference in implantation, pregnancy, and miscarriage rate whether the embryo transfer was performed in a fresh or in a subsequent frozen embryo replacement.

3.3 Progestin Primed Ovarian Stimulation (PPOS)

PPOS was initially described for fertility preservation in women with cancer; however, this protocol is not extensively studied in women with endometriosis. The rationale of using progestins was that they were equally effective in preventing the premature LH spike compared to antagonists. As this regimen could only be used in cycles where a fresh transfer was not done, these are less used for routine IVF stimulation. The advances of vitrification and equal or even superior results in FET cycles have made this option a strong candidate. This might be even more effective in women with endometriosis as a fresh transfer is less preferred due to the flare-up caused by gonadotropins.

Various progestin preparations have been tried and are found to be equally superior in stimulations. In a pioneer and recent study done by d'Argent et al., this PPOS protocol was compared to the antagonist protocol women with endometriosis. Women in the PPOS group were started on progestin desogestrel on the second day of their menstrual cycle and stimulation was started. The presence of deep versus superficial endometriosis alone, the location of endometriosis, the presence of

endometrioma during the stimulation, and the size of endometriomas were not associated with the number of retrieved oocytes. The study demonstrated that there were no significant differences in the oocytes retrieved and the mature oocytes between the groups [21].

This protocol combines the benefits of antagonist protocol in terms of lower stimulation and duration, while also giving additional benefits of a lower cost and fewer injections. The drawback is that a fresh transfer, which is as such generally a less preferred option in these women with endometriosis, is not possible (Fig. 2).

4 Conclusion

Infertile women with endometriosis frequently undergo OS for IVF due to its progressive nature, with or without corrective surgery. Evidence also suggests that decline in fertility in women with endometriosis is more related to quantitative damage than qualitative. OS was initially performed with GnRH agonists but then GnRH antagonists replaced almost completely the agonists due to its shorter duration. Available studies suggest that OS using antagonist or agonist protocols yield similar results in terms of oocyte quantity and usable embryos. If an FET is planned for different reasons, PPOS appear promising and can yield similar results. Overall,

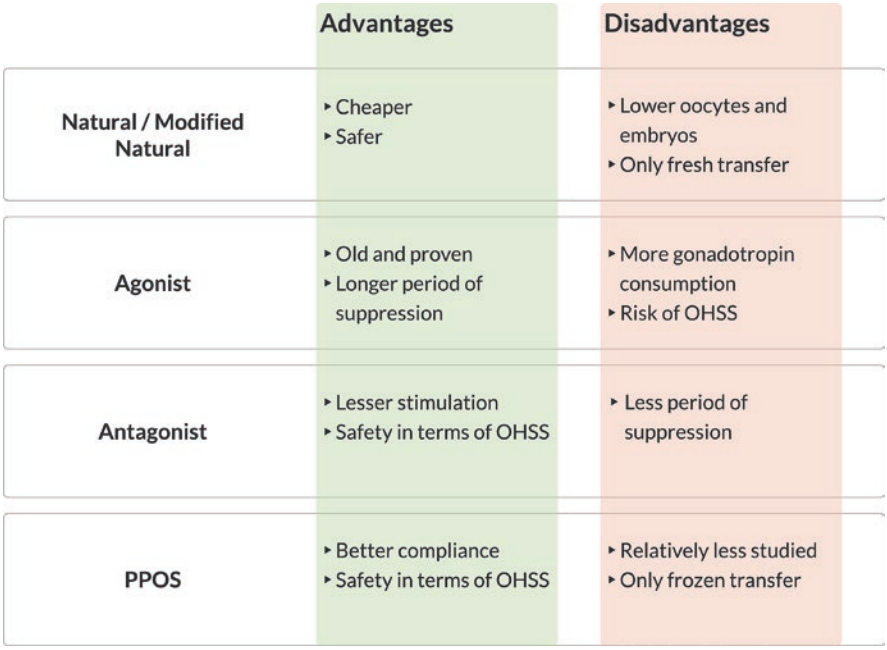

Fig. 2 Advantages and disadvantages of different OS protocols

available literature strongly suggests one thing – that it is early intervention with IVF for good results, irrespective of the OS protocol used.

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The Effect of Endometriosis on the Quality of Oocytes and Embryos Obtained by IVF

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1 Introduction

Endometriosis-induced infertility is a frequently encountered complication of aberrant endometrial tissue growth in ectopic sites, affecting 10% of women through their reproductive years. Extensive research has been produced for the investigation of disease causality, and findings mostly coalesce into the notion that its pathophysiology is a mosaic of both genetic [1] and epigenetic influences [2]. Hence, endometriosis, as a gynecological disorder being a multifaceted pathological modality, affects oocyte competence and embryonic development.

The decline of reproductive capacity in endometriosis has been attributed to molecular modifications, including oxidative stress (elevated concentrations of reactive oxygen species (ROS)), dysregulation of immune mechanisms, and altered cellular circles in terms of proliferation, differentiation, and apoptosis in the reproductive tissue. Several suggested mechanisms include chronic inflammation, adhesions, impaired folliculogenesis, disturbance of the luteal phase, and progesterone resistance. Abnormal oocyte morphology characteristics in endometriosis patients refer to darker cytoplasm, larger or thinner zona pellucida, and flat or fragmented polar bodies [3, 4]. Considering embryo competence, defects in embryonic implantation have been reported to be associated with alterations in hormone concentrations, delayed embryo growth, and blastocyst hatching [5].

While multiple theories have been proposed considering endometriosis pathophysiology [6, 7], definite answers need to be given, not only for prompt diagnosis but also for effective disease management and IVF success [8]. In this review, we attempt to outline the contributing molecular and pathophysiological mechanisms

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accountable for the impact on oocyte and embryo quality, along with future perspectives for effective management of endometriosis-induced infertility.

1.1 The Role of Oxidative Stress and Inflammatory Mechanisms in Oocyte and Embryo Quality

Chronic inflammation and oxidative stress correlate to endometriosis lesions in a causal and self-perpetuating manner. Augmented cellular proliferation in the endometrium results in ROS production, which in turn triggers and mediates inflammation processes. Hence, overproduction of ROS can directly impact on the microenvironment of the fallopian tubes, the follicles and on the oocyte and embryo development, or affect them indirectly through inflammation incitement [9, 10]. Interestingly, not only inflammatory events but also autophagy has been reported to occur in advanced endometriosis stages, with overexpression of BECN1 in granulosa cells leading to elevated concentrations of preovulatory progesterone and aggravating oocyte quality, and therefore, pregnancy outcome [11].

The production of ROS provokes lipid peroxidation, which enables membrane permeability and degradation/inactivation/impaired synthesis of enzymes and microtubule-associated proteins, hence hindrances in cellular cycle regulation, transport of metabolites, as well as nuclear DNA fragmentation and mitochondrial DNA damage could occur, negatively affecting embryonic development, as a part of enhanced apoptotic phenomena [10]. Results from transmission electron microscopy have revealed that the oocytes retrieved from women with minimal/mild endometriosis demonstrated impaired mitochondrial structure and shrinked mitochondria mass, with qRT-PCR analysis showing lower mtDNA copies for these cases [12].

By-products of oxygen metabolism have been studied for impairing oocyte and embryo quality in endometriosis patients. Oxidative stress is suggested to impair oocyte quality and trigger endometriosis-induced infertility, and cumulous cells act protectively for oocytes against cellular death induced by oxidative stress. In a case-control study consisting of 40 endometriosis patients undergoing ovarian stimulation and luteal phase supplementation for ICSI, *SOD1* (superoxide dismutase 1) gene was overexpressed in cumulous cells of women with moderate/severe endometriosis, compared to earlier disease stages [13]. Moreover, elevated concentrations of 8-hydroxy-2′-deoxyguanosine (8-OHdG) have been detected in the follicular fluid (FF) of infertile endometriosis patients [14]. Different results, however, have emerged in a study sample of 61 participants undergoing IVF. While the FF-identified 8-OHdG levels and the FF total non-enzymatic antioxidant capacity affected the count of good-quality embryos, the corresponding variables of serum samples did not demonstrate an effect [15].

Increased serum concentrations of glutathione, SOD and follicular vitamin E, and reduced serum concentrations of TAC have also been detected in endometriosis patients, suggesting that the presence of oxidative damage, induced by increased

8OHdG concentrations in the follicular milieu of patients, is associated with impaired oocyte quality [16].

In a total sample size of 89 women receiving IVF, concentrations of advanced oxidation protein products (AOPP) were significantly higher in the follicular fluid of endometriosis group compared to controls (51.5 ± 22.4 vs. 41.8 ± 18.3 μmol/L, $p < 0.05$). Moreover, a significant inverse correlation was observed between the follicular AOPP levels and blastocyst rate in endometriosis group compared to controls ((EM group: $r = -0.376$, $p = 0.012$; total: $r = -0.367$, $P < 0.001$). Thus, it can be deduced that AOPPs could be reliable biomarkers for the prediction of oocyte quality and outcomes of IVF in women with endometriosis-associated infertility [17]. In a similar mode, myeloperoxidase levels in the follicular fluid could also be deemed as potential oxidative stress targets for disease-induced infertility [18].

While oxygen-scavenging proteins protect the oocytes in vivo from the adverse effects of oxidative stress, the retention of this protective mechanism is not feasible during in vitro-fertilization [19].

Disruption of immunity mechanisms mostly pertains to increased production of inflammatory cytokines that instigate embryotoxicity and intercept proper oocyte maturation and embryo development. These factors include mostly interleukins (IL-1α, IL-1β, IL-2, IL-6, IL-8, and IL-10), TNF-α and prostaglandin, with the latter being reported to affect the function of the hypothalamic-pituitary-ovarian (HPO) axis [10]. Alterations in the intrafollicular thiol-redox system and overproduction of inflammatory cytokines in women with endometriosis-induced infertility could impact on oocyte and embryo quality. Among the analysis of follicular fluid of 65 women undergoing IVF-31, out of 65 were endometriosis patients-significantly reduced levels of glutathione and increased levels of thioredoxin-binding protein 2, IL-6, IL-8, and TNFα were identified in the follicular fluid of endometriosis group compared to infertile disease-free controls ($p < 0.001$). Particularly for TNFα, its levels in FF samples have been suggested to be negatively correlated with embryo quality (which is consistent with these results). Glutathione levels were positively associated with high-quality embryo count, glutathione peroxidase 3 and thioredoxin were inversely associated with oocyte maturity, the cumulative embryo score per embryo. Glutathione peroxidase 3 can induce hypoxia, producing ROS, which causes lipid peroxidation, inactivation of enzymes, irreversible cell damage, and apoptosis. It can be deduced that that the thiol-redox system and inflammatory cytokines could be contributors for achieving clinical pregnancy due to their affecting oocyte and embryo quality [20].

1.2 Meiotic Abnormalities and Chromosomal Misalignment

In the postovulatory stage, oocytes reach the metaphase II (MII) stage by termination of meiosis I, and stay in that stage by the time fertilization occurs. The MII spindle is a temporary structure consisting of microtubules that are formed by (de) polymerization depending on the cell cycle stage and is essential for proper

chromosomal segregation. It is evident, therefore, that oocyte quality is a critical prerequisite for successful implantation and depends upon unerring cytoplasmic and nuclear maturation. The MII spindle demonstrates sensitivity to factors associated with oxidative stress, leading to meiotic spindle abnormalities, chromosome instability, increased apoptosis rate, and impairment of embryonic pre-implantation [21].

The oocyte spindle demonstrates sensitivity to oxidative stress, which can result in endometriosis-induced infertility. It has been observed that the peritoneal fluid of women with endometriosis induces anomalies in the microtubules and chromosomes of murine oocytes and that these effects can be inhibited by the supplementation of the culture medium with an antioxidant factor [22, 23]. On the contrary, results from a pilot study with in vitro matured oocytes did not demonstrate MII abnormalities but revealed a higher rate of occurrence in mature oocytes in telophase I, which could lead to compromised oocyte quality and decreased fertilization rates for patients with endometriosis-induced infertility [24]. An increased incidence of telophase I mature oocytes has also been noted, leading to reduced fertilization rates for women with endometriosis [25].

Exposure to ROS and TNF-α has been suggested to cause modifications in the MII spindle architecture and chromosomal alignment, in a dose- and time-dependent pattern [19]. Indeed, the damage in DNA structure due to exposure to endometriosis-affected peritoneal fluid, leads to increased aneuploidy rates [10].

Cytokines regulate matrix metalloproteinases, which partake in extracellular matrix remodeling processes, such as ovulation and formation of corpus luteum, during proliferation and differentiation. In the peritoneal fluid of endometriosis patients, increased levels of metalloproteinases have been detected [26]. Inhibitors of metalloproteinases are released from endometriotic tissue to the peritoneal fluid and direct toward the theca of the preovulatory follicles, intercepting the function of metalloproteinases [27]. Post-ovulation, the oocyte becomes exposed to the peritoneal fluid which entails aberrant concentrations of metalloproteinase inhibitors, which affect oocyte development until preimplantation [28].

Multiple studies have underlined the impact of MII spindle abnormalities in oocyte and embryo quality in mild endometriosis cases. The follicular fluid is a core element for oocyte maturation; hence, alterations in the follicular microenvironment could affect oocyte quality, fecundity, and early embryonic development. It has been observed that follicular fluid from infertile women with mild endometriosis decreases the hatching rate of IVF-formed zygotes stemming from in vitro-matured bovine oocytes [29]. Interestingly, it has been observed that milder forms of the disease are more likely to intercept fertilization and earlier implantation processes [30]. The meiotic abnormalities of in vitro matured bovine MII oocytes, emerging due to endometriotic follicular fluid, could be prevented by N-acetyl-cysteine and L-carnitine [31]. Peritoneal endometriosis in 33 B6CBA/F1 mice has been associated with lower zygotes ((21.0 ± 3.8 vs. 35.5 ± 4.6; $p < 0.05$) and oocyte quality, with endometriotic rats presenting lower normal count of MII oocytes ((110/181 (60.8%) vs. 136/163 (83.4%); $p < 0.0001$) [32].

In small experimental study of 22 cases and controls, the percentage of meiotically normal mice oocytes was significantly higher ($p = 0.01$) for oocytes that underwent in vitro maturation in the absence of follicular fluid. Moreover, the oocyte count of oocytes in metaphase I stage was significantly higher in the follicular fluid of mild endometriosis patients compared to controls, and the percentage of meiotic abnormalities was significantly increased in oocytes matured with FF stemming from mild endometriosis than in FF retrieved from controls (55.8% vs 23.1%, $p = 0.01$). These findings suggest that the follicular milieu of infertile endometriosis patients might contain factors that could intercept nuclear oocyte maturation. Among the oocytes that achieved nuclear maturation and progressed to metaphase II stage with an analyzable spindle, the percentage of meiotic abnormalities was increased when oocytes underwent in vitro maturation in the presence of follicular fluid stemming from endometriosis patients. This can be interpreted that the follicular fluid of infertile women with mild endometriosis could instigate meiotic oocyte anomalies, thus impairing oocyte quality [33]. Congruous results have been yielded in another similar study, where the percentage of meiotically normal oocytes was significantly reduced for in vitro matured oocytes in the presence of 1% (62.50%) and 10% (56.25%) of peritoneal fluid from patients with endometriosis stage I/II than in the absence of peritoneal fluid (88.46%) and in the presence of 1% (78.57%) and 10% (84.61%) of peritoneal fluid from fertile women ($p < 0.001$) [34].

Regarding more advanced disease stages, in 100 patients who underwent IVF/ICSI with unilateral endometriomas, either recurrent or post-cystectomy, more immature oocytes of metaphase II and germinal vesicle stage were identified, compared to infertile controls ($p < 0.05$). Endometriomas greater than 3 cm in diameter were associated with impaired quality of obtained oocytes in endometrioma patients, with endometriomas post-cystectomy also affecting ovarian reserve [35]. In another study, women with recurrent unilateral endometriomas presented with decreased ovarian reserve markers (2.1 ± 1.75 vs. 3.2 ± 1.4, $p < 0.005$) and lower oocyte count (12.2 ± 1.8 and 10.2 ± 1.6 days, $p < 0.001$), with implantation rate being 1.5 times lower compared to controls (15.8% vs. 24.0% $p < 0.005$) [36]. As for patients having undergone endometrioma cyst surgery, the number of retrieved MII oocytes, high-quality embryos, implantation, and pregnancy rates appear to be significantly reduced compared with recurrent endometriomas or pelvic endometriosis [37]. On the contrary, a matched case-control study showed similar rates of matured oocytes, peak estrogen levels, fertilization, cleavage, high-quality embryo, viable embryo, cancellation, implantation, and clinical pregnancy rates between participants of advanced endometriosis stages, or endometrioma and cystectomy [38].

On a larger scale, a study with a total sample size of 1124 participants showed that women with endometriosis were associated with lower oocyte count (7.0 ± 4.3 vs. 9.7 ± 6.4, $p < 0.0001$) and metaphase II oocytes (4.8 ± 3.5 vs. 6.9 ± 5.0, $p < 0.0001$), as opposed to controls. Similarly, the total embryo number (3.5 ± 2.9 vs. 4.8 ± 3.9, $p = 0.0006$) and top-quality embryos (0.5 ± 0.8 vs. 0.7 ± 1.1, $p = 0.01$) was decreased in endometriosis patients. Top-quality embryos, however, were present in both groups (OR = 0.87; 95% CI [0.66–1.12]; $p = 0.3$) [39].

On the contrary, a retrospective trial of 328 patients [40] demonstrated similar aneuploidy rates, clinical pregnancy, pregnancy loss, and live-birth rates of endometriosis patients compared with controls. Therefore, it seems obscure whether endometriosis directly affects IVF outcomes through oocyte quality or the endometrium. In this study, age appeared to be the sole factor inducing higher aneuploidy rates. These results have been exhibited in previous research, as endometriosis patients receiving IVF presented in their blastocyst biopsy and preimplantation genetic testing similar aneuploidy rates compared with their age-matched peers [41]. A retrospective cohort study included 51 patients with endometriosis stage III-IV, divided into two groups depending on the location of lesions as identified during operative staging: a group ($n = 27$) with ovarian and a group ($n = 24$) with both ovarian and deep infiltrating endometriosis. Both groups showed diminished ovarian reserve regarding antral follicle count, though a significant decrease, age-dependent, was noted in patients with ovarian and pelvic infiltrating endometriosis compared to ovarian endometriosis alone. It can be deduced that deep infiltrating endometriosis influences ovarian reserve regarding antral follicle and retrieved oocyte count [42].

1.3 Biomarkers and Indicators of Oocyte and Embryo Quality

As an ongoing need for optimal management of endometriosis-induced infertility, researchers have sought suitable prognostic biomarkers that could be employed as a measure of IVF/ICSI success.

Differential gene expression patterns convey information on possible causative factors hindering fecundity. *BMP-6* and *SMAD4* genes are under-expressed, inducing alterations in cumulus cell function [43]. Lower expression of CYP19A1A in cumulus cells has also been reported for women with endometriosis-induced infertility [44]. *PTGS2* gene in cumulous cells is downregulated in a sample of 78 patients with endometriosis (7.2 ± 10.5 vs 12.4 ± 15.7, $p > 0.05$). *PTGS2* encodes cyclooxygenase 2 (COX-2), which is essential for oocyte competency. Hence, decreased COX-2 concentrations in cumulus cells could be responsible for compromised oocyte development [45]. In a retrospective study including 25 women less than 38 years old, 76% achieved good outcomes in live birth delivery after being treated with single embryo replacement. Expression levels of eight candidate genes (*HAS2*, *FSHR*, *SLC2A4*, *ALCAM*, *SFRP2*, *VCAN*, *NRP1,* and *PR*), corrected for the house-keeping gene *RPL19*, were measured in individual cumulus-cell masses. It was observed that the selection of MII oocytes according to relative ranking levels of a subset of cumulus cell-expressed genes provided a significantly increased chance of identifying a good quality oocyte, rather than randomly selecting MII oocytes [46].

Moderate to severe disease stages can significantly suppress GDF-9 mRNA expression in the granulosa cells of patients compared with disease-free controls. Despite the fact that GDF-9 in the follicular fluid of cases and controls was the same, mRNA expression in patients was significantly reduced, with the oocyte and

high-quality embryo count being positively associated with its expression in controls but not in the disease group. Moreover, no association for the endometriosis group was detected between GDF-9 mRNA expression and serum estrogen and progesterone concentrations, as opposed to a negative one present in controls [47].

Altered expression patterns of microRNAs have been associated with oocyte competence. Expression of microRNA-451 is suppressed in the follicular fluid of endometriosis patients and affects embryonic potential in both rodents and humans. The downregulated miR-451 in oocytes could impact on preimplantation embryogenesis through suppression of the Wnt signaling pathway [48]. In a prospective case-control study, miRNA profiling that had been performed considering cumulus cells for the investigation of possible differences between infertile patients with early (EI/II) and advanced endometriosis (EIII/IV), it was found that the miRNA miR-532-3p showed significant differences among the analyzed groups, being downregulated in the EIII/IV group compared to IC group, as well as compared to EI/II group. Enrichment analysis showed that several genes regulated by this miRNA partake in pivotal pathways for fidelity of oocyte competence, including oxytocin, calcium, Wnt, FoxO, ErbB, and Ras signaling pathways, as well as oocyte meiosis pathway [49].

Aberrant epigenetic modifications are involved in the pathogenesis of endometriosis. Transcript profiling of key chromatin-modifying enzymes on ovarian tissue has revealed significant differences in gene expression, in a disease-duration pattern. Pathway analysis has highlighted the abnormal regulation of chromatin-remodeling enzymes, with the most prominent being the arginine methyltransferases CARM1, PRMT2, and PRMT8. CARM1 is a core element of nuclear receptor-mediated transcription and preservation of pluripotency in the cleavage embryonic stage, thus, downregulation of CARM1 protein expression in endometriosis patients can justify the decreased oocyte competence. In the same study, the observed hypermethylation within the *PRMT8* promoter region is suggestive of transcriptional suggestion of the corresponding gene due to the occurrence of deregulated CpG methylation [50].

Transcriptome profiling has yielded commensurable results, as demonstrated through single-cell MII oocyte RNA sequencing. A total of 520 differential expressed genes were identified in patients with ovarian endometriosis compared to healthy individuals, 394 of them being up- and 126 downregulated. Some of the most significant overexpressed genes were *APOE*, *DUSP1*, *G0S2*, *H2AFZ*, *ID4*, *MGST1,* and *WEE1*, and *PXK* was one of the significantly suppressed genes. Outcomes from functional analysis displayed 31 enriched functions being deregulated in endometriosis patients, with 16 of them being involved in molecular processes of interest, including response to oxidative stress, cell growth, and steroid metabolism. Enrichment was also identified for mitochondria, as well as for functions relevant to processes pillar for embryonic development, as methylation and angiogenesis [51].

Another –omics technology that can offer insight regarding metabolic profile, inflammatory condition, and cellular damage is the utilization of metabolomics profiling of the follicular fluid, which depicts the ovarian milieu and can provide

guidance for a complex disease such as endometriosis. Metabolomic analysis of the patient's follicular fluid has shown elevated concentrations of phospholipids, lactate, insulin, PTX3, CXCL8, CXCL10, CCL11, and VEGF, while several fatty- and amino-acids, total LDH and LDH-3 isoform appeared in reduced levels. These differences in concentrations are suggestive of the impaired oocyte quality [52].

Since inflammation is an inherent process in the manifestation and course of disease, inflammatory biomarkers could be a promising tool for the assessment of oocyte and embryo quality in women with endometriosis. In a prospective case-controlled study of 340 subjects, IL-8, IL-12, and the angiogenic hormone adrenomedullin were significantly elevated in patients compared to controls ($p < 0.001$), being inversely associated with oocyte and embryo quality [53].

It could be hypothesized that variations in hormonal serum concentrations can reflect oocyte quality, thus delineating the efficacy of assisted reproduction. In a cross-sectional study of 749 patients, age and anti-Müllerian hormone were significantly associated with ovarian reserve in the group of poor IVF responders ($p < 0.001$). Multivariate analysis showed age to be the only significant predictor of ovarian response in the poor responder group ($p = 0.004$), which is an expected finding, as commented in the previous section [54]. In a retrospective study consisting of 50 patients with severe endometriosis, younger than 37 years of age, having undergone IVF, the count of oocytes retrieved (3.8 ± 2.6 vs 6.9 ± 4.6, $p < 0.001$) and the percentage of mature oocytes in metaphase II (70% vs 83%, $p < 0.001$) were significantly lower in patients with anti-Müllerian hormone (AMH) serum concentration equal or less than 1.1 ng/mL. On the contrary, implantation and pregnancy rate, as well as embryo morphology, were not associated with serum AMH levels; thus, they should probably not be employed as a tool for evaluating IVF/ICSI success [55]. In another analysis including 431 women aged equal to or less than 36 years, the obtained oocyte count (10.6 ± 21.2 vs. 14.6 ± 21.1, $p < 0.001$) was reduced among the patient group. Implantation rates (28.1% ± 38.9% vs. 33.9 ± 42.7%, $p < 0.001$) were decreased for patients with endometriosis; however, fertilization, pregnancy, miscarriage, and cycle cancelation rates were similar. The occurrence of extracytoplasmic, but not intra-cytoplasmic oocyte defects in patients also exhibited significance. While embryo quality (45.3% vs 47.3%, $p = 0.037$) in the endometriosis group was decreased, blastocyst formation rates demonstrated no difference [56].

AMH levels also exhibited no difference between infertile patients with endometriosis and infertility induced by other etiology; hence, endometriosis appears to not affect the primordial pool of follicles and oocytes but to impair the quality of the ovarian response to the hCG injection. Basal FSH concentrations, however, could predict the efficacy of assisted reproduction in endometriosis patients, independent of disease stage [57].

In a small prospective cohort study of 28 patients, reduced peak serum E2 (2068.8 ± 244.6 pg/mL vs. 2756.2 ± 205.0 pg/mL, $p = 0.047$) was detected in endometriosis group, as well as elevated apoptosis rates (80.0% vs. 22.2%, $p = 0.0054$) and increased frequency of cortical granule loss (83.3% vs. 24.0%, $p = 0.0132$), spindle disruption (66.7% vs. 16.0%, $p = 0.0258$) and zona pellucida dissolution

timing (133.8 ± 9.4 s vs. 90.5 ± 5.8 s, $p = 0.0021$) compared to disease-free controls. The augmented nitrate levels traced in the follicular fluid of patients suggest the involvement of oxidative stress through the dysregulation of nitric oxides in the impairment of oocyte quality [58].

Retinoids are substantial for multiple reproductive processes in both sexes, including ovarian somatic cell development, spermatogenesis, implantation, and embryonic development. In this study, it was found that all-trans retinoic acid (ATRA) is essential for oocyte development and quality, and that lower ATRA synthesis might lead to impaired fecundity of patients [59]. It has also been suggested that the brain-derived neurotrophic factor (BDNF) can serve as a biomarker for oocyte quality and fecundity. It is an estrogen effector and a crucial growth factor in endometrial cells [60].

1.4 Perspectives of Therapeutic Strategies

Multiple studies have focused on developing treatment strategies for endometriosis-induced infertility by taking advantage of the knowledge obtained by ongoing research on disease pathophysiology.

Freeze-all strategy could be an effective option for the improvement of assisted reproduction outcomes in advanced endometriosis patients, according to a matched retrospective cohort, as implantation, clinical pregnancy, and live birth rates demonstrated a statistically significant increase in the group having undergone the freeze-all method, compared to patients having received fresh transfer [61]. Moreover, morpho-kinetics analysis by time-lapse imaging can predict embryo implantation potential and can be deemed, thus, a useful tool for embryo selection. Time-lapse data by 72 women with endometriosis undergoing infertility treatment for IVF that were retrospectively collected showed deteriorated relative kinetics, which influenced embryo quality, independent of disease stage [62]. It should be pointed out that relative kinetics define cleavage synchrony and can therefore predict blastocyst quality.

Adjuvant interventions in hormonal mechanisms during IVF stimulation could be associated with better outcomes in terms of the success of assisted reproduction methods. Gonadotropin-releasing hormone agonist (GnRH-a) combined with transvaginal ultrasound-guided cyst aspiration has yielded improved treatment effects and pregnancy outcomes in women with ovarian endometriosis-associated infertility who underwent IVF–embryo transfer [63]. On the contrary, among 61 patients with mild peritoneal endometriosis who received IVF with an additional pituitary suppression by a 3-month administration of GnRH-a, no difference was observed in pregnancy rates and MII oocyte count, with FSH doses and stimulation period being lesser for controls [64]. Similarly, additional FSH surge at the time of hCG trigger was shown to not offer any benefit in IVF/ICSI outcomes regarding clinical pregnancy rates, number of obtained oocytes, rate of good quality embryos, and implantation rate [65].

2 Discussion

Impaired fecundity due to endometriosis occurs as a result of complex molecular interactions. The presence of ectopic endometrium deranges the endometrial hormonal, cellular, and immunological milieu, negatively influencing decidualization, placentation, and developmental programming of the embryo. Causative factors are the by-products of oxidative stress (ROS), which trigger inflammatory response, which in turn instigates perturbation of meiotic spindle structure, oocyte apoptosis, and abnormal chromosomal segregation. Moreover, epigenetic modifications and alterations in gene expression contribute further to impaired fertility.

An important issue regarding the effectiveness of assisted reproduction techniques is the still inconclusive research outcomes regarding the parameters of IVF success. Recent research has provided evidence that the number of cleavage-stage embryos obtained, the blastulation rate, as well as the percentage of good-quality embryos are the same in the endometriosis and control group, with only ongoing pregnancy rates exhibiting a decrease among patients [66]. Furthermore, a retrospective cohort study showed no difference in implantation of embryo/high-quality embryo ratio, fertilization implantation, and clinical pregnancy rates between cases and controls [67]. According to a systematic review and meta-analysis of 20,167 women with endometriosis, chances for clinical pregnancy and live birth after assisted reproduction were disease-stage independent [68]. While it has been suggested that ICSI could induce mechanical damage to the oocytes than conventional IVF, newer data did not support this notion for endometrioma patients, with the time of surgical removal being irrelevant to the assisted reproduction outcome [69].

For the successful management of patients with endometriosis-associated infertility undergoing IVF/ICSI, a thorough investigation of the molecular mechanisms of endometriosis would elucidate these complex pathophysiological interactions. In the era of precision medicine, the design of personalized therapeutic strategies according to patients' unique disease profiles is an effective tool for improving oocyte competence and achieving higher pregnancy rates. The identification and development of biomarkers would offer timely prediction and prognosis of disease progression and maximize the effects of assisted reproduction.

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Impact of Surgery for Deep Endometriosis on the Outcomes of In Vitro Fertilization

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1 Introduction

Laparoscopic excision of deep endometriosis (DE) effectively alleviates endometriosis-related pain [1]. However, the effect of surgery on the reproductive outcomes of infertile patients with DE undergoing in vitro fertilization (IVF) is controversial. Surgical treatment of DE is required in patients with severe pain symptoms who do not tolerate the interruption of hormonal therapies [2]. Furthermore, surgical excision of rectosigmoid endometriosis may prevent bowel occlusion or subocclusion during ovarian stimulation for IVF and pregnancy [3]. Moreover, the excision of deep endometriotic nodules may prevent rare complications during ovarian stimulation and pregnancy, such as spontaneous hemoperitoneum and bladder rupture [4–6]. However, surgical treatment of deep endometriosis may cause severe complications impacting fertility (such as a pelvic abscess). In addition, the excision of concomitant ovarian endometrioma may decrease ovarian

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reserve. Thus, we should be careful in interpreting data in cases where concomitant excision of endometrioma was performed in addition to the excision of DE.

This chapter will summarize the available data on the impact of surgery on the outcomes of IVF in infertile women with deep endometriosis.

2 Clinical Studies

The impact of surgical excision of DE on the outcomes of IVF was assessed in retrospective and prospective cohort studies with a relatively small sample size. No randomized controlled trial is available on this topic.

A retrospective study assessed the impact of surgery for endometriosis on IVF outcomes by comparing the outcomes of patients who underwent laparoscopy within 6 months of oocyte retrieval and those who underwent surgery between 6 and 60 months from oocyte retrieval [7]. There were no significant differences between the two groups with regards to age, extent of endometriosis, or results of ovarian stimulation and oocyte aspiration (dose and duration of gonadotropins required, number of oocytes obtained, fertilization rates, and embryo transferred).

Implantation rates and pregnancy rates were similar in the two study groups. Regression analysis revealed no correlation between implantation rates and surgery-oocyte aspiration interval. Therefore, the authors concluded that the time interval between surgical excision of endometriosis and IVF does not significantly affect IVF outcomes.

A Brazilian prospective study compared the outcomes of IVF in women who underwent extensive excision of endometriosis before IVF with those who underwent IVF only [8]. One hundred 79 consecutive infertile women were enrolled in the study; their ages ranged from 24 to 38 years, with a mean of 32 ± 3 years. After counseling, 105 women underwent IVF, and 66 chose to undergo laparoscopy to remove endometriosis before IVF. Eight women were lost to follow-up. The time interval between surgery and IVF was 14.6 ± 12.5 months, ranging from 3 to 18 months. Patients who spontaneously conceived were excluded from the study. 153 IVF cycles were performed in women who did not undergo surgery, and 86 IVF cycles were performed in women who underwent surgery. A significantly higher r-FSH dose was required to achieve adequate follicular development in women who underwent IVF after surgery. The number of oocytes retrieved was lower in patients who underwent surgery than in those who underwent only IVF. Fertilization rates, number of top-quality embryos, and number of embryos transferred did not differ between the two study groups. Patients who underwent surgery before IVF had higher implantation rates (32% vs. 19%) and higher pregnancy rates (41.0% vs. 24%). Pregnancy outcomes were similar in the two study groups.

A French cohort study investigated whether surgery for DE affects the outcomes of IVF/ICSI [9]. The study included 177 patients with DE that were divided into three groups: patients who underwent IVF without surgical treatment of DE ($n = 65$), patients who underwent complete excision of DE ($n = 49$), and patients who

underwent incomplete excision of DE to improve the accessibility of the ovary and to improve implantation ($n = 63$). The demographic characteristics and the severity of endometriosis were similar in the three study groups. The number of oocytes retrieved and the number of transferred embryos were similar in the three study groups. There was no significant difference in the pregnancy rate between the three study groups; the pregnancy rate per patient was 46.2% in women who did not undergo surgery, 51.0% in women who underwent complete excision of endometriosis and 41.3% in those who underwent incomplete excision of endometriosis. Therefore, the authors concluded that surgery for DE does not improve the pregnancy rate.

A French prospective longitudinal cohort study assessed fertility outcomes after IVF/ICSI in infertile women who previously underwent removal of colorectal endometriosis [10]. The study included 60 patients; 27 had prior surgery for endometriosis without removing colorectal endometriosis. Patients underwent conservative surgery (rectal shaving or full-thickness disc excision, $n = 18$) or segmental colorectal resection ($n = 42$). The median number of ICSI-IVF cycles per patient was one (range: 1–4). The median interval between surgery and the first IVF/ICSI cycle was 18 months (range, 6–120 months). Of the 60 women, 36 became pregnant; therefore, the overall pregnancy rate was 60%. The cumulative pregnancy rate was 41.7% after one ICSI-IVF cycle, 65% after two ICSI-IVF cycles, and 78.1% after three ICSI-IVF cycles. A decreased cumulative pregnancy rate was observed for women who underwent segmental colorectal resection compared to those who underwent rectal shaving or full-thickness disc excision. A trend for a reduced cumulative pregnancy rate was observed for women who received a first ICSI-IVF cycle more than 18 months following surgery. Five of the nine women with prior ICSI-IVF failure became pregnant after surgery.

In a retrospective study that included 155 women suffering from infertility for more than 1 year, fertility outcomes were compared between three groups: group A (60 women) consisted of patients who underwent surgery for endometriosis with colorectal segmental resection; group B consisted of 40 patients with evidence of bowel endometriosis underwent endometriosis removal without bowel resection; group C consisted of 55 women who underwent surgery for moderate or severe endometriosis with at least one endometrioma and deep infiltrating endometriosis but without bowel involvement. The number of patients who conceived after IVF was 5 out of 13 in group A, 1 out of 13 in group B, and 4 out of 6 in group C. These results suggest that the presence of bowel infiltration by endometriosis seems to negatively influence the reproductive outcome in women with endometriosis-associated infertility [11].

A French retrospective study investigated the impact of surgery for DE on the outcomes of IVF [12]. The study included only patients with DE without bowel involvement. The study included 72 patients; 35 underwent IVF after surgery, and 37 underwent IVF without previous surgical treatment. 58 IVF cycles were performed in women who underwent surgery and 54 in women who did not. There was no significant difference in the total FSH dose, number of oocytes retrieved, fertilization rate, total pregnancy rate, and clinical pregnancy rate between the two study groups. The clinical pregnancy rate (ultrasonographic visualization of a viable embryo) was similar in women who underwent surgery (40.0%) and in those who

underwent only IVF (40,5%). Based on these findings, the authors concluded that IVF does not impair the outcomes of IVF.

A retrospective observational study including 230 women investigated the factors associated with pregnancy during the first two IVF attempts in infertile women with posterior DE [13]. 48.7% of the women achieved pregnancy after two IVF attempts. The surgical treatment of DE did not affect the pregnancy rate.

A retrospective matched cohort study using propensity score matching analysis compared first-line IVF/ICSI and first-line surgery followed by IVF/ICSI in patients with colorectal endometriosis-associated infertility. The specific cumulative live birth rate at the first ICSI-IVF cycle in the first-line surgery group compared with the first-line ART was, respectively, 32.7% versus 13.0%, at the second cycle, 58.9% versus 24.8%, and at the third cycle, 70.6% versus 54.9%. The cumulative live birth rates were significantly higher for women who underwent first-line surgery followed by IVF/ICSI compared with first-line ART in the subset of women with good prognosis (age $\leq$ 35 years and AMH $\geq$ 2 ng/mL and no adenomyosis) and women with AMH serum level < 2 ng/mL [14]. A systematic review analyzed the reproductive outcomes in women who underwent IVF after surgery for endometriosis and those who underwent IVF only [15]. There was a statistically significant benefit for surgery for DE before IVF. The pregnancy rate per patient was 1.84 (95% C.I., 1.28–2.64) times more likely for patients treated with surgery than those who received IVF without surgery. The pregnancy rate per cycle was 1.84 (95% CI, 1.26–2.70) times more likely for patients with previous surgery than those receiving only IVF. The live birth rate per patient was 2.22 (95% C.I., 1.42–3.46) times more likely for patients with previous surgery than those receiving IVF only. First-line surgery improved the pregnancy rate per patient when there was DE with digestive involvement (OR 2.43; 95% C.I., 1.13–5.22) and DE without digestive involvement (OR 1.55; 95% C.I., 0.61–3.95).

In a recent retrospective study that included 84 women with DE who underwent IVF, the data did not show a substantially increased risk of recurrence/disease progression with IVF [16].

3 Discussion

The pathogenetic mechanisms of DE-associated infertility that would explain the improvement of IVF results after surgery are unclear [15]. Endometriosis increases the concentration of inflammatory cytokines in the pelvic cavity; these cytokines may affect oocyte production, ovulation, fertilization, and implantation. Endometriosis may increase oxidative stress, which may interfere with IVF. In addition, surgery may restore the normal pelvic anatomy, thus facilitating ovarian access during oocyte retrieval. Finally, surgery is required in some patients before IVF to treat hematosalpinx caused by endometriosis because the tubal fluid entering the endometrial cavity may alter the local environment or affect embryo implantation. Unfortunately, no randomized controlled trial investigated the impact of surgery for

DE before IVF. In general, it is challenging to establish the specific contribution of DE and its removal on IVF outcomes because deep endometriosis is frequently associated with other forms of the disease (such as ovarian endometrioma and superficial endometriosis), which are treated at the time of surgery. On the other hand, for reasons that are not fully understood, colorectal DE seems to contribute uniquely to infertility, and complete excision might offer improved outcomes.

Furthermore, DE may be associated with adenomyosis, affecting the implantation rate during IVF.

In this chapter, we summarized the available studies on the impact of surgery for DE on IVF outcomes. The literature is scarce, and there are few controlled studies with no randomized controlled trials. Currently, two clinical trials are focused on answering this important clinical question: the ENDO-FERT trial (ClinicalTrials.gov Identifier: NCT02948972) and the SVIDOE trial (ClinicalTrials.gov Identifier: NCT04743167). Based on this background, surgical excision of DE should not be offered as the first-line treatment in asymptomatic patients to improve the outcomes of IVF. However, surgical treatment of DE may be required before IVF in patients who do not tolerate pain after the discontinuation of hormonal therapies and during ovarian stimulation and in those at risk of bowel occlusion or subocclusion or hydroureter/hydronephrosis during IVF and pregnancy. Also, surgical management might be a suitable option for women who failed prior multiple IVF treatments. Future RCTs with adequate power and follow-up are required to define the role of surgery for DE before IVF.

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Impact of Surgery for Ovarian Endometriomas on the Outcomes of In Vitro Fertilization

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1 Introduction

Ovarian endometrioma (OE) is a clinical phenotype affecting 17–44% of females with endometriosis [1]. The OE is the common phenotype in women with endometriosis. The presence of OE is usually associated with a more advanced stage of the disease, stages III and IV of endometriosis, according to the American Society for Reproductive Medicine (ASRM) classification. The presence of OE normally relates to the overthrow of normal pelvic anatomy among these patients. Currently, OE is diagnosed by ultrasound through the identification of a persistent round-shaped, thick-wall cyst (>3 cm), which was filled with a low amount of echogenic fluid [2].

Although the pathogenesis of OE is not completely clear, three major theories have been developed to explain the possible origin of endometrioma. OE is described

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as a pseudocyst with progressive accumulation of menstrual debris by bleeding from active implants located at the site of inversion [3]. The opposite theory supposed that the OE has been originated from the metaplasia of the invaginated ovarian coelomic epithelium [4]. Finally, endometriomas can originate from the ovarian follicle formed by hematoma that causes invagination of the ovarian cortex, which is subsequently colonized by an endometrial surface with or without stroma, even though the origin of the fluid in the cysts remains unclear [5]. Based on clinical appearances and histology-related testing, the clinic-related types of endometriomas include i) Protopathic endometrioma-true endometrioma origin; and ii) secondary endometrioma-follicular or luteal ovarian cysts covered or invaded by cortical endometriotic implants or by major endometrioma [6].

Patients struggling with endometriosis often pursue assisted reproductive technologies (ART) due to fertility issues. Despite a relation between endometriosis and infertility being suggested by different cross-sectional studies, the risk of infertility is widely varied. Women with less than 35 years of laparoscopically confirmed endometriosis have been detected with a two-fold increased risk of infertility [7, 8]. The hyperestrogenism related to the presence of endometrioma can affect the implantation rate due to increased progesterone resistance.

The effect of endometrioma on women's fertility is still debated likewise, the management of ovarian endometrioma has been controversial, especially in the context of ART. Although surgery has been considered the primary treatment to improve IVF outcomes in patients with ovarian endometrioma, in the last years, the great advances in the scientific community have suggested a possible shift to a more conservative surgery in patients with OE.

2 The Impact of Endometrioma on Ovarian Physiology

In recent years, the evidence has focused on the potential effect of endometrioma on ovarian physiology and endometrial stroma. Several cytokines are altered in patients with endometriosis stage IV, causing a hyperinflammatory and hyperproliferative process related to the alteration in the "window of implantation," reducing the time of fecundability [9]. The presence of endometrioma in the ovary could cause direct damage to the structure of the ovary independently of the size through mechanical stretching [10]. The damage caused by endometrioma is demonstrated by the morphological and functional modification that affects the ovary compared to the normal tissue [11].

There are different explanations to support the possible damage to ovarian function by the endometrioma; the content of the cyst represents a potential source of 'toxicity' for the surrounding healthy tissue [12].

The concentration of proteolytic enzymes and inflammatory molecules inside the cyst is a hundred times higher compared to serum or in other types of cysts, and these molecules are responsible for the cellular damage in the ovarian cortex. The higher concentration of iron [13] and reactive species of oxygens (ROS) [14, 15] might damage the ovarian cells for direct contact. Additionally, with higher levels of

iron, there is also an increase in iron storage protein such as ferritin, in the endometriotic tissues of patients with endometriosis [16]. Ironbound to transferrin or other proteins can bind with high affinity to receptors on the surface of cells and the complex undergoes endocytosis.

The chocolate fluid content of the cyst causes critical alterations to the surrounding cells, including modifications in the expression of critical genes and genetic changes potentially initiating tumorigenesis [17, 18].

The healthy ovarian cortex surrounding the endometrioma has increased the oxidative stress compared to other benign cysts [19]. There is an impairment of the normal oxidative balance in the cortex surrounding the endometrioma, with decreased expression of the antioxidative mechanisms [20]. Granulosa cells from patients with endometriosis are characterized by increased apoptosis. Endoplasmic reticulum (ER) stress, a local factor closely associated with oxidative stress, has emerged as a critical regulator of ovarian function. The high oxidative stress in granulosa cells could activate ER stress in granulosa cells in ovaries, with endometrioma mediating the apoptosis [21].

A higher amount of ROS may promote a fibrogenic response together with transforming growth factor (TGF)-β and plasminogen activator inhibitor (PAI)-1 characterized by the expansion of mesenchymal elements, synthesis of collagen and fibronectin [11, 22], and collagen matrix remodeling.

The ovarian stroma supplies blood to the primordial follicles via capillaries and acts synergistically with other components to induce the transition from primordial to primary follicles [11]. Thus, the loss of a cortex-specific matrix could have potentially harmful effects on follicular development, reducing the blood supply to the follicles and decreasing the secretion of growth factors from stromal cells [23].

Oocytes from women with OE have a different transcriptomic profile than those from healthy women. In the oocytes from women with OE, 394 upregulated and 126 downregulated genes compared to oocytes from healthy donors. Several pathways involved in biological processes and molecular functions, such as steroid metabolism, response to oxidative stress, and cell growth regulation are impaired in the OE compared to the control healthy [24]. Mitochondrial function and other functions important in embryo development, such as angiogenesis and methylation are altered. Oxidative stress imbalance has been identified as a potential cause of oocyte apoptosis and necrosis in early follicles [25].

The follicular fluid of women with OE has an altered balance in favor of a pro-oxidative shift and a pro-inflammatory status [15]. The alteration in the mitochondria structures, with lower mitochondrial DNA copy number [26], combined with a significantly lower amount of ATP suggests that the reduced energy production has a role in the decrease of oocyte quality [27]. In the animal models, exposure to peritoneal fluid from women with endometriosis caused oocyte chromosome misalignment and spindle aberrations [28].

The presence of the OE changes the normal structure of the ovaries in fact in the surrounding cortex around OE there is a lower density of follicles after ovarian biopsy compared to a non-endometriotic cyst. Additionally, the vascular network was much less frequent in the ovarian tissue surrounding the endometrioma in comparison with other ovarian cysts.

Moreover, the high levels of ROS may inhibit ovarian angiogenesis and cause capillary loss, based on direct or indirect methods, which reflects a decrease in blood perfusion and impaired follicular maturation [29, 11]. The higher expression of a negative angiogenic factor, such as thrombospondin, associated with decreased microvessel are responsible for ovarian interstitial microvascular damage and a decrease in blood perfusion [30].

3 The Impact of Endometrioma on Ovarian Reserve

Antral follicle count (AFC) is a reliable indicator of ovarian reserve due to the close relationship with age-related declines in follicle counts and the ovarian response to the IVF stimulation cycle [31, 32]. AFC could also be considered a more reliable version of AMH because it measures the ovarian reserve of a single ovary. The main limitation of AFC is its intercycle variation of individual ovaries and the challenge of obtaining a high-resolution image of ovaries in the presence of an endometrioma. AFC might be underestimated in the presence of endometriomas [33].

In a retrospective study on 37 women with unilateral endometrioma who underwent an ART cycle, the authors concluded that the AFC in ovaries with endometrioma is underestimated since the number of oocytes retrieved was higher than expected based on the AFC [34]. The underestimation of AFC could be related to the impairment of the transvaginal probe resolution due to longer path length (the capsule of the cyst wall) and higher-frequency waves resulting in greater attenuation and making it difficult to detect smaller follicles [33].

In this context, AFC has been largely used as a reliable marker to estimate the ovarian reserve of women undergoing surgery for endometrioma. Two studies with unilateral endometrioma have studied the preoperative assessment of both the healthy and affected ovary [35, 36]. Women with endometrioma presented a lower number of antral follicles compared with contralateral, even though there was no statistical significance [37]. A prospective study involving 60 women with an average age of 30 years, reported that women with endometrioma had significantly lower AFC compared to healthy women with no endometriomas (9.73 ± 4.77 vs. 14.7 ± 4.1, respectively, $p < 0.01$). A recent meta-analysis analyzed the AFC in women with unilateral endometrioma, showing the AFC in the endometrioma was significantly lower than the contralateral one (mean difference − 2.09; 95% CI –3.46 to −0.73; $P = 0.003$) [38]. However, the studies included exhibited significant heterogeneity.

Serum AMH is a reliable marker of ovarian reserve, without the limitations of ultrasound and fewer intercycle variations than other hormonal serum parameters, such as follicle-stimulating hormone (FSH) levels. Several studies have analyzed the AMH levels in patients with unoperated ovarian endometriomas to assess the impact of the endometrioma on ovarian reserve [39–43]. A retrospective study on 141 women with endometrioma was compared with 1323 infertility patients

without endometrioma, which showed that the meanAMH concentration in the control group was significantly higher than in the endometrioma group [40].In a retrospective case-control study including 102 women with endometrioma versus 102 healthy matched control, the AMH levels were lower even though there wasn't statistical significance [42]. Similar findings were reported in another prospective study conducted by Chen et al., who evaluated the impact of the presence of endometrioma and laparoscopic cystectomy on ovarian reserve as assessed by serum AMH levels. Before surgery, the endometrioma group had significantly lower AMH levels compared with the other benign ovarian cyst group and the tubal factor infertility group [41]. Conversely, a large retrospective demonstrated that endometrioma does not influence AMH levels. AMH levels are decreased in women with previous surgery for endometriotic cysts independently from the presence of current endometriomas [44].

In a recent systematic review and meta-analysis, a total of 968 women with endometrioma and 1874 controls were pooled together. Women with endometrioma have been presented with a significantly lower level of AMH compared to non-endometriotic benign ovarian [45]. In the secondary analyses, women with endometriomas were separately compared with women with non-endometriotic ovarian cysts and women with normal ovaries. Both comparisons showed significantly lower AMH in endometrioma patients. In this context, the only presence of an endometrioma is associated with decreased AMH levels. The AMH levels analyzed in women with endometrioma, compared to healthy control over 6 months, demonstrated a faster decline of AMH in women with endometrioma [46].

4 Implications on the Response to Ovarian Stimulation after Surgical Excision of Endometrioma

Women with endometrioma presented a progressive decline in serum AMH levels, faster than that in age-matched healthy females [46]. Considering the pathogenesis of endometrioma and the possible effects on the ovarian reserve, early diagnosis, and subsequent treatment are the options to avoid further ovarian injury and preserve ovarian reserve function [47, 48], although the exact efficacy of this strategy has not been elucidated.

In the past, the option mostly suggested to the patients in case of ovarian endometrioma was the surgical excision. In patients with ovarian endometriosis visible to the ultrasound with a concurrent history of infertility, surgery has been considered as the first approach [49]. Since the introduction of laparoscopic cystectomy, it has become the gold standard treatment for ovarian endometrioma with surgical indication [50]. The procedure of laparoscopic cystectomy using the stripping technique, in over 50% of cases, results in the removal of healthy ovarian parenchyma [33], with several implications on the ovarian response during ovarian stimulation. The damage to the healthy ovarian parenchyma is directly proportional to the

endometrioma size [51]. This frequently leads to follicle pool loss, mostly during hilar dissection, in which primary and secondary follicles have been observed in 85% of the specimens [52, 53]. It remains unclear whether these follicles are fully functional, and if their capacity to grow is preserved in such a fibrotic area. Furthermore, several aspects relating to the surgical management of endometriomas remain controversial, and the implications on future fertility represent an open debate. According to the ESHRE guidelines in women with ovarian endometrioma before laparoscopic surgery, the physician should provide a clear explanation of the possible impact on ovarian reserve before performing the surgery [54].

In the women with unilateral endometrioma removed with laparoscopic surgery, there is a decrease in the AFC, using women who had a unilateral endometriotic lower number of developing follicles, and an increased risk of no response to the ovarian stimulation compared to the contralateral healthy ovary [2, 55, 56]. In women with endometrioma, the normal ovarian tissue along with the excised endometrioma was 10 times more frequently noted than with other cyst types [57]. In addition, cysts from young patients are more frequently characterized by a fibroblastic capsule compared with older women. Therefore the more severe follicular depletion observed in the younger group may be related not only to the higher preexisting follicular pool but also to a more "inflammatory" typology of endometriosis [58].

In 2014, the European Society of Human Reproduction and Embryology (ESHRE) Guideline Development Group recommended that clinicians only consider cystectomy before ART to improve endometriosis-associated pain or the accessibility of follicles [54]. In a small study published in 2014 by Coccia, 52 women with endometrioma less than 3 cm undergoing IVF were compared to 12 women with endometrioma greater than 3 cm [22]. While follicles greater than 16 mm, retrieved oocytes, mature oocytes, and transferred embryos were similar, implantation rate was 50% less in the larger endometrioma group (6.5% vs. 13%) and pregnancy rate was also less (16.7% vs. 26.2%) [59].

In this context, the role of surgical excision of endometrioma has been challenged by evidence indicating that the damage resulting from cystectomy may negatively affect the postoperative ovarian reserve [60, 61]. The use of bipolar coagulation rather than suturing seems to be involved in the damage of the ovarian reserve [62], even though the damage caused by electrocautery remains controversial. The method used for hemostasis [63], the surgeon's experience, and the techniques [35] used for the cystectomy are related to ovarian damage. In a comprehensive systematic review regarding the excision of endometriomas and ovarian reserve, there was an evident decline of AMH in women undergoing endometrioma surgery, with a decreased level of AMH in patients with bilateral endometrioma surgery. Interestingly, the major part of the study included used bipolar energy which seems associated with a higher risk of devascularization and thus, negatively impacting AMH [64].

Some authors have proposed to combine the advantages of excisional therapy and drainage and ablation [65, 66]. The endometrioma was approached with excisional cystectomy, but in the area close to the hilus was used the bipolar coagulation

except for the area close to CO_2 laser. The objective of this technique maintain the functional ovarian parenchyma. A randomized control trial (RCT) compared the laparoscopic cystectomy with a combined approach. Women with bilateral endometriomas underwent conventional cystectomy in one ovary and combined surgery in the contralateral gonad with random assignment. Independently by the techniques used, there was a similar rate of recurrences and ovarian reserve after 6 months of follow-up were overlapping [67]. Despite the results of the RCT showing no difference in the technique used, the sample size was small, with a limited follow-up period, and the ovarian reserve was not the primary outcome. Finally, the ovarian reserve was asses using only the AFC. Women who received laparoscopic cystectomy for endometrioma presented diminished serum AMH concentrations [10, 35, 40]. After surgical treatment, the AMH levels progressively decreased in 6–9 months, and it is related to the endometrioma size. Even though Muzi et al. showed in a meta-analysis no postsurgical declination of AFC, the only use of AFC to assess the ovarian reserve should be used cautiously because the AFC could be underestimated in this population [37].

The factor that could affect the decrease of ovarian reserve in women with endometrioma treated with surgery is still debated. The age of the surgery did not seem to be involved in the progressive decline of AMH.

Several studies have reported that there is a positive correlation between preoperative (baseline) AMH states and postoperative AMH decline. Women with higher ovarian reserve presented an increased primordial follicle intensity. During the surgery, due to removal of the cortex or direct damage by the surgery, numerous follicles could be lost, with consequent downregulation of AMH. Although patients with high baseline AMH concentrations may lose a higher proportion of primordial follicles, they may still conserve a higher residual compared with women with low AMH levels preoperatively. Likewise, bilaterality is a significant factor in predicting surgery-related ovarian reserve impairment. Women with recurrent unilateral endometrioma present a higher risk of ovarian failure compared to primary unilateral cases. Likewise, bilaterality is a significant factor in predicting surgery-related ovarian reserve impairment [68, 69]. Although the influence of surgery appears mitigated when only one ovary is involved, Ferrero et al. [70] identified a higher risk of ovarian failure during surgery for recurrent unilateral endometrioma when compared with primary unilateral cases. The severity of endometrioma, based on the revised American Society for Reproductive Medicine scoring, is likely to predict the decline of postoperative serum AMH levels [53]. The ovarian damage was directly proportional between cyst diameter and removed tissue during cystectomy [51]. The deleterious effect is more significant following the excision of larger endometriomas [71].

Wang et al. found that the decrease in the levels of AMH following cystectomy was a short-term effect, with some recovery observed within 1 year [72]. Nevertheless, not every patient presented with a fully restored ovarian reserve, revealing several risk factors of permanent damage: AMH concentrations decreased noticeably after 1 year in patients with bilateral endometriomas, in individuals with cyst size >7 cm and in stage IV groups. In most cases, the effect of endometrioma

excision on the ovarian reserve is unpredictable [72]. Timepoint selection for ovarian reserve testing after intervention may be critical to assessing any harmful effects. The long-term effect of surgical treatment of endometriomas on serum AMH levels requires in-depth studies.

The most recent meta-analyses have demonstrated a progressive decline of AMH from 6 to 9 months post-surgery [73, 74], with a worse decline in women with bilateral endometrioma but not statistically significant [43]. In patients with bilateral endometriomas, the AMH decrease was higher after 9 months from the surgery.

5 Endometrioma and Fertility Outcomes

Endometriosis has been considered to independently cause damage over time, the major part of the studies considering endometriosis the principal cause of the shorting reproductive window [75]. Although clinically-recognized associations have been reported, the definite cause-effect relationship between endometrioma and infertility remains unclear. In the past, surgery has been considered the primary treatment for infertility in cases of endometrioma [54]. Nevertheless, patients who have received cystectomy for endometrioma may experience a further reduction in ovarian reserve, prompting concern about reduced fecundity following surgery [69]. Several aspects associated with infertility management in an endometrioma setting remain controversial, and consequences to future fertility (spontaneous and assisted pregnancy) require further clarification. Clinicians in the setting of IVF should consider carefully the opportunities to perform surgical treatment before IVF. Extensive evidence indicates that surgery can lead to quantitative and qualitative injuries. Moreover, numerous reports describe a poorer ovarian response to controlled ovarian hyperstimulation (COH) among women who have undergone surgery for ovarian endometrioma [76, 77]. Tang et al. found a statistically significant decrease in the number of oocytes retrieved after surgery for ovarian endometrioma, but only when the operated endometriotic cyst was larger than 4 cm [78]. Other investigations show similar COH responses between women with previous surgery versus those with intact endometrioma and between operated versus nonoperated gonads from the same patient [79, 80]. However, a complete lack of COH response is reported in up to 13% of operated patients [81]. No statistical differences are reported in ovarian response to COH in the two groups [82]; another meta-analysis found that women undergoing surgery required higher gonadotropin doses with fewer oocytes collected [83], the last meta-analysis only demonstrated the need for increased gonadotropin doses after surgery [84]. Only one RCT has investigated whether pre-IVF cystectomy improved reproductive outcomes, and it did not demonstrate any benefit of surgery, instead reporting that surgery was associated with similar fertilization, implantation, and pregnancy rates along with a decreased ovarian response to COH [85].

5.1 Impact of Endometrioma and Surgical Excision on Unassisted Conception

To date, a clear association has not been found between endometrioma and fertility. Although several studies have reported a decrease in ovulation in women with endometriosis, the number of studies on ovulation cycles is insufficient [81, 86]. In more than [62] 1000 menstrual cycles, there were similar ovulatory rates among affected ovaries, regardless of the size and number of endometriotic cysts. The ovulatory function can be reasonably assumed to be preserved in patients with endometrioma [87, 88]. Since endometrioma is not found to have an impact on ovulation, existing concerns have focused on its adverse effects on ovarian reserve [87]. Furthermore, the ovarian reserve reveals the reproductive potential of a patient, both qualitatively and quantitatively [88]. Fertility is likely to be reduced by the presence of endometrioma alone, since the association between endometriotic ovarian cysts and a decreased ovarian reserve has been extensively established [37, 41, 43]. However, the extent to which this impacts pregnancy in females with endometrioma is not well understood. The endometrioma usually coexists with pelvic endometriosis, and it is rarely isolated; the role of endometrioma in female infertility is therefore assumed to be overestimated [89]. Moreover, more than half of females with small endometriomas have pelvic adhesions and adenomyosis that could reduce fertility [89], which affects the judgment on the correlation between endometrioma and infertility. In a population of patients with histologically proven endometriosis, endometrioma showed no relation to the presence of infertility. Nevertheless, to further clarify this, additional research is required. There is controversy regarding the surgical excision of endometriomas in females who have received infertility treatment. There are high variations in the rate of pregnancy after laparoscopic excision of endometriomas, considering the length of follow-up times [87, 90]. In the studies, there are many confounds factor and methoological biases; for this reason, the beneficial effect of surgery remains unknow. Even though surgical excision of endometrioma could improve spontaneous pregnancy rates by restoring the ovarian functional anatomy, some data indicated that surgical excision alone does not seem to affect fertility [91]. There are many concerns regarding the safety of surgery due to the reduction of AMH levels after surgery. According to the guidelines from the European Society of Human Reproduction and Embryology, 2005, the American Society for Reproductive Medicine, 2012, and the National Health Service, 2010, laparoscopic cystectomy through excisional surgery for an endometrioma $\geq$4 cm is felt to improve fertility (spontaneous pregnancy) compared with drainage and coagulation [92, 93]. Surgical excision of endometrioma may cause ovarian reserve to be reduced in a short time, which may delay achieving pregnancy [49, 94, 95]. In cases of bilateral endometrioma, there is a higher risk of premature ovarian insufficiency following cystectomy [96, 97]. Repeated surgery for endometrioma has a higher risk of complications compared with primary endometrioma [98], and accumulation of postoperative adhesions over a lifetime may affect future fertility. Moreover, low

AMH levels following surgery are predictive of earlier menopause and a shorter reproductive lifespan [99, 100].

5.2 *Impact of Endometrioma and Surgical Excision on the IVF Outcomes*

Patients with endometrioma scheduled for ART presented a lower number of oocytes retrieved from the affected gonads compared with the contralateral ovaries [101, 102]. Several studies demonstrated that the presence of endometrioma in the ovary affected ovarian responsiveness to superovulation during ART [84, 103]. In women with surgery for endometrioma was observed decreased oocyte retrieval rate compared with no operated women [81]. Hamdan et al. [84] also found similar CPR rates and live birth rates (LBR) in patients with endometrioma and control subjects. The endometrioma causes prevalently quantitative rather than qualitative damage to the ovarian reserve. Even if patients with endometrioma presented a decreased number of oocytes retrieved, the clinical pregnancy rate was similar [81, 104]. The opportunity to remove an endometrioma in the patients scheduled for ART should be evaluated carefully by a physician. Several studies indicated that ovarian surgery for endometrioma may cause damage to ovarian reserve [49, 94, 95]. Moreover, much evidence [105–107] suggests that surgery for OMA harms the ovarian response, with a reported reduction of oocytes retrieved following surgery. Yang et al. performed a meta-analysis in 2015, including 1039 cases, the number of oocytes retrieved (mean difference [MD] −1.50; 95% CI, −2.84 to −0.15; $p = 0.03$), metaphase II oocytes retrieved (MD −3.61; 95% CI, −4.44 to −2.78; $p < 0.001$), and total embryos formed (MD −0.66; 95% CI, −1.13 to −0.18; $p = 0.007$) were significantly lower in the endometrioma group. However, the number of good-quality embryos, embryo implantation rate, and clinical pregnancy rate were similar [108].

A recent meta-analysis including 28 studies demonstrated no benefit of ovarian surgery in patients with endometrioma compared to no treated patients, similar CPR and LBR in patients undergoing cystectomy than the control group [106]. Additionally, evidence from other studies demonstrated that females having undergone surgery for endometrioma before in vitro fertilization (IVF) or intracytoplasmic sperm injection exhibited similar fertility results compared with controls [105, 107, 109, 110]. In 2020, a meta-analysis [111] reported no significant difference in the live birth rate between the endometrioma and control groups [odds ratio (OR) 1.23; 95% CI 0.37, 4.06] ($p = 0.74$). No difference in clinical pregnancy rate was identified between the endometrioma and control groups (OR 1.29, 95% CI 0.83–2.0) ($p = 0.26$). The implantation rate did not differ significantly between the endometrioma and the control groups (OR 1.04, 95% CI 0.69–1.56) ($p = 0.86$). Endometrioma could be a physical barrier that may hinder access to the ovary, consequently decreasing the number of oocytes that can be retrieved [59]. Overall, this

seems to suggest that the detrimental influence of endometriomas on ovarian function does not seem to influence fertility outcomes in the context of assisted conception, once an embryo is fertilized [11].

However, other studies have different results, demonstrating that patients with ovarian endometrioma presented a lower number of pregnancies [112]. Another study reported that the CPR rate and LBR in ART cycles were lower in females with reduced ovarian reserve secondary to OMA cystectomy compared with females with idiopathic diminished ovarian reserve [113]. Furthermore, Maignien et al. [114] conducted a multivariate logistic regression analysis to identify the prognostic factors that affected pregnancy in IVF cycles and suggested that surgery for OMA was independently associated with lower pregnancy rates. Importantly, these studies [112–114] are limited as they are surgical, and did not control for any confounding factors including, but not limited to postoperative duration, differing surgical procedure, surgeon's expertise, endometrioma diameter, and laterality. Based on the available evidence, the European Society of Human Reproduction and Embryology guidelines concluded that cystectomy for endometrioma before ART treatment does not improve pregnancy rates [37]. The association between endometrioma and infertility has been extensively reported in the literature; however, the causal relationship between the two is still not identified. Patients with endometrioma and no history of infertility presented a high rate of spontaneous pregnancy, demonstrating the possibility of natural conception before ART treatment [87]. An important question is whether females with endometrioma require ART. When scheduling ART for infertile patients with endometrioma, clinicians must carefully consider whether to perform surgical excision before ART. Currently, the evidence indicates that surgery performed before ART treatment does not improve reproductive outcomes and otherwise increases the risks and costs compared with conservative management [115]. Surgical excision of endometrioma using any technique significantly affects ovarian reserve, particularly in cases of bilateral and recurrent endometriomas [69, 70]. In addition to unintentionally removing healthy ovarian tissue [89], other probable mechanisms affecting ovarian reserve consist of the effect of bipolar electrocoagulation on parenchyma and blood supply of residual healthy ovarian tissue and surgery-related inflammatory response [68]. Patients scheduled to receive ART treatments should only undergo prior surgical treatment in cases of severe pelvic pain, where malignancy cannot be excluded, or reduced accessibility of follicles; conversely, surgery should not be offered to each patient with endometrioma [76, 116]. To reduce the possible effect adverse on the ovarian reserve, surgery should be performed by a gynecologist with specific expertise in endometriosis and fertility. In cases of young females who are not yet planning to become pregnant, it would be appropriate to offer fertility preservation such as oocyte freezing and embryo and ovarian tissue cryopreservation before surgery [117]. Fertility preservation offers a valid treatment option to clinicians and their patients with endometriosis to help them increase their reproductive chances in the future. The detrimental effect of surgical excision of ovarian endometriomas in young women could be indicated surgery after ovarian stimulation for fertility preservation [118].

6 Impact of Ovarian Endometriosis on Donor Oocyte Cycles

Lower implantation rates have been reported in patients with endometriosis undergoing IVF and ET cycles [119]. Some investigators suggest an altered embryo quality [120, 121] maybe due to aberrant events in morphological embryogenesis [122] or a higher in vitro embryo blockage in patients with endometriosis [123].

Women with endometriosis have experimented with lower implantation and pregnancy rates; the oocytes from patients with endometriosis have a differential gene expression pattern, with deregulation involved in abnormal oocyte and embryo development [24]. Several studies have assumed that the negative effect on oocyte development, embryogenesis, and implantation in women with ovarian endometriosis could be related to impaired folliculogenesis, exposure to a hostile environment of macrophages, cytokines, and vasoactive substances in the peritoneal fluid, and anatomical dysfunction of the fallopian tube and ovary [124]. Several pathways are altered in oocytes from women with endometriosis when compared to oocytes from healthy donors, regardless of whether oocytes were retrieved from an affected or unaffected ovary. Conversely, few pathways are altered between affected and unaffected ovaries. Women with ovarian endometriosis presented alteration in steroid metabolism, response to oxidative stress, and cell growth regulation implicated in the decreased oocyte quality [28]. Ovarian endometriosis exhibits a global effect on oocyte quality, independent of whether the oocyte comes from the affected or unaffected ovary [24].

In the oocyte donor cycles, patients with endometriosis had the same chances of implantation and pregnancy compared to when they came from donors without known endometriosis [120]. Patients with advanced endometriosis stage III/IV, according to ASRM classification, have not decreased implantation when the oocyte came from donors, suggesting that infertility in these patients is not related to an unsuitable peritoneal and/or endometrial environment affecting endometrial receptivity [125, 126]. In contrast, patients who received embryos derived from endometriotic ovaries showed a significantly reduced implantation rate as compared to the remaining groups ($p < 0.05$) and hypothesized that this observation was related to oocyte quality [120]. The poor IVF outcome in cases of advanced stages of endometriosis may be related to a reduced number of retrieved oocytes, leading to a reduced number of selected embryos available to be transferred. There is a strong body of evidence that embryo morphology correlates with implantation rates and IVF success. Furthermore, in a prospective matched case-control study by Diaz et al., 2000, IVF outcomes of women with or without endometriosis who received "siblings" oocytes from the same "healthy" donor were evaluated in an attempt to avoid the bias of assigning oocytes of different quality to the different groups. Pregnancy, implantation, and miscarriage rates were not affected by moderate/severe endometriosis when compared with the control group [125].

Similarly, a slightly earlier study [127] retrospectively analyzed 239 oocyte recipients who were divided into two groups: patients with and without endometriosis. The women with endometriosis were further subdivided according to the stage

of the disease into mild and severe. There were no differences regarding pregnancy rates (28% versus 29%) or implantation rates (12% and 13%) in women with and without endometriosis, nor according to the endometriosis stage. These results support a similar conclusion reached by Sung et al., that the adverse effect of endometriosis on reproductive outcomes is not related to implantation [127]. These findings implicated that in patients with severe endometriosis, the poor outcomes and lower implantation rate are more likely due to oocyte and embryo quality in these patients [128].

In a previous large study, almost 9000 couples and more than 35,000 ETs were obtained using donated oocytes in successive cycles, demonstrating that the success of oocyte donation cycles can be predominantly achieved within the first five embryos transferred. The couples failing to achieve a newborn within this range have a diminished but reasonable likelihood of fulfilling a newborn by increasing the number of EmbR to 10. The rate of success in oocyte donation was independent by oocyte donation indication and recipient age, patients with ovarian endometriosis have the same opportunity to reach a newborn with five embryos transferred [129].

Jones et al. has also reported favorable results of IVF in patients with endometriosis. During 3 years, follicular stimulation was initiated for 600 cycles in 319 patients, with endometriosis being the primary diagnosis in 20 cycles. The results show good IVF outcomes among patients with endometriosis who did not become pregnant after surgical and/or endocrine therapy [130]. Furthermore, the findings highlight the fact that endometriosis does not influence the sperm/egg interface or the implantation mechanism. Endometrial receptivity is similar between women with and without endometriosis, and across the different stages of endometriosis.

Frequently, in patients with ovarian endometriosis, may coexist a condition of uterine adenomyosis. The gene expression profile of the samples obtained on LH + 7 (window of implantation) did not differ between women with adenomyosis and healthy subjects using parametric tests. With nonparametric tests, only 34 genes were found to be differentially expressed (dysregulated) in women with adenomyosis. The genes involved in the endometrial receptivity are not altered in patients with adenomyosis in donor oocyte cycles, even though there is a higher miscarriage rate, probably due to the early invasive process [131].

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Endometriosis Progression and In Vitro Fertilization

Ginevra Mills and Michael H. Dahan

1 Introduction

Endometriosis represents one of the most common causes of chronic pelvic pain, dysmenorrhea, and infertility in reproductive-age women. Among infertile women, the prevalence of endometriosis is 15–55% [1, 2] and assisted reproductive technology (ART) is required in at least half of these women to achieve a successful pregnancy [3]. Endometriosis is a sex-steroid hormone-dependent condition. As such, many successful medical treatment options for women with endometriosis involve the suppression of menstruation through the use of gonadotropin-releasing hormone (GnRH) agonists or antagonists, progestins, and oral contraceptives.

In women seeking pregnancy, management options for their endometriosis are severely limited. Moreover, ART use to assist in achieving pregnancy has been thought to negatively impact the symptomatology or actual expression of the disease [4]. Early case reports of women with endometriosis undergoing in vitro fertilization (IVF) suggested a detrimental and even harmful effect of IVF on disease progression. However, ongoing investigations assessing the impact of ART on endometriosis progression have presented mixed results. The aim of this chapter, therefore, is to review the evidence in the literature regarding the effects of IVF on the recurrence and progression of endometriosis.

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2 Endometriosis, Menstrual Tissue, and Hormonal Responsiveness

Endometriosis is a chronic, progressive condition that is characterized by the abnormal growth of endometrial tissue outside the endometrial cavity. Endometrial deposits can be located throughout the peritoneal cavity, on the ovaries, the rectovaginal fascia, the uterosacral ligaments, and the bladder. In rare occurrences, endometrial tissue can be found in such distant regions as the gastrointestinal tract, the lungs, or the thoracic cavity.

Deposits of ectopic endometrial tissues are influenced by the menstrual cycle, as they contain both endometrial glands and stroma, which are hormone-sensitive. The balance of estrogen and progesterone activity that directs the function of normal endometrium during the menstrual cycle also affects endometriosis deposits. Therefore, with each menstruation, endometriotic lesions can proliferate and bleed, and in the process, cause inflammation and fibrosis in the nearby tissues.

3 Estrogen Responsiveness

Estrogen production and metabolism are altered in the endometrial tissue of women with endometriosis, which is believed to play a role in disease establishment and promotion [5]. In general, in non-pregnant women, estrogen is produced in two major forms: Estradiol, a more potent estrogen, is produced in the granulosa cells of the ovary and minimally by the adrenal glands and secreted into the circulation and directly released into the peritoneal cavity at ovulation; and estrone, a weaker estrogen, is produced by adipose tissue via the conversion of circulating androgens and minimally by the ovary and adrenal gland [5]. However, in women with endometriosis, endometriotic tissue is known to express a complete set of steroidogenic enzymes, including aromatase, which leads to a substantial amount of estrogen being synthesized locally within endometriotic tissue itself [5, 6].

Although the effects of local estrogen synthesis within ectopic endometrium are not fully elucidated, the abnormal production of estrogen in endometriotic stromal cells is linked to the high local production of pain-inducing prostaglandins. This mechanism is believed to be implicated in both the menstrual and non-menstrual pelvic pain that is the hallmark symptom of endometriosis. Prostaglandins play a further role in the induced inflammatory response that perpetuates the abnormal establishment and growth of ectopic endometrial tissue, as well as the subsequent damage to surrounding normal tissues [5, 7].

The responsiveness of endometriosis to fluctuating levels of estrogen throughout the menstrual cycle forms the basis of the theory that ART, particularly ovarian hyperstimulation, could accelerate the progression of the disease. During ovarian hyperstimulation, systemic estrogen levels rise significantly, with peak levels sometimes reaching more than ten-fold higher than physiological levels. However,

estrogen is not the only hormone involved in the pathogenesis of endometriosis, nor is it the only hormone whose expression is altered during ART.

4 Progesterone Responsiveness

Together with estrogens, progesterone controls the cellular composition and normal function of the endometrium during the menstrual cycle. While estrogen stimulates proliferation and endometrial thickening in the first half of the menstrual cycle, after ovulation and throughout the duration of the menstrual cycle, progesterone counteracts the effects of estrogens by inhibiting proliferation and further inducing the decidualization of stromal cells. Although circulating progesterone levels in women with and without endometriosis are similar, ectopic endometriotic lesions do not appear to respond appropriately to progesterone [8].

Abnormal response to progesterone activity by ectopic endometrium has been attributed to alterations in the expression of the progesterone receptors (PR). Endometriotic tissues contain a lower density of PRs than normal endometrium, which leads to a phenotype of effective progesterone resistance. Therefore, the downstream effects of progesterone on normal endometrial tissues, including its anti-estrogen effects, are only variably observed in ectopic endometriotic tissues [3, 8]. Progesterone resistance in endometriosis, like the abnormal local expression of estrogen, appears to be related to aberrant gene expression in the endometrium of women with endometriosis. There are highly variable levels of expression of these abnormal genes related to both estrogen overproduction and progesterone resistance, which manifests as the wide phenotypic variations seen in women with endometriosis.

Treatment of endometriosis with progestogens and oral contraceptives is common and often yields acceptable results for patients. This suggests that even in the presence of abnormal progesterone response in endometriosis tissue, progesterone exposure has a favorable effect on symptomatology and disease progression [9]. Furthermore, during pregnancy, a progesterone-dominated hormonal environment, endometriosis lesions and their resultant symptoms often disappear or are improved [8]. Such predictable improvement further supports the positive role of progesterone in modifying the disease process. Although IVF often results in periods of significantly elevated estrogen levels, these periods are often followed by longer intervals of elevated progesterone exposure. Therefore, the effect of progesterone supplementation used in IVF and endogenous progesterone produced by the ovary cannot be overlooked when assessing the possible role ART plays in the progression and recurrence of endometriosis.

5 Changes in Endometriosis Over Time

Endometriosis is, by nature, a progressive condition. Unfortunately, factors that cause endometriosis to progress, remain stable, or even regress in some patients remain elusive. The progression and recurrence of endometriosis in the setting of medical and surgical treatments pose an arduous challenge for women as well as the physicians managing their care.

Given that surgery is the gold standard for the diagnosis and treatment of endometriosis, studies on the recurrence rates of the condition almost exclusively rely on the analysis of women who have undergone at least one surgery for endometriosis management. Feasibly, recurrence after surgery can occur for multiple reasons, including the growth of residual endometriotic lesions not completely removed at surgery, the growth of microscopic endometriosis undetected at the time of surgery, the development of de novo lesions, or a combination of these factors. Despite the proven efficacy of surgical treatment of endometriosis in the short term, about 40–45% of patients will have a recurrence of the disease within 5 years after their primary surgery [10].

The cumulative recurrence rate of endometriosis varies greatly within the published literature as a direct result of inconsistencies in the definition of recurrence as well as the varied methodologies of studies. It is well-accepted that the endometriosis recurrence rate increases as the length of follow-up increases. Therefore, various studies may derive vastly different recurrence rates depending on the duration of follow-up. The definition of recurrence also differs greatly between studies. While some studies assess progression or recurrence of disease with objective clinical instrumentation, such as surgical or sonographic observation, other studies rely on the more subjective feelings of new, recurrent, or worsening pain. Furthermore, variables including the type of endometriosis, disease severity, method of surgery, type of hospital where the surgery is performed, the skills of the surgeon performing the surgery, and the usage of medical interventions will all impact the rate of endometriosis recurrence [10].

Regardless of the challenges in assessing the true rates of progression of endometriosis, there is a wealth of evidence that illustrates the progressiveness of the disease with time. Despite surgical removal of endometriotic lesions, as well as medical treatment options, the disease continues to develop, change, and progress with the passage of time.

6 Changes in Endometriosis with IVF

6.1 Initial Case Reports

The first evidence to suggest that ovarian stimulation during ART could cause the progression of endometriosis was published as a case report in 1995. In this report, the authors documented a case of a woman who had undergone previous surgery for the management of endometriosis and was then diagnosed with left hydronephrosis and complete ureteral stenosis 26 days after oocyte retrieval. The woman required distal resection of the left ureter, which allowed for histological diagnosis of ureteral endometriosis, defined by complete transmural and intramural invasion of the ureter by endometriotic tissue [11]. The temporal relationship between the ART cycle and the onset of the woman's symptoms led the authors to suspect a causal relationship between ovarian hyperstimulation and acute worsening of her endometriosis.

Subsequent to this initial case report, two small case series and one case report were published that described ten additional IVF-related cases of worsening endometriosis. In 2000, a small case series described four women with a surgically confirmed diagnosis of deep infiltrating endometriosis who required segmental bowel resections after undergoing IVF [12]. Although the time between ovarian stimulation and the occurrence of the women's symptoms was not clearly reported, at least one case reported the onset of symptoms during the course of stimulation. This report attributed the need for bowel resections in these women to be related to the rapid growth of sigmoid endometriosis as a result of exposure to ovarian hyperstimulation.

Again, in 2007, another case series described five women who experienced new onset or worsening pelvic pain symptoms while undergoing ovarian hyperstimulation for IVF. Four of the five women had surgical confirmation of endometriosis prior to undergoing IVF, and one woman had only a history of dysmenorrhea. After cycle completion in all cases, a diagnosis of endometriosis was confirmed (or reconfirmed) in all the women [13]. However, no mention was made regarding the specific forms or stages of endometriosis at the confirmatory surgery, nor was there a comparison to the type of stage of endometriosis seen at the initial surgery for diagnosis.

Finally, in 2012, an interesting case report was published, which described symptomatic thoracic endometriosis that presented immediately after an IVF procedure. The woman presented was initially presumed to have ovarian hyperstimulation syndrome three days after the retrieval of 30 oocytes. On presentation, she was found to have a significant amount of free pelvic fluid as well as bilateral hydrothoraxes requiring bilateral thoracenteses. After an uneventful recovery and subsequent pregnancy from a frozen embryo transfer, she underwent surgical repair of a previously undiagnosed congenital diaphragmatic agenesis, which incidentally revealed the presence of thoracic endometriosis. In light of this new information, the authors

re-evaluated their earlier diagnosis of Ovarian hyperstimulation syndrome (OHSS) and reclassified the diagnosis as thoracic endometriosis syndrome [14].

Assessing single case reports and small case series of adverse outcomes after relatively common procedures poses interesting analytical challenges (Table 1). When considering the possibility of a causal relationship between ovarian stimulation in IVF procedures and the worsening of endometriosis, the most notable challenge is trying to understand how many women with endometriosis underwent IVF without experiencing any worsening of symptoms or adverse outcomes. While the above-described case reports do not provide information regarding these null cases, there was one study published in 1998 that sought to assess the frequency of adverse outcomes related to IVF treatment, including in women with a confirmed diagnosis of endometriosis [15]. Out of 1500 women who underwent IVF treatment for various indications, 143 women with endometriosis underwent 311 IVF cycles. Out of these cycles, they described only two cases of worsening of endometriosis necessitating surgery and subsequent bowel resection (2/311; 0.16%) [15]. When considering the results presented in this study, one can determine that the 10 total described cases of adverse events attributed to worsening endometriosis in the setting of IVF

Table 1 Case reports on the progression of endometriosis during IVF

Authors, Year	*Time between IVF and Symptoms*	*Adverse Outcome*	*Endometriosis Stage (ASRM)*	*Presence of DIE*
Renier, et al., *1995*	26 days	Left Hydronephrosis; ureteral stenosis	Not reported	Yes
Govaerts et al., *1998*	2 months	Rectorrhagia; bowel resection	Not reported	Yes
	2 months	Rectorrhagia; bowel resection	Not reported	Yes
Anaf et al., *2000*	3 cycles	Rectorrhagia; suboclussion	IV	Yes
	3 cycles	Rectorrhagia; suboclussion	IV	Yes
	1 cycle	Rectorrhagia; suboclussion	IV	Yes
	7 cycles	Rectorrhagia; suboclussion	IV	Yes
Jun and Lathi, 2007	During stimulation	Increased pelvic pain	II	Not reported
	During stimulation	Increased pelvic pain	IV	Yes
	During stimulation	Increased pelvic pain	II	Not reported
	During stimulation	Increased pelvic pain	Not reported	Not reported
	During stimulation	Increased pelvic pain	I	Not reported
Halvorson et al., *2012*	3 days	Bilateral hydrothorax	Not reported	Yes

treatment remain relatively rare. Furthermore, it begs one to question whether these few cases of worsening endometriosis would have happened regardless of whether the patient underwent ovarian stimulation, given the natural tendency of endometriosis to progress with time.

Nevertheless, the possible impact that ovarian stimulation may have on the progression or recurrence of endometriosis is clinically relevant. Particularly, the occurrence of adverse events and their temporal relationship to ovarian hyperstimulation for IVF treatments raised an important scientific question regarding the correlation between IVF treatment and endometriosis progression, which led to the publication of larger studies aimed at elucidating this relationship.

7 Pain as a Surrogate for Endometriosis Recurrence

Pelvic pain is the most common symptom encountered by women with endometriosis. While up to 82% of women with chronic pelvic pain are diagnosed with endometriosis, it has also been well-established that some women with endometriosis experience no pain at all [7]. Moreover, there is a poor correlation between the pain experienced by women and the type, severity, or location of the endometriosis that is found at laparoscopy [16].

Surgery is not without its inherent risks; therefore, utilizing laparoscopic evaluation of disease progression in prospective study designs poses an ethical dilemma. For this reason, researchers have conducted prospective studies looking at the possible progression of endometriosis by assessing changes or recurrence of pain in the time following IVF treatments in women with endometriosis. Regardless of the modest relationship between pain and endometriosis, it is of value to assess these studies and the information they present.

The first well-designed prospective study on symptom progression in women with endometriosis was published in 2011 by Benaglia et al. [17]. Specifically, this study evaluated women with a surgical or sonographically documented diagnosis of endometriosis prior to starting an IVF cycle. Women who did not get pregnant were then re-evaluated 3–6 months later. Eventually, 64 women were assessed at both time points. The before and after intra-patient comparisons of the severity of dysmenorrhea, deep dyspareunia, and non-menstrual pelvic pain did not demonstrate significant differences. The majority of women in the study ($n = 43$; 67%) subjectively judged their symptoms as unchanged after IVF. Seven women (11%) reported worsening of their symptoms, while 14 women (22%) reported improvement in their symptoms.

In 2014, Van der Houwen et al. performed a similar prospective study that assessed the overall satisfaction of women with a previous surgical diagnosis of stage III-IV endometriosis who were initiating intrauterine insemination (IUI) ($n = 25$), classical IVF ($n = 25$), or IVF with an ultra-long protocol (which involved between one and 3 months of GnRH agonist administration before initiating ovarian stimulation) ($n = 25$) [18]. As part of the satisfaction assessment, women were asked

to subjectively rate their pain before and after their specified ART cycle. For the whole cohort of 75 women, the number of women with an improvement or deterioration of visual analog scores was as follows: for dysmenorrhea (affecting 31 women), improvement and deterioration were seen in 7 (23%) and 8 (12%), respectively. For dyspareunia (affecting 49 women), this occurred in 7 (14%) and 5 (10%) women, respectively; and for chronic pelvic pain (69 women), this occurred in 9 (13%) and 10 (15%) women, respectively. In addition to there being no significant difference in pain modification throughout the entire cohort of women, there were also no statistically significant differences when comparing these pain modifications within the three separate ART study groups [18]. Furthermore, the inclusion of 25 women who received several months of GnRH agonist downregulation, a known treatment for endometriosis prior to care, may have affected the results.

In 2016, Santulli et al. conducted a prospective study comparing pain symptoms and modifications between women with endometriosis and unaffected women during an IVF cycle [19]. Subjective assessments of pain were scheduled at four time-points throughout the IVF cycle: prior to initiation of oral contraceptive synchronization, during synchronization, at the time of oocyte retrieval, and 3 weeks after oocyte retrieval. At all time-points, the scores for gastrointestinal symptoms, chronic pelvic pain, dyspareunia, and dysmenorrhea were higher in women with endometriosis. However, when compared to the baseline evaluation, there was a significant increase in pain during the IVF cycle for women without endometriosis while women with endometriosis reported an overall improvement in pain symptoms post-retrieval when compared to their initial scores [19].

Although pain symptom modification and endometriosis recurrence are distinctive aspects, they are frequently associated with the medical literature. Analysis of the above, prospective studies on the effect of IVF on endometriosis-associated pain reveals that there is not a consistent or reproducible change in subjective pain experiences before and after IVF treatment in women with endometriosis. Regardless of the poor correlation between the severity of pain and the presence or extent of endometriosis, if worsening pelvic pain were to be used as a surrogate assessment for the progression of endometriosis, it would appear as though IVF treatment has little impact on the progression or recurrence of the disease.

Special consideration should be given to the third study by Santulli et al., which compared subjective pain experience between women with and without endometriosis [19]. This study alludes to the chronic effects of endometriosis on pain modulation in affected women [7]. Despite experiencing worse pain at baseline, women with endometriosis do not appear to experience the normal or expected increases in pain that are usually associated with IVF. This observation further supports the notion that subjective changes in pain experienced by women with endometriosis cannot be correlated to the presence, severity, or extent of the disease.

8 Surgical Evaluation of Endometriosis Recurrence

Surgical evaluation of endometriosis is the gold standard for diagnosis as it allows for an objective assessment of the extend of disease, as well as an opportunity to obtain a biopsy for tissue diagnosis. Ideally, surgical assessment after IVF treatment would provide the most accurate evidence regarding the recurrence of the progression of endometriosis. Although a second-look surgery to evaluate recurrence for the sole purpose of a research study induces ethical considerations, it is possible to assess the differences in endometriotic disease in women who undergo a second surgery for recurrence of endometriosis-associated pain and symptoms after IVF.

There are multiple studies that have retrospectively assessed the recurrence of endometriosis based on the need for a second surgery. Although a second surgery was used as the definition of recurrence in these studies, the reason for requiring surgery in the study participants is not explicitly described. In 2006, D'Hooghe et al. studied the recurrence of endometriosis, as defined by requiring a repeat surgery, in 67 women undergoing IVF after an initial surgery [20]. Of those who did not get pregnant and who were not lost to follow-up, there were 11 women who underwent a second surgery because of suspected endometriosis recurrence. The revised American Society for Reproductive Medicine (rASRM) endometriosis scores from both the primary and the second surgery were compared in the 11 patients. The scores were unchanged in five women, decreased in three women, and increased in three women. The results of this study did not support the authors' assertion that stimulation from ART would worsen endometriosis [20].

A second retrospective study assessing endometriosis recurrence after IVF treatment based on the need for surgery (the cause of which was not clear) was conducted in 2010 by Benaglia et al. [21]. Here, 189 women with surgically confirmed endometriosis who underwent IVF at one center over a 5-year time period were contacted and asked about endometriosis recurrence. The study defined endometriosis recurrence as the need to undergo repeat surgery or to start a hormone treatment despite the desire to achieve pregnancy. Forty-one women (22%) experienced a recurrence of pain symptoms, with 21 of them having undergone a second surgery. The rASRM classification at the most recent surgery prior to IVF intervention was stage II in one case, stage III in nine cases, and stage IV in 11 cases. At surgery for post-IVF recurrence, rASRM classification was stage III in 6 cases and stage IV in 15 cases. The authors noted that there was documented evidence of endometriosis progression in all surgical cases. They also acknowledged that women in the recurrence group had significantly more severe endometriosis stage at the previous surgery and were more likely to have an endometriosis cyst diagnosed on ultrasound at the time of IVF treatment than women without recurrence. Despite subjective surgical evidence of recurrence in 21 women, the median (IQR) duration of follow-up from treatment to recurrence was 34 (21–52) months. The authors concluded that the 36-month cumulative recurrence rate of endometriosis in women undergoing IVF was 20%, which was comparable to previously published reports of pain recurrence and disease relapse rates of 24% over 36 months [16, 21].

The most recent study on surgical assessment of endometriosis relapse, conducted by Crochet et al. in 2016, is in agreement with the previously described studies [22]. In this study, the authors retrospectively recruited women who had undergone two surgeries for endometriosis within their institution with the intent to compare changes in the stage and extend of endometriosis observed at both surgeries. Of 57 women recruited, 21 women underwent an IVF cycle in the period of time between their two surgeries, and the remaining 36 women who did not undergo IVF acted as a control group. Although the women in the IVF intervention group had higher rASRM scores at both surgeries, intra-patient comparison of the rASRM scores from the first surgery and the second surgery revealed worsening endometriosis in all women, with no difference in the rate of progression in either group. Interestingly, the authors noted a non-significant trend toward longer time to repeat surgery in the women who underwent IVF compared to the control group 24.7 vs. 17.7 months. They considered the irony of this difference given that initial rASRM scores were worse in the IVF group and they were less likely to undergo hormonal management for symptoms while pursuing fertility treatments: Either the patients were delaying surgical management because of fertility plans, or these findings offer support to the contention that rASRM scores do not always correlate well with symptoms.

Laparoscopic evaluation is required to diagnose and evaluate the true extent of endometriosis. Therefore, subsequent surgical assessment represents the best objective measure for endometriosis recurrence or progression after IVF treatment. Although the number of studies in the literature that utilize a second surgery to observe recurrence is low, the findings of each study are concordant. The available evidence suggests that IVF treatment does not affect the recurrence or progression of endometriosis beyond the baseline progression that is known to occur with the passage of time.

9 Endometrioma and Deep Lesion Growth During IVF

Although surgery is required for the diagnosis and monitoring of superficial endometriosis, the use of ultrasonography in the diagnosis of ovarian endometrioma and deep invasive endometriosis has become highly reliable [4]. The use of transvaginal ultrasound to monitor endometriosis lesions during IVF cycles represents a reliable, well-tolerated, and ethically appropriate way to assess for objective changes in endometriosis over time. There are four studies in the literature that used transvaginal ultrasound evaluation of ovarian endometriomas and/or deep infiltrating peritoneal lesions to assess for endometriosis progression.

The first prospective study assessing changes in endometrioma size after an IVF cycle was published by Benaglia et al. in 2009 [23]. This group evaluated 70 endometriomas in 48 women both before IVF and at 3–6 months after a failed IVF cycle (women who became pregnant were excluded from the study). The median volume (interquartile ranges, IQR) of the cysts before and after IVF was 3.9 (2.9–7.9) ml

and 4.9 (2.4–9.9) mL, respectively, with no significant difference detected. When a subgroup analysis according to the initial size of the cysts was conducted to analyze the change in size in conjunction with the responsiveness to ovarian stimulation, the group was unable to identify a subgroup that was at higher risk of significant growth. Although one woman out of 48 was diagnosed with an additional endometrioma at the second ultrasound, this was not determined to be significant.

This same group, as a secondary analysis in their study on pain modification before and after IVF cycles (described above), also reported data on the changes in endometriomas and deep infiltrating lesions. In the same population of women, they identified 35 women with 45 cysts, and 9 women with 10 deep infiltrating lesions. The median (IQR) diameter of the endometriomas before IVF was 20 (12–27) mm and after IVF was 20 (17–27) mm, with no significant difference. Similarly, they observed no significant difference in the size of the deep infiltrating lesions before and after IVF treatment, with mean (IQR) diameters of 10 (5–18) mm and 10 (5–18) mm, respectively [17].

In 2018, Seyhan et al. used 3D ultrasonography to assess the change in size and volume of endometrioma cysts during IVF cycles [24]. Their study included 25 women with 28 cysts, with initial dimensions of the cysts measured on the first day of ovarian stimulation. The final measurements were obtained on the day of ovulation trigger. The median (IQR) volume of the cysts increased from 22 (12–30) ml to 25 (11–37) mL ($p < 0.001$), which corresponds to a median increase of 14%. This study also demonstrated a positive correlation between the baseline volume of the endometriomas and endometrioma growth. However, there was no correlation between cyst growth and responsiveness to stimulation. Although a statistically significant difference in the volume of endometriomas was observed during ovarian stimulation, the authors suggested that a 3 ml average growth could not be regarded as clinically significant [24].

Finally, the most recent study assessing changes in endometriotic lesions was published in 2019 by Berlanda et al. [25]. In this retrospective study, women with a documented history of deep infiltrating endometriotic lesions by either surgery or ultrasound who underwent IVF cycles were reviewed for the occurrence of endometriosis-related adverse outcomes, changes in pain symptoms, and changes in lesion size. Although 84 women were included in the study, 24 had complete surgical excision of their deep infiltrating lesions, leaving only 60 women with lesions that were identified on ultrasound examination. Of these women, 35 had documented follow-up ultrasonographic evaluation 3 to 6 months after IVF. In these women, the mean diameter of the endometriotic lesions was 19 ± 6 mm before IVF and 18 ± 7 mm after IVF ($p = 0.06$) [25]. Out of the entire cohort, only one woman experienced a possible endometriosis-related adverse outcome, corresponding to an overall rate of 1.2% (95% CI: 0.05–5.5%) and 1.7% (95% CI: 0.08–7.6%) for the women with ultrasound evidence of endometriotic lesions.

Endometriotic lesions that can be visualized by transvaginal ultrasonography provide a convenient and practical means to assess the progression of endometriosis temporally and in relation to IVF treatment. Multiple studies in the literature have utilized this method of assessment to study if and how endometriotic cysts and deep

infiltrating nodules change during and after an IVF cycle. Although the studies described above have relatively small sample sizes and short follow-up durations, they provide reassuring evidence that ovarian hyperstimulation in the setting of IVF has little, if any, impact on the growth and modification of endometrioma cysts and deep infiltrating endometriosis.

10 Dose Responsiveness in IVF and Endometriosis Recurrence

Endometriosis is an estrogen-dependent condition, which serves as the basis for the notion that a state of hyperestrogenism could result in an expedited progression or recurrence of the disease [6]. During IVF, supraphysiological levels of estradiol are achieved, albeit for a short period of time, supporting the contention that IVF can worsen endometriosis. Studies in the literature have also considered this concept in the development of study designs intended to assess the correlation between peak estradiol levels in IVF cycles or the level of ovarian responsiveness (a surrogate for high estradiol levels) and the rate of progression of endometriosis.

In their 2006 study, D'Hoogue et al. were interested in the relationship between exposure to high levels of estradiol and the recurrence of endometriosis [20]. For each patient who underwent ovarian stimulation for intrauterine insemination (IUI) or IVF, total estradiol exposure over the duration of their study involvement was calculated as follows: Peak estradiol levels from each stimulate cycle were added to a standard preovulatory level of estradiol (250 pg/ml) for each natural cycle during the time the patient was enrolled in the study. This total level of estradiol exposure was then compared against the rate of endometriosis recurrence for all patients, and no correlation was found between the cumulative peak estradiol per patient and the recurrence of endometriosis [20].

Another study directly assessing peak estradiol levels in the context of endometriosis progression during IVF was undertaken by Seyhan et al. in 2018 [24]. This study, which tracked the change in volume of endometriomas during the course of ovarian stimulation, also measured peak estradiol levels of women participating in the study, while performing IVF. Although the authors observed a statistically significant increase in the volume of endometriomas during IVF stimulation, there was no correlation between the change in endometrioma volume and the peak estradiol levels. These findings, similar to those of the previous study described, do not support the assertion that higher estradiol exposure hastens the progression of endometriosis in an ART setting.

Direct evaluation of estradiol levels does not represent the only method for assessing estrogen exposure as a result of IVF treatment. In multiple studies by Benaglia et al., ovarian responsiveness to stimulation, as measured by the number of stimulated follicles, and the number of started IVF cycles, was considered in the evaluation of endometriosis progression [21, 23]. Poor ovarian response, often

characterized by lower levels of estrogen and the development of fewer ovarian follicles, would represent an overall lower level of estradiol exposure during the course of treatment. Alternatively, a higher number of cycle starts would result in higher cumulative estradiol exposures. Their earlier study, which documented that IVF does not induce a significant volume increase of endometriomas, also failed to identify a correlation between changes in endometrioma volume and responsiveness to stimulation [23]. Similarly, in their subsequent study, stratification of their results based on either the number of the cycle starts or on ovarian responsiveness (categorized as normal vs. poor responders with less than four oocytes retrieved) revealed no correlation based on a possible gradient of exposure [21].

Estrogen exposure plays an important role in the pathophysiology of endometriosis. However, there is a lack of evidence to suggest that the short-term elevations in estradiol levels achieved during IVF treatments are enough to accelerate the natural progression of endometriosis. Although evidence in the literature is limited, it was postulated that a causal relationship between estradiol exposure in IVF and disease may manifest as a positive correlation between gradient of estradiol exposure and the rate of progression. This relationship was not observed, however. Therefore, a transient, short-term increase in estrogen exposure may be insufficient to result in a clinically relevant progression of disease. These results are further complicated by the fact that women stimulating more follicles to develop would subsequently have higher peak secreted progesterone levels. Elevated progesterone levels could act to counterbalance any endometriosis lesion growth that may be caused by estrogen production.

11 Controlled Ovarian Hyperstimulation for Intrauterine Insemination

Ovarian hyperstimulation is not only used in IVF treatment but can also be applied in a more controlled manner to induce ovulation of multiple follicles in conjunction with intrauterine insemination (IUI). While the goal of ovarian hyperstimulation in IVF is to safely induce the growth of many follicles for aspiration before ovulation, the goal of controlled ovarian stimulation with IUI is to grow two or three follicles meant to ovulate. Similar to the theory of dose responsiveness of endometriosis to high estradiol levels obtained in IVF cycles, it has been postulated that because stimulation for IUI results in lower ovarian response and peak estradiol levels, there would be little effect on endometriosis progression in women undergoing IUI with controlled ovarian hyperstimulation. While ovulation induction for IUI can be achieved through the use of oral agents or gonadotropins; and indeed, IUI can be performed without ovarian stimulation, for the purposes of this section, IUI will refer to the use of gonadotropin stimulation in conjunction with IUI.

Multiple studies attempted to characterize the effect of IUI on the rate of endometriosis progression compared to that seen with IVF treatment. D'Hooghe et al.

(2006) retrospectively selected 67 women with surgically confirmed stage III-IV endometriosis who subsequently underwent either IUI alone ($n = 17$), IUI followed by IVF ($n = 11$), or IVF alone ($n = 39$). Recurrence was defined by surgical evaluation of lesions or cytological confirmation of fluid aspirated from endometriomas seen on ultrasound. After 21 months of follow-up, the recurrence rate was significantly higher in the IUI alone group as compared to the IUI and IVF group and the IVF alone group (84%, 7%, and 43%, respectively, $p = 0.002$ for both) [20]. Subsequent studies, however, provided inconsistent results.

A study by Coccia et al. from 2010 failed to show a significant difference in the endometriosis recurrence observed in groups of women undergoing different types of ART treatment [26]. This study included women with laparoscopically confirmed endometriosis of any stage and at least 1 year history of infertility. Recurrence was defined by the presence of endometriotic cysts or nodules seen on transvaginal ultrasound in the period following ART. Similar to the previous study, women who underwent IUI only ($n = 34$), IVF only ($n = 36$), or IUI followed by IVF ($n = 20$) were compared regarding their cumulative endometriosis recurrence rate. Recurrence rates were not found to be significantly different between the three groups (IUI – 18%, IVF – 19%, IUI and IVF – 25%).

A third study evaluating the effects of IUI in women with surgically confirmed stage III-IV endometriosis was published in 2014 by van der Houwen et al. [27]. Unlike the previous two publications, this study compared the recurrence of endometriosis between women who underwent up to three cycles of natural cycle IUI (without any ovarian stimulation) followed by up to three cycles of gonadotropin-stimulated IUI ($n = 45$) with women who only underwent up to six cycles of gonadotropin stimulated IUI ($n = 20$). Endometriosis recurrence was defined as a recurrence or increase in the patient's complaints within 12 months of the last IUI cycle in women who did not become pregnant. The cumulative recurrence rate was 35% in the group of women who underwent at least one natural IUI cycle, and 72% in the group of women who only underwent gonadotropin-stimulated IUI ($p = 0.03$) [27].

The results of these three studies present conflicting data regarding the effect of ovarian stimulation on the rate of progression of endometriosis. The different comparisons in each publication, as well as the varying inclusion criteria and the inconsistent definition of recurrence between the studies, make a global interpretation difficult. The two publications that included women with moderate to severe endometriosis suggested that, unlike stimulation for IVF, IUI stimulation may have a detrimental effect on endometriosis [20, 27]. The one study that did not appreciate the difference between types of ART treatment included women with mild endometriosis diagnosed at surgery, but they utilized a follow-up method that is unable to detect superficial forms of endometriosis (transvaginal ultrasound) [26]. Therefore, it is possible that recurrences of these milder forms of endometriosis remain undetected.

Considering the findings and the methodologies of these three studies on the effects of ovarian stimulation for IUI, a detrimental effect of ovarian stimulation in the setting of IUI on endometriosis progression cannot be ruled out. When this

possibility is considered within the context of the relative lack of evidence to support the enhanced progression of endometriosis after IVF treatment, it becomes evident that the relationship between ovarian stimulation for ART and endometriosis is complex.

12 Emerging Issues and Ideas

12.1 Ovulation and Endometriosis Progression

The possibility that ovarian stimulation for IUI may negatively impact the progression of endometriosis in the absence of effects of IVF is a curious finding in the literature. Considering the lower estradiol levels and the lower number of developing follicles compared to IVF cycles, the opposite finding would have been expected. However, a review of current theories regarding the formation of endometriomas may provide a mechanism for this occurrence, separate from that of increased peripheral estrogen levels.

The *ovulation theory* is currently the most widely accepted theory regarding the formation of endometriomas. According to this theory, cells from superficial endometriotic deposits on the surface of the ovary invade a newly forming corpus luteum that is within close proximity to each other [28]. Inflammatory signaling involved in the attachment and growth of ectopic endometrial deposits is similar to those involved in the mechanism of follicular dehiscence required for ovulation [29]. This shared inflammatory mechanism preferentially promotes follicular dehiscence in close proximity to endometriotic lesions. Based on this theory, therefore, ovulation, or true follicular dehiscence, is required for endometrioma formation.

In IVF, multiple follicles are punctured and aspirated before spontaneous ovulation occurs [4]. This prevents follicular dehiscence and circumvents the invasion of developing corpus lutea by endometriotic cells. In IUI, however, follicular growth is enhanced through controlled ovarian hyperstimulation while the mechanism of ovulation proceeds normally. Therefore, where there might have only been one ovulatory site physiologically, stimulation for IUI may double or triple the number of areas of rupture that could permit the invasion of endometriotic cells.

Although the ovulatory theory of endometrioma formation provides a convenient and plausible explanation for the suggested increase in endometriosis progression seen in patients undergoing IUI, there is still very little evidence to support this relationship. Therefore, more studies investigating this association must be performed. It may also be possible that increased risk observed in IUI cycles may simply be related to the confounding effect of time. Women who undergo IUI usually allow more time to pass in their fertility journey than women who proceed straight to IVF, and this passage of time, therefore, would naturally result in a higher rate of endometriosis recurrence.

12.2 Protective Effects of Progesterone

Significant attention has been given to the increase in estrogen levels during IVF cycles and this potential impact on the progression and recurrence of endometriosis. However, while the rise in peripheral estrogens may be considerable during ovulation stimulation, these concentrations are only elevated for a few days [4]. Immediately following oocyte retrieval, peripheral progesterone concentrations rise considerably above physiological levels and remain elevated for the typical 14-day length of the luteal phase, and longer if pregnancy is achieved. As progesterone has been shown to have a protective effect regarding endometriosis, not only could this rise in progesterone counteract any short-term detrimental effect of hyperestrogenism from ovarian hyperstimulation, but it may actually serve to improve symptomatology and prevent progression given the longer exposure time.

The effect of prolonged progesterone exposure during IVF treatment has not been directly studied in the literature. However, the absence of de novo endometrioma development during prolonged oral contraceptive pill (OCP) and progestin use has been reported, and post-operative OCP exposure is associated with a reduction in the risk of endometrioma recurrence [6, 28]. The successful medical treatment of endometriosis with progestins and oral contraceptives further supports the assertion that supraphysiological progesterone levels during ART treatment can offer a protective advantage to women with endometriosis.

While the possibility of a protective effect of progesterone in ART has not been studied in the literature, it does provide a plausible explanation for why IVF treatments in women with endometriosis do not worsen symptom progression, and in some cases lead to subjective improvements in pain levels. Furthermore, there is a substantial variation in the administration of progesterone in the luteal phase of IUI cycles when compared to IVF cycles. Until quite recently, supplemental progesterone for luteal support was not provided in gonadotropin IUI cycles as it is in IVF cycles. Moreover, there can be variations in the endogenous levels of progesterone within both IUI and IVF cycles depending on the number of stimulated follicles that develop. Therefore, the trend for worsening endometriosis after IUI cycles in the absence of worsening IVF cycles may potentially be attributable to these differences in luteal phase progesterone exposure. The important role that progesterone exposure may play in mitigating endometriosis progression appears to have been severely overlooked in the literature and may, in fact, be an important consideration when assessing the overall effects of ART treatment in women with endometriosis. Without robust and well-designed studies assessing the effect of luteal progesterone administration in ART on endometriosis progression, definitive conclusions cannot be made. However, the effect of progesterone exposure in ART should not be overlooked.

13 Conclusion

Endometriosis is an estrogen-dependent disease, which lead to a postulation that the substantially elevated estrogen levels seen during IVF treatment would have a negative effect on the disease. In fact, this postulation was initially supported by alarming case reports in the literature, which correlated adverse endometriosis-related complications with recent IVF treatments. However, examination of the available studies that have been published since these initial case reports provides a moderate level of evidence to suggest that IVF has a rather unremarkable effect on endometriosis disease recurrence or progression.

The major premise linking the estrogen dependence of endometriosis to a detrimental effect of IVF simply because of transient elevations in estrogen levels is ostensibly simplistic and omits other critical concepts. These include the relationship between other pathophysiological etiologies of endometriosis, such as the role of ovulatory events and the effects of progesterone exposure, on endometriosis symptomatology and disease progression. Furthermore, the natural tendency for endometriosis to recur complicates the interpretation of the findings presented in the literature. It is difficult to discern between recurrences that are caused by ovarian stimulation or those that just coincidentally occurred after, or in close relation to, ART.

Regardless of the multiple factors that deserve consideration in the relationship between endometriosis and IVF treatment, a review of the current literature provides reassuring data on the limited impact of IVF on endometriosis recurrence or progression. Data regarding the effect of IVF on endometrioma growth is clinically unremarkable, and this evidence can be used to reassure women with endometriomas who are about to undergo IVF. However, data relating to the progression of deep invasive endometriosis is more limited. The emerging evidence is reassuring, but considering that call case reports of serious endometriosis-related complications occurring after IVF involve women with deep infiltrating lesions or rare, atypical forms of endometriosis, caution should be taken when such disease is present.

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Endometriosis-Related Complications in Women Undergoing In Vitro Fertilization

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1 Introduction

Of women with endometriosis with a childbearing desire, a considerable amount requires in vitro fertilization (IVF) due to primary infertility or subfertility [1]. A possible and neglected concern in this field is the potential risk of progression of the disease due to IVF treatment [2]. Physiopathologically, controlled ovarian hyperstimulation (COH) leads to the development of multiple follicles and a reasonable increase in serum estradiol levels. Endometriosis is an estrogen-related pathology, and the number of ovulatory events has been related to critically acting in the formation of ovarian endometriomas. To date, it cannot be excluded or even confirmed that IVF favors the progression of the disease [3].

Notably, although the impact of endometriosis on IVF outcome has attracted the interest of researchers for many decades, scanty evidence has been carried out to analyze the impact of IVF on endometriosis progression [4]. Actually, the available evidence is still contradictory.

As a consequence of high estrogen levels related to COH and IVF, endometriotic lesions may undergo an inflammatory process, which is supposed to lead to severe complications such as bowel occlusion in patients with intestinal lesions [5]. Furthermore, IVF procedures, including oocyte retrieval, have been linked to increased risk of infection, pelvic inflammatory disease (PID), and pelvic or tubo-ovarian abscess (TOA) [6]. Such conditions in women with endometriosis tend to be more severe and prolonged compared with those without endometriosis (Fig. 1) [7].

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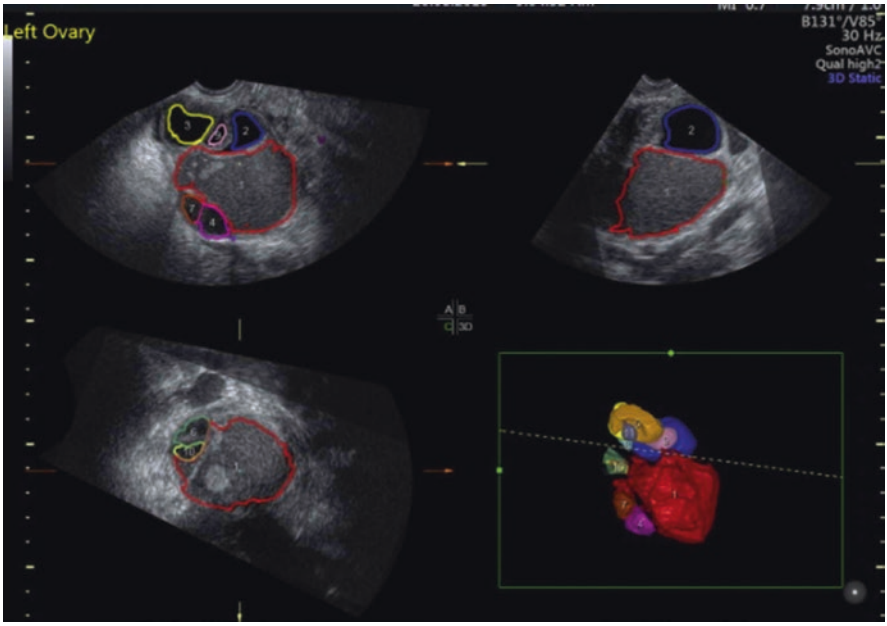

Fig. 1 Ultrasonographic view of an ovarian endometrioma during ovarian stimulation

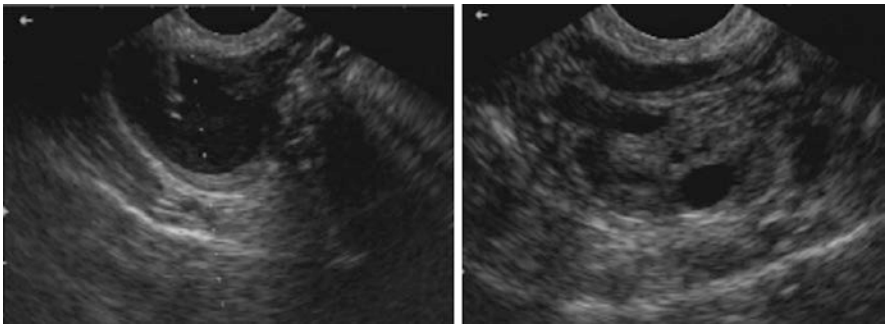

Fig. 2 Aspiration/sclerotization of ovarian endometrioma

This chapter will describe the potential endometriosis-related complications in patients undergoing IVF, and it will provide information on the counseling of patients at higher risk of complications.

2 ART with Endometrioma Present

The existence of one or more tiny endometriomas generates various concerns about a poor ART outcome; however, recent research has shown that these fears are unfounded. Surgery for endometrioma, on the other hand, severely decreases ovarian reserve, especially if endometriomas are bilateral [8].

Leaving endometriomas in place can make oocyte retrieval more difficult, as well as increase the risk of infection [9]. As a result, some writers propose aspirating the endometrioma before starting ART, stating that it improves outcomes (Fig. 2). However, several articles describe rapid endometrioma recurrence; therefore, this viewpoint is far from universal. Others have suggested sclerosing the endometrioma with alcohol injection for this reason, but this has not gained much traction [10].

Efforts should be made to avoid perforating the endometrioma during the oocyte retrieval technique. The endometriotic liquid is toxic to the oocyte without affecting fertilization rates directly, but it interferes with blastulation and embryo implantation. If endometriotic fluid is found in follicular fluid, the laboratory should be notified to avoid contaminating the entire cohort of oocytes extracted [10, 11].

3 Infections

The exact risk of an infectious complication following transvaginal oocyte retrieval in the presence of ovarian endometriomas is unknown [12]. The risk of infectious complications from ART administered while endometriomas are present is both under- and over-reported, according to a recent study. Over a 4-year period, women with endometriosis had an increased incidence of all acute pelvic infections, including tubo-ovarian abscesses and salpingitis, according to a retrospective analysis. Only three of the individuals who had had an ART procedure developed such disorders. As a result, in the absence of ART, an acute infectious complication of ovarian endometriosis might emerge spontaneously [12].

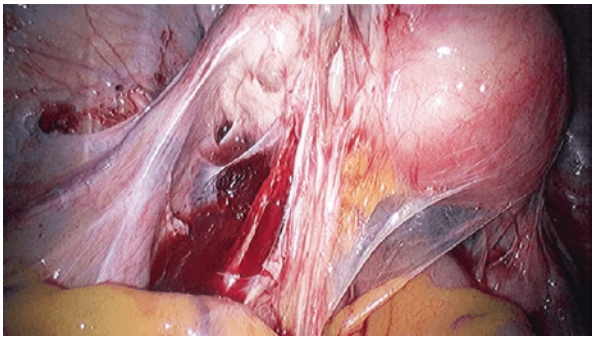

Fig. 3 Laparoscopic view of peritoneal adhesions related to PID

Late-onset infections in ART patients may therefore represent naturally occurring infections unrelated to ART, leading to an overreporting of problems [13].

However, a recent study indicated that women with endometriosis have more severe pelvic infections, which necessitate lengthy hospitalization and surgical intervention. PID and TOA were more likely to develop in endometriosis-affected women after infertility treatments, particularly IVF (Fig. 3) [12]. PID is more common in women with endometriosis than in the general population, according to Grammatikakis et al. [14], but their study only looked at women who had endometriotic ovarian cysts and were operated on. Their study cohort had a greater frequency of endometriosis (14%) than the reported prevalence of pelvic endometriosis (6–10%). One possible explanation is that the study participants were hospitalized women who are likely to have more severe endometriosis than the general population [14, 15].

Endometriosis' function in the development of pelvic infection could be explained in a variety of ways. TOA has been linked to ovarian endometriomas in the past. This could be related to the endometrioma's bleeding content, which acts as a culture medium for bacteria and aids infection spread [16]. Furthermore, because endometriosis is linked to infertility, many women with endometriosis undergo reproductive therapies, including IVF. These operations raise the chances of a pelvic infection. Indeed, it has been found that up to 4 weeks prior to their admission, 45% of hospitalized women with endometriosis had undergone some type of fertility technique, particularly IVF. This emphasizes the relevance of reproductive techniques and therapies in women with endometriosis as a risk factor for PID [17].

In a patient with US-suspected endometrioma, Benaglia et al.found an extraordinarily low incidence of TOA after IVF treatment and egg retrieval. The majority of the individuals in his study had unilateral small (3 cm) endometriomas. The severity of the condition was unknown because none of the ladies had undergone laparoscopies [18].

It is usually best to avoid puncturing the endometrioma during oocyte aspiration to reduce the possibility of endometrioma infection. Disinfecting the vaginal canal with povidone-iodine and sterile isotonic saline solution, as well as giving antibiotics throughout the surgery, could be explored [19, 20].

Second, in women with severe endometriosis, significant pelvic adhesions and the obliteration of the cul-de-sac may produce technical issues during oocyte retrieval. During follicle aspiration from a fixed ovary, there is also a risk of intestinal puncture. Prior to IVF, surgical excision of endometriosis implants and thorough adhesiolysis should theoretically reduce this problem. However, surgical morbidity and the high likelihood of endometriosis recurrence after surgery should be taken into account. As a result, the effectiveness of surgery in reducing the incidence of pelvic infection remains debatable [19, 20].

Interestingly, it is more difficult to understand the etiology in the absence of previous surgical procedures. We hypothesize two explanations for this.

First, these women may have had some kind of medical procedure more than 4 weeks prior to their admittance, resulting in a slowly growing illness with a

delayed clinical presentation. Second, the bloody contents of endometriosis implants, particularly endometriomas, may act as a culture medium for bacteria, allowing infection to spread without the need for an intrusive procedure. Escherichia coli colony development in menstrual blood from women with endometriosis was previously found to be greater than in control women. As a result, endometriosis-affected women's menstrual blood could be an additional source of infection. Furthermore, endometriosis "flare ups" may have symptoms that are similar to PID and could be a contributing factor in the increased incidence of endometriosis in PID patients [6].

PID is treated by starting broad-spectrum antibiotics against the most frequent pathogens as soon as possible, as stated in the preceding paragraphs. Clinical or microbiological cure in short-term studies determined the efficacy of these regimens, not the prevention of long-term sequelae. Outpatient oral antibiotics produced clinical outcomes equivalent to inpatient IV antibiotics in women with mild or moderate PID [6].

Because of the risk of abscess rupture and sepsis, women with TOA should be kept in a hospital for 24 h. Patients with clinically severe PID or who satisfy the aforementioned criteria should be hospitalized to the hospital and given antibiotics by parenteral injection. Medications for pain relief, nausea or vomiting, and fever should also be started as soon as possible after admission. If a patient is unable to accept oral intake, fluid resuscitation should be considered. The Centers for Disease Control and Prevention recommends the intravenous (IV) antibiotics listed below, which have been demonstrated to cure individuals with acute PID in more than 90% of cases:

- IV cefotetan or IV cefoxitin plus oral or IV doxycycline.
- IV clindamycin plus IV gentamicin.
- Alternative: ampicillin/sulbactam plus doxycycline.

In general, whether antibiotic therapy is combined with drainage or surgical excision of the TOA is determined by the patient's condition and the size of the abscess. As soon as the diagnosis of TOA is made, antibiotics should be started. Because of the morbidity and mortality associated with a ruptured TOA, immediate surgical intervention is necessary when it is suspected. Patients with signs of sepsis, such as hypotension, tachycardia, and tachypnea, as well as an acute abdomen, should be taken to the operating room very far away for surgical exploration [21].

4 ART and Endometriosis-Associated Pain

The impact of IVF on endometriosis-related pain symptoms and on ovarian endometriomas were the issues studied in most detail, both being investigated with at least two independent prospective studies. However, the data are not fully consistent. Even if the observational studies on pain symptoms failed to identify

detrimental effects (moderate-quality evidence), it could not be excluded that there may be a worsening of pain in some particular cases [22].

The effects of IVF on endometriosis-related pain symptoms and ovarian endometriomas were the most thoroughly researched topics, with at least two independent prospective studies conducted on each. The data, on the other hand, is not entirely consistent. Even if observational studies on pain symptoms failed to find negative effects (moderate-quality data), it is impossible to rule out the possibility of pain worsening in some circumstances [23]. The five women documented by Jun and Lathi (2007) who experienced discomfort increasing after ovarian stimulation support this theory [24].

Women with endometriosis did not have worsening pain or quality of life (QoL) during ART, according to a recent prospective study, when compared to women without endometriosis and women with endometriosis who did not have ART [23].

The worst nonmenstrual pelvic discomfort increased in both groups who had ART. Women without endometriosis became weary throughout ART, and nonmenstrual pelvic pain increased in general compared to the other groups. The relationship between pain and regulated ovarian stimulation or the presence of the ovarian corpus luteum in women with endometriosis could be one explanation [25].

Women without endometriosis had a worsening quality of life, whereas those with endometriosis had a slight improvement. This disparity could be attributed to the groups' differing approaches to EHP30, as well as the fact that EHP30 has not been validated for women without endometriosis. The variations in numeric rating scale (NRS) are not always clinically significant. These paradoxical changes, however, do not suggest a worsening of QoL in endometriosis patients following ART [23].

Santulli et al. discovered a comparable or lower level of pain indices when compared to a control group [25]. The non-endometriosis-specific FertiQoL questionnaire (Cardiff University, Cardiff, UK) was used to assess QoL at one time point, and no significant differences were detected when compared to women without endometriosis. It was not indicated whether the majority of women with deep infiltrating endometriosis had previously undergone surgery; therefore, it is unclear whether the lack of advancement in symptoms was related to earlier endometriosis excision. Several instances with an uncontrolled design have described dramatically worsened endometriosis with ART, notably in terms of intestinal endometriosis [25].

5 IVF and Worsening of the Pathology

When it comes to endometriomas, IVF does not appear to change their size significantly, although the evidence is not conclusive [26]. Indeed, two investigations from the same research group found no changes, while a third, unrelated study found a small but statistically significant increase in size. It can be inferred that the impact of IVF on endometrioma size is modest, if present at all, although the quality of data is poor, and more research is needed [4].

Independent studies back the data on the unremarkable effects of IVF on the rate of recurrences and those on the negative effects of intrauterine insemination (IUI), although the study designs have some flaws (none of them was prospective), and the data for IUI is not conclusive. For IVF and IUI, the evidence quality might be classified as moderate and low, respectively [4, 27].

Finally, it is worth mentioning that the most concerning potential side effect of ovarian stimulation, deep invasive endometriosis, is only substantiated by case reports (very low-quality evidence). More information about this subject is required [21, 28].

Because endometriosis is an estrogen-dependent illness, the lackluster results of IVF are surprising. Endometriosis growth may be aided by estrogen exposure, according to research. As a result, modern medical therapy for endometriosis still focuses on reducing blood estrogen levels. This conceptual discrepancy is difficult to explain, but the syllogism linking endometriosis' estrogen reliance to IVF's negative effects due to a marked rise in estrogens is apparently oversimplified. The most logical answer, in our opinion, is connected to the period of exposure. During ovarian stimulation, peripheral estrogens rise dramatically, reaching concentrations up to ten times greater (2000–4000 pg/ml) than in normal cycles [29–32].

However, these levels are only maintained for a few days, and progesterone levels often rise dramatically shortly after egg retrieval. It is possible that this could successfully and quickly counterbalance hyper-earlier oestrogenism's short-term negative effects, especially since high-dose progesterone is often recommended following ovarian stimulation to support the luteal phase [33].

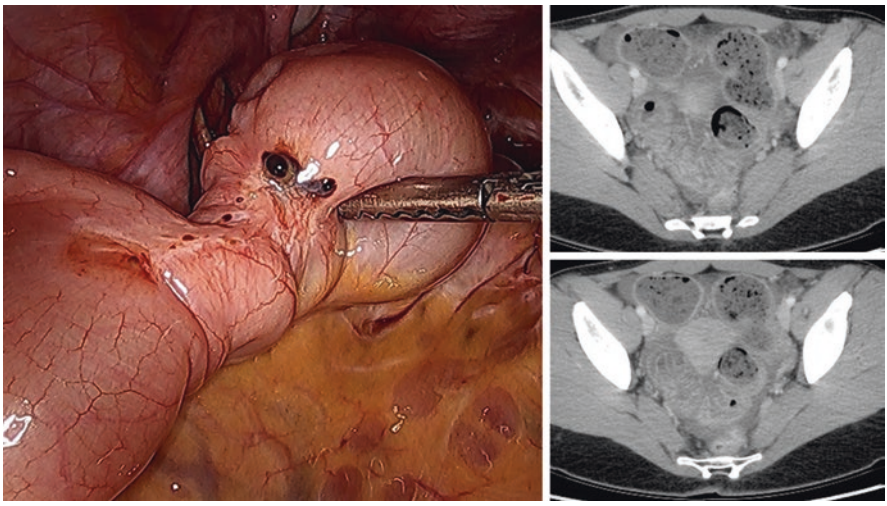

Fig. 4 Laparoscopic and magnetic resonance imaging view of bowel endometriosis

6 ART and Bowel Endometriosis

We can only hypothesize that severe decidualization after ART might weaken the intestinal wall and induce injuries during uterine growth because of the accompanying adhesions. Furthermore, it has been hypothesized that there is a subtype of deep endometriosis that reacts differently to the hormonal environment of pregnancy. If this is true, we believe that other organs known to be badly impacted by endometriosis, such as the bladder, ureter, small bowel, diaphragm, and lungs, could be harmed in the same way during pregnancy (Fig. 4) [34].

Because this is a rare but dangerous problem, we strongly urge using an international database to collect cases. This should lead to a better understanding of the problem and its prevention. Women with profound endometriosis should be aware that significant bowel issues can occur during the third trimester of pregnancy. Because it is likely underreported, the prevalence of this consequence is unknown. It is uncertain whether infertile women should undergo additional testing before starting ovulation induction or IVF because of this issue [34].

7 Endometriosis Recurrence after ART

The research on the effects of ovarian stimulation and ART on endometriosis development and recurrence is limited. Endometriosis' natural tendency to recur hampers the interpretation of the findings due to the intrinsic difficulties of distinguishing between recurrences triggered by stimulation and those that just happened to develop after ART treatment [35].

However, several concepts have emerged that should be considered in clinical practice. The positive results on IVF's low impact on endometriosis recurrence or pain symptom development, in particular, is backed up by moderate-quality research. Furthermore, the effect (if any) on the size of endometriomas may be clinically insignificant. This material can be utilized to reassure endometriosis patients who are considering IVF and are concerned about the potential hazards of ovarian stimulation [36].

There is currently insufficient evidence to suggest a negative effect. Interestingly, despite the small sample size, Benaglia's prospective investigation found no evidence of a significant increase in these lesions [29]. On this premise, the requirement for prophylactic surgery to halt advancement in women with deep invasive endometriosis appears to be unfounded and possibly inappropriate. Surgery for deep invasive endometriosis is technically challenging and potentially dangerous; it should only be performed if there is strong clinical evidence. In this light, it is also worth mentioning that there's no proof that preventive surgery improves the chance of getting pregnant after ART. Surgery to boost the chances of conception should currently only be considered if IVF fails [37, 38].

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Fertility Preservation in Endometriosis

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1 Introduction

Endometriosis typically affects women of reproductive age, and surgery may be required to relieve pain symptoms. However, the excision of ovarian endometriomas, even in the hands of expert surgeons, may reduce ovarian reserve [1]. Serum antimullerian hormone (AMH) significantly decreases after surgery [2, 3], and whenever necessary, second surgery for endometriomas significantly impairs ovarian reserve [4, 5]. Response to ovarian stimulation (OS) for IVF treatments is decreased after surgical treatment of endometriomas [6, 7]. Patients operated for bilateral endometriomas enter menopause earlier [8], and postsurgical ovarian failure may occur following the excision of bilateral endometriomas [9, 10]. Finally, endometriosis per se may have a detrimental effect on ovarian reserve [11–13].

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Fertility preservation (FP) aims to enhance a woman's chance of having biological children. It has been widely used in patients undergoing gonadotoxic treatment for malignant diseases [14]; however, a multitude of non-gynecological or gynecological conditions may impair the ovarian reserve, therefore requiring FP [15]. The techniques available for women seeking to preserve their fertility are oocyte cryopreservation (OOC), embryo cryopreservation (EC), and ovarian tissue cryopreservation (OTCP).

2 Oocyte Cryopreservation

OOC aims to obtain oocytes that can be cryopreserved and warmed later when the patient is ready to have offspring. It was initially developed for women requiring gonadotoxic treatments, but it is an efficient option for elective FP in women with benign gynecological diseases, such as endometriosis. The main advantages are the relatively low invasiveness and lack of detrimental effects on the ovarian reserve; however, compared with EC, it also allows patients to preserve unfertilized oocytes giving more freedom for the future partner decision. OOC, once deemed experimental, has been increasingly used in the last ten years with the use of cryoprotectants and cryotools in combination with rapid cryopreservation techniques and fertilization with intracytoplasmic sperm injection (ICSI). In fact, vitrification led to an improvement in the OOC. It consists of ice-free solidification of an aqueous solution by ultra-rapid cooling, and it has several advantages over the slow freezing procedure, including quickness, ease, cost-effectiveness, and no ice crystal formation usually associated with physical and mechanical cell injury [16].

OOC in endometriosis was initially proposed by Elizur et al. in a 25-year-old nulliparous woman with persisting pain despite four previous surgeries (including right oophorectomy). The patient underwent three ovarian OS and oocyte aspiration; 21 mature oocytes were vitrified and cryopreserved [17]. A retrospective, multicenter, observational study investigated the results of OS for oocyte vitrification in 560 non-oncological patients and 475 oncological patients. Thirty-eight out of 560 (6.8%) women with a non-oncological indication for FP suffered from endometriosis. Five patients returned to use their own oocytes; however, the precise characteristics of the patients and the outcome of the five thawed cases were not reported in detail. A retrospective observational cohort study published as a letter to the editor investigated the results of oocyte vitrification in women with endometriosis [18]. Forty-nine women who underwent 70 OS cycles were included in the study. The mean patient age was 33.9, and OS was performed using GnRH antagonist or long agonist protocols. Thirty-three patients had one cycle, and 16 had $\geq$2 cycles, leading to a mean number of oocytes cryopreserved of 10.7 ± 4.9 per patient (median 9, range 1–25). The mean number of recovered and mature oocytes per cycle was 9.5 ± 6.1 and 7.2 ± 4.9, respectively. These parameters were significantly lower in patients reporting previous endometrioma excision when compared with those without ovarian surgery. No differences were noted in the mean duration of OS and

total dose of gonadotropin used between patients with different endometriosis phenotypes.

Another multicenter retrospective observational study investigated FP outcomes using vitrified oocytes in patients with endometriosis [19]. One thousand 44 women had their oocytes vitrified, and 485 women were included in the study because they attempted pregnancy using their own vitrified oocytes (return rate 46.5%). This high rate of women thawing their gametes is one of the most striking results of the study [20]. The criteria for inclusion in the study were age up to 42 years, endometrioma larger than 1 cm in mean diameter with apparently healthy ovarian tissue visible at the ultrasound, antimullerian hormone (AMH) level > 0,5 ng/mL; and more than three antral follicles. The mean age at the time of vitrification was 35.7 ± 3.7 years. 97.7% of the patients had stage III-IV endometriosis. 47.8% of the patients had FP after removing their endometrioma (34.9% had bilateral cystectomy, and 65.1% had unilateral surgery). The most frequently used protocol for OS was the GnRH antagonist. A mean of 7.1 ± 6.5 oocytes was retrieved per cycle, and 12.0 ± 8.1 oocytes were retrieved per patient. The number of vitrified oocytes per cycle (6.2 ± 5.8) was higher for the nonsurgical patients compared with the unilateral (5.0 ± 4.5) or bilateral (4.5 ± 4.4) surgery groups but was comparable among the surgical patients. The mean storage time was 1.7 ± 0.4 years. The mean age at warming was 37.3 ± 2.1 years. The overall oocyte survival rate was 83.2%. 22% of the embryo transfers were canceled because of the absence of chromosomally normal embryos, absence of viable embryos, fertilization failure, deferred embryo transfer, and oocyte survival failure. Two hundred 25 babies were born with a 46.4% cumulative live birth rate (CLBR) per patient. There was no significant difference in the antral follicle count, number of oocytes retrieved and MIII vitrified, embryo quality, survival rate, pregnancy rate, and CLBR between patients with stage I-II endometriosis and those with stage III-IV. However, these parameters were significantly different when analyzed based on patient age at oocyte vitrification (≤ 35 vs. > 35 years). The number of oocytes retrieved and MIII oocytes vitrified was higher in women without ovarian surgery before FP. Despite the statistically significantly lower number of oocytes obtained in the surgical group, the survival rates and clinical outcomes (including the CLBR) were comparable between surgical and nonsurgical patients. Also, the authors compared their findings with cancer patients' findings [14]. In the younger group of women aged ≤35, the oocyte survival, implantation, pregnancy, and CLBR were significantly lower for endometriosis patients than for young cancer women. The authors concluded that young women are the best candidates for FP before surgery because they would need fewer OS cycles due to a better ovarian reserve and a better reproductive prognosis. Conversely, FP in older women is not as effective, regardless of whether they had surgery. A retrospective study investigated the clinical characteristics and cycle outcome of OOC for FP in women with ovarian endometriosis before ovarian cystectomy [21]. Thirty-four women were included in the study. The mean age of the study population was 30.7 ± 5.9 years. The mean basal AMH was 1.85 ± 1.14 ng/mL. The mean diameter of the largest endometrioma was 6.0 ± 2.5 cm; multiple endometriotic cysts were present in 17.6% of patients. 13 patients (38.2%) underwent OS more than once. The mean number of oocytes

retrieved was 6.3 ± 4.3, the mean number of mature oocytes retrieved was 4.1 ± 3.1, and the mean number of oocytes cryopreserved was 4.8 ± 3.2. The percentage of mature and cryopreserved oocytes was 65.8% and 77.3%, respectively. Overall, 18 women with bilateral endometrioma underwent 28 OS, and 16 women with unilateral endometrioma underwent 22 OS cycles. The percentage of mature oocytes was significantly lower in patients with bilateral endometriomas than those with unilateral endometriomas. The number of oocytes cryopreserved was lower in the bilateral endometrioma group compared with the unilateral endometrioma (4.1 ± 2.9 versus 5.7 ± 3.4), but the difference did not reach statistical significance. Repeated OS in women with endometrioma did not affect the number of oocytes cryopreserved. A retrospective observational study investigated how the number of oocytes used affects the CLBR in endometriosis patients who have their oocytes vitrified for FP [22]. The study included 485 patients with endometriosis who underwent 840 cycles. The CLBR increased as the number of oocytes used per patient rose, reaching 89.5% using 22 oocytes. Higher outcomes were observed in young women (≤35 years old versus >35 years old). The cumulative live-birth rate in the younger group was 95.4% using approximately 20 oocytes, versus 79.6% in older women. An observational cohort study investigated the prognostic factors related to high oocyte yield in FP for women with endometriosis [23]. The study included 146 women who underwent 258 OS cycles. The mean age of the study participants was 31.5 ± 4.4 years. Eighty-two women (56.2%) underwent more than one OS cycle. Fourteen OS cycles (5.4%) were canceled due to the absence of an adequate ovarian response. A mean of 8.4 ± 6.8 oocytes was retrieved after the first cycle. The mean total number of oocytes retrieved per woman was 13.6 ± 8.2. The study showed that previous history of surgery for endometriosis, women's age, and total dose of gonadotropin were associated with a reduced number of oocytes retrieved. In contrast, serum AMH level and gravidity positively correlated with an increase in the number of oocytes retrieved. BMI, smoking habits, history of infertility, absenteeism from school during menstruation, oral contraceptive use, and the endometriosis phenotype were not associated with the number of oocytes retrieved. Another retrospective study evaluated the efficacy of FP in women with endometrioma before planned ovarian surgery [24]. Ninety-five cycles were performed in 62 patients with endometrioma. OS was performed using a GnRH antagonist protocol. The maximum number of cycles performed in a single patient was 4, and 34.7% of cases were treated with OS more than once. The median number of retrieved oocytes was 5.0, the median number of mature oocytes was 3.0, and the maturation rate was 58.3%. Patients with unilateral endometriomas had better embryo quality than those with bilateral cysts. One more retrospective cohort study investigated the outcome of FP in women with endometriomas [25]. Seventy-one women with ovarian endometriomas underwent 138 FP cycles. The median age of patients was 31 years (range 29–35). Forty out of 71 (56%) women underwent at least one surgery for endometrioma before FP treatment. Women who underwent endometrioma surgery before FP treatment had a 51.7% reduction in the number of MII oocytes compared with women with endometrioma who did not undergo surgery. The median AMH concentration was significantly lower in those who had prior surgery; they required

significantly higher doses of gonadotrophins during OS, and peak estradiol concentrations at ovulation induction were also lower. Among a subgroup who did not undergo surgery, those with an endometrioma larger than 4 cm had similar AMH concentration, number of oocytes retrieved, and number of MII oocytes compared with women with an endometrioma of 4 cm or less. A further observational cross-sectional study investigated factors and patient symptomatology affecting ovarian response in women with endometriosis who seek FP [26]. Eighty-one women were included in the study; their mean patient age at the time of FP was 35.2 (± 4.9) years. Endometrioma was present in 72% of the women, of whom 63% had bilateral endometrial cysts, and 37% had unilateral endometrial cysts. Endometriomas were excised before FP in 45.6% of the women. The examined reported symptoms were lethargy, chronic pelvic pain, dyschezia, dyspareunia, bowel-associated symptoms, and urinary tract symptoms. Dysmenorrhea was the most common symptom, presenting in 81% of the participants. The GnRH antagonist protocol was the most frequently used OS protocol (97.5% of cycles). The mean accumulated number of oocytes vitrified per patient was 16.7 (± 12.1) oocytes. The correlation coefficient assessed between the number of oocytes vitrified per cycle, and AMH was significantly positive. The authors observed a significant negative association between the number of clinical symptoms and the number of vitrified oocytes. AMH was found to have the highest correlation with treatment success in patients with endometriosis undergoing FP.

2.1 Ovarian Stimulation for Fertility Preservation

FP in women with endometriosis has been performed using OS protocols initially developed for assisted reproduction technology. Women treated with hormonal therapies to alleviate pain symptoms may suffer a reduction in the number of oocytes retrieved after OS and from a suboptimal response to gonadotropin-releasing hormone agonist trigger [27, 28]. There is insufficient data to determine if women should discontinue hormonal therapies before the OS. Some authors pragmatically suggested starting OS for FP 2–3 months after stopping hormonal therapies [29].

No specific OS protocol is recommended for FP in women with endometriosis [29]. When the GnRH antagonist protocol is used, the ovulation can be triggered with the GnRH agonist, which has two advantages over the hCG-trigger of the ovulation: first, a reduced risk of ovarian hyperstimulation syndrome and, second, a lesser impact on pain symptoms due to rapid luteinization phase [30]. A recent prospective cohort study compared a GnRH antagonist protocol with a progestin-primed ovarian stimulation (PPOS) in terms of FP outcome (retrieved and vitrified oocytes) and cost-effectiveness [31]. Patients on long-term oral progestin treatment who chose the antagonist protocol had to stop their progestin treatment. The protocol was started on the first or second day of a natural cycle. For the PPOS protocol, patients could continue their long-term oral progestin treatment and start the protocol when they wished. Patients in the PPOS protocol without long-term oral

progestin treatment started an oral treatment by desogestrel at the same time as OS on the first day of a natural cycle. The study included 108 women who had a single OS performed with either a GnRH antagonist ($n = 54$) or a PPOS protocol ($n = 54$). No significant differences were observed between the PPOS and GnRH antagonist protocols regarding the total dose of gonadotrophin, duration of treatment, trigger method, E2, and progestin levels on the trigger day. Furthermore, the two protocols had no significant difference in the number of oocytes retrieved and vitrified. In the PPOS group, different types of progestin were used, but there was no statistical analysis between subgroups. The PPOS protocol was significantly cheaper than the other one.

3 Embryo Cryopreservation

EC consists of the cryopreservation of embryos. It requires the male gamete (rather than the partner or sperm donor) and has ethical and legal implications for death or separation. A retrospective cohort study analyzed the outcome of a combination treatment of preoperative EC and laparoscopic surgery in 39 infertile women with decreased ovarian reserve with uterine fibroids ($n = 36$) and/or ovarian endometriomas ($n = 16$) [32]. Patients underwent embryo freezing, 2–4 months of preoperative treatment with GnRH analogs, laparoscopy, and embryo transfer. One patient obtained no embryo after oocyte retrieval and gave up conceiving. Fourteen patients experienced childbirth, and 24 patients experienced implantation failure or miscarriage. Preoperative frozen embryos were significantly higher in the success than failure groups. The 14 women who underwent successful surgery-assisted reproductive technology (ART) hybrid therapy were younger and had a larger number of cryopreserved embryos than the 24 who experienced hybrid therapy failure. There were no significant differences in the size of endometriomas and severity of endometriosis between the patients who conceived and those who did not conceive. A case report described a live birth following FP in a young woman with iatrogenic infertility due to endometriosis mistaken for rectosigmoid cancer [33]. A 30-year-old patient had a 5 cm rectosigmoid lesion diagnosed as locally advanced rectal cancer. The patient was recommended neoadjuvant chemotherapy with capecitabine and high-dose pelvic radiotherapy. Before treatment, the patients underwent OS; 5 oocytes were obtained, and four embryos were cryopreserved. Following neoadjuvant therapy, the patient underwent laparotomy with anterior rectum resection and bilateral salpingo-oophorectomy. Histopathologic examination of the resected specimen revealed no evidence of malignancy but clear signs of endometriosis and fibrosis. The patient attempted a transfer of a frozen-thawed embryo 2.5 years after surgery. At the third transfer, the patient conceived.

4 Ovarian Tissue Cryopreservation

OTCP is currently used to preserve fertility in young women facing chemotherapy or radiotherapy who are at high risk of losing ovarian function and cannot delay treatment and undergo OS. The technique usually involves one-sided surgical removal of ovarian cortical tissue or complete oophorectomy. The harvested cortical tissue is dissected into thin (1–2 mm) strips measuring 0.5×1 cm^2, which are frozen for future transplantation. Primordial follicles are located in a poorly vascular environment and are relatively resistant to ischemia. After thawing, the ovarian cortical strips may be grafted into orthotopic sites (such as the atrophic ovary), allowing recovery of endocrine function and spontaneous pregnancy, or into heterotopic sites (such as the subcutaneous space of the abdominal wall), allowing recovery of endocrine function. Approximately 20 years ago, Schubert et al. demonstrated the feasibility of OTCP surrounding benign ovarian cysts, including endometrioma [34]. This approach is not recommended in patients with endometriosis because it may deteriorate ovarian reserve and carries significant risks in patients with distortion of pelvic anatomy and adhesions caused by endometriosis [35]. However, healthy ovarian cortex fragments can be isolated and cryopreserved during the surgical removal of endometriomas. The tissue surrounding the cyst or the pseudocapsule should not be stored because of the oocytes' poor number and low quality.

Few reports described ovarian cortex freezing in women with endometriosis. In 2005, Donnez et al. first reported two cases of fresh ovarian tissue orthotopic transplantation in two patients with severe endometriosis who underwent left oophorectomy for recurrent endometriosis [36]. Before removal of the ovary, strips of 3–4 by 12 mm of ovarian cortex were taken from residual healthy ovarian tissue. A peritoneal window was created beneath the contralateral ovarian hilus close to ovarian blood vessels. Two strips of fresh ovarian cortex were placed in the peritoneal window and covered with interceed. Three months after the graft, the patients underwent second-look laparoscopy. In the grafted area, macroscopically viable-looking ovarian tissue was visible. Biopsies were taken; the histological exam revealed primordial follicles and active angiogenesis. One patient became pregnant on the third IVF attempt. In a prospective longitudinal analysis of 59 women who underwent OTCP, Oktay, and Oktem reported a single case of a woman who underwent OTCP at 28 years of age and who subsequently had the ovarian fragments transplanted into the left pelvic peritoneum 1 year later when she was in menopause. The woman had regular ovulatory cycles at her last follow-up 9 months after the intervention [37]. A case report described the outcomes of cryopreserved ovarian tissue transplantation performed in a patient affected by struma-ovarii associated with mature cystic teratoma, recurrent endometriotic cysts, and diffuse peritoneal malignant struma-ovarii implants [38]. The patient was submitted to radioiodine therapy for metastases and experienced premature ovarian failure. Ten years after cryopreservation, the first orthotopic transplantation was performed in the left ovary and in a peritoneal pocket, but there was no recovery of ovarian function. Then, three years later, a second transplantation was performed heterotopically in abdominal subcutaneous sites.

Few small follicles were observed at ultrasound, but hormonal levels remained at menopausal values. In a recent report, ovarian cortex freezing was performed in two women aged 21 who underwent excision of large endometriotic cysts [39].

5 Conclusions

The number of original studies looking at FP technologies' effectiveness in women with endometriosis is relatively low [40]. EC and OOC are the most common FP techniques used in patients with endometriosis. OOC preserves a woman's reproductive autonomy, allowing a patient to procreate with a chosen partner in the future [41]. Clinical data on OTCP in women with endometriosis are very scanty [36, 37]. It requires two surgical procedures: the first to harvest the ovarian tissue and the second for transplantation. Therefore, OOC and EC should be preferred to OTCP because of more solid evidence. In addition, OOC and EC do not harm ovarian reserve and have low morbidity compared with ovarian cortex freezing.

A European survey investigated the existence of local guidelines regarding endometriosis surgery and FP to evaluate if centers/hospitals offer this possibility, and if so, which techniques are used and how these are performed [42]. Of 58 responses, 45 (77.6%) in 11/13 countries had endometriosis management guidelines, of which 37/45 (82.2%) included treatment recommendations for infertile patients. Most centers (51.7%) reserved fertility counseling for patients with severe endometriosis (large endometriomas with or without deep endometriosis), while 15.5% did not offer FP for endometriosis.

There is a lack of clinical consensus and guidelines for identifying patients with endometriosis who should be counseled to consider FP [41]. The recent ESHRE guideline state that, in the case of extensive ovarian endometriosis, clinicians should discuss FP in women with endometriosis, although the true benefit of FP in women with endometriosis remains unknown [43]. The National Specialty Commission in Endometriosis of the Brazilian Federation of Gynecology and Obstetrics Associations (FEBRASGO) suggests that FP (EC and OOC) should be considered in women with unoperated bilateral endometriomas and those who have previously removed unilateral endometriomas and need surgery for a contralateral recurrence [44]. The Collège National des Gynécologues et Obstétriciens Français (CNGOF) and the Haute Autorité de santé (HAS) propose that FP by OOC should be performed in case of recurrent endometriomas, repetitive surgery for endometriosis, bilateral endometriomas (independently from their diameter), and large unilateral endometriomas ($\geq$ 5 cm) [45].

Drawing clear recommendations for FP in women with endometriosis represents a complex task. Reproductive counseling should be an integral part of endometriosis management, and it should be performed in patients with advanced disease and those with early/mild stages of disease [29]. However, no evidence exists that FP should be systematically recommended to all women with endometriosis. Predicting future fertility and the likelihood of requiring ART for conceiving are complex tasks

in the context of endometriosis, especially in women who have never tried to conceive [29]. Size of endometrioma, bilaterality, previous surgery, and age are the obvious candidates for determining the risk of infertility [46]. In patients undergoing surgery, the risk of ovarian reserve impairment differs widely between women undergoing surgery for small unilateral endometriomas and those with large bilateral cysts [47].

However, some limiting points for FP with endometriosis, a relatively common condition [48], are that egg banking is expensive, and women undergoing FP may be exposed to some clinical risks [20]. Furthermore, oocyte retrieval can be challenging in patients with advanced endometriosis because of anatomical distortion and adhesions, and it may be associated with a theoretical risk of pelvic abscess [49]. While a pelvic abscess may not significantly damage the spontaneous fertility of an infertile woman, it may harm the spontaneous fertility of a woman with unknown fertility status. Therefore, including all patients diagnosed with endometriosis in an FP program would have profound clinical, logistic, and financial effects [15].

It would be relevant to understand the magnitude of the benefit of performing FP in various subgroups of women with endometriosis. Some women may spontaneously conceive [50, 51], and other patients may conceive by intrauterine insemination (IUI) or IVF without egg banking. FP will be unnecessary for many patients, particularly young ones with normal or high ovarian reserve [52]. Thus, many young women undergoing FP might never need their oocytes. Individualized counseling should include the patient's age, familial history of premature ovarian insufficiency, markers of ovarian reserve, smoking, presence of endometriomas, previous surgery for endometriomas, extent and progression of endometriosis, the need for extensive surgery involving the ovaries [18]. FP should be offered before surgery [18, 19, 22, 25]. In fact, after the excision of endometriomas, because of the decline in ovarian reserve, the patients may require several OS to achieve a satisfying number of oocytes [29]. Repeated preservation cycles for oocyte accumulation can be costly and could potentially have a physical and psychological impact. EC and OOC should not be proposed before surgery in women with sub-occlusive bowel endometriosis and hydronephrosis because the OS may worsen these conditions [53].

FP is particularly indicated for women facing a consistent risk of bilateral ovarian damage (i.e., women with bilateral endometriomas and those operated unilaterally with a contralateral recurrence) [15]. The age of the patient is particularly relevant in choosing to perform FP. As female age increases, more oocytes are required to reach live birth [54]. Women diagnosed with ovarian endometriosis at a young age, who are not expected to seek pregnancy in the short-medium term, may be good candidates for FP because the quality of the banked oocytes is expected to be higher. Financial aspects also influence the decision when FP is not reimbursed (by national health systems or insurance).

Patients must be informed that FP is no guarantee of pregnancy and that the procedure's success depends on the number of mature oocytes cryopreserved and the age of the patients at the time of OS.

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